Endocrinology
Series Editor: Andrea Lenzi
Series Co-Editor: Emmanuele A. Jannini

SPRINGER
REFERENCE

Felice Petraglia · Bart C. Fauser
Editors

Female Reproductive Dysfunction

Springer

Endocrinology

Series Editor

Andrea Lenzi
Department of Experimental Medicine
Section of Medical Pathophysiology
Food Science and Endocrinology
Sapienza University of Rome
Rome, Italy

Series Co-Editor

Emmanuele A. Jannini
Department of Systems Medicine
University of Rome Tor Vergata
Rome, Italy

Within the health sciences, Endocrinology has a unique and pivotal role. This old, but continuously new science is the study of the various hormones and their actions and disorders in the body. The matter of Endocrinology are the glands, i.e., the organs that produce hormones, active on the metabolism, reproduction, food absorption and utilization, growth and development, behavior control, and several other complex functions of the organisms. Since hormones interact, affect, regulate, and control virtually all body functions, Endocrinology not only is a very complex science, multidisciplinary in nature, but is one with the highest scientific turnover. Knowledge in the Endocrinological sciences is continuously changing and growing. In fact, the field of endocrinology and metabolism is one where the highest number of scientific publications continuously flourishes. The number of scientific journals dealing with hormones and the regulation of body chemistry is dramatically high. Furthermore, Endocrinology is directly related to genetics, neurology, immunology, rheumatology, gastroenterology, nephrology, orthopedics, cardiology, oncology, gland surgery, psychology, psychiatry, internal medicine, and basic sciences. All these fields are interested in updates in Endocrinology. The aim of the MRW in Endocrinology is to update the Endocrinological matter using the knowledge of the best experts in each section of Endocrinology: basic endocrinology, neuroendocrinology, endocrinological oncology, pancreas with diabetes and other metabolic disorders, thyroid, parathyroid and bone metabolism, adrenals and endocrine hypertension, sexuality, reproduction, and behavior.

More information about this series at http://www.springer.com/series/14021

Felice Petraglia • Bart C. Fauser
Editors

Female Reproductive Dysfunction

With 41 Figures and 40 Tables

Springer

Editors
Felice Petraglia
Department of Biomedical, Experimental and Clinical Sciences, Division of Obstetrics and Gynecology
University of Florence
Firenze, Italy

Bart C. Fauser
Department of Reproductive Medicine and Gynecology, University Medical Center Utrecht
University of Utrech
Utrecht, The Netherlands

ISSN 2510-1927 ISSN 2510-1935 (electronic)
ISBN 978-3-030-14781-5 ISBN 978-3-030-14782-2 (eBook)
ISBN 978-3-030-14783-9 (print and electronic bundle)
https://doi.org/10.1007/978-3-030-14782-2

This Springer imprint is published by the registered company Springer Nature Switzerland AG.
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland.

Series Preface

Is there an unmet need for a new MRW series in endocrinology and metabolism? It might not seem so! The vast number of existing textbooks, monographs and scientific journals suggest that the field of hormones (from genetic, molecular, biochemical and translational to physiological, behavioral, and clinical aspects) is one of the largest in biomedicine, producing a simply huge scientific output. However, we are sure that this new Series will be of interest for scientists, academics, students, physicians and specialists alike.

The knowledge in endocrinology and metabolism almost limited to the two main (from an epidemiological perspective) diseases, namely hypo/hyperthyroidism and diabetes mellitus, now seems outdated and closer to the interests of the general practitioner than to those of the specialist. This has led to endocrinology and metabolism being increasingly considered as a subsection of internal medicine rather than an autonomous specialization. But, endocrinology is much more than this.

We are proposing this series as the manifesto for "Endocrinology 2.0", embracing the fields of medicine in which hormones play a major part but which, for various historical and cultural reasons, have thus far been "ignored" by endocrinologists. Hence, this MRW comprises "traditional" (but no less important or investigated) topics: from the molecular actions of hormones to the pathophysiology and management of pituitary, thyroid, adrenal, pancreatic and gonadal diseases, as well as less common arguments. Endocrinology 2.0 is, in fact, the science of hormones, but it is also the medicine of sexuality and reproduction, the medicine of gender differences and the medicine of well-being. These aspects of Endocrinology have to date been considered of little interest, as they are young and relatively unexplored sciences. But, this is no longer the case. The large scientific production in these fields coupled with the impressive social interest of patients in these topics is stimulating a new and fascinating challenge for endocrinologists.

The aim of the MRW in Endocrinology is thus to update the subject with the knowledge of the best experts in each field: basic endocrinology, neuroendocrinology, endocrinological oncology, pancreatic disorders, diabetes and other metabolic disorders, thyroid, parathyroid and bone metabolism, adrenal and endocrine

hypertension, sexuality, reproduction and behavior. We are sure that this ambitious aim, covering for the first time the whole spectrum of Endocrinology 2.0, will be fulfilled in this vast Springer MRW in Endocrinology Series.

Andrea Lenzi, M.D.
Series Editor
Emmanuele A. Jannini, M.D.
Series Co-Editor

Volume Preface

The female reproductive system is made up of a complex of anatomical and physiological structures that have the purpose of controlling the menstrual cycle and the mechanism of reproduction.

At the base of the female reproductive system is the hypothalamic-pituitary-ovary axis. The hypothalamus releases GnRH (gonadotropin-releasing hormone) which in turn stimulates the secretion of LH (luteinizing hormone) and FSH (follicle-stimulating hormone) at the pituitary level. These hormones then induce the gonadal secretion of estradiol, progesterone, inhibins, and testosterone, which play an important role in the regulation of menstrual cycle.

The disorders of the menstrual cycle may be changes in its rhythm or in the flow (bleeding, pain), which may be episodic, caused by periods of excess fatigue, stress, or emotional tension, or repeat periodically and be an indication of possible hormonal or systemic problems.

The present volume describes the dysfunction of the menstrual cycle on hormonal basis: polycystic ovary syndrome (PCOS), premature ovarian insufficiency (POI), prolactin secreting pituitary adenoma, and thyroid disorders. Also systemic disorders like coagulation defects or metabolic diseases may explain menstrual cycle dysfunction. The therapeutic use of oral hormonal contraceptives is discussed.

Endometriosis is the major disease characterized by menstrual cycle-related pain (dysmenorrhea) and its clinical management is described in a separate chapter. Abnormal uterine bleeding (AUB) is another topic treated in great detail, from the classification to the clinical management. Infertility is visited in all the diagnostic and therapeutic approaches, assisted reproductive technologies (ART).

Menopause is the physiological period of a woman which corresponds to the cessation of the childbearing age and symptoms are linked to the reduction of estrogen production, such as palpitations, intolerance to heat, sudden redness, anxiety, and depression, leading in the long term to a greater risk of cardiovascular diseases and alterations of bone metabolism.

The present book also treats the endocrinology of maternal-placental axis. The hormonal production of the placenta is fundamental for the establishment and maintenance of pregnancy. The trophoblast secretes into the maternal circulation steroids and trophic peptides that are essential for fetal development, for pregnancy

maintenance, and parturition. A chapter is also dedicated to the breast disorders and lactation.

We hope that gynecologists/obstetricians and endocrinologists will benefit from the present book, entering into the exciting field of physiology and pathology of the main reproductive pathologies, also encouraging future research in the field.

Firenze, Italy — Prof. Felice Petraglia
Utrecht, The Netherlands — Prof. Bart C. Fauser
May 2020

Contents

About the Editors

Felice Petraglia is Professor and Chairman of Obstetrics and Gynecology and Director of the Residency Program in Obstetrics and Gynecology at the University of Florence (Florence, Italy).

Prof. Petraglia is President of the Society of Endometriosis and Uterine Disorders (SEUD) and fellow ad eundem of the Royal College of Obstetricians and Gynaecologists (RCOG). He was President of the Society for Gynecologic Investigation (SGI) (2008–2009), member of the Executive Board of the International Society of Gynecological Endocrinology (ISGE), and member of the International Federation of Gynecology and Obstetrics (FIGO) and of the European Board and College of Obstetrics and Gynecology (EBCOG).

Prof. Petraglia has received the award for the best publications of the Endocrine Society (2010) and the Award of the Society Reproductive Investigation (2014).

He is Editor of *RBMO* and is Associate Editor of *Reproductive Science and Faculty 1000 Medicine* (Section Head in Reproductive Endocrinology).

He was Editor-in-Chief of *Journal of Human Reproduction Update* (2013–2018) and of *Journal of Endometriosis* (2009–2012).

Prof. Petraglia is author or co-author of more than 920 peer-reviewed papers in Scientific International Journals (IF: 19886, HI:69) and co-editor of 27 scientific volumes.

Bart C. Fauser is a gynecologist and Em. Professor of Reproductive Medicine, former Head of the Department for Reproductive Medicine and Gynaecology and Chair of the Division of Woman and Baby at the University of Utrecht and the University Medical Center Utrecht, The Netherlands.

He is a Fellow ad eundum of the Royal College of Obstetrics and Gynaecology, Honorary member of ESHRE, Knight in the order of the Netherlands Lion, member of the (US) National Academy of Medicine, member of the board of the Dutch Medical Research Counsil (ZonMW), Editor-in-Chief of *Reproductive BioMedicine Online* (RBMO), and Executive Board Member of International Federation of Fertility Societies (IFFS).

He previously held posts as a Fulbright scholar at the University of California, San Diego; visiting professorships at Stanford University School of Medicine, California, USA; Free University, Brussels, Belgium; University of Siena, Italy; University of Southampton, UK; and the University of Adelaide, Australia. He was a past chair of the World Health Organization (WHO) steering committee for infertility guidelines and past Editor-in-Chief of *Human Reproduction Update*.

His major research interests include the pathophysiology of human ovarian function, PCOS, POI, IVF, and women's health. He has published over 450 peer-reviewed articles (Hirsch factor > 100), and his work has been widely covered in the national and international lay press.

Contributors

Sarah L. Berga Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, University of Utah School of Medicine, Salt Lake City, UT, USA

Nicoletta Biglia Gynaecology and Obstetrics, Umberto I – Mauriziano Hospital, Turin, Italy

Gynaecology and Obstetrics, University of Turin School of Medicine, Turin, Italy

Valentina Elisabetta Bounous Gynaecology and Obstetrics, Umberto I – Mauriziano Hospital, Turin, Italy

M. L. Brandi FirmoLab Fondazione F.I.R.M.O, Florence, Italy

University of Florence, Metabolic Bone Diseases Unit, University Hospital of Florence, Florence, Italy

Vincenzina Bruni University of Florence, Florence, Italy

Dante Campana Department of Biomedical, Experimental and Clinical Sciences, Division of Obstetrics and Gynecology, University of Florence, Florence, Italy

Marta Caretto Department of Clinical and Experimental Medicine, Division of Gynecology and Obstetrics, University of Pisa, Pisa, Italy

F. Carmona Department of Gynecology, Hospital Clínic of Barcelona, Barcelona, Spain

Irene Cetin Department of Obstetrics and Gynecology, Ospedale dei Bambini "Vittore Buzzi", University of Milano, Milano, Italy

John Challis Simon Fraser University, Vancouver, BC, Canada

Murdoch University, Murdoch, WA, Australia

Rohan R. Chodankar MRC Centre for Reproductive Health, The Queen's Medical Research Institute, The University of Edinburgh, Edinburgh, UK

Sara Clemenza Department of Biomedical, Experimental and Clinical Sciences, Division of Obstetrics and Gynecology, University of Florence, Florence, Italy

Maria Elisabetta Coccia Azienda Ospedaliero-Universitaria Careggi Ostetricia e ginecologia, Florence, Italy

Department of Clinical and Experimental Biomedical Sciences, University of Florence, Florence, Italy

Larissa Milani Coutinho Department of Maternal and Child Health, Universidade Federal de Juiz de Fora, Juiz de Fora, Brazil

Department of Obstetrics and Gynecology, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

Hilary O. D. Critchley MRC Centre for Reproductive Health, The Queen's Medical Research Institute, The University of Edinburgh, Edinburgh, UK

Laura Cucinella Research Center for Reproductive Medicine, Gynecological Endocrinology and Menopause, Obstetrics and Gynecology Unit, IRCCS San Matteo Foundation, Department of Clinical, Surgical, Diagnostic and Pediatric Sciences, University of Pavia, Pavia, Italy

Marta D'Alonzo Gynaecology and Obstetrics, Umberto I – Mauriziano Hospital, Turin, Italy

Konstantinos Dafopoulos Department of Obstetrics and Gynecology, School of Medicine, University of Thessaly, Larissa, Greece

Metella Dei Italian Society Pediatric Adolescent Gynecology, Florence, Italy

L. Di Medio University of Florence, Metabolic Bone Diseases Unit, University Hospital of Florence, Florence, Italy

Mariarosaria Di Tommaso Department of Health Sciences, Division of Obstetrics and Gynecology, University of Florence, Florence, Italy

Bart C. Fauser Department of Reproductive Medicine and Gynecology, University Medical Center Utrecht, University of Utrecht, Utrecht, The Netherlands

M. Gracia Division of Gynecology, Institute Clinic of Gynecology, Obstetrics and Neonatology, Hospital Clinic, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Faculty of Medicine, University of Barcelona, Barcelona, Spain

M. N. Gunning Department of Reproductive Medicine and Gynecology, University Medical Center Utrecht, University of Utrecht, Utrecht, The Netherlands

F. J. Janse Department of Reproductive Medicine and Gynecology, University Medical Center Utrecht, University of Utrecht, Utrecht, The Netherlands

Antonio La Marca Mother-Infant Department, Obstetrics and Gynecology, University of Modena and Reggio Emilia, University Hospital of Modena, Modena, Italy

Joop S. E. Laven Erasmus University Medical Centre, Rotterdam, The Netherlands

Yvonne V. Louwers Erasmus University Medical Centre, Rotterdam, The Netherlands

S. Luisi Department of Molecular and Developmental Medicine, University of Siena, Siena, Italy

Roberto Maggi Department of Pharmaceutical Sciences (DISFARM), Università degli Studi di Milano, Milan, Italy

Elisa Maseroli Andrology, Women's Endocrinology and Gender Incongruence Unit, Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Florence, Florence, Italy

Elisa Mastellari Mother-Infant Department, Obstetrics and Gynecology, University of Modena and Reggio Emilia, University Hospital of Modena, Modena, Italy

Salvatore Andrea Mastrolia Department of Obstetrics and Gynecology, Ospedale dei Bambini "Vittore Buzzi", University of Milano, Milano, Italy

Błażej Męczekalski Department of Gynecological Endocrinology, Poznan University of Medical Sciences, Poznan, Poland

Costanzo Moretti Department of Systems' Medicine, Section of Reproductive Endocrinology, University of Rome TorVergata, Rome, Italy

Rossella E. Nappi Research Center for Reproductive Medicine, Gynecological Endocrinology and Menopause, Obstetrics and Gynecology Unit, IRCCS San Matteo Foundation, Department of Clinical, Surgical, Diagnostic and Pediatric Sciences, University of Pavia, Pavia, Italy

Giulia Orlandi Azienda Ospedaliero-Universitaria Careggi Ostetricia e ginecologia, Florence, Italy

Department of Clinical and Experimental Biomedical Sciences, University of Florence, Florence, Italy

Francesca Pampaloni Pediatric and Adolescent Gynecology Unit Careggi Hospital Florence, Florence, Italy

Francisco A. N. Pereira Division of Human Reproduction, Hospital das Clínicas, and Department of Obstetrics and Gynecology, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

Felice Petraglia Department of Biomedical, Experimental and Clinical Sciences, Division of Obstetrics and Gynecology, University of Florence, Florence, Italy

Agnieszka Podfigurna Department of Gynecological Endocrinology, Poznan University of Medical Sciences, Poznan, Poland

Fernando M. Reis Division of Human Reproduction, Hospital das Clínicas, and Department of Obstetrics and Gynecology, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

Francesca Rizzello Azienda Ospedaliero-Universitaria Careggi Ostetricia e ginecologia, Florence, Italy

Assisted Reproduction Center, Careggi University Hospital, Florence, Italy

Viola Seravalli Department of Health Sciences, Division of Obstetrics and Gynecology, University of Florence, Florence, Italy

Tommaso Simoncini Department of Clinical and Experimental Medicine, Division of Gynecology and Obstetrics, University of Pisa, Pisa, Italy

Anna Szeliga Department of Gynecological Endocrinology, Poznan University of Medical Sciences, Poznan, Poland

Basil C. Tarlatzis Department of Obstetrics and Gynecology, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece

L. Troìa Department of Molecular and Developmental Medicine, University of Siena, Siena, Italy

Silvia Vannuccini Department of Biomedical, Experimental and Clinical Sciences, Division of Obstetrics and Gynecology, University of Florence, Florence, Italy

Department of Molecular and Developmental Medicine, University of Siena, Siena, Italy

Linda Vignozzi Andrology, Women's Endocrinology and Gender Incongruence Unit, Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Florence, Florence, Italy

Michela Villa Gynaecology and Obstetrics, Umberto I – Mauriziano Hospital, Turin, Italy

Andrea Villasco Gynaecology and Obstetrics, Umberto I – Mauriziano Hospital, Turin, Italy

Part I

Hypothalamic-Pituitary-Ovary Axis Disorders

The Hypothalamus-Pituitary-Ovary Axis

1

Roberto Maggi

Contents

Abstract

Human reproduction depends on an intact hypothalamic-pituitary-gonadal axis. Hypothalamic gonadotropin-releasing hormone (GnRH) has been recognized as the central regulator of the production and release of the pituitary gonadotropins that, in turn, regulate the gonadal functions and the production of sex steroids. The peculiar development, distribution, and episodic activity of GnRH-producing neurons have solicited an interdisciplinary interest on the etiopathogenesis of several reproductive diseases. Moreover, growing knowledge on a new more

R. Maggi (✉)
Department of Pharmaceutical Sciences (DISFARM), Università degli Studi di Milano, Milan, Italy
e-mail: roberto.maggi@unimi.it

F. Petraglia, B. C. Fauser (eds.), *Female Reproductive Dysfunction*, Endocrinology,
https://doi.org/10.1007/978-3-030-14782-2_1

complex interplay among integrative centers (hypothalamus and pituitary) and the effector (ovary) open to a new perception of the mechanisms controlling the reproductive axis.

Keywords

Hypothalamus · Pituitary · GnRH · Gonadotropin releasing hormone · Hormonal cycle · Ovarian hormones

Introduction

From a general point of view, the function of the endocrine system is based on the functional relationship among the endocrine hypothalamus, the pituitary gland, and the peripheral endocrine glands.

The hypothalamic regulatory functions are exerted by the secretion of peptidic releasing hormones in the pituitary portal vessels to control of the production of hypophysiotropic hormones, which act on several endocrine glands by promoting their development and functions. This biological architecture is the basis of the definition of the endocrine axes as well as of the "endocrine orchestra" first described by Hubble in 1961.

The Hormonal Reproductive Axis

The mammalian reproductive functions are regulated by a complex hormonal hypothalamo-pituitary-ovarian axis (HPO), which involves the functional interaction of hypothalamic gonadotropin-releasing hormone (GnRH), the two pituitary gonadotropins (follicle stimulating hormone, FSH, and luteinizing hormone, LH), and ovary-derived hormones.

Intricate time and space scales also characterize the HPO axis. The physiological processes characterizing HPO axis occur in millisecond (neuronal) timescale or with a circannual rhythmicity and spacing from molecules to organs. This complexity can be solved by a multiscale mathematical modeling description of the control of reproductive functions (Clement 2016). Therefore, instead of the "romantic" graphical illustration of the HPO axis (Fig. 1a), a more scientific flowchart may be more representative of the functional interplay of these physiological processes (Fig. 1b).

From this new point of view, the signals, from internal and external environment, elaborated by high brain centers and impinging on GnRH neurons are integrated at hypothalamic level in the neuronal circuits forming the "GnRH system." The generation of alternative responses, characterized by low and high frequency pulse generator, forward the instructions to the pituitary gland. These signals are once more integrated through the activation of GnRH receptors with consequent differential production and release of FSH and LH, which, in turn, induce the response of

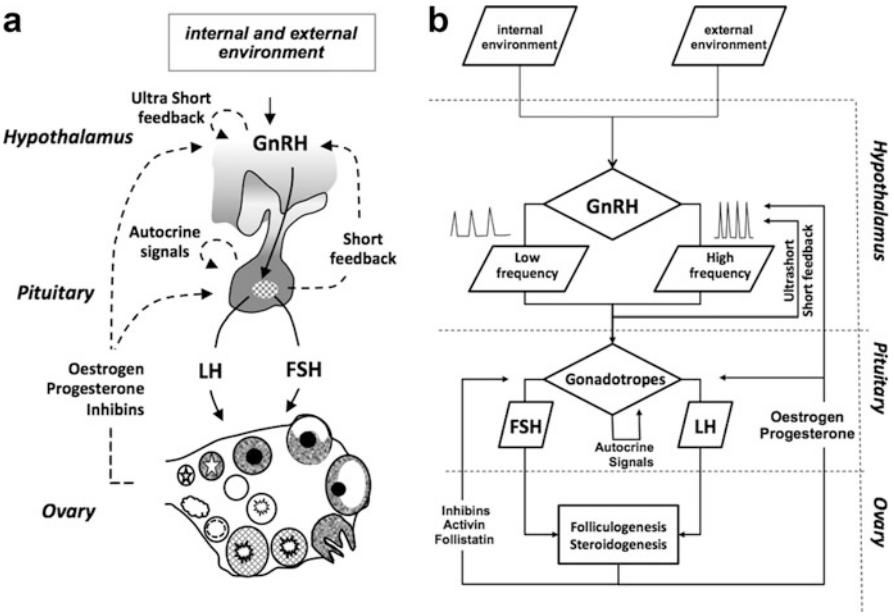


Fig. 1 Overview of the hormonal female hypothalamo-pituitary-ovarian (HPO) axis by a classical representation (**a**) or by a flowchart illustration of the functional interplay of the centers involved (**b**)

the ovaries (effectors) with the activation of specific processes, like folliculogenesis, follicle maturation, and steroidogenesis.

The ovarian responses generate further information flow by sex steroid (oestrogens and progesterone) and polypeptidic (inhibits, activins, follistatin) hormones that feed back in negative and positive modalities at the different integrative centers.

The Hypothalamus

The hypothalamus is a complex brain structure involved in fundamental homeostatic mechanisms and controls (reproduction, food intake, thermoregulation, etc.) governed by a complex interaction of neuronal networks, neurotransmitter, and neuromodulator systems.

Gonadotropin-releasing hormone (GnRH, also indicated as Luteinizing Hormone-Releasing Hormone) represents the key factor for the control of reproductive functions. It operates a differential regulation of the synthesis and release of the two pituitary gonadotropins, LH and FSH, which, in turn, promote the gonadal tropism and functions, as well as the production of gonadal sex steroid and polypeptidic hormones (Millar 2005).

Gonadotropin Releasing Hormone

Discovered in 1971, GnRH is a decapeptide of sequence pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH2 (Schally et al. 1971).

The human GnRH gene is located on the short arm of chromosome 8 (8p21-p11.2) and it is organized into four exons and three introns (Fig. 2). The gene encodes for a prohormone of 92 amino acid enzymatically modified into the cell secretory granules. The ProGnRH consists of a signal peptide of 23 amino acids, to direct intracellular packaging and secretion, the GnRH decapeptide sequence, a three amino acid (Gly-Lys-Arg) proteolytic processing site, and a 56 amino acid GnRH-Associated Protein (GAP) which is secreted with GnRH but whose physiological function is still not clear. The amino acid sequence of the C-terminal domain of native GnRH is involved in the binding of the peptide to its membrane receptors, while the amino acid sequence of the N-terminal domain is crucial for both the receptor binding and activation.

In addition to the classical form of GnRH (also named GnRH-I), isoforms of the decapeptide, with conserved N- (Glp-His-Trp-Ser) and C-terminal (Pro-Gly-NH_2) amino acid sequences, have been characterized in mammalian and nonmammalian vertebrates (Fig. 2).

In humans, the genes coding for only GnRH-I and GnRH-II isoforms have been surely identified. GnRH-II (also *chicken* GnRH-II) shows a structure uniquely conserved from fish to mammals. Human GnRH-II is encoded by a gene located on chromosome 20 and it shows a 70% similarity at the amino acid level to GnRH-I

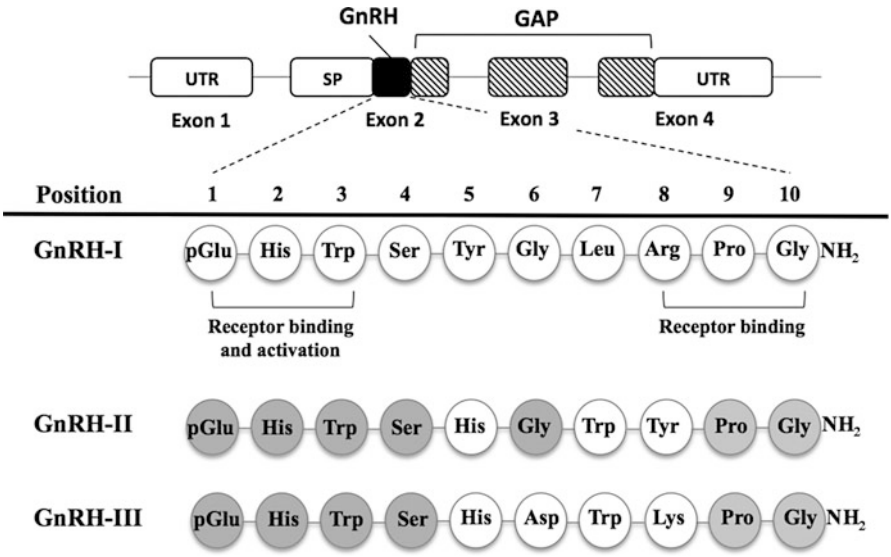


Fig. 2 Structure of the proGnRH gene and the aminoacid sequence of GnRH-I and its isoforms GnRH-II and III

with three different aminoacid substitutions (His^5, Trp^7, Tyr^8) (Chen et al. 1998; White et al. 1998; Leung et al. 2003). GnRH-II isoform is widely expressed in the central nervous system, where may act as a regulator of sexual behavior (Chen et al. 1998; Millar 2005), and in different peripheral tissues, including endometrium, ovary, and placenta.

A GnRH-III isoform has been isolated in the sea lamprey, where it is involved in the control of the reproductive functions. In mammals, GnRH-III exerts a very low activity on gonadotropin secretion; however, the observation of a significant anti-tumor action increased the scientific interest to this isoform (Kovacs et al. 2002).

Loss-of-function mutations of GNRH1 gene are a very rare cause of autosomal recessive HH possibly due the small size of the translated peptide or that they are rapidly eliminated from the population. Mutations in *GNRH1* gene were reported in few patients with severe HH (Quaynor et al. 2011). Indeed, patients with mutations in *PCSK1*, which encodes prohormone convertase 1/3, exhibit HH because of abnormal processing of the GnRH decapeptide from its prohormone precursor (Jackson et al. 1997).

GnRH Neurons

The GnRH involved in the regulation of HPO axis is synthesized in a subset of hypothalamic neurons able to secrete the neurohormone in a pulsatile fashion into the hypothalamo-pituitary portal vessels, through which it is transported to the anterior pituitary gland.

In mammals, including humans, endocrine GnRH neurons originate in the embryonic nasal compartment (olfactory placode/vomeronasal organ) (Cho et al. 2019); however, populations of "nonendocrine" GnRH neurons with different origin have been also reported in different species (Forni et al. 2011).

In human, newly generated GnRH neurons undergo rapid migration towards the forebrain, in association with olfactory, vomeronasal, and terminal nerves, during early embryogenesis, and become fully differentiated between 39 and 44 days of gestation. The process of migration begins about at day 39 and completes at birth.

A total of at least 10,000 immunoreactive GnRH neurons have been detected in human fetal brain during gestation; about 2000 GnRH neurons are located in the hypothalamus and the remaining widely distributed in brain areas not involved in the control of the hypothalamic-pituitary-gonadal axis (i.e., olfactory bulb, hippocampus, and cerebral cortex) (Casoni et al. 2016).

Once reached the hypothalamic region, endocrine GnRH neurons dissociate from their guiding axons to disperse in the septo-hypothalamic region, their final destination. These GnRH neurons are essential for the establishment and maintenance of reproduction and defects in their migration lead to hypogonadotropic hypogonadism (HH) due to lack of pituitary gonadotropin release (Sykiotis et al. 2010). To date a number of different genes affecting the migration, differentiation and function of the GnRH neuron have been implicated in causing HH (Topaloglu 2017).

GnRH Secretion

Approaching to the correct anatomical region, GnRH neurons undergo terminal differentiation and activate the episodic release of the decapeptide with different patterns of secretion according to gender and age of life.

In rodents, only 10–30% of hypothalamic GnRH neurons are required to attain a normal fertility (Herbison et al. 2008), but they require complex control interaction to synchronize the release of the GnRH in a pulsatile manner into the hypothalamo-hypophyseal portal vessels, a prerequisite to induce an efficient secretion of pituitary gonadotropins (Tsutsumi and Webster 2009).

Actually, pulsatile administration of GnRH results in pituitary GnRH receptors (GnRHR, see next chapter) upregulation; on the contrary, continuous infusion of GnRH exerts a reversible suppressive action on both LH and FSH secretion mainly due to a rapid receptor uncoupling from its intracellular effectors followed by GnRHR downregulation (Janjic et al. 2017). The biological role of the pulsatile secretion of GnRH is therefore the prevention of GnRHR downregulation in the gonadotrope cells. This unique feature of the GnRH system has a clinical relevance for the pharmacological shutdown of the reproductive axis by administration of long-acting GnRH analogues (see below).

The direct detection of levels and pulsatility of GnRH in pituitary portal vessels is not feasible in human and its low peripheral blood concentrations prevent an accurate measure of hypothalamic GnRH secretion. The measure of serum LH level fluctuations is accepted as a marker of the GnRH pulses; in particular, the measure of free gonadotropin α-subunit (FAS) levels is a reliable indicative measure of fast GnRH pulses due to its shorter half-life.

GnRH/LH pulses occur approximately every 2 h in adult male; in female, the pattern of pulsatile GnRH release is more complicated and shows variations during the development of reproductive functions (from puberty to adult), where influence the development of sex functions, and during the ovulatory cycle (Fig. 3b).

In vitro and in vivo observations showed that the pulsatile release of decapeptide from GnRH neurons might be cell-autonomous, synchronized by an extensive inter-cellular communication and affected by several afferent neuronal systems (Hrabovszky and Liposits 2013).

In particular, the neuronal inputs coming from the hypothalamic infundibular/arcuate nucleus (INF) and preoptic (POA) region are considered the main candidates to the regulatory functions of the GnRH pulsatile release in human (Piet et al. 2015; Plant 2015; Fig. 3a).

Since the first observation of gene mutation-dependent infertility, it was proposed the involvement of the product of *KISS1* gene (kisspeptin), as a main regulator of GnRH release and of the mammalian reproductive axis.

Kisspeptin neurons are mainly present in the human INF region and in a lesser extent in POA. GnRH neurons express kisspeptin receptors (Piet et al. 2015) and kisspeptin neuron projections affect GnRH release playing a critical role in the onset of puberty and in the control of ovarian cycle and fertility in adulthood (Terasawa et al. 2013; Skorupskaite et al. 2014).

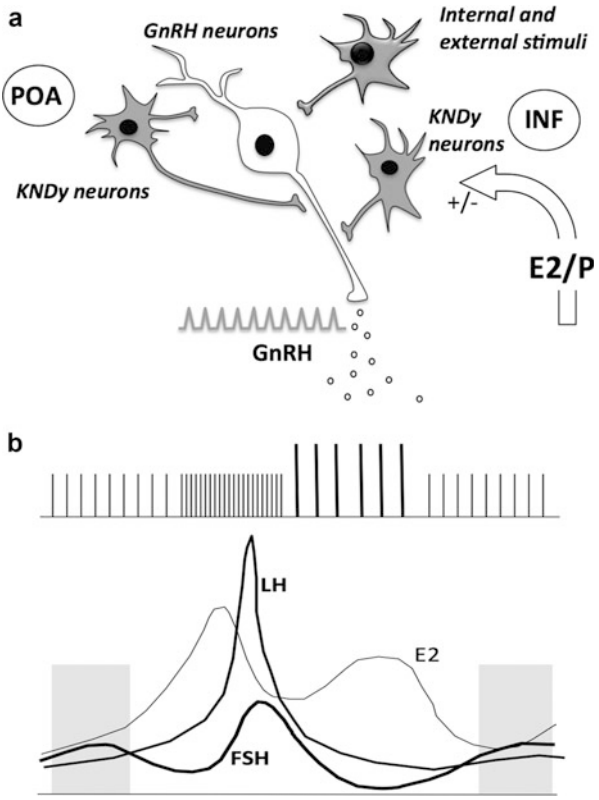


Fig. 3 Summary of the physiological mechanisms possibly involved in the control of GnRH secretion (**a**) and schematic representation of the frequency and amplitude of pulsatility of GnRH-induced gonadotropin release in pituitary portal vessels during the human hormonal reproductive cycle (**b**). (*POA* preoptic area, *INF* infundibular region, *KNDy* neurons releasing kisspeptin, neurokinin B and dynorphin, *E2* estradiol, *P* progesterone)

The control exerted by kisspeptin on GnRH release is subjected to negative and positive feedback of gonadal steroids and to metabolic/environmental signals (Skorupskaite et al. 2014; Simonneaux 2018; Evans and Anderson 2018). INF kisspeptin neurons may also co-express other peptides as neurokinin B and, rarely in humans, the endogenous opioid peptide dynorphin; this led to the acronym KNDy to indicate this neuronal population (Skrapits et al. 2015). Such peptides may exert a fine-tuning of kisspeptin secretion through a paracrine/autocrine action. In non-human primates, neurokinin B was found to exert a kisspeptin-mediated stimulatory action on GnRH release, whereas dynorphin may inhibit the release of GnRH.

Mutations of KISS1 or KISS1 receptor (KISS1R) gene lead to HH; similarly, loss-of-function mutations in the gene for neurokinin B (TAC3) or for its receptor (TAC3R) are associated with hypogonadism and delayed puberty (Topaloglu et al. 2009).

Overall, the pattern of GnRH release from the hypothalamic neurons is under the control of a number central and peripheral signals which may exert stimulatory (i.e., kisspeptin, norepinephrine, and neuropeptide Y) or inhibitory (i.e., endogenous opioids, interleukin-1, progesterone) actions, including GnRH itself that, acting by an ultra-short feedback loop, may modify its own secretion.

The function of GnRH neurons may be modulated by a peptide recognized in the quail as a gonadotropin inhibitory hormone (GnIH) (Tsutsui et al. 2000) and identified in humans as RFamide-related peptide-3 (RFRP-3). RFRP-3 interact with GPR147 receptors and its action is subjected to feedback by gonadal steroids and are influenced by stress hormones; recently, it has been reported a possible modulatory effect of RFRP-3 on the regulation of human pubertal development (Ubuka et al. 2009; Lima et al. 2014). These findings may outline a new perception of the HPO axis in which the release of pituitary gonadotropin could be positively regulated by GnRH and negatively by GnIH/RFRPs.

Finally, it is recognized that environmental factors, as stress, changes in energy stores as well as epigenetic modifications may affect both the release of GnRH and the activity of the hypothalamic pulse generator (Evans and Anderson 2018; Toro et al. 2018).

In primates, including humans, the onset of puberty is driven by the dynamic activity of the GnRH neuronal system. For a hormonal point of view, it has been proposed that the development of the neuroendocrine reproductive axis may be distinguished in three phases (on-off-on) (Plant et al. 2005), with the first phase, occurring from fetal (16th week) to postnatal life (4th and 10th week), characterized by a sustained GnRH pulsatility (on) with gonadotropin and steroid hormone secretion (the so-called "mini puberty"). This is followed by a phase (juvenile) of low GnRH pulsatility and gonadal quiescence (off) and by a true "pubertal" phase in which GnRH pulsatility is reactivated (on).

The differential activation of the GnRH pulse generator may result from the interplay among developmental events, occurring in the brain, and the feedback control exerted by gonadal steroids.

KNDy neurons seem to be involved in the mini-puberty, since a pulsatile release of kisspeptin, resulting in a corresponding pattern of GnRH secretion, is observed during this phase. Conversely, the decrease of GnRH secretion during late infancy and juvenile development seems due to both a neurobiological central "brake" and the negative feedback signals coming from the ovary (Witchel and Plant 2014). The reactivation of GnRH pulses could be due to internal and external signals that could weak the central brake; hypothalamic pubertal sensor could detect circulating signals of somatic development to reactivate HPO axis to the attainment of the adequate adult age. The further increased production of ovarian hormones can then exert the first negative feedback phase on GnRH/gonadotropin secretion, responsible of the delay between the menarche and the first ovulation (Plant 2015).

The Pituitary

GnRH Receptors

GnRH exerts its biological actions by binding to specific membrane receptors (gonadotropin-releasing hormone receptors, GnRHRs, also called "type I GnRHR") on pituitary gonadotropes.

A gene located on chromosome 4 (4q13) encodes for the type I GnRHR, a highly conserved 328-amino acid protein belonging to the GPCR (rhodopsin-like G protein-coupled receptors) receptor family. It is composed of three exons and two introns and the encoded protein is characterized by a 35-amino acid extracellular N-terminus, with two putative glycosylation sites, followed by seven transmembrane domains (Millar 2005). The peculiar feature of this receptor is the very short (1–2 amino acids) intracellular tail present at C-terminus. The C-terminal part of the GPCR receptors is involved in the association with the specific G proteins, receptor internalization, and desensitization; the observed slow processing of GnRHR after ligand-induced activation could be due to this short C-terminal sequence.

Once activated, GnRHR activate an intracellular signaling involving the $G\alpha_{q/11}$ protein and leading to the formation of the second messengers diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP_3) with a consequent activation of protein kinase C (PKC) and the release of Ca^{2+} from the endoplasmic reticulum stores. The increase of intracellular Ca^{2+} levels is responsible of the GnRH-induced gonadotropin release, while PKC activation is responsible for the activation of a MAPK pathway leading to transcription factors phosphorylation (such as c-Jun, c-Fos, Elk1) responsible for upregulating the gene expression of the gonadotropins α subunits and of the FSHβ subunit (Maggi et al. 2016; Limonta et al. 2018).

Beside of the main role of $G\alpha_{q/11}$ isoform in GnRHR transduction system, the $G\alpha_s$/adenylate cyclase/PKA signaling pathway may also be involved in the physiological responses of pituitary gonadotropes to GnRHR stimulation. A specific cross talk between the different intracellular pathways activated by GnRHR plays a regulatory role to shape gonadotropin synthesis and secretion (Kraus et al. 2001).

The expression of pituitary GnRHR undergoes fluctuations according to the levels of secretion of the two gonadotropins and is regulated by circulating levels of gonadal steroids (i.e., estradiol and progesterone) (Yu et al. 2011). More than 25 different mutations of GnRHR gene causing autosomal recessive HH have been reported; about 40–50% of familial autosomic HH and 15–18% of sporadic HH show mutations of GnRHR (Topaloglu 2017).

A specific GnRH-II receptor (referred to as type II GnRHR) was initially identified in nonhuman primates as a classical GPCR. In humans, the gene coding for the type II GnRHR reveals a frameshift in coding exon 1 and a premature internal stop codon in the sequence coding its extracellular loop suggesting that a functional full-length type II GnRHR protein is not expressed in humans (Millar 2005).

Therefore, in humans GnRH-II and GnRH-III isoforms seem to exert its effects through the activation of the classical form of GnRHRs (Montagnani Marelli et al. 2015).

Control of Gonadotropin Expression and Secretion

The control of the differential expression and secretion of LH and FSH is based on the decodification of the pulsatile GnRH signals by GnRHR at the level of pituitary gonadotrope cells. According to different receptor activation, the intracellular

signals will drive gonadotropin expression (Perrett and McArdle 2013; Stamatiades and Kaiser 2018). Although the mechanisms by which gonadotropes decode GnRH pulse frequency are still to be completely clarified, studies using in vitro models provide some suggestions.

It has been reported that the frequency and the amplitude of GnRH pulses may lead to differential activation $G_{\alpha q/11}$ and $G_{\alpha s}$ subunits coupled to GnRHR. A pulsatile GnRH regimen induces a stable response mediated by the $G_{\alpha s}$/cAMP signaling pathway while the $G_{\alpha q/11}$ pathway shows an initial pulsatile response followed by a rapid decrease of pulse amplitude (desensitization), suggesting that $G_{\alpha s}$ and the $G_{\alpha q/11}$ pathways may mediate an adaptive and desensitization response, respectively, to pulses of GnRH (Tsutsumi and Webster 2009).

On the other hand, studies carried out in in vitro models revealed that after a continuous GnRH exposure the $G_{\alpha s}$/cAMP pathway shows a rapid initial transient response followed by a rapidly deactivation, whereas $G_{\alpha q/11}$ pathway remains constantly activated (Liu et al. 2002).

Apart of to the different receptor activation by GnRH, several intracellular signal cascades (e.g., MAPKs) able to independently modulate gonadotrope-specific expression of LHβ and FSHβ genes have been identified (Lim et al. 2009; Tsutsumi and Webster 2009).

High frequency GnRH pulses induce LHβ expression, mainly by the activation of steroidogenic factor 1 (SF1) and early growth response 1 (EGR1) (Fortin et al. 2009) transcription factors, and the inhibition of FSHβ expression due to the activation of co-repressors. Conversely, low frequency GnRH pulses lead to a decrease of EGR1 levels, with a prevalent action of co-repressors for LHβ promoter (Luxardi et al. 2007), while a parallel decrease of co-repressors allows the activation of FSHβ promoter, driven by AP-1 transcription factor family members (c-fos, c-jun) (McGillivray et al. 2007).

The differential pulsatile secretion of the two gonadotropins is also regulated by GnRH pulse frequency, with increasing frequencies resulting in preferential secretion of LH and decreasing frequencies in greater FSH release, and mediated by PKC activation and Ca^{++} influx (Stojilkovic et al. 1988).

The key role of GnRH in the control of the reproductive functions and the relevance of its possible clinical applications for the treatment of reproductive-related diseases led to the development of GnRH synthetic analogues (agonist and antagonists) (Table 1) to restore fertility in GnRH-deficient conditions or to suppress the pituitary-gonadal axis in selective clinical situations (Maggi et al. 2016; Limonta et al. 2018).

The analogues were designed considering that natural GnRH has a half-life of 2–4 min due to degradation of the glycine-leucine bond between amino acids 6 and 7. Thus, GnRH agonists with a modification of Gly^6, which is usually replaced by a D-amino acid, to increase the plasma half-life have been developed; moreover, the deletion of Gly^{10}-amide with the addition of an ethylamide residue to Pro^9 was introduced to increase the affinity for GnRHR. A characteristic of a sustained administration of high doses GnRH agonists is the so called after "flare effect," an initial stimulation of gonadotropes followed by a suppression the activity of the pituitary-gonadal axis, mediated by a downregulation of GnRHR. This therapeutic regimen is indicated, for instance, for the treatment of central precocious puberty, the endometriosis, and the polycystic ovarian disease.

Table 1 Amino acid sequence of main GnRH agonists and antagonists

GnRH agonists										
Buserelin	pGlu	His	Trp	Ser	Tyr	DSer (tBu)	Leu	Arg	Pro	Net
Goserelin	pGlu	His	Trp	Ser	Tyr	DSer (tBu)	Leu	Arg	Pro	azaGly NH2
Leuprolide	pGlu	His	Trp	Ser	Tyr	DLeu	Leu	Arg	Pro	Net
Triptorelin	pGlu	His	Trp	Ser	Tyr	DSer (tBu)	Leu	Arg	Pro	Gly NH2
GnRH antagonists										
Abarelix	DNal	DCpa	DPal	Ser	NMe Tyr	DAsn	Leu	Lys(iPr)	Pro	DAla NH2
Cetrorelix	DNal	DCpa	DPal	Ser	Tyr	DCit	Leu	Arg	Pro	DAla NH2
Dagarelix	DNal	DCpa	DPal	Ser	Aph (Hor)	DAph (Cba)	Leu	Lys(iPr)	Pro	DAla NH2
Ganirelix	DNal	DCpa	DPal	Ser	Tyr	DhArg (Et2)	Leu	hArg (Et2)	Pro	DAla NH2

D dextro, *Net* ethylamide, *tBu* O-tert butyl, *Nal* 2-naphtylalanine, *Cpa* 4-chlorophenylalanine, *Pal* 3-pyridylalanine, *N Me* N Methyl, *iPr* isopropyl, *Cit* citrulline, *Aph* 4-aminophenylalanine, *Hor* L-hydroorotyl, *Cba* carbamoyl, *DhArg* D-homoarginine, *Et2* diethyl. Gray boxes indicate the aminoacid residues conserved from GnRH-I

GnRH antagonists exert their effects by competitively binding to GnRHR, blocking gonadotropin synthesis and secretion (Copperman and Benadiva 2013). Antagonists presented a very complex structure; they contain modifications (e.g., Ac-D-Nal-D-Cpa-D-Pal) in the N-terminal of the peptide and D-Ala in position 10; in addition, they present different amino acid substitutions in positions 5, 6, and 8. GnRH antagonists have different clinical applications in reproductive medicine and in gynecology (e.g., in the control of ovarian hyperstimulation protocols) (Coccia et al. 2004; Al-Inany et al. 2011).

The Ovary

The Gonadotropin Receptors

The pituitary gonadotropins released into the general circulation exert their main actions on the ovary stimulating gametogenesis, gonadal steroidogenesis, and peptide hormone secretion.

LH and FSH are heterodimeric glycoproteins (molecular mass, 30–40 kDa) consisting of a common 92-aminoacid residues alpha subunit and a unique beta subunit; alpha subunit is also shared with human chorionic gonadotropin (hCG) and thyrotropin (TSH). FSHβ and LHβ possess *N*-glycosylation (carrying *N*-acetylgalactosamine) sites with a variable content of sialic acid, which may characterize different isoforms of the same hormone, having higher plasma half-life those with the high sialic acid content. In general, the FSH secretion look likes to be constitutive, whereas LH secretion is induced by stimulation, LH pulse frequency in the women shows a variability linked to the phase of the menstrual cycle, with low-frequency pulses during the luteal phase of the cycle and higher-frequency pulses in the follicular phase.

The gonadotropins exert their effects on the HPO axis through interaction with and activation of specific membrane receptors. The LH receptors (LHR) play a crucial role in the regulation of reproductive functions including ovulation and ovarian steroidogenesis; because of its ability to bind both LH and hCG with high affinity, LHR is designated as LH/hCG receptor. The FSH receptors (FSHR) bind only FSH and it is crucial in the regulation of ovarian function through its action in follicle development and stimulation of estrogen production (Dias et al. 2002) but also in the steroidogenesis facilitating the aromatase-dependent conversion of androgen to estrogen in the granulosa cells.

FSHR activation additionally induces the production of the ovarian polypeptides activin and inhibin, which may feed back to the pituitary and hypothalamus to regulate FSH production (de Kretser et al. 2002; Namwanje and Brown 2016).

LH and FSH receptors contain an N-terminal extracellular domain that binds the respective ligands with high affinity and specificity, and seven transmembrane domains responsible for signal transduction.

Both LHR and FSHR are glycosylated, at asparagine residues presumably required for the proper folding of the protein, as well as they may be phosphorylated, possibly after the interaction of the receptor with its ligand. While the exact role of phosphorylation has not been determined, it is thought to facilitate internalization of the ligand-bound receptor to the intracellular sites.

The LH and FSH receptors may be coupled to Gαs protein resulting, upon activation, in elevation of intracellular cAMP levels. In experimental models, these receptors were found able to activate other signal transduction pathways leading to increased phosphatidylinositide turnover, elevated intracellular Ca^{2+}, and activation of mitogen-activated protein kinases have been found (Themmen and Huhtaniemi 2000).

Upon hormonal stimulation, the LH and FSH receptors desensitize due to uncoupling of the receptor from the intracellular transducing G proteins, and by internalization of the receptor, even though these mechanisms have not been confirmed in reproductive human tissues.

Several naturally occurring mutations with reproductive phenotypes have been reported for human LHR and FSHR linked to oligo-amenorrhea, primary amenorrhea, primary ovarian failure, and infertility (Menon and Menon 2012).

The key role exerted by the gonadotropins on ovarian functions rose the need of analogues with an efficient pharmacological profile. Several gonadotropin analogues/

preparations have then become available for clinical use. To date, many efforts are addressed to develop low molecular weight, orally active molecules with activity at gonadotropin receptors; such molecules may show a reduced risk usually associated with gonadotropin treatments (as ovarian hyperstimulation syndrome) and a possible wider range of therapeutic applications (e.g., contraception, polycystic ovary syndrome, treatment of ovarian, and prostate cancers) (Anderson et al. 2018).

The Ovarian Hormones

The hormonal cascade activated by hypothalamic GnRH is regulated by ovarian-derived hormones, like sex steroids (estrogen and progesterone) and polypeptidic hormones (inhibin, activin, follistatin). Differential regulatory effects of the ovarian steroids on GnRH secretion and pulsatility have been assessed in many mammals and in women: progesterone exerts an inhibitory action while estradiol can have both stimulatory and inhibitory effects, depending upon the stage of the menstrual cycle.

The FSH released during the late-luteal/early-follicular phases of the cycle is responsible of the recruitment of Graafian follicles into the ovarian cycle and of the follicular development. The increasing circulating levels of estradiol produced by developing follicle suppress, at first, LH secretion by a negative-feedback mechanism possibly acting at both hypothalamic and pituitary levels (Shaw et al. 2010). At the end of the follicular phase, a rapid elevation of plasma concentrations of estradiol is produced by maturation of the preovulatory follicle. Once a critical threshold is reached, estradiol action switches to a positive feedback increasing GnRH pulse frequency and amplitude and the pituitary sensitivity to GnRH pulses, by induction of GnRH receptor expression; this reflects a midcycle increase in LH pulse frequency, a rapid LH surge, and the induction of ovulation and follicle luteinization. The subsequent production of progesterone, from luteinized follicle, feeds backs negatively at the hypothalamus suppressing LH secretion with consequent atrophy of the corpus luteum.

The biological action of the ovarian steroid hormones on central integration centers has been extensively investigated. Estrogen and progesterone drive the variation in frequency and amplitude of the GnRH pulses through the menstrual cycle. A tonic pulsatile GnRH secretion, present throughout the ovulatory cycle, is maintained by the negative feedback actions of ovarian steroids with an inhibition of the pulse amplitude induced by estradiol and of the pulse frequency by progesterone. During the luteal phase, a lower frequency and increased GnRH pulse amplitude is mainly mediated by progesterone (McCartney et al. 2007).

However, while estrogen receptor beta (ERβ) was detected in a subset of GnRH neurons, the presence of ERα and of progesterone receptors have not been completely clarified (Hu et al. 2008). Therefore, it has been proposed an indirect action of ovarian steroids on GnRH pulsatility, possibly mediated by peptidergic neurons afferent to GnRH cells.

Actually, the different modalities of steroid feedback appear to be linked to the amount of kisspeptin released by INF/POA neurons (Lehman et al. 2010). Therefore,

the increasing production of ovarian estradiol, during the follicular phase of the ovulatory cycle, activates kisspeptin neurons to induce the expression of progesterone receptors (Mittelman-Smith et al. 2015). This overrides the normal negative feedback effect of sex steroids leading to the increase of GnRH pulse frequency and amplitude. The increase of progesterone production by luteinized granulosa cells downregulates kisspeptin expression that slows GnRH pulse frequency with a consequent decrease of LH and a progressive increase of FSH production and release to induce a next cycle of ovulation (Rance 2009).

An additional control of the HPO axis is mediated by the action of a series of ovarian polypeptidic hormones, namely, activins, inhibins, and follistatin.

These gonadal hormones exert a selective effect on pituitary follicle-stimulating hormone (FSH) production and release.

Inhibins are polypeptides mainly produced by the ovary, but both activins and follistatin are also produced in the pituitary and may affect FSH secretion through autocrine-paracrine mechanisms. These factors are members of transforming growth factor β (TGFβ) superfamily and are implicated in several biological processes, including embryonic development and cell differentiation (Namwanje and Brown 2016; Bloise et al. 2019).

Inhibins are disulfide-linked dimers sharing a common α-subunit and different β-subunit that characterize the two isoform A and B. Inhibin B originates from the ovary in response to FSH stimulation of granulosa cells; it is the major circulating form of inhibin and it may selectively suppress FSH secretion by a negative feedback action at the pituitary levels. Since inhibins have the capacity to suppress FSH secretion in concert with estradiol, both factors are responsible for the feedback regulation of FSH secretion from the pituitary gland.

Activins (A and B) share common β subunits with inhibins but activins forms ββ homodimers. Activin B is produced by gonadotropes themselves in the anterior pituitary and exerts an autocrine/paracrine effects on gonadotropes, enhancing GnRH-induced FSHβ expression and FSH release; pituitary activin B expression is controlled by the GnRH pulse frequency (Burger et al. 2002).

The increase primarily in FSH in younger women with decreased ovarian function or menopausal women is due to a lack of inhibin to antagonize autocrine activin (Santoro and Randolph 2011).

Follistatin, originally isolated from ovarian follicular fluid, is synthesized by the pituitary (folliculostellate cells and gonadotropes) and it represses FSH synthesis by binding activin at aminoacid residues involved in the binding to its receptor, thus inhibiting its function, antagonizing endogenous autocrine activin (Makanji et al. 2014). Follistatin also diminishes the effect of GnRH on the induction of FSHβ gene transcription (McGillivray et al. 2007).

These evidences strongly indicate that while ovarian inhibin acts as a negative feedback regulator of FSH secretion, activin and follistatin are more likely to exert an effect on FSH secretion through paracrine mechanisms within the pituitary gland. Moreover, it became clear that inhibin and follistatin regulate FSH primarily by antagonizing the activin-mediated FSH expression and release.

The expression of GnRH in the hypothalamus and of GnRHR in the pituitary gonadotropes is also enhanced by activins, and blocked by follistatin (Norwitz et al. 2002). Moreover, these polypeptides, including inhibins, may also exert local effects on ovarian folliculogenesis.

Conclusions

In conclusion, the HPO axis is characterized by a really complex interplay of hierarchical and coordinated hormonal signals.

The accumulating findings on the structure and the function of HPO axis will lead in a new era with a better knowledge of the physiology of reproduction and new options for the treatment of related disorders.

Cross-References

► Hormonal Treatments in the Infertile Women
► Infertility
► The Menstrual Cycle and Related Disorders

Acknowledgments This work was supported by the Italian Ministry for Universities and Research (MIUR) and by the Università degli Studi di Milano (Research Support Plan, line 2, action B 2017–18).

References

Al-Inany HG, Youssef MA, Aboulghar M, Broekmans F, Sterrenburg M, Smit J, Abou-Setta AM. Gonadotrophin-releasing hormone antagonists for assisted reproductive technology. Cochrane Database Syst Rev. 2011;5:CD001750. https://doi.org/10.1002/14651858.CD001750.pub3.

Anderson RC, Newton CL, Anderson RA, Millar RP. Gonadotropins and their analogs: current and potential clinical applications. Endocr Rev. 2018;39(6):911–37. https://doi.org/10.1210/er.2018-00052.

Bloise E, Ciarmela P, Dela Cruz C, Luisi S, Petraglia F, Reis FM. Activin A in mammalian physiology. Physiol Rev. 2019;99(1):739–80. https://doi.org/10.1152/physrev.00002.2018.

Burger LL, Dalkin AC, Aylor KW, Haisenleder DJ, Marshall JC. GnRH pulse frequency modulation of gonadotropin subunit gene transcription in normal gonadotropes-assessment by primary transcript assay provides evidence for roles of GnRH and follistatin. Endocrinology. 2002;143(9):3243–9. https://doi.org/10.1210/en.2002-220216.

Casoni F, Malone SA, Belle M, Luzzati F, Collier F, Allet C, Hrabovszky E, Rasika S, Prevot V, Chedotal A, Giacobini P. Development of the neurons controlling fertility in humans: new insights from 3D imaging and transparent fetal brains. Development. 2016;143(21):3969–81. https://doi.org/10.1242/dev.139444.

Chen A, Yahalom D, Ben-Aroya N, Kaganovsky E, Okon E, Koch Y. A second isoform of gonadotropin-releasing hormone is present in the brain of human and rodents. FEBS Lett. 1998;435(2–3):199–203. S0014-5793(98)01064-3 [pii].

Cho HJ, Shan Y, Whittington NC, Wray S. Nasal Placode development, GnRH neuronal migration and Kallmann syndrome. Front Cell Dev Biol. 2019;7:121. https://doi.org/10.3389/fcell.2019.00121.

Clement F. Multiscale mathematical modeling of the hypothalamo-pituitary-gonadal axis. Theriogenology. 2016;86(1):11–21. https://doi.org/10.1016/j.theriogenology.2016.04.063.

Coccia ME, Comparetto C, Bracco GL, Scarselli G. GnRH antagonists. Eur J Obstet Gynecol Reprod Biol. 2004;115(Suppl 1):S44–56. https://doi.org/10.1016/j.ejogrb.2004.01.033. S0301211504000181 [pii].

Copperman AB, Benadiva C. Optimal usage of the GnRH antagonists: a review of the literature. Reprod Biol Endocrinol. 2013;11:20. https://doi.org/10.1186/1477-7827-11-20.

de Kretser DM, Hedger MP, Loveland KL, Phillips DJ. Inhibins, activins and follistatin in reproduction. Hum Reprod Update. 2002;8(6):529–41.

Dias JA, Cohen BD, Lindau-Shepard B, Nechamen CA, Peterson AJ, Schmidt A. Molecular, structural, and cellular biology of follitropin and follitropin receptor. Vitam Horm. 2002;64:249–322.

Evans MC, Anderson GM. Integration of circadian and metabolic control of reproductive function. Endocrinology. 2018;159(11):3661–73. https://doi.org/10.1210/en.2018-00691.

Forni PE, Taylor-Burds C, Melvin VS, Williams T, Wray S. Neural crest and ectodermal cells intermix in the nasal placode to give rise to GnRH-1 neurons, sensory neurons, and olfactory ensheathing cells. J Neurosci. 2011;31(18):6915–27. https://doi.org/10.1523/jneurosci.6087-10.2011.

Fortin J, Lamba P, Wang Y, Bernard DJ. Conservation of mechanisms mediating gonadotrophin-releasing hormone 1 stimulation of human luteinizing hormone beta subunit transcription. Mol Hum Reprod. 2009;15(2):77–87. https://doi.org/10.1093/molehr/gan079.

Herbison AE, Porteous R, Pape JR, Mora JM, Hurst PR. Gonadotropin-releasing hormone neuron requirements for puberty, ovulation, and fertility. Endocrinology. 2008;149(2):597–604. https://doi.org/10.1210/en.2007-1139.

Hrabovszky E, Liposits Z. Afferent neuronal control of type-I gonadotropin releasing hormone neurons in the human. Front Endocrinol (Lausanne). 2013;4:130. https://doi.org/10.3389/fendo.2013.00130.

Hu L, Gustofson RL, Feng H, Leung PK, Mores N, Krsmanovic LZ, Catt KJ. Converse regulatory functions of estrogen receptor-alpha and -beta subtypes expressed in hypothalamic gonadotropin-releasing hormone neurons. Mol Endocrinol. 2008;22(10):2250–9. https://doi.org/10.1210/me.2008-0192.

Jackson RS, Creemers JW, Ohagi S, Raffin-Sanson ML, Sanders L, Montague CT, Hutton JC, O'Rahilly S. Obesity and impaired prohormone processing associated with mutations in the human prohormone convertase 1 gene. Nat Genet. 1997;16(3):303–6. https://doi.org/10.1038/ng0797-303.

Janjic MM, Stojilkovic SS, Bjelobaba I. Intrinsic and regulated gonadotropin-releasing hormone receptor gene transcription in mammalian pituitary gonadotrophs. Front Endocrinol (Lausanne). 2017;8:221. https://doi.org/10.3389/fendo.2017.00221.

Kovacs M, Seprodi J, Koppan M, Horvath JE, Vincze B, Teplan I, Flerko B. Lamprey gonadotropin hormone-releasing hormone-III has no selective follicle-stimulating hormone-releasing effect in rats. J Neuroendocrinol. 2002;14(8):647–55. 828 [pii].

Kraus S, Naor Z, Seger R. Intracellular signaling pathways mediated by the gonadotropin-releasing hormone (GnRH) receptor. Arch Med Res. 2001;32(6):499–509. S0188-4409(01)00331-9 [pii].

Lehman MN, Coolen LM, Goodman RL. Minireview: kisspeptin/neurokinin B/dynorphin (KNDy) cells of the arcuate nucleus: a central node in the control of gonadotropin-releasing hormone secretion. Endocrinology. 2010;151(8):3479–89. https://doi.org/10.1210/en.2010-0022. [pii].

Leung PC, Cheng CK, Zhu XM. Multi-factorial role of GnRH-I and GnRH-II in the human ovary. Mol Cell Endocrinol. 2003;202(1–2):145–53. S0303720703000765 [pii].

Lim S, Pnueli L, Tan JH, Naor Z, Rajagopal G, Melamed P. Negative feedback governs gonadotrope frequency-decoding of gonadotropin releasing hormone pulse-frequency. PLoS One. 2009;4(9):e7244. https://doi.org/10.1371/journal.pone.0007244.

Lima CJ, Cardoso SC, Lemos EF, Zingler E, Capanema C, Menezes LD, Vogado G, Dos Santos BT, de Moraes OL, Duarte EF, de Brito VN, Latronico AC, Lofrano-Porto A. Mutational analysis of the genes encoding RFamide-related peptide-3, the human orthologue of gonadotrophin-inhibitory hormone, and its receptor (GPR147) in patients with gonadotrophin-releasing hormone-dependent pubertal disorders. J Neuroendocrinol. 2014;26(11):817–24. https://doi.org/10.1111/jne.12207.

Limonta P, Marelli MM, Moretti R, Marzagalli M, Fontana F, Maggi R. GnRH in the human female reproductive axis. Vitam Horm. 2018;107:27–66. https://doi.org/10.1016/bs.vh.2018.01.003.

Liu F, Usui I, Evans LG, Austin DA, Mellon PL, Olefsky JM, Webster NJ. Involvement of both G(q/11) and G(s) proteins in gonadotropin-releasing hormone receptor-mediated signaling in L beta T2 cells. J Biol Chem. 2002;277(35):32099–108. https://doi.org/10.1074/jbc.M203639200. [pii].

Luxardi G, Galli A, Forlani S, Lawson K, Maina F, Dono R. Glypicans are differentially expressed during patterning and neurogenesis of early mouse brain. Biochem Biophys Res Commun. 2007;352(1):55–60. https://doi.org/10.1016/j.bbrc.2006.10.185.

Maggi R, Cariboni AM, Marelli MM, Moretti RM, Andre V, Marzagalli M, Limonta P. GnRH and GnRH receptors in the pathophysiology of the human female reproductive system. Hum Reprod Update. 2016;22(3):358–81. https://doi.org/10.1093/humupd/dmv059.

Makanji Y, Zhu J, Mishra R, Holmquist C, Wong WP, Schwartz NB, Mayo KE, Woodruff TK. Inhibin at 90: from discovery to clinical application, a historical review. Endocr Rev. 2014;35(5):747–94. https://doi.org/10.1210/er.2014-1003.

McCartney CR, Blank SK, Marshall JC. Progesterone acutely increases LH pulse amplitude but does not acutely influence nocturnal LH pulse frequency slowing during the late follicular phase in women. Am J Physiol Endocrinol Metab. 2007;292(3):E900–6. https://doi.org/10.1152/ajpendo.00371.2006.

McGillivray SM, Thackray VG, Coss D, Mellon PL. Activin and glucocorticoids synergistically activate follicle-stimulating hormone beta-subunit gene expression in the immortalized LbetaT2 gonadotrope cell line. Endocrinology. 2007;148(2):762–73. https://doi.org/10.1210/en.2006-0952.

Menon KM, Menon B. Structure, function and regulation of gonadotropin receptors – a perspective. Mol Cell Endocrinol. 2012;356(1–2):88–97. https://doi.org/10.1016/j.mce.2012.01.021.

Millar RP. GnRHs and GnRH receptors. Anim Reprod Sci. 2005;88(1–2):5–28. https://doi.org/10.1016/j.anireprosci.2005.05.032. S0378-4320(05)00129-6 [pii].

Mittelman-Smith MA, Wong AM, Kathiresan AS, Micevych PE. Classical and membrane-initiated estrogen signaling in an in vitro model of anterior hypothalamic kisspeptin neurons. Endocrinology. 2015;156(6):2162–73. https://doi.org/10.1210/en.2014-1803.

Montagnani Marelli M, Manea M, Moretti RM, Marzagalli M, Limonta P. Oxime bond-linked daunorubicin-GnRH-III bioconjugates exert antitumor activity in castration-resistant prostate cancer cells via the type I GnRH receptor. Int J Oncol. 2015;46(1):243–53. https://doi.org/10.3892/ijo.2014.2730.

Namwanje M, Brown CW. Activins and inhibins: roles in development, physiology, and disease. Cold Spring Harb Perspect Biol. 2016;8(7). https://doi.org/10.1101/cshperspect.a021881.

Norwitz ER, Xu S, Jeong KH, Bedecarrats GY, Winebrenner LD, Chin WW, Kaiser UB. Activin A augments GnRH-mediated transcriptional activation of the mouse GnRH receptor gene. Endocrinology. 2002;143(3):985–97. https://doi.org/10.1210/endo.143.3.8663.

Perrett RM, McArdle CA. Molecular mechanisms of gonadotropin-releasing hormone signaling: integrating cyclic nucleotides into the network. Front Endocrinol (Lausanne). 2013;4:180. https://doi.org/10.3389/fendo.2013.00180.

Piet R, de Croft S, Liu X, Herbison AE. Electrical properties of kisspeptin neurons and their regulation of GnRH neurons. Front Neuroendocrinol. 2015;36:15–27. https://doi.org/10.1016/j.yfrne.2014.05.006.

Plant TM. Neuroendocrine control of the onset of puberty. Front Neuroendocrinol. 2015;38:73–88. https://doi.org/10.1016/j.yfrne.2015.04.002.

Plant TM, Ramaswamy S, Simorangkir D, Marshall GR. Postnatal and pubertal development of the rhesus monkey (*Macaca mulatta*) testis. Ann N Y Acad Sci. 2005;1061:149–62. https://doi.org/10.1196/annals.1336.016.

Quaynor SD, Kim HG, Cappello EM, Williams T, Chorich LP, Bick DP, Sherins RJ, Layman LC. The prevalence of digenic mutations in patients with normosmic hypogonadotropic hypogonadism and Kallmann syndrome. Fertil Steril. 2011;96(6):1424–1430 e1426. https://doi.org/10.1016/j.fertnstert.2011.09.046.

Rance NE. Menopause and the human hypothalamus: evidence for the role of kisspeptin/neurokinin B neurons in the regulation of estrogen negative feedback. Peptides. 2009;30(1):111–22. https://doi.org/10.1016/j.peptides.2008.05.016.

Santoro N, Randolph JF Jr. Reproductive hormones and the menopause transition. Obstet Gynecol Clin N Am. 2011;38(3):455–66. https://doi.org/10.1016/j.ogc.2011.05.004.

Schally AV, Arimura A, Baba Y, Nair RM, Matsuo H, Redding TW, Debeljuk L. Isolation and properties of the FSH and LH-releasing hormone. Biochem Biophys Res Commun. 1971;43(2):393–9.

Shaw ND, Histed SN, Srouji SS, Yang J, Lee H, Hall JE. Estrogen negative feedback on gonadotropin secretion: evidence for a direct pituitary effect in women. J Clin Endocrinol Metab. 2010;95(4):1955–61. https://doi.org/10.1210/jc.2009-2108.

Simonneaux V. A kiss to drive rhythms in reproduction. Eur J Neurosci. 2018. https://doi.org/10.1111/ejn.14287.

Skorupskaite K, George JT, Anderson RA. The kisspeptin-GnRH pathway in human reproductive health and disease. Hum Reprod Update. 2014;20(4):485–500. https://doi.org/10.1093/humupd/dmu009.

Skrapits K, Borsay B, Herczeg L, Ciofi P, Liposits Z, Hrabovszky E. Neuropeptide co-expression in hypothalamic kisspeptin neurons of laboratory animals and the human. Front Neurosci. 2015;9:29. https://doi.org/10.3389/fnins.2015.00029.

Stamatiades GA, Kaiser UB. Gonadotropin regulation by pulsatile GnRH: signaling and gene expression. Mol Cell Endocrinol. 2018;463:131–41. https://doi.org/10.1016/j.mce.2017.10.015.

Stojilkovic SS, Chang JP, Izumi S, Tasaka K, Catt KJ. Mechanisms of secretory responses to gonadotropin-releasing hormone and phorbol esters in cultured pituitary cells. Participation of protein kinase C and extracellular calcium mobilization. J Biol Chem. 1988;263(33):17301–6.

Sykiotis GP, Pitteloud N, Seminara SB, Kaiser UB, Crowley WF. Deciphering genetic disease in the genomic era: the model of GnRH deficiency. Sci Transl Med. 2010;2(32):32rv32. https://doi.org/10.1126/scitranslmed.3000288.

Terasawa E, Guerriero KA, Plant TM. Kisspeptin and puberty in mammals. Adv Exp Med Biol. 2013;784:253–73. https://doi.org/10.1007/978-1-4614-6199-9_12.

Themmen APN, Huhtaniemi IT. Mutations of gonadotropins and gonadotropin receptors: elucidating the physiology and pathophysiology of pituitary-gonadal function. Endocr Rev. 2000;21(5):551–83. https://doi.org/10.1210/edrv.21.5.0409.

Topaloglu AK. Update on the genetics of idiopathic hypogonadotropic hypogonadism. J Clin Res Pediatr Endocrinol. 2017;9(Suppl 2):113–22. https://doi.org/10.4274/jcrpe.2017.S010.

Topaloglu AK, Reimann F, Guclu M, Yalin AS, Kotan LD, Porter KM, Serin A, Mungan NO, Cook JR, Ozbek MN, Imamoglu S, Akalin NS, Yuksel B, O'Rahilly S, Semple RK. TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for neurokinin B in the central control of reproduction. Nat Genet. 2009;41(3):354–8. https://doi.org/10.1038/ng.306. [pii].

Toro CA, Aylwin CF, Lomniczi A. Hypothalamic epigenetics driving female puberty. J Neuroendocrinol. 2018;30(7):e12589. https://doi.org/10.1111/jne.12589.

Tsutsui K, Saigoh E, Ukena K, Teranishi H, Fujisawa Y, Kikuchi M, Ishii S, Sharp PJ. A novel avian hypothalamic peptide inhibiting gonadotropin release. Biochem Biophys Res Commun. 2000;275(2):661–7. https://doi.org/10.1006/bbrc.2000.3350.

Tsutsumi R, Webster NJ. GnRH pulsatility, the pituitary response and reproductive dysfunction. Endocr J. 2009;56(6):729–37.

Ubuka T, Morgan K, Pawson AJ, Osugi T, Chowdhury VS, Minakata H, Tsutsui K, Millar RP, Bentley GE. Identification of human GnIH homologs, RFRP-1 and RFRP-3, and the cognate receptor, GPR147 in the human hypothalamic pituitary axis. PLoS One. 2009;4(12):e8400. https://doi.org/10.1371/journal.pone.0008400.

White RB, Eisen JA, Kasten TL, Fernald RD. Second gene for gonadotropin-releasing hormone in humans. Proc Natl Acad Sci U S A. 1998;95(1):305–9.

Witchel S, Plant T. Puberty: gonardarche and adrenarche. In: Strauss JF, Barbieri RL, editors. Yen and Jaffe's reproductive endocrinology. Philadelphia: Saunders Elsevier; 2014. p. 382–421.

Yu B, Ruman J, Christman G. The role of peripheral gonadotropin-releasing hormone receptors in female reproduction. Fertil Steril. 2011;95(2):465–73. https://doi.org/10.1016/j.fertnstert.2010.08.045. S0015-0282(10)02459-3 [pii].

The Menstrual Cycle and Related Disorders

2

Sarah L. Berga

Contents

Abstract

The menstrual cycle refers to an orderly progression of events that produces a mature ovum ready for fertilization and an endometrium primed for implantation. Colloquially, the common use of the term menstrual cycle also refers to the periodic shedding of the endometrium that occurs when fertilization and implantation do not occur after ovulation is often termed "menses" by the medical profession and "a period" by the lay public. The tightly orchestrated sequence of events that comprises the menstrual cycle requires appropriate input or drive from the hypothalamic gonadotropin-releasing hormone (GnRH) neuronal network. The decapeptide GnRH is released into the portal circulation in a pulsatile manner, and the pulses must be of sufficient frequency and magnitude to drive pituitary release of the gonadotropins, LH, and FSH. Understanding what is a "normal" menstrual cycle is critical to promoting women's health, including fertility, and to diagnosing and treating disorders or disturbances of the menstrual

S. L. Berga (✉)
Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, University of Utah School of Medicine, Salt Lake City, UT, USA
e-mail: sberga1@aol.com; sberga1@aol.com

F. Petraglia, B. C. Fauser (eds.), *Female Reproductive Dysfunction*, Endocrinology,
https://doi.org/10.1007/978-3-030-14782-2_2

cycle, including those commonly associated with infertility and other health consequences. Since there are many medical conditions that cause anovulation and luteal insufficiency, it is imperative that physicians and other health practitioners who care for women as patients understand the physiology of cyclic ovarian function and its impact on women's health and disease.

Keywords

Menstrual cycle · GnRH · Ovarian reserve · Amenorrhea · Eumenorrhea

Introduction

The menstrual cycle refers to an orderly progression of events that produces a mature ovum ready for fertilization and an endometrium primed for implantation. Colloquially, the common use of the term menstrual cycle also refers to the periodic shedding of the endometrium that occurs when fertilization and implantation do not occur after ovulation is often termed "menses" by the medical profession and "a period" by the lay public. The tightly orchestrated sequence of events that comprises the menstrual cycle requires appropriate input or drive from the hypothalamic gonadotropin-releasing hormone (GnRH) neuronal network. The decapeptide GnRH is released into the portal circulation in a pulsatile manner, and the pulses must be of sufficient frequency and magnitude to drive pituitary release of the gonadotropins, LH, and FSH. Understanding what is a "normal" menstrual cycle is critical to promoting women's health, including fertility, and to diagnosing and treating disorders or disturbances of the menstrual cycle, including those commonly associated with infertility and other health consequences. Since there are many medical conditions that cause anovulation and luteal insufficiency, it is imperative that physicians and other health practitioners who care for women as patients understand the physiology of cyclic ovarian function and its impact on women's health and disease. In essence, every tissue has estrogen, progesterone, and androgen receptors, so the typical hormonal excursions of the menstrual cycle impact not only organs and tissues traditionally associated with reproductive function but also those not specific to or essential for reproduction. Likewise, alterations in ovarian function impact more than reproductive function. This chapter presents a synopsis of our current appreciation of the ontogeny of the menstrual cycle and reproductive aging with the goal of enhancing the diagnosis and treatment of menstrual cycle disorders to achieve fertility when desired and promote overall general health.

Physiology of the Normal Menstrual Cycle

Technically, a normal menstrual cycle starts with appropriate hypothalamic GnRH drive and also requires physiological responses from the pituitary, ovaries, and endometrium to that input. Any deviations from appropriate function at any level

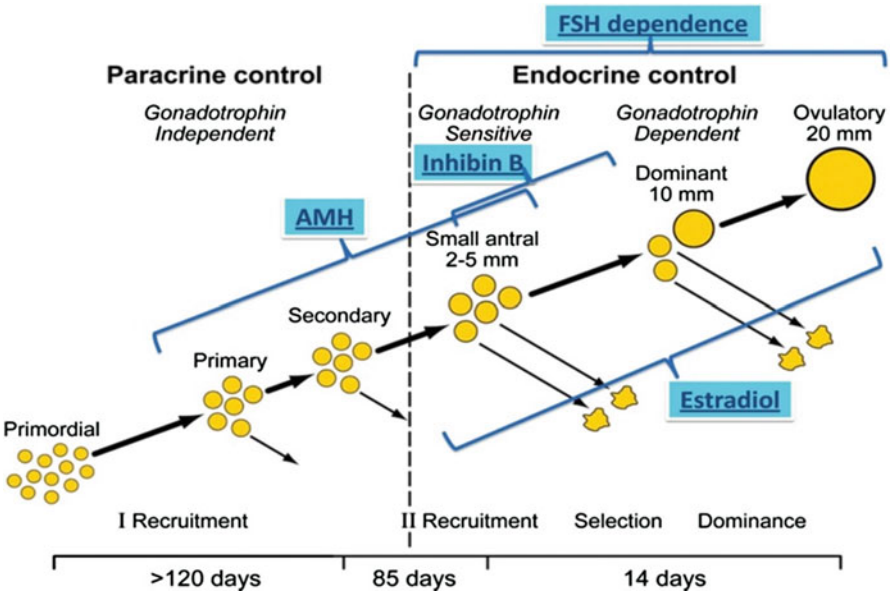


Fig. 1 Schematic representation of follicle development emphasizing that AMH is produced in early stages of follicle development (characterized by gonadotrophin-independent growth), as opposed to inhibin B and estradiol which are produced by follicles at later stages of development when growth depends on FSH. (Adapted from McGee and Hsueh 2000 in Broer et al. 2014)

of the hypothalamic-pituitary-ovarian-endometrial (HPOE) axis can result in a menstrual cycle disorder. Sex steroid excursions associated with cyclic ovarian function including ovulation not only prime the endometrium for implantation but also leave their molecular and cellular imprint on every tissue in the body, including bone, brain, breast, cardiovascular tree, skin, hair, and fat. Indeed, one signature of cyclic ovarian function is gynecoid fat deposition and altered metabolism.

The menstrual cycle is generally viewed as being composed of two halves, the follicular and the luteal phases when referring to ovarian stages and the proliferative and secretory phases when referring to endometrial events. During the follicular phase, in response to FSH levels that exceed the required threshold for a sufficient number of days, one or more oocytes grow from an antral follicle to a preovulatory follicle, and this progression is accompanied by a progressive increase in estradiol. The stages of oocyte growth and development, termed recruitment, selection, and dominance, are shown in Fig. 1 (Broer et al. 2014).

The exponential rise in estradiol associated with the final stage of follicular maturation triggers a LH surge that causes the ovum or ova to be released from the follicle. The increase in estradiol causes the endometrium to proliferate, hence the designation “proliferative phase.” The rapid rise of LH can be detected 12–24 h later in the urine, and this is the basis for most “ovulation predictor kits” that are manufactured for home use.

The post-ovulatory or luteal phase begins when the follicle becomes a progesterone-secreting corpus luteum. Absent implantation, this phase lasts approximately 14 days, but the luteal phase can be shorter than 14 days when gonadotropin stimulation is insufficient to support ongoing corpus luteal function or if the follicular apparatus that housed the oocyte developed suboptimal. The former generally reflects insufficient GnRH drive to support full folliculogenesis, while the latter often reflects ovarian aging with low antral follicle count and/or poor-quality oocytes. This is often termed diminished ovarian reserve and is a common cause of infertility. Progesterone exposure of sufficient amount and duration causes the endometrium to transform into a secretory pattern that then permits implantation or shedding if implantation does not occur.

By convention, the first day of menstrual shedding, termed menses, is cycle day 1. However, if the day of the LH surge is known, that day may be designated day 0 and the preceding follicular days assigned −1, −2, and so on, while the luteal phase may be numbered +1, +2, and so on. A typical menstrual cycle lasts 28–29 days, but the interval between cycle day 1 of menses and onset of cycle day 1 of next menses can be of any duration. Short and long duration cycle intervals typically are a clinical sign of altered ovarian function. At menarche, the menstrual interval is longer, around 30–32 days, and initially the menstrual interval is more erratic (Treloar et al. 1967). The menstrual cycle interval shortens progressively across with advancing

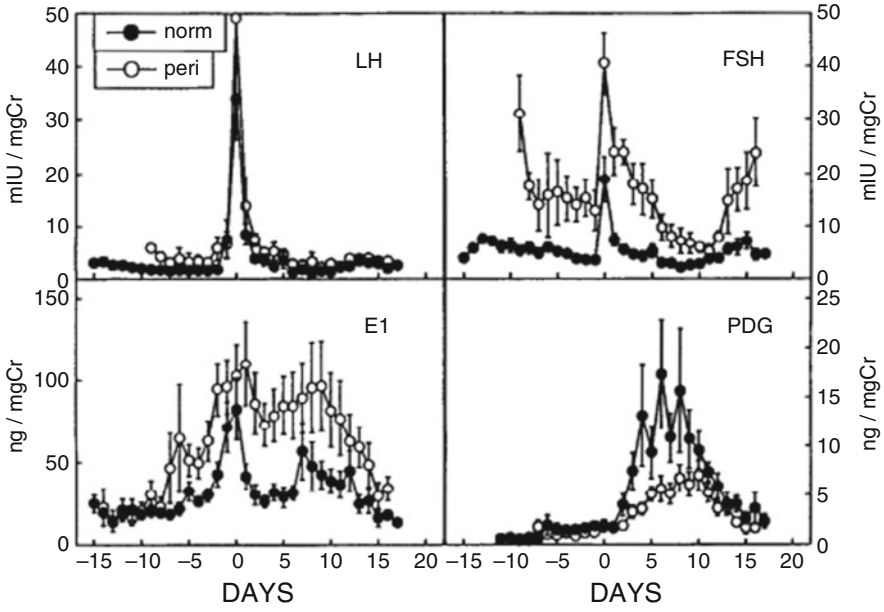


Fig. 2 Mean ± SEM daily urinary gonadotropin and sex steroid excretion patterns in 11 perimenopausal women aged 43–52 yr. (open circles) compared to 11 midreproductive aged women aged 19–38 yrs. (closed circles). Data are standardized to day 0, the putative day of ovulation. E1 = estrone conjugates and PDG = pregnanediol glucuronide (Santoro et al. 1996)

gynecologic age until the perimenopause ensues, when again cycles become more erratic. Gynecologic age refers to years since menarche and in the absence of a menstrual cycle disorder relatively constant from 20 to 40 years of age. As shown in Fig. 2, older and/or lower-quality oocytes yield both higher estradiol levels and lower progesterone levels (Santoro et al. 1996) and are associated with lower fertility. The excess of estrogen and decrement in progesterone may be associated with endometrial hyperplasia and fibroid growth.

The Role of Hypothalamic GnRH Drive

The complex regulation of the hypothalamic-pituitary-ovarian-endometrial axis aligns ovarian function and fertility with internal and external circumstance. The initiation of ovulation requires appropriate GnRH input. Ovarian dysfunction either is caused by or causes disordered GnRH drive. Gonadotropin levels and patterns primarily reflect GnRH input, although some factors, particularly estrogens, alter pituitary function independently of GnRH. Factors which modulate GnRH drive fall into categories, including factors which convey information about (1) the age and developmental stage of an individual; (2) ovarian function, including stage of folliculogenesis; (3) energy balance, body composition, and metabolism; (4) circadian and circannual position; (5) stress and emotional states; and (6) cognitive assessments. At the level of the hypothalamus, specialized neurovascular cells transport peripheral signals across the blood-brain barrier to communicate the individual's state (Prevot et al. 2007), including metabolic state, while central signals communicate via neuronal circuits and synapses and cerebrospinal fluid. GnRH drive can be increased, decreased, disordered, or appropriate. During the menstrual cycle, what is the appropriate GnRH pulse pattern depends on the stage of follicular development. In the follicular phase, GnRH pulsatility as reflected in LH pulsatility is approximately one pulse every 86 minutes (Leondires and Berga 1998). Hypothalamic drive is subject to feedback; stress, including undernutrition, and prepubertal status make the GnRH "pulse generator" more sensitive to estradiol negative feedback (Michopoulos et al. 2009). Increased sensitivity to estradiol negative feedback is one reason why stressed women do not readily ovulate in response to antagonism of estrogen action by agents such as clomiphene and letrozole.

Many of the factors that modulate GnRH secretion also modulate hypothalamic function more generally. Indeed, insufficient GnRH drive is rarely an isolated disruption of hypothalamic function except when GnRH neurons fail to migrate into or develop appropriately within the hypothalamus. Classically, idiopathic hypothalamic hypogonadism presents as primary amenorrhea and developmental delay. Pathogenetic studies have revealed several genetic causes (Caronia et al. 2011). In practice, it can be difficult to distinguish organic from functional causes of hypothalamic hypogonadism unless there are other associated developmental cues such as anosmia. More typically, insufficiency of GnRH drive to the reproductive axis is variable and functional, that is, not due to organic causes. The greater the suppression or disruption of GnRH drive, the more likely is amenorrhea to result and the

greater the likelihood of clinical detection. The term "functional hypothalamic amenorrhea" (FHA) is commonly understood to indicate that there is insufficient GnRH drive of prolonged duration that results in anovulation and manifests as amenorrhea. The term implies that no organic cause has been detected. Other terms for FHA include stress-induced anovulation (SIA), exercise amenorrhea when observed in an athletically active individual, and psychogenic amenorrhea (Reifenstein Jr 1946). The term functional hypothalamic hypogonadism connotes a spectrum of hypothalamic-pituitary-gonadal insufficiency that presents in women as a spectrum ranging from luteal insufficiency to complete anovulation and amenorrhea and in men as "low testosterone syndrome" and oligoasthenozoospermia.

The notion that GnRH neuronal pulsatility could be disrupted by stress is often met with disbelief among physicians and patients alike. However, over the last three decades, we and others have established that FHA is associated with increased cortisol in the circulation, urine, saliva, and cerebrospinal fluid (Brundu et al. 2006) that reflects activation of the hypothalamic-pituitary-adrenal (HPA) axis. Further, if stress is the cause, then behavioral changes that reduce stress should result in return of ovarian function and menses and a decrease in HPA activation. IN a randomized trial, we found that about 75% of women with FHA treated with cognitive behavior therapy (CBT) to address problematic attitudes and behaviors recovered ovarian function and menses as compared to those who were observed (Berga et al. 2003). Those who recovered following CBT showed a decrease in nocturnal cortisol secretion and an increase in TSH and leptin independent of weight gain (Michopoulos et al. 2013). Further, we found that women with FHA who recovered spontaneously displayed a concomitant increase in LH pulsatility and decrease in circulating cortisol and that women with other forms of anovulation did not have increased circulating cortisol (Berga et al. 1997).

To fully appreciate the mechanisms that underlie the alignment of reproduction with social determinants of health and environmental cues such as daylength requires recognizing that FHA is more than an isolated disruption of GnRH drive associated with modest increases in cortisol. There is also suppression of the hypothalamic-pituitary-thyroidal axis that manifests as reduced circulatory thyroxine and thyronine (Berga et al. 1989;s Loucks et al. 1992). Other neuroendocrine secretory patterns, including melatonin, are also altered (Berga et al. 1988). We have taken this constellation of neuroendocrine aberrations as evidence that stressors cause subtle, but pervasive, alterations in brain function. Indeed, in support of this view, using state-of-the-art neuroimaging techniques in humans, Hermans et al. found that stress-related noradrenergic activity prompted a large-scale neural network reconfiguration (Hermans et al. 2011). The shift was elicited by a fear-related acute stressor that provoked robust cortisol responses and involved both cortical (frontoinsular, dorsal anterior cingulate, inferotemporal, and temperoparietal) and subcortical (amygdala, thalamus, hypothalamus, and midbrain) regional as a function of stress response magnitudes. This shift in brain state allows strategic reallocation of resources to functions that are vital to survival and away from the energetic and psychological demands of reproductive function.

One difficulty in understanding the pathogenesis of FHA is recognizing the stressors that have elicited the stress response. Often, the stressors are not objective or quantifiable, but are subjective and idiosyncratic (Berga and Girton 1989; Giles and Berga 1993; Marcus et al. 2001). Indeed, particularly for humans, what is stressful entails cognitive interpretations and valences about what is important rather than simply behaviors that are easily quantified such as miles run per week or caloric intake. Women with FHA may not report stressors and may not recognize that they are stressed and clinicians may not ask. Indeed, we found that women with FHA often display low awareness of psychological status and often have unrealistically high expectations of self and others. It takes an astute clinician to recognize that seemingly mundane stressors can cause large-scale neural network reconfiguration that then activates the HPA axis while suppressing hypothalamic GnRH and TRH release.

Other types of anovulation are also associated with alterations in GnRH drive. Diminished ovarian reserve results in altered patterns of folliculogenesis including follicles that secrete only estradiol but in increased amounts relative to a normal menstrual cycle. Declines in the follicle pool result in elevated levels of FSH and a maximal GnRH-LH pulse frequency and amplitude as well as an insensitivity to estradiol negative feedback (Weiss et al. 2004). Polycystic ovary syndrome appears to be associated with a masculinization of the GnRH "pulse generator" with an increase in LH pulse frequency and amplitude and a decrease in FSH that leads to chronic anovulation and thecal-stromal ovarian hyperstimulation (Berga et al. 1993). Thus, GnRH-LH pulse frequency that is chronically too slow to support full folliculogenesis results in hypothalamic hypogonadism, while GnRH-LH that is chronically too rapid results in an altered LH:FSH ratio with anovulation due to insufficient FSH and hyperandrogenism from sustained LH stimulation of the ovarian stromal-theca cells that secrete androstenedione.

Ovarian Events

The menstrual cycle starts with the development of a cohort of follicles, one of which will become dominant. Follicles are comprised of an oocyte encircled by granulosa cells that are in turn surrounded by theca cells. Follicular development requires the sustained release of GnRH at a pulse frequency of about once every 90 minutes for approximately 14 days. GnRH stimulates the release of the pituitary gonadotropins LH and FSH. LH stimulates theca cells to synthesize and release androgens, whereas FSH induces granulosa cell development that includes the induction of the enzyme aromatase. Aromatase converts androstenedione produced by theca cells into estradiol. This is termed the "two-cell, two-gonadotropin" hypothesis of ovarian folliculogenesis (McNatty et al. 1979). In the presence of sustained GnRH drive, the secretion of FSH is regulated primarily by estradiol feedback at the level of the pituitary. As the follicles develop, there is a progressive rise in estradiol that suppresses FSH below the level needed for the less mature follicles to continue to grow, and one or two follicles continue to grow because they have become less

dependent on FSH and more dependent on LH (Zeleznik 2001). Selection of the dominant follicle occurs about day 8 or 9 of the follicular phase. The dominant or lead follicles continue to mature because of the development of LH receptors on its granulosa cells (Sullivan et al. 1999). Superovulation protocols used in infertility therapies work by providing sustained FSH stimulation of the ovaries that results in multifollicular development primarily based on egg count. Egg count or ovarian reserve can now be assessed by measuring AMH as shown in Fig. 1.

In the late follicular phase, the exponential rise in estradiol secreted by the nearly mature dominant follicle triggers a LH surge. The LH surge lasts about 36 h and depletes the pituitary of LH. Ovulation ensues 36–40 h after the start of the LH surge. Thereafter, granulosa cells transform into progesterone-secreting luteal cells, and the residual follicular apparatus becomes the corpus luteum. The progesterone secreted by the corpus luteum causes the endometrium to transform from a proliferative state to a secretory state capable of sustaining implantation. Progesterone also has other actions. It causes myometrial smooth muscle relaxation to facilitate implantation should the ovum be fertilized. The combined exposure to estradiol and progesterone alters central neuroregulation and slows GnRH and LH pulse frequency to about one pulse every 4 h. This slowing of GnRH pulse frequency further ensures that FSH levels do not rise, thereby constraining follicular development of the next cohort of follicles.

The process by which a primordial follicle leaves the resting pool and starts to grow is termed recruitment. For follicles to grow beyond the antral stage, they need sustained FSH stimulation. Of note, recruitment and selection are the final phases of ovum maturation. Prior to follicles becoming ready for recruitment, there is a largely gonadotropin-independent stage that lasts at least 120 days (McGee and Hsueh 2000). Germ cells arrive in the gonadal ridge from the yolk sac endoderm by the 7th week of gestation, and then the oogonia transform into oocytes and enter the first stage of meiosis until about the 20th week of gestation. Thereafter, oogonia form primordial follicles, a process that lasts until shortly after birth. Primordial follicles are defined by their simple structure consisting of an ovum surrounded by a layer of flattened epithelial cells. The endowment of primordial follicles is fixed in utero, and after birth the number of primordial follicles declines, a process termed atresia. The determinants of the rate of oocyte atresia are under investigation. To date, 88 genes have been correlated with follicle count and/or rates of atresia (Desai and Rajkovic 2017; Qin et al. 2015). Examples of accelerated rates of atresia related to the X chromosome include fragile X mutation (*FMR1*) and Turner syndrome (45,XO).

Across time, a certain percentage of resting follicles become atretic (die) regardless of the level of FSH. Follicles that remain in the resting state do so because of active inhibition that requires oocyte-granulosa cell communication. Very high levels of FSH may accelerate rates of atresia. Before puberty, primordial follicles initiate growth up to the FSH-independent stage, and then they undergo atresia due to insufficient FSH to support recruitment. It takes about 120 days for a primordial follicle to grow to the secondary stage and about 85 days for it to grow from a secondary follicle to a FSH-sensitive antral follicle. Antral follicles contain oocytes that have acquired a zona pellucida and are competent to undergo germinal vesicle breakdown to complete meiosis. As noted previously, selection of the dominant

follicle occurs when the lead follicle secretes enough estradiol to suppress FSH below the threshold needed for follicular development and the dominant follicle escapes demise by developing LH receptors and becoming FSH independent and responsive to the LH surge triggered by the exponential rise in estradiol primarily from the lead follicle(s).

The process of ovulation involves a series of tightly orchestrated events. Meiosis is arrested until just before ovulation occurs, when oocytes undergo nuclear progression from the dictyate of the first meiotic prophase (4X the haploid DNA complement) to metaphase I (2X the haploid DNA complement). Germinal vesicle breakdown and extrusion of the first polar body accompany the first meiotic division. If fertilization occurs, meiosis is completed with reduction to a single DNA haploid complement and extrusion of the second polar body. The release of the mature ovum from the follicle requires the degeneration of the follicle wall by proteolytic enzymes, including plasminogen activator. Prostaglandins are formed in the follicular fluid, and the concentrations peak at the time of ovulation and participate in the proteolysis of the follicle as well as extrusion of the oocyte-cumulus cell mass by smooth muscles in the ovary.

Characterization of Menstrual Patterns

Altered menstrual patterns are a readily identifiable indicator of altered ovarian function. The causes of altered menstrual patterns are many and include both ovarian and nonovarian causes. Common ovarian causes include (1) polycystic ovary syndrome, sometimes referred to as hyperandrogenic anovulation; (2) premature ovarian insufficiency which is due to reduced or accelerated loss of oocytes and is often accompanied by autoimmune disorders such as juvenile diabetes and autoimmune thyroiditis and by chromosomal abnormalities such as 45,XO or 46,XX/45,XO mosaicism or microdeletions within the long arm of the X chromosome; (3) stress-induced hypothalamic hypogonadism, which is accompanied by a constellation of neuroendocrine aberrations including hypercortisolism and hypothalamic hypothyroidism; (4) syndromal psychiatric syndromes such as depression, schizophrenia, and eating disorders, including anorexia nervosa and bulimia, which may alter neuroendocrine function and result in hypothalamic hypogonadism; (5) other systemic medical conditions and disorders including thyroidal conditions, hyperprolactinemia, and adrenal disorders to name but a few; (6) environmental and other unintentional exogenous hormonal exposures; (7) hormone use such as oral contraceptives; (8) drug use and abuse, including alcohol, marijuana, and opiates; (9) medications including antipsychotics and neuroleptics, selective estrogen receptor modulators (clomiphene, tamoxifen, raloxifene), and aromatase inhibitors; and (10) food-based phytoestrogens.

Women with eating disorders often have severe nutritional compromise and significant hypercortisolemia. There is a paucity of studies on the long-term health of women with all forms of hypothalamic hypogonadism. Greater investigative attention has been given to women with polycystic ovary syndrome due to

accompanying features of metabolic syndrome than women with premature ovarian insufficiency and functional hypothalamic hypogonadism.

Stressors produce hypothalamic hypogonadism in both women and men, with attendant reductions in sex-specific hormones such as estradiol and progesterone in women and testosterone in men (Stephens et al. 2015). Women may be more sensitive to certain types of psychosocial stressors than men, while men appear to be more sensitive to metabolic stressors such as undernutrition than women (Berga et al. 2001; Cameron et al. 1991; Davis 2000). During lactation, women are much less sensitive to metabolic stressors (Altemus et al. 1995). Furthermore, while stressors increase cortisol secretion in women and men, the molecular signature and other effects of cortisol upon various tissues differ by sex (Duma et al. 2010). Cortisol antagonizes the physiological actions of estrogen through co-regulation of the same molecular pathways, suggesting that stress-induced hypogonadism cannot be counteracted by hormone replacement regimens that simply supply the missing gonadal hormones (Whirledge and Cidlowski 2013).

Recognizing and diagnosing the cause of altered menstrual cycle function is rarely straightforward, but it is important to establish the etiology and to recognize that cyclic ovarian function or its absence affects nonreproductive tissues (Berga et al. 2001).

The first step in detecting altered ovarian function is to determine the bleeding pattern. There are many causes of abnormal uterine bleeding. An evaluation of abnormal uterine bleeding (AUB) must determine the presence or absence of endometrial and cervical polyps, fibroids, malignancy, coagulopathy, endometrial infection, adhesions, and scarring and exogenous exposure to hormones and hormonal mimetics (Munro et al. 2011). Once the above causes have been identified or excluded, the next step is to determine the presence or absence of ovulation and ovarian dysfunction. Ovarian patterns can be classified as fully ovulatory, anovulatory, and luteal insufficiency with either long or short follicular phases (Sherman and Korenman 1975). There is a spectrum of ovarian function ranging from fully ovulatory to fully anovulatory. The intermediate states are the most difficult to identify and characterize and result from a combination of variable hypothalamic GnRH drive and varying oocyte quantity and quality. If ovarian dysfunction is detected, the cause must then be sought. Functional hypothalamic hypogonadism is likely the most common cause of AUB and amenorrhea, but it is a diagnosis of exclusion. To make matters more complex, ovarian function changes with age as the egg reserve diminishes and oocyte responses become increasingly less predictable. Classification schemes such as STRAW (Stages of Reproductive Aging Workshop) aim to partition the transition from normative ovarian function to menopause but cannot fully define an individual woman's endogenous hormonal exposures (Harlow et al. 2012) nor predict timing of menopause. The lack of a simple, robust methodology for classifying ovarian function and dysfunction is intrinsic to the reproductive system and likely explains, at least in part, the lack of clarity about the contribution of sex hormones to overall health.

Strategies exist for characterizing alterations in gonadal function, but most of the strategies require serial blood or urine samples taken across time and often defined pragmatically as the interval between day of first menstrual bleeding and the day of

next first menstrual bleeding. When menstrual cycles occur with predictable regular intervals, a single blood sample that is appropriately timed allow for characterization of gonadal function (Berga et al. 2001). For instance, in women whose menstrual cycle interval is regularly 27–31 days, the presence or absence of ovulation can be easily monitored with (1) over-the-counter urinary LH surge detector kits and (2) measuring progesterone during the midluteal phase (days 20–23 from day of first menses) or day +7 after LH surge. A progesterone >10 ng/ml is considered "fully ovulatory," a level < 1 ng/ml signals anovulation, and an intermediate value indicates either luteal insufficiency or a delayed or advanced luteal phase relative to cycle day 1. Tracking urinary LH with home kits helps to establish the presence and length of the luteal phase and allows for better timing of the measurement of progesterone to determine luteal sufficiency. How often to sample blood, saliva, urine, or any other bodily tissue depends on the need to accurately define ovarian function. The more frequent the sampling, the greater the accuracy of assessment but

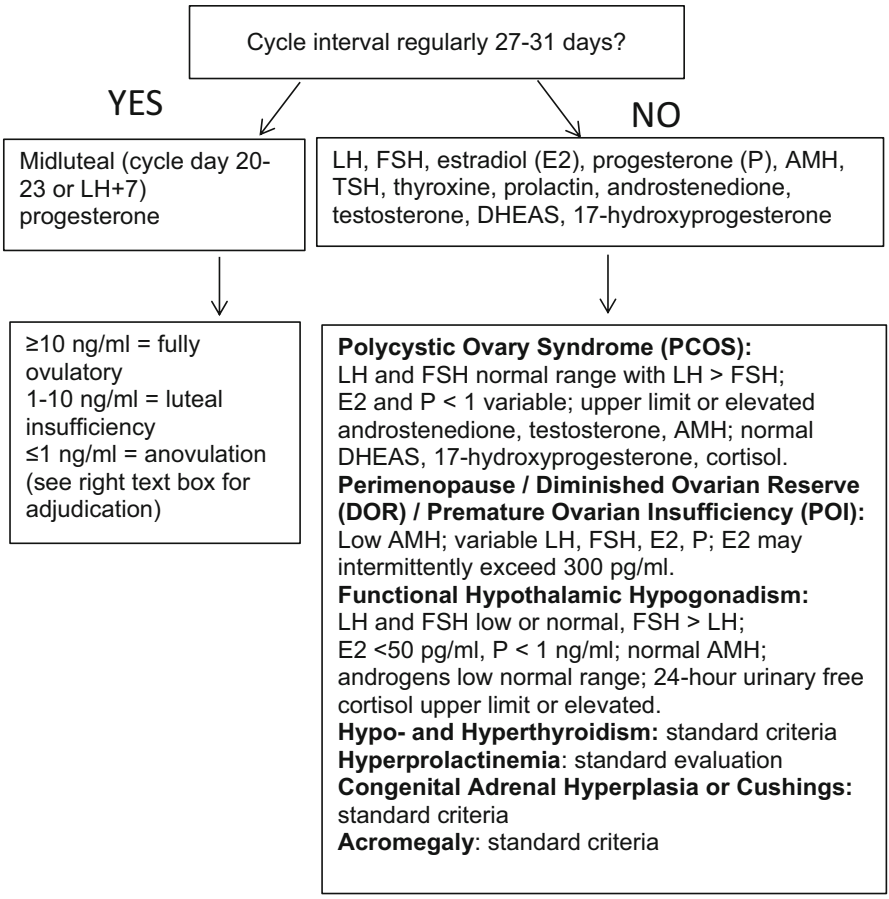


Fig. 3 Algorithm for characterizing ovarian function

the greater the subject burden and cost. The gold standard for measurement of sex steroids such as testosterone and androstenedione in blood or other tissues is liquid chromatography linked with mass spectroscopy. This method reduces the possibility of measuring similar steroids (greater specificity) and allows for greater sensitivity. This method may not be cost-effective or feasible in all research or clinical environments, although it is becoming more affordable and more widely available (Rosner et al. 2010, 2013). Various immunoassay methodologies are commercially available (Rosner et al. 2013; Wierman et al. 2006; Miller et al. 2004). Figure 3 provides an algorithm for characterizing ovarian function.

Interpretation of hormonal levels requires considering the overall pattern including neuroendocrine signals. The complexity is often frustrating for clinical investigators hoping for simple classification schemes. Menopausal typically presents as a change in menstrual pattern with associated symptoms such as hot flashes. The WISE study developed an algorithm using menstrual, surgical, and reproductive history with serum hormone assays to determine reproductive stage (Johnson et al. 2004). AMH has provided a new window for determining ovarian reserve, perimenopausal ovarian function, and menopausal status (Broer et al. 2014).

Clinical Considerations

The hormonal excursions associated with a typical menstrual cycle serve as important physiological benchmarks for understanding the health benefits of ovulation and eumenorrhea. The health consequences of anovulation depend on the cause. We are beginning to appreciate the pros and cons of hormone use for gender-affirming hormone therapy, contraception, postmenopausal hormone use, and many other therapeutic uses. Hormones affect all tissues and organs, including those not traditionally classified as reproductive. An understanding of the normal menstrual cycle can help us refine our therapeutic uses and assess risk for dementia, depression, osteoporosis, cardiovascular disease, diabetes, and many other medical conditions.

Certain mythologies deserve clarification. The first is that the luteal phase is always 14 days. In fact, the corpus luteum has the inherent capacity to sustain progesterone secretion for about 14 days but only if there is sufficient LH secretion and a normal corpus luteum developed. Another common myth is that withdrawal bleeding signals that the preceding cycle was ovulatory. Menstrual bleeding occurs whenever there is a decline in estradiol and hence the presence or absence of bleeding is not always a good indicator of ovulatory ovarian function.

The excursions in the levels of estradiol and progesterone associated with ovulatory ovarian function are a physiological constant. Menstrual cycle disorders are associated with deviations from the physiological standard and carry health consequences such as increased cardiovascular disease, osteoporosis, hirsutism, depression, and anovulation. When fertility is the goal, the clinical focus is on determining if there is ovulatory ovarian function and appropriate endometrial development. When considering how to replicate physiology for therapeutic purposes such as menopausal hormone therapy and endometrial preparation for embryo transfer, it is

critical to recognize that not all estrogens and progestins elicit the same molecular and cellular responses. Further it is important to understand interactions among hormones. For instance, glucocorticoids block estrogen action (Whirledge and Cidlowski 2013), so stress-induced anovulation is more than lack of ovarian function, and treatment requires more than hormone replacement (Gordon et al. 2017). In devising rational treatment strategies for postmenopausal women, it is important to know the levels of estradiol and progesterone associated with a normal menstrual cycle. For estradiol, the mean level across a normal menstrual cycle is approximately 100 pg/ml with a low of 20–30 pg/ml on day 2–3 and a peak of 300–400 pg/ml at midcycle. Progesterone levels in the luteal phase peak in the range of 10–20 ng/ml. Estradiol delivered topically best approximates physiology. To approximate physiological progesterone levels requires a topical or intramuscular route of administration. For ease, oral micronized progesterone is often administered.

Conclusion

In summary, understanding the normal menstrual cycle provides the necessary foundation for guiding hormone use, diagnosing disorders of menstruation and anovulation, and delineating disease risk. Given the profound effects of ovarian cyclicity and the associated sex steroid excursions, medical professionals are advised to ask patients about their menstrual cycle patterns. Indeed, menstrual dates are an important vital sign for premenopausal women.

Cross-References

- ▶ Abnormal Uterine Bleeding
- ▶ Diagnostic Protocols for Infertility
- ▶ Infertility
- ▶ Menstrual Disorders Related to Endocrine Diseases
- ▶ Premature Ovarian Insufficiency
- ▶ The Hypothalamus-Pituitary-Ovary Axis
- ▶ The Menstrual Disorders Related to Systemic Diseases
- ▶ The Polycystic Ovary Syndrome (PCOS)

References

Altemus M, Deuster PA, Galliven E, Carter CS, Gold PW. Suppression of hypothalamic-pituitary-adrenal axis responses to stress in lactating women. J Clin Endocrinol Metab. 1995;80:2954–9.

Berga SL. Systemic benefits of cyclic ovarian function. J Soc Gynecol Investig. 2001;8:S3–6.

Berga SL, Girton LG. The psychoneuroendocrinology of functional hypothalamic amenorrhea. Psychiatr Clin North Am. 1989;12:105–16.

Berga SL, Mortola JF, Yen SS. Amplification of nocturnal melatonin secretion in women with functional hypothalamic amenorrhea. J Clin Endocrinol Metab. 1988;66:242–4.

Berga SL, Mortola JF, Girton L, Suh B, Laughlin G, Pham P, Yen SS. Neuroendocrine aberrations in women with functional hypothalamic amenorrhea. J Clin Endocrinol Metab. 1989;68:301–8.
Berga SL, Guzick DS, Winters SJ. Increased luteinizing hormone and alpha-subunit secretion in women with hyperandrogenic anovulation. J Clin Endocrinol Metab. 1993;77:895–901.
Berga SL, Daniels TL, Giles DE. Women with functional hypothalamic amenorrhea but not other forms of anovulation display amplified cortisol concentrations. Fertil Steril. 1997;67:1024–30.
Berga SL, Loucks TL, Cameron JL. Endocrine and chronobiological effects of fasting in women. Fertil Steril. 2001;75:926–32.
Berga SL, Marcus MD, Loucks TL, Hlastala S, Ringham R, Krohn MA. Recovery of ovarian activity in women with functional hypothalamic amenorrhea who were treated with cognitive behavior therapy. Fertil Steril. 2003;80:976–81.
Broer SL, Broekmans FJ, Laven JS, Fauser BC. Anti-Mullerian hormone: ovarian reserve testing and its potential clinical implications. Hum Reprod Update. 2014;20:688–701.
Brundu B, Loucks TL, Adler LJ, Cameron JL, Berga SL. Increased cortisol in the cerebrospinal fluid of women with functional hypothalamic amenorrhea. J Clin Endocrinol Metab. 2006;91:1561–5.
Cameron JL, Weltzin TE, McConaha C, Helmreich DL, Kaye WH. Slowing of pulsatile luteinizing hormone secretion in men after forty-eight hours of fasting. J Clin Endocrinol Metab. 1991;73:35–41.
Caronia LM, Martin C, Welt CK, Sykiotis GP, Quinton R, Thambundit A, Avbelj M, Dhruvakumar S, Plummer L, Hughes VA, Seminara SB, Boepple PA, Sidis Y, Crowley WF Jr, Martin KA, Hall JE, Pitteloud N. A genetic basis for functional hypothalamic amenorrhea. N Engl J Med. 2011;364:215–25.
Davis SN, Galassetti P, Wasserman DH, Tate D. Effects of gender on neuroendocrine and metabolic counterregulatory responses to exercise in normal man. J Clin Endocrinol Metab. 2000;85 (1):224–30. https://doi.org/10.1210/jcem.85.1.6328.
Desai S, Rajkovic A. Genetics of reproductive aging from gonadal dysgenesis through menopause. Semin Reprod Med. 2017;35:147–59.
Duma D, Collins JB, Chou JW, Cidlowski JA. Sexually dimorphic actions of glucocorticoids provide a link to inflammatory diseases with gender differences in prevalence. Sci Signal. 2010;3(143):ra74. https://doi.org/10.1126/scisignal.2001077.
Giles DE, Berga SL. Cognitive and psychiatric correlates of functional hypothalamic amenorrhea: a controlled comparison. Fertil Steril. 1993;60:486–92.
Gordon CM, Ackerman KE, Berga SL, Kaplan JR, Mastorakos G, Misra M, Murad MH, Santoro NF, Warren MP. Functional hypothalamic amenorrhea: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2017;102:1413–39.
Harlow SD, Gass M, Hall JE, Lobo R, Maki P, Rebar RW, Sherman S, Sluss PM, de Villiers TJ. Straw +10 collaborative group. Executive summary of the stages of reproductive aging workshop +10: addressing the unfinished agenda of staging reproductive aging. J Clin Endocrinol Metab. 2012;97:1159–68.
Hermans EJ, van Marle HJF, Ossewaarde L, Henckens MJAG, Qin S, van Kesteren MTR, Schoots VC, Cousijn H, Rijpkema M, Oostenveld R, Fernandez G. Stress-related noradrenergic activity prompts large-scale neural network reconfiguration. Science. 2011;334:1151–3.
Johnson BD, Merz CN, Braunstein GD, Berga SL, Bittner V, Hodgson TK, et al. Determination of menopausal status in women: the NHLBI-sponsored Women's ischemia syndrome evaluation (WISE) study. J Womens Health (Larchmt). 2004;13:872–87.
Leondires MP, Berga SL. Role of GnRH drive in the pathophysiology of polycystic ovary syndrome. J Endocrinol Investig. 1998;21:476–85.
Loucks AB, Laughlin GA, Mortola JF, Girton L, Nelson JC, Yen SSC. Hypothalamic-pituitary-thyroidal function in eumenorrheic and amenorrheic athletes. J Clin Endocrinol Metab. 1992;75:514–8.
Marcus MD, Loucks TL, Berga SL. Psychological correlates of functional hypothalamic amenorrhea. Fertil Steril. 2001;76:310–6.

McGee EA, Hsueh AJW. Initial and cycle recruitment of ovarian follicles. Endocr Rev. 2000;21:200–14.
McNatty MA, DeGrazia G, Osathanondh R, Ryan KJ. The production of progesterone, androgens, and estrogens by granulosa cell, thecal tissue, and stromal tissue form human ovaries in vivo. J Clin Endocrinol Metab. 1979;49:687–99.
Michopoulos V, Berga SL, Kaplan JR, Wilson ME. Social subordination and polymorphisms in the gene encoding the serotonin transporter enhance estradiol inhibition of luteinizing hormone secretion in female rhesus monkeys. Biol Reprod. 2009;81:1154–63.
Michopoulos V, Mancini F, Loucks TL, Berga SL. Neuroendocrine recovery initiated by cognitive behavioral therapy in women with functional hypothalamic amenorrhea: a randomized, controlled trial. Fertil Steril. 2013;99:2084–91.
Miller KK, Rosner W, Lee H, Hier J, Sesmilo G, Schoenfeld D, Neubauer G, Klibanski A. Measurement of free testosterone in normal women and women with androgen deficiency: comparison of methods. J Clin Endocrinol Metab. 2004;89:525–33.
Munro MG, Critchley HO, Fraser IS, Group FMDW. The FIGO classification of causes of abnormal uterine bleeding in the reproductive years. Fertil Steril. 2011;95:2204–8.
Prevot V, Dehouck B, Poulain P, Beauvillain JC, Buée-Scherrer V, Bouret S. Neuronal-glial endothelial interactions and cell plasticity in the postnatal hypothalamus: implications for the neuroendocrine control of reproduction. Psychoneuroendocrinology. 2007;32(Suppl 1):S46–51.
Qin Y, Jiao SJL, Chen Z-J. Genetics of primary ovarian insufficiency: new developments and opportunities. Hum Reprod Update. 2015;21:787–808.
Reifenstein EC Jr. Psychogenic or hypothalamic amenorrhea. Med Clin North Am. 1946;30:1103–14.
Rosner W, Vesper H, Endocrine S. American Association for Clinical C, American Association of Clinical E, androgen excess PS et al. toward excellence in testosterone testing: a consensus statement. J Clin Endocrinol Metab. 2010;95:4542–8.
Rosner W, Hankinson SE, Sluss PM, Vesper HW, Wierman ME. Challenges to the measurement of estradiol: an endocrine society position statement. J Clin Endocrinol Metab. 2013;98:1376–87.
Santoro N, Brown JR, Adel T, Skurnick JH. Characterization of reproductive hormonal dynamics in the perimenopause. J Clin Endocrinol Metab. 1996;81:1495–501.
Sherman BM, Korenman SG. Hormonal characteristics of the human menstrual cycle throughout reproductive life. J Clin Invest. 1975;55:699–706.
Stephens MA, Mahon PB, McCaul ME, Wand GS. Hypothalamic-pituitary-adrenal axis response to acute psychosocial stress: effects of biological sex and circulating sex hormones. Psychoneuroendocrinology. 2015;66:47–55.
Sullivan MW, Stewart-Akers A, Krasnow JS, Berga SL, Zeleznik AJ. Ovarian responses in women to recombinant follicle-stimulating hormone and luteinizing hormone (LH):a role for LH in the final stages of follicular maturation. J Clin Endocrinol Metab. 1999;84:228–32.
Treloar AE, Boynton RE, Behn BG, Brown BW. Variation of the human menstrual cycle through reproductive life. Int J Fertil. 1967;12:77–126.
Weiss G, Skurnick JH, Goldsmith LT, Santoro NF, Park SJ. Menopause and hypothalamic-pituitary sensitivity to estrogen. JAMA. 2004;292:2991–6.
Whirledge S, Cidlowski JA. A role for glucocorticoids in stress-impaired reproduction: beyond the hypothalamus and pituitary. Endocrinology. 2013;154:4450–68.
Wierman ME, Basson R, Davis SR, Khosla S, Miller KK, Rosner W, et al. Androgen therapy in women: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2006;91:3697–710.
Zeleznik AJ. Follicle selection in primates: “many are called but few are chosen”. Biol Reprod. 2001;65:655–9.

The Polycystic Ovary Syndrome (PCOS)

3

Yvonne V. Louwers and Joop S. E. Laven

Contents

Abstract

PCOS is the commonest endocrine abnormality in women of reproductive age. It represents the major cause of anovulatory infertility and is also associated with hirsutism and acne. The typical biochemical features are elevated serum levels of testosterone and luteinizing hormone (LH) along with metabolic disturbances

Y. V. Louwers · J. S. E. Laven (✉)
Erasmus University Medical Centre, Rotterdam, The Netherlands
e-mail: j.laven@erasmusmc.nl

F. Petraglia, B. C. Fauser (eds.), *Female Reproductive Dysfunction*, Endocrinology,
https://doi.org/10.1007/978-3-030-14782-2_3

including insulin resistance and abnormalities of energy expenditure. PCOS is now recognized as a major risk factor for the development of type 2 diabetes (T2DM) and cardiovascular disease in later life. At least in part, this reflects the strong associations between PCOS and obesity, with the latter being an amplifier of PCOS.

The etiology of PCOS is unclear, and it seems to be a complex disease resulting from a complex interplay between genetic and environmental factors. Moreover, there is evidence for familial clustering of endocrine and metabolic features of PCOS. Environmental factors such as diet and obesity might similarly contribute to the phenotype. Due to its heterogeneous nature, there have been historical disagreements about the definitions and how to diagnose PCOS.

Treatment should be tailored to the complaints and needs of the patient and involve restoring fertility, treatment of metabolic complaints, treatment of androgen excess, and providing endometrial protection.

The complexity of the disorder, and the impact on quality of life, requires timely diagnosis, screening for complications, and management strategies of the long-term health issues associated with PCOS. The syndrome remains underdiagnosed, and women experience significant delays to diagnosis.

Keywords

Polycystic ovary syndrome (PCOS) · Oligomenorrhea · Amenorrhea · Anovulation · Hyperandrogenism · Polycystic ovarian morphology (PCOM) · Hirsutism · Ovulation induction · Medical treatment · Health risks

Introduction

Polycystic ovary syndrome (PCOS) is a significant public health issue with reproductive, metabolic, and psychological features. PCOS is the most common endocrine disease affecting 5–20% of reproductive-aged women with up to 70% of affected women remaining undiagnosed (Azziz et al. 2016; Teede et al. 2018). Presentation varies by ethnicity, and the prevalence of more complete phenotypes in PCOS was higher in subjects identified in referral versus unselected populations, suggesting the presence of significant referral bias (Lizneva et al. 2016). Women with PCOS present with diverse features including psychological issues such as anxiety, depression, and disturbed bodily images. Moreover, PCOS is associated with reproductive disorders such as irregular menstrual cycles, hirsutism, infertility, and pregnancy complications. Finally, metabolic features such as insulin resistance, metabolic syndrome, prediabetes, type 2 diabetes, and several cardiovascular risk factors are also associated with the syndrome (Teede et al. 2018). PCOS is a diagnosis of exclusion, based primarily on the presence of hyperandrogenism, ovulatory dysfunction, and PCOM.

Treatment should be tailored to the complaints and needs of the patient and involves induction of ovulation in order to restore fertility either by oral drugs or

by administering gonadotropins. Treatment targeting metabolic abnormalities includes lifestyle changes, medication, and potential surgery for the prevention and management of excess weight. In case restoring fertility is not the ultimate goal, androgen suppression to treat hirsutism and/or alopecia as well as providing endometrial protection in order to avoid endometrial cancer is a necessity. Last but not least, physicians should pay more attention to the detection and treatment of psychological features associated with the syndrome (Azziz et al. 2016; Teede et al. 2018).

The complexity of the disorder, and the impact on quality of life, requires timely diagnosis, screening for complications, and management strategies of the long-term health complications such as type 2 diabetes mellitus (T2DM), hypertension, and possibly cardiovascular disease. This is especially important since PCOS remains underdiagnosed and women experience significant delays to diagnosis (Neven et al. 2018).

Diagnostic Features

The diagnosis of PCOS remains a controversial issue with challenges defining individual components within the diagnostic criteria, significant clinical heterogeneity generating a range of phenotypes with or without obesity, ethnic differences, and variation in clinical features across the life course (Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group 2004). This culminates in delayed diagnosis, poor diagnosis experience, and dissatisfaction with care reported by women internationally. These challenges are exacerbated by a lack of recognition of the diverse features of PCOS, inadequate research, and a lack of comprehensive international evidence-based guidelines (Teede et al. 2018).

The Rotterdam criteria for PCOS have been endorsed by the National Institutes of Health (NIH) (Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group 2004). There is a general agreement that the diagnosis of PCOS must be based on the presence of at least two of the following three criteria: chronic anovulation, hyperandrogenism (clinical or biological), and polycystic ovaries (PCOM). Moreover, other disorders that might mimic these clinical features of PCOS are to be excluded. These include thyroid disease, hyperprolactinemia, and nonclassical congenital adrenal hyperplasia (Legro et al. 2013). The Androgen Excess Society emphasizes the importance of clinical and/or biochemical hyperandrogenism and places less importance on polycystic ovary morphology (PCOM) (Goodman et al. 2015). However, the most recent consensus meeting endorsed by the NIH has decided that for clinical purposes the Rotterdam criteria should be used.

Irregular Menstrual Cycles

Ovulatory dysfunction is a key diagnostic feature of PCOS with irregular menstrual cycles reflecting ovulatory dysfunction, as reflected in the Rotterdam criteria. If the

interval between two consecutive menses exceeds 35 days, it can be assumed that chronic anovulation is present. Such cycle intervals are generally referred to as oligomenorrhea. However, if the interval between subsequent menstrual bleedings is only slightly longer than normal (ranging from 32 to 35 days), or if cycles are slightly irregular, ranging from 32 to 35 to 36 days, ovulation should be assessed (Goodman et al. 2015). Several studies have shown that 10–15% of hyperandrogenic women with apparently normal cycles are anovulatory. In contrast, the finding of anovulation is very uncommon in normo-androgenic women with normal menses (Goodman et al. 2015). On the contrary, in women reporting oligomenorrhea, incidental ovulatory cycles might be recorded in up to 15% of cases (Burgers et al. 2010). Alternatively one might use the number of menstrual cycles per year to define oligomenorrhea. The Rotterdam consensus does suggest that if this number is less than eight, chronic anovulation should be suspected (Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group 2004). Amenorrhea is arbitrarily defined as a menstrual cycle interval exceeding 188 days or being longer than half a year. Whenever irregular or absent menstrual cycles occur, a diagnosis of PCOS should be considered because approximately 85–90% of women with oligomenorrhea and 30–40% of women with amenorrhea will have PCOS (Neven et al. 2018).

Irregular cycles and ovulatory dysfunction are also a normal component of the pubertal and menopausal transitions, and defining cycle abnormalities at these life stages remains challenging. Indeed, the greatest controversy in this diagnostic criteria is during the pubertal transition. Irregular menstrual, and therefore anovulatory, cycles are common during the first year post menarche. Intermenstrual cycle intervals exceeding 90 days are mostly anovulatory in girls being between 1 and 3 years post menarche. Overall, irregular cycles (>35 or <21 days) that continue for more than 3 years post-menarche are likely to have oligo- or anovulation. With increasing gynecologic age, fewer females experience cycles exceeding 45 days (Teede et al. 2018).

Measurement of serum progesterone during the midluteal phase (days 21 to 22) is the best way to assess ovulation. Alternatives to a single progesterone measurement such as basal body temperature charts, urinary luteinizing hormone kits, or timed endometrial biopsies may be used, but they do not give sufficient information about the luteal phase, are elaborately costly, and should therefore not be used as first-line assessments tools (Legro et al. 2013).

Hyperandrogenism

Hyperandrogenism is a key diagnostic feature of PCOS affecting between 60% and 100% with the condition with both clinical (hirsutism, alopecia, and acne) and biochemical hyperandrogenism. Both features of hyperandrogenism are challenging to assess and vary by methods of assessment, ethnicity, and confounding factors including excess weight and life stage. Methods for directly assessing total circulating testosterone levels (e.g., direct radioimmunoassays or chemiluminescence

immunoassays) are of insufficient precision, sensitivity, and specificity to be used for the accurate assessment of total testosterone levels in women and female adolescents, including those with PCOS. There are also currently no reliable direct assays for total or free testosterone. However, laboratories can provide calculated bioavailable testosterone, calculated free testosterone, or free androgen index (FAI = 100 × (total testosterone/sex hormone-binding globulin (SHBG))). Androstenedione and dehydroepiandrostenedione sulfate (DHEAS) have only a limited role and can increase the probability of detecting hyperandrogenemia, yet they are arguably more useful in exclusion of other causes of hyperandrogenism. DHEAS is predominantly an adrenal androgen, and mild elevation may be seen in conjunction with PCOS. Significant elevations of any androgen and/or virilization require investigation for possible androgen secreting adrenal or ovarian tumors. Androstenedione is elevated in 21-hydroxylase-deficient nonclassical congenital adrenal hyperplasia (Teede et al. 2018). PCOS is a diagnosis of exclusion. Hence, non-classic congenital adrenal hyperplasia, Cushing's syndrome, and androgen secreting tumors need to be excluded.

Signs and symptoms of severe androgen excess can result in virilization (e.g., male pattern balding, severe hirsutism, and clitoromegaly). Virilization is rare. Clinical evidence of mild to moderate androgen excess is more common including hirsutism, acne, and androgen-related alopecia. The interrelationship of these clinical features remains unclear, varies by ethnicity, and requires clinician training, vigilance, and skill to assess. These features impact considerably on quality of life in women with PCOS, and treatment burden including cosmetic therapies can be significant (Teede et al. 2018). When evaluating symptoms of hyperandrogenism, hirsutism should be assessed using the modified Ferriman-Gallwey score (mFG), assessing the existence and growth of terminal pigmented and medullated hair in nine masculine body areas. Each area is scored from 0 (no terminal hair) to 4 (terminal hair consistent with a well-developed male individual). A level exceeding 4–6 indicates hirsutism. The prevalence of hirsutism is the same across ethnicities, yet the mFG cutoff scores for defining hirsutism and the severity of hirsutism vary by ethnicity (Teede et al. 2018). Alopecia can be assessed using the Ludwig visual score. For acne, no universally accepted visual assessment is available (Neven et al. 2018).

PCOM

Polycystic ovarian morphology (PCOM) was incorporated into the diagnosis of PCOS in 2003 in the Rotterdam criteria, as a common feature associated with clinical and endocrine features of the condition (Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group 2004). This introduced arguably milder phenotypes into PCOS with limited data on natural history, prompting calls for phenotype identification and more research (Goodman et al. 2015). The definition of PCOM in the Rotterdam criteria is 12 or more follicles measuring 2–9 mm throughout the entire ovary or an ovarian volume $\geq$10 mL. This was based on a single report on

sensitivity and specificity in PCOS compared to controls (Balen et al. 2003). Factors that mandate revision of this diagnostic criteria include advances in ultrasound technology with greater resolution, variable operator skill level, lack of standard reporting, skill-defined cutoffs between normal ovaries and PCOM, the impact of the chosen approach (e.g., transvaginal vs. transabdominal), body habits, and age (Teede et al. 2018). Using newer ultrasound technology to count follicles, a substantially higher cutoff for PCOM than 12 follicles is required to distinguish between women with PCOS and healthy women from the general population (Lujan et al. 2013). Using the transvaginal approach, ultrasound transducers with a frequency bandwidth that includes 8 MHz, the threshold for PCOM should be on either ovary, a follicle number per ovary of $\geq$20 and/or an ovarian volume $\geq$10 ml, ensuring no corpora lutea, cysts, or dominant follicles are present. When using older equipment with transducer frequencies less than 8 MHz, the threshold for PCOM could be an ovarian volume $\geq$10 ml on either ovary (Teede et al. 2018).

In adolescents PCOM can also be detected without any of the accompanying features of PCOS, making it a common normal finding in these youngsters. Due to the high incidence of PCOM and its non-specificity in those with a gynecological age of less than 8 years post menarche, ultrasound is not recommended at this life stage for the purposes of diagnosis (Neven et al. 2018).

Women with PCOM have generally higher serum concentrations of anti-Mullerian hormone (AMH) and higher levels of AMH are associated with anovulation and hyperandrogenism (Mulders et al. 2004). Current research is evaluating whether serum AMH can replace ultrasound in the diagnosis of PCOM but is also hampered by poorly defined patient and control populations, variable quality between commercially available assays, and no international agreed standards (Victoria et al. 2019).

Prevalence of Phenotypes

The Rotterdam criteria for PCOS recognize four different phenotypes (Table 1). A large meta-analysis including 41 studies reported on the prevalence of the 4 different phenotypes in referral versus unselected populations. Phenotype A was identified in up to 50% in patients referred to a hospital versus being present in only 19% in unselected populations. Similarly, phenotype B is 13% as prevalent in referred populations, whereas it is seen in up to 25% in unselected population-based samples. Phenotype C is present in about 14% of women referred to clinics versus 34% in unselected populations. Finally phenotype D is found in up to 17% of women attending clinics for PCOS, whereas a nearly similar (19%) number is found in unselected populations. Differences between referral and unselected populations were statistically significant for phenotypes A, B, and C. Moreover, it was noted that referred PCOS subjects had a greater mean body mass index (BMI) than local controls. This latter difference in BMI was not observed when unselected PCOS patients were compared to healthy controls. Hence, it seems to matter whether a woman is seeking medical assistance for her PCOS (Lizneva et al. 2016). There is

Table 1 Phenotypes of PCOS according to the Rotterdam criteria

	Diagnostic features of PCOS		
	Oligo or amenorrhea	Clinical and/or biochemical hyperandrogenism	PCOM
Phenotype A	Present	Present	Present
Phenotype B	Present	Present	Absent
Phenotype C	Absent	Present	Present
Phenotype D	Present	Absent	Present

PCOS polycystic ovary syndrome, *PCOM* polycystic ovarian morphology

still some controversy about whether or not the phenotypes A, B, and C are more often associated with metabolic derangements such as insulin resistance and obesity and dyslipidemia (Neven et al. 2018).

It should be noted that these three criteria for diagnosis of PCOS are subject to changes over time naturally occurring with increasing age and therefore impacting on the phenotype and presenting challenges in diagnosis (Brown et al. 2011). Overall it is acknowledged that there is inadequate evidence of the natural history of PCOS, and the concept of whether PCOS resolves and/or persists remains unclear pending better longitudinal studies. Postmenopausal phenotypes of PCOS are poorly defined, with limited longitudinal natural history studies. Uncertainty in assessment and diagnosis at this life stage leads to confusion for health professionals and women on long-term health risks and screening recommendations (Teede et al. 2018). However, postmenopausal persistence of PCOS could be considered likely with continuing evidence of hyperandrogenism after menopause. Similarly, a diagnosis of PCOS postmenopausal could be considered if there is a past diagnosis of PCOS, a long-term history of irregular menstrual cycles and hyperandrogenism and/or PCOM, during the reproductive years (Teede et al. 2018). Interestingly, women with PCOS generally enter menopause later in life compared to those who are not suffering from the syndrome. This is mainly due to a selective enrichment of menopause postponing genetic variants in women with PCOS (Day et al. 2015).

Clinical Presentation

Reproductive Features

PCOS is the most common cause of medically treatable infertility, and it accounts for up to 70% of cases of anovulatory infertility (Brassard et al. 2008). In a similar large study from Australia, anovulatory infertility was noted by 72% in women reporting to have been diagnosed with PCOS compared to an incidence of only 16% in those not reporting PCOS. Infertility was 15-fold higher in women reporting PCOS and was independent of BMI. In those women reporting infertility, there was greater use of fertility hormone treatment in women with PCOS although the incidence of ART use was similar in women with and without PCOS. Notably, the women with PCOS had a similar number of children as those without PCOS (Joham et al. 2015).

Women with PCOS had a four times increased risk of developing gestational diabetes compared with the reference group of pregnant women without PCOS. Moreover, the children born from mothers with PCOS were again nearly four times more often small for gestational age (de Wilde et al. 2017). In a subgroup analysis, maternal complications were statistically significantly more often present in hyperandrogenic PCOS women compared with those without hyperandrogenemia (de Wilde et al. 2017). In another study from the same group of investigators, a prediction model was designed to predict the chance of developing gestational diabetes in women with PCOS. First-degree relatives with T2DM, serum levels of fasting glucose, fasting insulin, androstenedione, and sex hormone-binding globulin before conception were identified as predictors (de Wilde et al. 2014). Primary disease characteristics of PCOS, chiefly hyperandrogenism and impaired glucose metabolism, seem to predict suboptimal obstetric and neonatal outcomes. According to these authors, increased surveillance during pregnancy in women with PCOS should focus on these clinical features, and this might help to mitigate obstetrical and neonatal risk (Christ et al. 2019).

A recent large meta-analysis examined the incidence of gynecological cancers in younger women with PCOS compared with controls of similar age. Current data suggest that women of all ages with PCOS are at an increased risk of endometrial cancer due to unopposed estrogen exposure due to their anovulatory status. In contrast the risk of ovarian and breast cancer was not significantly increased overall (Barry et al. 2014). It should however be noted that most studies addressing the risk of endometrial cancer did not take into account obesity, infertility, T2DM, and metabolic syndrome, all proven risk factors for endometrial cancer. In those studies where BMI was considered, associations with PCOS and endometrial cancer are less consistent (Harris and Terry 2016).

Because the menstrual cycle length shortens with increasing age in normal women in those suffering from PCOS, a similar shortening is noticed over time rendering a substantial number of these women eumenorrheic. Since menopause is also occurring later on in life in women with PCOS, regular ovulatory cycles are more frequent toward the end of their reproductive life span (Brown et al. 2011; Elting et al. 2003).

Psychosocial Features

Several meta-analyses reported up to four times increased depressive symptom scores in women suffering from PCOS compared to healthy controls which persisted in BMI-matched studies. Although some studies reported increased scores that might not have been clinically significant, others showed increased moderate to severe depressive symptoms with a prevalence of depression of up to nearly 40% in women with PCOS opposed to an incidence of only around 15% in controls. Again these increased scores were independent of obesity and seen in both clinic and community recruits (Veltman-Verhulst et al. 2012; Dokras et al. 2011; Barry et al. 2011; Cooney et al. 2017).

Similarly, anxiety symptoms are also increased in women with PCOS. Several meta-analyses reported higher anxiety scores in PCOS compared to controls (Veltman-Verhulst et al. 2012; Barry et al. 2011). Another four studies reported a sevenfold increase in abnormal anxiety scores in PCOS (Dokras et al. 2012). Again there was considerable heterogeneity between studies in all meta-analyses. A recent rigorous meta-analysis showed increased moderate/severe anxiety symptoms in PCOS with a prevalence of 42% in women with PCOS compared to only 9% in controls (Cooney et al. 2017). A large population-based study of 24,385 women with PCOS matched for sex, age, and country of birth to 10 controls showed increased anxiety disorder (Cesta et al. 2016). A large hospital database showed anxiety in PCOS at 14%, compared to 6% of those without a diagnosis of PCOS (Hart and Doherty 2015). Collectively, these studies indicate increased anxiety symptoms and anxiety disorders in women with PCOS, across diverse ethnic groups. Interestingly, a large study in a Swedish cohort of twins revealed common genetic factors between neuroticism, PCOS, and major depressive disorder. That study concludes that neuroticism shares approximately half of the genetic and environmental components behind the phenotypic correlation between PCOS and major depression disorder and thus provides some etiological evidence behind the comorbidity between PCOS and depression (Cesta et al. 2017). In the context of PCOS, identification of psychological features and mental health disorders is crucial to address gaps in care identified by affected women, to improve well-being and quality of life, facilitate appropriate referral and care, and optimize engagement with lifestyle and preventive strategies. However, overdiagnosis of depression and anxiety should also be avoided (Teede et al. 2018).

In a recent meta-analysis, significant small effect sizes were found on sexual function subscales such as arousal, lubrication, satisfaction, and orgasm, indicating impaired sexual function in women with PCOS. Larger effect sizes were seen on sexual function and feelings of sexual attractiveness. Satisfaction with sex life was impaired as well; however, sexual satisfaction was rated equally important in women with PCOS and controls. Physical PCOS symptoms such as hirsutism, obesity, menstrual irregularity, and infertility may cause loss of feminine identity and a feeling of being unattractive which may impact on sexuality (Pastoor et al. 2018).

Surveys in PCOS show mixed results across the different disorders, but overall a recent systematic review and meta-analysis suggest an increased prevalence of eating disorders and disordered eating in women with PCOS. Women with PCOS also have more identified risk factors for eating disorders such as obesity, depression, anxiety, self-esteem, and poor body image (Lee et al. 2018).

Metabolic Features

Obesity is neither necessary nor sufficient for the PCOS phenotype, and the association of PCOS with obesity is not universal with national, cultural, and ethnic differences. However, the incidence of obesity in women with PCOS is increased

compared to women without PCOS. Particularly visceral adiposity is the common entity in obese and nonobese women with PCOS, which amplifies and worsens all metabolic and reproductive outcomes. Hence, obesity increases insulin resistance and the resulting hyperinsulinemia, which in turn increases adipogenesis and decreases lipolysis. Obesity also sensitizes thecal cells to LH stimulation and thereby causes functional ovarian hyperandrogenism. Obesity also impacts on inflammatory adipokines which, in turn, increase insulin resistance and adipogenesis (Glueck and Goldenberg 2019).

PCOS is associated with insulin resistance and hyperinsulinemia (Dumesic et al. 2015). A recent meta-analysis of clamp assessments of insulin action in PCOS found a decrease in insulin sensitivity of nearly 30% in women with PCOS compared with controls. This decrease in insulin sensitivity was independent of BMI, age, or diagnostic criteria. BMI exacerbated insulin resistance by 15% in women with PCOS and had a greater impact on insulin resistance in PCOS than in controls (Cassar et al. 2016). PCOS is associated with impaired glucose tolerance in up to 30% and T2DM in up to 10% of women with PCOS (Legro et al. 1999). When followed up over 10 years, the age-standardized prevalence of diabetes was nearly 40% in women with PCOS compared to only 5% in controls of similar age (Gambineri et al. 2012). Different phenotypes show different levels of insulin resistance being the most prevalent in the full-blown clinical picture (phenotype A), whereas phenotype C is not associated with insulin resistance (Panidis et al. 2012). It is estimated that about 2% of women with PCOS progress directly from baseline normoglycemia to T2DM. Moreover, every year about 16% progress from impaired glucose tolerance to T2DM. The prevalence of T2DM in PCOS continues to increase during the late reproductive years (Gambineri et al. 2012; Norman et al. 2001; Hudecova et al. 2011). Glycemic status should be assessed (using an oral glucose tolerance test [OGTT], fasting plasma glucose, or HbA1c) at baseline in all women with PCOS and should be repeated every 1 to 3 years depending on other individual risk factors for diabetes present. 5 A 75-g OGTT is recommended for women with additional risk factors and preconception and during pregnancy (Teede et al. 2018).

Nonalcoholic fatty liver disease seems to be as prevalent as 50% in patients with PCOS compared to controls. Women with PCOS had significantly higher values for waist circumference, lipid accumulation products, insulin and HOMA-IR, total cholesterol, and triglycerides than controls (Macut et al. 2016).

Women with PCOS are generally more overweight and obese compared to their age-matched counterparts without the syndrome. Because they also suffer from insulin resistance and the resulting hyperinsulinemia, dyslipidemia, and hypertension, they also are much more often affected by the so-called metabolic syndrome (Azziz et al. 2016; Dumesic et al. 2015). Also, metabolic syndrome, hypertension, and dyslipidemia are all reported to be significantly increased in first-degree relatives of women diagnosed with PCOS. It might therefore be that these families are already a priori more at risk and more susceptible for metabolic syndrome (Yilmaz et al. 2018; Laven 2018).

Cardiovascular disease (CVD) remains one of the leading causes of death in women, and any condition further increasing CVD risk will have significant public health impact. CVD primarily affects postmenopausal women in the later decades of life; however, CVD development and risk factors are present in early adulthood. A meta-analysis found no statistical difference between PCOS and non-PCOS groups in terms of myocardial infarction, stroke, CVD-related death, and coronary artery/heart disease (Heida et al. 2016; de Groot et al. 2011). Some age classes where apparently more at risk than others in some studies. However, when all age groups were combined, there was no difference in risk between women with and without PCOS, for either myocardial infarction or angina, regardless of where the control population was sourced (Heida et al. 2016). Given the methodological and reporting limitations and small sample sizes of these observational studies, all findings should be interpreted with caution. Furthermore the relatively young age of women included in most studies limits the interpretation of the available data (Teede et al. 2018). One large population-based Dutch study did assess the impact of hyperandrogenemia, assessed around their perimenopausal transition, on CVD outcome in women currently aged between 70 and 80 years of age. This prospective study was not able to show any relationship between androgens and CVD risk. Moreover, in a subpopulation with PCOS, they recorded a similar incidence of CVD, stroke, and coronary heart disease compared to age and BMI-matched women without PCOS (Meun et al. 2018). A second large hospital-based Danish population study did report an increased event rate of CVD including hypertension and dyslipidemia which was higher in women with PCOS compared to controls. They included hospital-referred PCOS patients and compared them to population-based controls which might have caused ascertainment bias. Also their definition of CVD was very broad including both prevalent and incident events (Glintborg et al. 2018). The only prospectively designed study with sufficiently long follow-up was published by a Swedish group. That study reported an increased incidence of neither myocardial infarction nor stroke or ischemic heart disease in women with PCOS compared to age-matched controls without PCOS (Schmidt et al. 2011).

It is acknowledged that metabolic syndrome and CVD risk factors are clearly increased in PCOS and that cardiovascular health overall needs to be considered; however, given the limited current data on clinical events, overall CVD risk and optimal screening for additional risk factors remains highly controversial (Teede et al. 2018).

Pathophysiology

PCOS is a complex multifactorial disease where genetic, endocrine, environmental, and behavioral factors are intertwined. The interplay between these mechanisms results in and perpetuates the clinical features of PCOS, including ovulatory dysfunction, hyperandrogenism, and PCOM, in addition to the associated mood disturbances, psychosexual dysfunction, and long-term morbidities (Azziz et al. 2016) (Fig. 1).

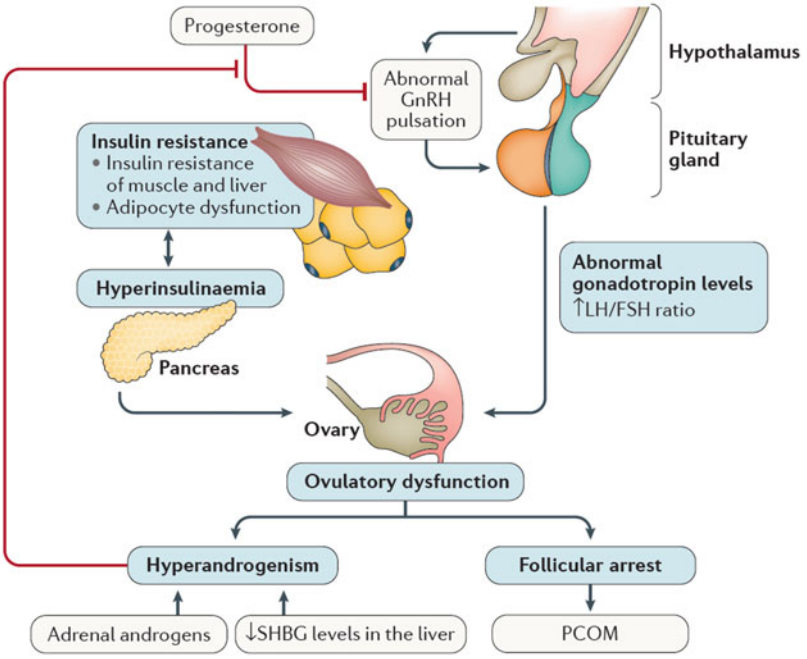


Fig. 1 The pathophysiology of PCOS. The pulsatile release of gonadotropin-releasing hormone (GnRH) from the hypothalamus is often disturbed in polycystic ovary syndrome (PCOS), leading to luteinizing hormone (LH) hypersecretion by the pituitary gland, which induces ovulary dysfunction and hyperandrogenism. This perturbed secretion of LH seems to arise early in puberty and is related to disturbed inhibition of GnRH secretion by progesterone. Although serum follicle-stimulating hormone (FSH) levels are generally normal, follicles seem to be more resistant to FSH in women with PCOS than in controls. This effect might be due to increased levels of intra-ovarian anti-Müllerian hormone (AMH). Notably, genetic and epigenetic variants contribute considerably to susceptibly for most of these alterations. Environmental factors contribute somewhat less, most by exacerbating insulin resistance and dysregulated gonadotropin secretion. PCOM, polycystic ovarian morphology; SHBG, sex hormone-binding globulin. Source: Azziz et al. (2016)

Genetic Factors

Several hundreds of candidate genes have been studied; however, the majority of these genetic variants have not been replicated in sufficiently large case control studies. Genetic variants in the *fibrillin* gene, the androgen receptor, *FTO* gene, the insulin receptor, the *FSHR* gene, the *TNF alpha* gene, and some variants in the *IL-6* gene do confer a certain risk for PCOS and have been replicated in sufficiently large studies or meta-analyses (Azziz et al. 2016).

More recently, GWAS has identified up to 20 genetic variant genes involved in neuroendocrine, metabolic, and reproductive pathways. These studies also provided evidence for shared biologic pathways between PCOS and a number of metabolic disorders, menopause, depression, and male-pattern balding, a putative male phenotype (Day et al. 2015, 2018; Hayes et al. 2015; Chen et al. 2011). There is not much

of overlap between GWAS findings and most functional molecular studies. Most of the identified SNPs seem to play a role in a pathway responsible for trafficking and recycling of large protein transmembrane receptors (McAllister et al. 2015). Moreover, some promising SNPs involved in gonadotropin action have been identified which do not only constitute risk factors for PCOS but also seem to influence response to ovulation induction treatment (Valkenburg et al. 2015; Laven 2019).

Last but not least, evidence is accumulating that epigenetic mechanisms might as well play a role in the pathogenesis of PCOS either during fetal programming or in later life via factors as obesity and diet composition (Mykhalchenko et al. 2017).

Endocrine Factors

Pathophysiology of PCOS as well as potential mechanisms underlying anovulation in PCOS are explained in Figures 1 and 2. In women with PCOS, there is an increased production of LH from the pituitary concomitant with a decrease in the secretion of follicle-stimulating hormone (FSH) leading to an increased LH/FSH ratio. The reason for this seems to be a perturbed gonadotropin-releasing hormone (GnRH) pulse generator in the hypothalamus. This increased LH pulsing is already obvious in young girls during their pubertal transition (Burt Solorzano et al. 2012). Moreover, neurokinin B receptor (NK3-R) antagonist specifically reduced LH pulse frequency and subsequently serum LH and testosterone concentrations, thus presenting NK3-R antagonism as a potential approach to treating the central neuroendocrine pathophysiology of PCOS (George et al. 2016). Interestingly, more recently AMH type II receptors were found on GnRH neurons in the midbrain in rodents as well as in humans. By administering AMH directly to these GnRH neurons in rodents, as well as in vitro and in vivo, they responded with an increase in LH

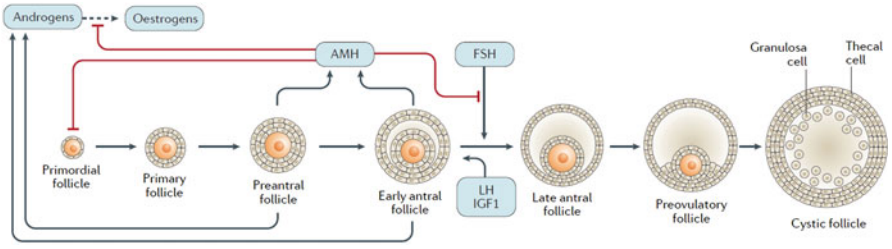


Fig. 2 Anovulation in PCOS explained. **Ovarian follicular maturation arrest in PCOS.** Normal ovulation is the result of synchronized signalling between centrally released gonadotropins and factors produced in the developing follicle of the ovary. Anovulation in women with polycystic ovary syndrome (PCOS) is characterized by arrested follicle growth at the early antral stage. Hypersecretion of luteinizing hormone (LH) and insulin-like growth factor 1 (IGF1) lead to hyperandrogenism, which results in follicular maturation arrest93. In addition, high levels of anti-Müllerian hormone (AMH) in PCOS block follicle-stimulating hormone (FSH) action contributing to hyperandrogenism and inhibiting the recruitment of further primordial follicles. Dashed line indicates androgen to oestrogen conversion. (Source: Azziz et al. (2016))

release and a decrease in FSH release leading to an increased LH/FSH ratio. These findings raise the intriguing hypothesis that AMH-dependent regulation of GnRH release could be involved in the pathophysiology of fertility and could hold therapeutic potential for treating PCOS (Cimino et al. 2016). In a second study, the same group showed that excessive intrauterine exposure of offspring to AMH induced a neuroendocrine-driven androgen excess with perturbed GnRH pulsatility leading to follicular arrest. Treatment with a GnRH agonist did restore their neuroendocrine phenotype to a normal state (Tata et al. 2018).

Hyperinsulinemia might also contribute to the clinical picture since theca cells seem to be more sensitive to LH and insulin causing them to produce more androgens upon stimulation compared to theca cells of normal women. Moreover, insulin does inhibit the production of SHBG in the liver, thereby even further increasing the amount of free androgen serum levels (Azziz et al. 2016). Similarly, although women with PCOS can show little difference in fat distribution and possibly in overall BMI, strong evidence supports that adipocytes and adipocyte function are aberrant in PCOS, favoring insulin resistance and subclinical inflammation which in turn might contribute to the perturbed endocrine environment in women with PCOS (Azziz et al. 2016).

Environmental and Behavioral Factors

Regardless of the specific diagnosis, patients with eating disorders (ED) have several features in common with women with PCOS. Both groups are at higher risk of depression and anxiety, body image disturbances, and significant detrimental effects on quality of life. The binge eating disorder (BED) is particularly relevant to PCOS, as it is independently associated with diabetes mellitus, obesity, and hypertension, and all comorbidities are also associated with PCOS (Dokras et al. 2012; Lee et al. 2018). Of note, women with PCOS often report that weight loss is more challenging for them than for women without PCOS. This may place them at risk for disordered eating behaviors, such as severe restricting, binge eating, and/or inappropriate compensatory behaviors. Therefore, both the potential effect of ED on treatment of PCOS and the possible increased risk of ED in those with PCOS drive the need to evaluate the precise prevalence of ED in women with PCOS (Lee et al. 2018). It is undeniably important to promote weight loss in women with PCOS given the impact on insulin sensitivity and reproductive function; however, the potential paradoxical harm in overemphasizing the value of weight loss cannot be dismissed (Lee et al. 2018).

Obstructive sleep apnea (OSA) is characterized by repetitive occlusions of the upper airway during sleep with futile ventilatory efforts, oxygen desaturations, sleep arousal, and the resumption of ventilation, fragmenting sleep and causing daytime sleepiness. Hence, OSA might affect quality of life, mood, and productivity. OSA appears more common in PCOS and in obesity, a common corollary of PCOS. In PCOS an increased prevalence has been reported which was not only explained by obesity (Mokhlesi et al. 2012). Hyperandrogenism may also contribute to OSA and

there are links to metabolic syndrome (Tasali et al. 2011). Although treatment studies in PCOS are very limited, successful treatment of OSA improves insulin sensitivity, decreases sympathetic output, and reduces diastolic blood pressure. The magnitude of these beneficial effects is modulated by the hours of CPAP use and the degree of obesity (Tasali et al. 2011).

Treatment

Reproductive Disorders

The ultimate goal in treating fertility disorders in PCOS is restoring normal mono-ovulation. Indeed many physicians do not discriminate between ovarian hyper-stimulation and ovulation induction both being different treatment protocols with different treatment goals (The Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2008). There are several ways to achieve mono-ovulation: either one uses drugs that increase the endogenous levels of FSH or one might directly administer FSH to the patients. The first group consists of the antiestrogens or aromatase inhibitors. Antiestrogens, such as clomiphene citrate, decrease the negative feedback on the hypothalamus and pituitary leading to an increase in the release of endogenous FSH which in turn might induce ovulation. Aromatase inhibitors, such as letrozole, reduce the conversion of androgens into estrogens, thereby again reducing the negative feedback of estrogens on the hypothalamic pituitary axis and thereby increasing the endogenous FSH release from the pituitary. Several trials have compared letrozole with clomiphene citrate, and a recent network meta-analysis concluded that in normogonadotropic normo-estrogenic anovulatory women, letrozole is superior to clomiphene citrate. Compared with clomiphene alone, letrozole is the only treatment showing a significantly higher rate of ovulation, higher pregnancy rates, and higher live birth rates. Moreover, letrozole and the combination of clomiphene citrate with metformin were superior to clomiphene citrate alone in terms of ovulation and pregnancy rates. There was no difference between letrozole and clomiphene citrate for multiple pregnancy rate per patient and miscarriage rate per patient (Wang et al. 2017; Franik et al. 2018). The balance of benefits in terms of improved live births with letrozole and less hot flushes was considered to currently outweigh the adverse effects of relatively increased fatigue and dizziness, multiple pregnancy, and unconfirmed concerns about higher congenital anomalies (Teede et al. 2018; Franik et al. 2018). In most countries, letrozole can only be used off label in those instances one might consider to use clomiphene citrate alone in anovulatory PCOS women to improve ovulation and pregnancy rates. Similarly, metformin could be used alone in anovulatory women with PCOS to improve ovulation, pregnancy, and live birth rates, although women should be informed that there are more effective ovulation induction agents. Clomiphene citrate seems to be superior to metformin in anovulatory PCOS women in case these women have a BMI of over 30 kg/m^2 (Teede et al. 2018).

In case the first-line treatment is not successful or patients are resistant to the oral medication because they do not ovulate, gonadotropin therapy might be initiated. Again the goal here is induction of one dominant follicle. Gonadotrophin therapy is suitable for improving infertility in women with PCOS in specialist care, with close monitoring with ultrasound and strict criteria to cancel cycles in case of multiple follicle development (The Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2008). Gonadotropin therapy provides better per cycle and cumulative pregnancy and live birth rates compared with the use of oral antiestrogens and/or no therapy at all (Wang et al. 2017). An extra advantage is that there is no evidence of teratogenicity although the risk of multiple pregnancy is increased as is the cost of medication when compared to the oral agents (Teede et al. 2018). Another second-line treatment option is laparoscopic ovarian drilling, in order to reduce the number of follicles, an intervention that can lead to a singleton birth in women with PCOS. There is no convincing evidence of inferiority over other common ovulation induction agents. An extra advantage is that there is no need for monitoring and only a background risk of multiple pregnancy. However, it is important to note that laparoscopic ovarian drilling is an invasive surgical intervention. Moreover, there is a small risk of reduced ovarian reserve or loss of ovarian function afterward and an increased chance of adhesion formation (Lepine et al. 2017). A recent meta-analysis indicated that a unilateral procedure might be as effective as bilateral drilling (Abu Hashim et al. 2018).

In the absence of an absolute indication for IVF with or without ICSI, women with PCOS and anovulatory infertility could be offered IVF as third-line therapy where first- or second-line ovulation induction therapies have failed. IVF should be considered after failed ovulation induction treatment with high pregnancy rates per cycle, especially in younger women. Given the risks and the high costs that can be prohibitive for many patients, IVF should be considered third-line medical therapy. Indeed, the use of IVF is effective, and when elective single embryo transfer is used, multiple pregnancies can be minimized (Teede et al. 2018). It seems that in case a short protocol with a GnRH antagonist is used in combination with an agonist trigger, the chances for ovarian hyperstimulation syndrome are reduced. However, GnRH agonist triggers are associated with lower pregnancy rates, primarily in fresh embryo transfers, which can be overcome in frozen cycles. Similarly, in vitro maturation (IVM) of oocytes limits or omits ovarian stimulation prior to oocyte retrieval, with maturation of oocytes post-retrieval, avoiding OHSS risk (Teede et al. 2018).

Combined contraceptives, including oral contraceptive pills, are commonly prescribed for adults and adolescents with PCOS without wish to conceive to ameliorate the clinical symptoms and associated hormonal disturbances. The effects of COCPs on menstrual cycle, hirsutism, weight loss, waist/hip ratio, testosterone concentrations, lipid profile, and blood sugar levels are variably reported and depend on the type of COCP used, duration of use, severity of presentation/phenotype, and adherence to the regimen, among other factors. Different combinations of COCPs are available with heterogeneous estrogen and progestin preparations with varying pharmacological and clinical properties. Thus, the efficacy and consequences of

COCPs in PCOS may vary. Some preparations also comprise natural estrogen instead of synthetic ethinylestradiol with benefits and contraindications considered similar (Teede et al. 2018). The use of antiandrogens in combination with COCP should only be considered in women with PCOS to treat hirsutism and androgen-related alopecia exclusively in case COCP and cosmetic therapy have failed to adequately improve symptoms. Where COCPs are contraindicated or poorly tolerated, in the presence of other effective forms of contraception, antiandrogens could be considered to treat hirsutism and androgen-related alopecia (Azziz et al. 2016; Teede et al. 2018).

Metabolic Disorders

Given the gaps in evidence in some areas in PCOS, the relevant literature on metformin in other populations was reviewed to inform recommendations. Metformin works by decreasing gluconeogenesis and lipogenesis and enhancing glucose uptake in the liver, skeletal muscle, adipose tissue, and ovaries (Teede et al. 2018). It is known in other populations to prevent weight gain and appears to assist with weight loss, to prevent and manage T2DM and gestational diabetes (GDM), and to reduce microvascular and cardiovascular disease (Naderpoor et al. 2015). Side effects are not uncommon, yet these are primarily gastrointestinal and appear mild and self-limiting (Naderpoor et al. 2015). Concerns on vitamin B12 deficiency with longer-term metformin use have also emerged; however, more research is needed. Data from other populations suggests that side effects can be minimized with lower metformin starting dose, extended release preparations and/or administration with food (Teede et al. 2018).

The most recent literature would suggest that inositol could represent an important therapeutic strategy for the improvement of metabolic aspects of PCOS (Gateva et al. 2018). Similarly, inositol might also improve reproductive outcomes. Moreover, inositol seems effective in preventing and treating GDM although larger cohort studies are needed to better clarify these results (Gateva et al. 2018).

Lifestyle intervention, preferably multicomponent including diet, exercise, and behavioral strategies, should be recommended in overweight or obese women with PCOS to effectively reduce weight, central obesity, and insulin resistance (Moran et al. 2011).

A recent meta-analysis including 29 studies revealed that in severely obese patients submitted to bariatric surgery, obesity-associated gonadal dysfunction was very prevalent. PCOS was present in nearly 40% of cases. After bariatric surgery, resolution of PCOS was found in nearly all cases of affected women. SHBG concentrations increased after bariatric surgery in women, whereas serum estradiol concentrations decreased in women with PCOS. Similarly, total testosterone serum levels decreased in women. The latter was accompanied by resolution of hirsutism in nearly half of the cases and a reduction in the incidence of menstrual dysfunction in nearly all cases of women showing these symptoms before surgery (Escobar-Morreale et al. 2017).

Psychosocial Disorders

Although there is no compelling evidence that behavioral modifications might work in women with PCOS, they do so however in other high cardiometabolic risk populations. In those studies behavioral change strategies and/or behavioral/cognitive interventions in combination with diet and exercise improved weight loss and were more effective than diet and/or physical activity alone. Emphasis on self-management components enhances weight loss and healthy lifestyle behavior change and is incorporated into advice on lifestyle interventions for the general population. Skill levels among health professionals may vary, presenting implementation challenges (Moran et al. 2011; Wing et al. 2008). The effectiveness of lifestyle programs seems to be improved when incorporating behavioral strategies such as goal setting, self-monitoring, stimulus control, problem-solving, assertiveness training, slower eating, reinforcing changes, and relapse prevention, to optimize weight management, healthy lifestyle, and emotional well-being (Neven et al. 2018).

Although extensive information on diet types in women with PCOS is lacking, there is no benefit of any one diet type and that hormone levels including insulin do not predict responses. There is currently no evidence that modifying dietary macronutrient composition offers additional benefits over conventional dietary approaches for weight loss, and further research is needed (Moran et al. 2009).

Exercise should be encouraged and advised in PCOS. It was considered that exercise interventions and physical activity do not require clinical centers, expensive gyms, and fitness centers. They can be delivered in community centers, sporting grounds/facilities, in groups, and with minimal equipment. Low-cost e-health (electronic health) and m-health (mobile health) options may also be used (Teede et al. 2018; Moran et al. 2009).

Detection of negative body image provides the opportunity to address both psychological aspects such as self-esteem and self-acceptance and working on the physical aspects of the condition such as hirsutism, overweight, and acne, if appropriate. Evidence specific to PCOS models of care is limited; however, existing evidence suggests integrated multidisciplinary services, support groups, and nurse-led education can address identified gaps, increase understanding of PCOS, and improve lifestyle change (Neven et al. 2018). Needs differ by individual and life stage. Cultural influences need to be considered in PCOS in the context of both care and information needs. Culturally appropriate care involves more than linguistic considerations (Teede et al. 2018).

Some of the PCOS-related treatments including lifestyle modification, OCPs, laser treatment, and insulin sensitizers have shown favorable effects on depression or anxiety symptoms. Generally, these interventions are also well tolerated with a favorable side effect profile and thus can be continued in women diagnosed with depression or anxiety disorder. In addition, women with a clinically confirmed diagnosis of depression or anxiety should be treated based on standard guidelines. Future studies should focus on understanding the mechanisms that lead to the increased risk of depression and anxiety in women with PCOS and the best interventions in this population which is already at risk for several comorbidities (Cooney and Dokras 2017).

Conclusions

PCOS is a complex disease where genetic, endocrine, environmental, and behavioral factors are intertwined with each other giving rise to a heterogeneous phenotype with reproductive, metabolic, and psychological characteristics that affect women's health and quality of life across the life course. Physicians should be aware of the clinical features and risks for women with PCOS and screen and manage them accordingly. Clinicians should focus on lifestyle adjustments as the first-line management to improve reproductive, metabolic, cardiovascular, and psychosocial outcomes focusing on weight management and physical exercise as well as on cognitive behavioral interventions. In addition, pharmacological therapy in the form of COCPs and metformin may be useful. Similarly, for anovulatory infertility, lifestyle modification is also recommended as first-line treatment. If this is unsuccessful, ovulation induction using letrozole is the first-line medical management, whereas clomiphene citrate and metformin may have an additive effect. Gonadotrophins and laparoscopic drilling are second-line treatment options, whereas IVF constitutes the third-line therapy option only indicated when other fertility treatments have failed.

The latest international evidence-based guideline for PCOS details these treatments along with providing translation resources for health professionals or women with PCOS. Together these are designed to provide a valuable resource aiding clinicians in optimal assessment and management of women with PCOS.

Cross-References

- ▶ Endocrinology of Maternal-Placental Axis
- ▶ Hormonal Contraception
- ▶ Hormonal Treatments in the Infertile Women
- ▶ The "Great Obstetrical Syndromes"
- ▶ The Hypothalamus-Pituitary-Ovary Axis
- ▶ The Menstrual Cycle and Related Disorders
- ▶ The Menstrual Disorders Related to Systemic Diseases

References

Abu Hashim H, Foda O, El Rakhawy M. Unilateral or bilateral laparoscopic ovarian drilling in polycystic ovary syndrome: a meta-analysis of randomized trials. Arch Gynecol Obstet. 2018;297(4):859–70.

Azziz R, Carmina E, Chen Z, Dunaif A, Laven JS, Legro RS, et al. Polycystic ovary syndrome. Nat Rev Dis Primers. 2016;2:16057.

Balen AH, Laven JS, Tan SL, Dewailly D. Ultrasound assessment of the polycystic ovary: international consensus definitions. Hum Reprod Update. 2003;9(6):505–14.

Barry JA, Kuczmierczyk AR, Hardiman PJ. Anxiety and depression in polycystic ovary syndrome: a systematic review and meta-analysis. Hum Reprod. 2011;26(9):2442–51.

Barry JA, Azizia MM, Hardiman PJ. Risk of endometrial, ovarian and breast cancer in women with polycystic ovary syndrome: a systematic review and meta-analysis. Hum Reprod Update. 2014;20(5):748–58.

Brassard M, AinMelk Y, Baillargeon JP. Basic infertility including polycystic ovary syndrome. Med Clin North Am. 2008;92(5):1163–92.. xi

Brown ZA, Louwers YV, Fong SL, Valkenburg O, Birnie E, de Jong FH, et al. The phenotype of polycystic ovary syndrome ameliorates with aging. Fertil Steril. 2011;96(5):1259–65.

Burgers JA, Fong SL, Louwers YV, Valkenburg O, de Jong FH, Fauser BC, et al. Oligoovulatory and anovulatory cycles in women with polycystic ovary syndrome (PCOS): what's the difference? J Clin Endocrinol Metab. 2010;95(12):E485–9.

Burt Solorzano CM, Beller JP, Abshire MY, Collins JS, McCartney CR, Marshall JC. Neuroendocrine dysfunction in polycystic ovary syndrome. Steroids. 2012;77(4):332–7.

Cassar S, Misso ML, Hopkins WG, Shaw CS, Teede HJ, Stepto NK. Insulin resistance in polycystic ovary syndrome: a systematic review and meta-analysis of euglycaemic-hyperinsulinaemic clamp studies. Hum Reprod. 2016;31(11):2619–31.

Cesta CE, Mansson M, Palm C, Lichtenstein P, Iliadou AN, Landen M. Polycystic ovary syndrome and psychiatric disorders: co-morbidity and heritability in a nationwide Swedish cohort. Psychoneuroendocrinology. 2016;73:196–203.

Cesta CE, Kuja-Halkola R, Lehto K, Iliadou AN, Landen M. Polycystic ovary syndrome, personality, and depression: a twin study. Psychoneuroendocrinology. 2017;85:63–8.

Chen ZJ, Zhao H, He L, Shi Y, Qin Y, Shi Y, et al. Genome-wide association study identifies susceptibility loci for polycystic ovary syndrome on chromosome 2p16.3, 2p21 and 9q33.3. Nat Genet. 2011;43(1):55–9.

Christ JP, Gunning MN, Meun C, Eijkemans MJC, van Rijn BB, Bonsel GJ, et al. Pre-conception characteristics predict obstetrical and neonatal outcomes in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2019;104(3):809–18.

Cimino I, Casoni F, Liu X, Messina A, Parkash J, Jamin SP, et al. Novel role for anti-Mullerian hormone in the regulation of GnRH neuron excitability and hormone secretion. Nat Commun. 2016;7:10055.

Cooney LG, Dokras A. Depression and anxiety in polycystic ovary syndrome: etiology and treatment. Curr Psychiatry Rep. 2017;19(11):83.

Cooney LG, Lee I, Sammel MD, Dokras A. High prevalence of moderate and severe depressive and anxiety symptoms in polycystic ovary syndrome: a systematic review and meta-analysis. Hum Reprod. 2017;32(5):1075–91.

Day FR, Hinds DA, Tung JY, Stolk L, Styrkarsdottir U, Saxena R, et al. Causal mechanisms and balancing selection inferred from genetic associations with polycystic ovary syndrome. Nat Commun. 2015;6:8464.

Day F, Karaderi T, Jones MR, Meun C, He C, Drong A, et al. Large-scale genome-wide meta-analysis of polycystic ovary syndrome suggests shared genetic architecture for different diagnosis criteria. PLoS Genet. 2018;14(12):e1007813.

de Groot PC, Dekkers OM, Romijn JA, Dieben SW, Helmerhorst FM. PCOS, coronary heart disease, stroke and the influence of obesity: a systematic review and meta-analysis. Hum Reprod Update. 2011;17(4):495–500.

de Wilde MA, Veltman-Verhulst SM, Goverde AJ, Lambalk CB, Laven JS, Franx A, et al. Preconception predictors of gestational diabetes: a multicentre prospective cohort study on the predominant complication of pregnancy in polycystic ovary syndrome. Hum Reprod. 2014; 29(6):1327–36.

de Wilde MA, Lamain-de Ruiter M, Veltman-Verhulst SM, Kwee A, Laven JS, Lambalk CB, et al. Increased rates of complications in singleton pregnancies of women previously diagnosed with polycystic ovary syndrome predominantly in the hyperandrogenic phenotype. Fertil Steril. 2017;108(2):333–40.

Dokras A, Clifton S, Futterweit W, Wild R. Increased risk for abnormal depression scores in women with polycystic ovary syndrome: a systematic review and meta-analysis. Obstet Gynecol. 2011;117(1):145–52.

Dokras A, Clifton S, Futterweit W, Wild R. Increased prevalence of anxiety symptoms in women with polycystic ovary syndrome: systematic review and meta-analysis. Fertil Steril. 2012; 97(1):225–30. e2.

Dumesic DA, Oberfield SE, Stener-Victorin E, Marshall JC, Laven JS, Legro RS. Scientific statement on the diagnostic criteria, epidemiology, pathophysiology, and molecular genetics of polycystic ovary syndrome. Endocr Rev. 2015;36(5):487–525.

Elting MW, Kwee J, Korsen TJ, Rekers-Mombarg LT, Schoemaker J. Aging women with polycystic ovary syndrome who achieve regular menstrual cycles have a smaller follicle cohort than those who continue to have irregular cycles. Fertil Steril. 2003;79(5):1154–60.

Escobar-Morreale HF, Santacruz E, Luque-Ramirez M, Botella Carretero JI. Prevalence of 'obesity-associated gonadal dysfunction' in severely obese men and women and its resolution after bariatric surgery: a systematic review and meta-analysis. Hum Reprod Update. 2017;23(4):390–408.

Franik S, Eltrop SM, Kremer JA, Kiesel L, Farquhar C. Aromatase inhibitors (letrozole) for subfertile women with polycystic ovary syndrome. Cochrane Database Syst Rev. 2018;5: CD010287.

Gambineri A, Patton L, Altieri P, Pagotto U, Pizzi C, Manzoli L, et al. Polycystic ovary syndrome is a risk factor for type 2 diabetes: results from a long-term prospective study. Diabetes. 2012; 61(9):2369–74.

Gateva A, Unfer V, Kamenov Z. The use of inositol(s) isomers in the management of polycystic ovary syndrome: a comprehensive review. Gynecol Endocrinol. 2018;34(7):545–50.

George JT, Kakkar R, Marshall J, Scott ML, Finkelman RD, Ho TW, et al. Neurokinin B receptor antagonism in women with polycystic ovary syndrome: a randomized, placebo-controlled trial. J Clin Endocrinol Metab. 2016;101(11):4313–21.

Glintborg D, Rubin KH, Nybo M, Abrahamsen B, Andersen M. Cardiovascular disease in a nationwide population of Danish women with polycystic ovary syndrome. Cardiovasc Diabetol. 2018;17(1):37.

Glueck CJ, Goldenberg N. Characteristics of obesity in polycystic ovary syndrome: etiology, treatment, and genetics. Metab Clin Exp. 2019;92:108–120.

Goodman NF, Cobin RH, Futterweit W, Glueck JS, Legro RS, Carmina E, et al. American association of clinical endocrinologists, American college of endocrinology, and androgen excess and Pcos society disease state clinical review: guide to the best practices in the evaluation and treatment of polycystic ovary syndrome – part 2. Endocr Pract. 2015;21(12):1415–26.

Harris HR, Terry KL. Polycystic ovary syndrome and risk of endometrial, ovarian, and breast cancer: a systematic review. Fertil Res Pract. 2016;2:14.

Hart R, Doherty DA. The potential implications of a PCOS diagnosis on a woman's long-term health using data linkage. J Clin Endocrinol Metab. 2015;100(3):911–9.

Hayes MG, Urbanek M, Ehrmann DA, Armstrong LL, Lee JY, Sisk R, et al. Genome-wide association of polycystic ovary syndrome implicates alterations in gonadotropin secretion in European ancestry populations. Nat Commun. 2015;6:7502.

Heida KY, Bots ML, de Groot CJ, van Dunne FM, Hammoud NM, Hoek A, et al. Cardiovascular risk management after reproductive and pregnancy-related disorders: a Dutch multidisciplinary evidence-based guideline. Eur J Prev Cardiol. 2016;23(17):1863–79.

Hudecova M, Holte J, Olovsson M, Larsson A, Berne C, Poromaa IS. Diabetes and impaired glucose tolerance in patients with polycystic ovary syndrome–a long term follow-up. Hum Reprod. 2011;26(6):1462–8.

Joham AE, Teede HJ, Ranasinha S, Zoungas S, Boyle J. Prevalence of infertility and use of fertility treatment in women with polycystic ovary syndrome: data from a large community-based cohort study. J Womens Health (Larchmt). 2015;24(4):299–307.

Laven JSE. Family ties. Fertil Steril. 2018;109(2):254–5.

Laven JSE. Follicle stimulating hormone receptor (FSHR) polymorphisms and polycystic ovary syndrome (PCOS). Front Endocrinol. 2019;10:23.

Lee I, Cooney LG, Saini S, Sammel MD, Allison KC, Dokras A. Increased odds of disordered eating in polycystic ovary syndrome: a systematic review and meta-analysis. Eat Weight Disord. 2018;24(5):787–797.

Legro RS, Kunselman AR, Dodson WC, Dunaif A. Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective, controlled study in 254 affected women. J Clin Endocrinol Metab. 1999;84(1):165–9.

Legro RS, Arslanian SA, Ehrmann DA, Hoeger KM, Murad MH, Pasquali R, et al. Diagnosis and treatment of polycystic ovary syndrome: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2013;98(12):4565–92.

Lepine S, Jo J, Metwally M, Cheong YC. Ovarian surgery for symptom relief in women with polycystic ovary syndrome. Cochrane Database Syst Rev. 2017;11:CD009526.

Lizneva D, Kirubakaran R, Mykhalchenko K, Suturina L, Chernukha G, Diamond MP, et al. Phenotypes and body mass in women with polycystic ovary syndrome identified in referral versus unselected populations: systematic review and meta-analysis. Fertil Steril. 2016; 106(6):1510–20.e2.

Lujan ME, Jarrett BY, Brooks ED, Reines JK, Peppin AK, Muhn N, et al. Updated ultrasound criteria for polycystic ovary syndrome: reliable thresholds for elevated follicle population and ovarian volume. Hum Reprod. 2013;28(5):1361–8.

Macut D, Tziomalos K, Bozic-Antic I, Bjekic-Macut J, Katsikis I, Papadakis E, et al. Non-alcoholic fatty liver disease is associated with insulin resistance and lipid accumulation product in women with polycystic ovary syndrome. Hum Reprod. 2016;31(6):1347–53.

McAllister JM, Legro RS, Modi BP, Strauss JF 3rd. Functional genomics of PCOS: from GWAS to molecular mechanisms. Trends Endocrinol Metab. 2015;26(3):118–24.

Meun C, Franco OH, Dhana K, Jaspers L, Muka T, Louwers Y, et al. High androgens in postmenopausal women and the risk for atherosclerosis and cardiovascular disease: the Rotterdam study. J Clin Endocrinol Metab. 2018;103(4):1622–30.

Mokhlesi B, Scoccia B, Mazzone T, Sam S. Risk of obstructive sleep apnea in obese and nonobese women with polycystic ovary syndrome and healthy reproductively normal women. Fertil Steril. 2012;97(3):786–91.

Moran LJ, Pasquali R, Teede HJ, Hoeger KM, Norman RJ. Treatment of obesity in polycystic ovary syndrome: a position statement of the androgen excess and polycystic ovary syndrome society. Fertil Steril. 2009;92(6):1966–82.

Moran LJ, Hutchison SK, Norman RJ, Teede HJ. Lifestyle changes in women with polycystic ovary syndrome. Cochrane Database Syst Rev. 2011;2:CD007506.

Mulders AG, Laven JS, Eijkemans MJ, de Jong FH, Themmen AP, Fauser BC. Changes in anti-Mullerian hormone serum concentrations over time suggest delayed ovarian ageing in normogonadotrophic anovulatory infertility. Hum Reprod. 2004;19(9):2036–42.

Mykhalchenko K, Lizneva D, Trofimova T, Walker W, Suturina L, Diamond MP, et al. Genetics of polycystic ovary syndrome. Expert Rev Mol Diagn. 2017;17(7):723–33.

Naderpoor N, Shorakae S, de Courten B, Misso ML, Moran LJ, Teede HJ. Metformin and lifestyle modification in polycystic ovary syndrome: systematic review and meta-analysis. Hum Reprod Update. 2015;21(5):560–74.

Neven ACH, Laven J, Teede HJ, Boyle JAA. Summary on polycystic ovary syndrome: diagnostic criteria, prevalence, clinical manifestations, and management according to the latest international guidelines. Semin Reprod Med. 2018;36(1):5–12.

Norman RJ, Masters L, Milner CR, Wang JX, Davies MJ. Relative risk of conversion from normoglycaemia to impaired glucose tolerance or non-insulin dependent diabetes mellitus in polycystic ovarian syndrome. Hum Reprod. 2001;16(9):1995–8.

Panidis D, Tziomalos K, Misichronis G, Papadakis E, Betsas G, Katsikis I, et al. Insulin resistance and endocrine characteristics of the different phenotypes of polycystic ovary syndrome: a prospective study. Hum Reprod. 2012;27(2):541–9.

Pastoor H, Timman R, de Klerk C, W MB, Laan ET, Laven JS. Sexual function in women with polycystic ovary syndrome: a systematic review and meta-analysis. Reprod Biomed Online. 2018;37(6):750–60.

Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod. 2004;19(1):41–7.

Schmidt J, Landin-Wilhelmsen K, Brannstrom M, Dahlgren E. Cardiovascular disease and risk factors in PCOS women of postmenopausal age: a 21-year controlled follow-up study. J Clin Endocrinol Metab. 2011;96(12):3794–803.

Tasali E, Chapotot F, Leproult R, Whitmore H, Ehrmann DA. Treatment of obstructive sleep apnea improves cardiometabolic function in young obese women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2011;96(2):365–74.

Tata B, Mimouni NEH, Barbotin AL, Malone SA, Loyens A, Pigny P, et al. Elevated prenatal anti-Mullerian hormone reprograms the fetus and induces polycystic ovary syndrome in adulthood. Nat Med. 2018;24(6):834–46.

Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, et al. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Hum Reprod. 2018;33(9):1602–18.

The Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Consensus on infertility treatment related to polycystic ovary syndrome. Hum Reprod. 2008;23(3):462–77.

Valkenburg O, van Santbrink EJ, Konig TE, Themmen AP, Uitterlinden AG, Fauser BC, et al. Follicle-stimulating hormone receptor polymorphism affects the outcome of ovulation induction in normogonadotropic (World Health Organization class 2) anovulatory subfertility. Fertil Steril. 2015;103(4):1081–8.e3.

Veltman-Verhulst SM, Boivin J, Eijkemans MJ, Fauser BJ. Emotional distress is a common risk in women with polycystic ovary syndrome: a systematic review and meta-analysis of 28 studies. Hum Reprod Update. 2012;18(6):638–51.

Victoria M, Labrosse J, Krief F, Cedrin-Durnerin I, Comtet M, Grynberg M. Anti Mullerian Hormone: more than a biomarker of female reproductive function. J Gynecol Obstet Hum Reprod. 2019;48(1):19–24.

Wang R, Kim BV, van Wely M, Johnson NP, Costello MF, Zhang H, et al. Treatment strategies for women with WHO group II anovulation: systematic review and network meta-analysis. BMJ (Clin Res Ed). 2017;356:j138.

Wing RR, Papandonatos G, Fava JL, Gorin AA, Phelan S, McCaffery J, et al. Maintaining large weight losses: the role of behavioral and psychological factors. J Consult Clin Psychol. 2008; 76(6):1015–21.

Yilmaz B, Vellanki P, Ata B, Yildiz BO. Metabolic syndrome, hypertension, and hyperlipidemia in mothers, fathers, sisters, and brothers of women with polycystic ovary syndrome: a systematic review and meta-analysis. Fertil Steril. 2018;109(2):356–64.e32.

Menstrual Disorders Related to Endocrine Diseases

4

Costanzo Moretti

Contents

Abstract

Endocrine disturbances affecting the menstrual cycle and fertility in women can interfere with the coordinated stimulatory and inhibitory effects that lead to the release of a single mature oocyte from the ovarian pool of primordial oocytes. The female reproductive system may be susceptible to dysfunction generated by internal and external forces that include disorders in alimentation, excess exercise, psychophysical stress, and pathologies. This chapter examines the influence of neuroendocrine and pituitary dysfunctions and adrenal, thyroid, local, and systemic pathologies on the female reproductive axis. These disorders can modify a variety of factors that contribute to abnormal uterine bleeding, and they include steroidal and nonsteroidal hormones and paracrine, autocrine, and intracrine factors. All these substances can alter the cyclic changes in the major pituitary and gonadal hormones and the related feedback mechanism involved in the normal menstrual cycle. Pathologies of the hypothalamus can cause pituitary dysfunction, neuropsychiatric and behavioral disorders, and resulting menstrual disorders. The anterior

C. Moretti (✉)
Department of Systems' Medicine, Section of Reproductive Endocrinology, University of Rome TorVergata, Rome, Italy
e-mail: moretti@med.uniroma2.it

F. Petraglia, B. C. Fauser (eds.), *Female Reproductive Dysfunction*, Endocrinology,
https://doi.org/10.1007/978-3-030-14782-2_4

pituitary gland controls the secretion of essential hormones from other endocrine glands including gonads, thyroid, and the adrenal cortex, and its disorders – partial or complete – can greatly influence the normal menstrual cycle. The ovarian sex steroids and their cognate receptors regulate an array of local factors within the endometrium that lead to important paracrine, autocrine, and intracrine actions in the regulation of menstruation. Endometrial intracrinology, associated with altered expression of key enzymes within the cells, has recently opened up new routes in understanding the development of menstrual disorders mediated by endocrine diseases.

Keywords

Female reproductive axis · Menstrual disorders · Ovary · Hypothalamus · Pituitary · Adrenal · Thyroid · PCOS · Gonads · Neuroendocrine system · LH · FSH · Estradiol · Gonadotropins

Introduction

The normal menstrual cycle is a coordinated series of systemic and local effects that results in ovulation and the decidualization of endometrial stromal cells to prepare for embryo implantation. By tradition, the cycle is divided into two periods: the proliferative follicular phase, which begins with the onset of menses and ends on the day before the LH surge, and the secretive luteal phase, which starts on the day of the LH surge and ends on the first day of menstruation, for an average of 28–35 days (Barbieri 2014). The gonadotropins secreted by the anterior pituitary control the ovarian activity through feedback mechanisms mediated by estradiol and progesterone. Evidence has been provided that even nonsteroidal substances, such as inhibin A and B, and the recently described gonadotropin surge-attenuating factor (GnSAF), participate in the negative feedback (Messinis et al. 2014), playing a role in the control of LH secretion during the follicular phase and at midcycle (Jabbour et al. 2006).

Over the last decade, genetic and genomic research has added information about the neuroendocrine control of the menstrual cycle (Genazzani et al. 1997; Leng and MacGregor 2018). The hypothalamic gonadotropin-releasing hormone (GnRH), the main neuropeptide controlling the reproductive axis, is regulated upstream in its pulsatile activity mainly by neurokinin B, kisspeptin, and the inhibiting makorin ring finger protein 3 (MKRN3), which negatively controls the release of GnRH from the hypothalamus, modulating the onset of puberty (Tsutsui and Ubuka 2018; Livadas and Chrousos 2016). Thus, abnormal uterine bleeding may arise from the imbalance of many clinical pathologies and cause significant reproductive disorders. Many endocrine disorders can affect the complex network of neuroendocrine and pituitary function, modifying ovarian steroid hormone production which regulates endometrial function and human menstruation.

Once ovulation has occurred, the corpus luteum secretes progesterone. In the mid-luteal phase, the action of progesterone upon the estrogen-primed endometrium

promotes decidualization, converting the elongated endometrial stromal cells into more spherical decidual cells. This induces the expression of two hemostatic proteins in decidualized endometrial stromal cells: the tissue factor (TF), a 46 kDa cell membrane-bound glycoprotein that acts as a receptor for the active form of coagulation factor VII, and the plasminogen activator inhibitor-1 (PAI-1) that controls trophoblast invasion and cooperates in maintaining anti-fibrinolytic and anti-proteolytic properties together with angiopoietin-1, an angiostatic agent that stabilizes the vessels blocking uncontrolled angiogenesis. Simultaneously with the corpus luteum regression, there is a reduction in TF, PAI-1, and angiotensin-1, and the endometrium becomes infiltrated by leukocytes, cytokines, and matrix metalloproteinases (Gellersen and Brosens 2014).

Therefore, during the late secretory phase of the menstrual cycle, progesterone withdrawal creates a pro-hemorrhagic environment around the endometrial blood vessels, provoking endometrial inflammation and local cytokine presence and the initiation of menstruation. Matrix metalloproteinases (MMPs) are considered the main mediators of endometrial breakdown, considering that they have the ability to degrade all components of the extra cellular matrix (ECM). In addition to chemokine release, an important point is the endometrial vascular effects activated by progesterone withdrawal and the intense vasoconstriction observed which induces endometrial shrinkage. Progesterone withdrawal is associated with upregulation of interleukin-8 and macrophage chemoattractant protein-1. Hypoxia may occur at menstruation in human endometrial tissue, and, even if its function must be still fully clarified, it has been demonstrated that it enhances angiogenesis by inducing endometrial stromal cells to express the vascular endothelial growth factor (VEGF) and endometrial endothelial cells to express angiopoietin-2 participating in the MMP release and tissue destruction (Maybin and Critchley 2015).

A higher expression of TNFα, a pro-inflammatory cytokine, has been described in the flaked endometrium of women affected by menstrual disorders compared with women with a normal menstrual period (Malik et al. 2006). Glucocorticoids may alter the inflammatory response by limiting the cytokine production, increasing both macrophage phagocytosis and the transcription of anti-inflammatory transcription factors that repress the pro-inflammatory machinery. The glucocorticoid receptor is expressed on stroma cells, endometrial leukocytes, and endothelial cells, and the local availability of glucocorticoids may be important in endometrial physiology.

To summarize, during the luteal and gestational phase, the action of factors inducing stabilization of the stromal and underlying vascular extracellular matrices prevents endometrial shrinkage and cleavage. In contrary fashion, perimenstrual progesterone withdrawal in the absence of fertilization is associated with increased expression of prostaglandins and chemokine release, promoting leukocyte infiltration, vascular effects inducing hypoxia and increased expression of VEGF, and MMP activation and release in a general proteolytic milieu that causes tissue destruction (Malik et al. 2006).

Beyond the effects of endocrine dysregulation, menstrual disorders can be generated by a disruption of the well-ordered and highly regulated autocrine-paracrine/intracrine processes underlying endometrial physiology, inducing excessive tissue

damage and/or prolonged inflammatory response at the time of menstruation. In this area, menstrual disorders may be related to lifestyle and physical and emotional stress and traumas. Menstrual dysfunction includes menorrhagia; dysmenorrhea; irregular, frequent, and prolonged periods; oligomenorrhea; amenorrhea; and abnormal uterine bleeding (AUB) caused by both local and systemic pathologies or related to medication (Lockwood 2011).

The most common etiologies encompass uterine pathologies (like adenomyosis, leiomyomas, polyps, endometrial hyperplasia, endometriosis, endometrial cancer, or, albeit rarely, intrauterine adhesions or synechiae). Many of these disorders are associated with a dysfunction of the hypothalamic-pituitary-ovarian axis, and they induce morphological changes within the uterus that are not related to modifications in the activity of endometrial local factors. Menstrual disorders can originate from primary or secondary ovarian failure, the dysregulation of gonadotropin secretion, and polycystic ovary syndrome (PCOS) (Gray 2013). The aim of this chapter is to focus mainly on neuroendocrine and pituitary dysfunctions and adrenal and thyroid pathologies that can induce menstrual disorders.

Menstrual Disorders and Hypothalamic Pathologies

After the gonadotropin-releasing hormone (GnRH) – the regulator of the production and release of the pituitary gonadotropins – was isolated and characterized, the central role of this decapeptide in the reproductive function was established. GnRH is synthesized in a small subset of hypothalamic neurons which differentiate in the olfactory placode and migrate to the medial basal hypothalamus where they establish a close morphofunctional connection with the pituitary portal system in the median eminence as part of the hypothalamic portal system. The discovery of a gonadotropin-inhibitory hormone (GnIH), highly conserved among vertebrates, that acts by inhibiting gonadotropin synthesis and release by acting on gonadotropes and GnRH neurons (Tsutsui et al. 2018) indicates that GnRH is not the only hypothalamic neuropeptide able to regulate gonadotropin release. GnRH in the anterior pituitary gland binds to specific receptors (GnRHRs) expressed on gonadotroph cells, triggering an intracellular signaling cascade that involves the α_q-subunit of G-protein which activates phospholipase Cβ leading to an intracytoplasmatic increase in diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP_3), and therefore, through the activated protein kinase C (PKC), to the release of Ca^{2+} from intracellular stores (Maggi et al. 2016). To be activated, pituitary GnRHRs require a pulsatile stimulation by GnRH. In fact, the presence both in females and in males of a physiological reproductive function that is dependent on the pulsatile pattern of GnRH secretion and many neuromodulators has been identified as being involved in this mechanism (Stamadiates and Kaiser 2018). The distribution and episodic activity of GnRH-producing neurons and the network of neurotransmitters and neuropeptidergic inputs, modulating its pulsatile secretion, drive many events connected to the onset of the menstrual cycle and to the regulation of the

physiological ovarian function (Maggi et al. 2016). Developmental defects of GnRH neuron migration or the inability of the hypothalamus to release GnRH in the correct pulsatile manner can lead to a wide range of disorders, identified overall as central hypogonadotropic hypogonadism (Fourman and Fazeli 2015).

Genetic studies to date have identified at least 16 genes affecting the development and function of the GnRH neurons, and in patients with isolated GnRH deficiency, they have highlighted either forms with olfactory dysfunctions (Forni and Wray 2015), referred to as Kallmann syndrome, or forms occurring with a normal sense of smell (Valdes-Socin et al. 2014). Female patients with isolated GnRH deficiency rarely develop clinical features at birth, but they have them at the onset of puberty (amenorrhea and a failure to establish a pubertal growth spurt), demonstrating inappropriately low or high (but also normal) luteinizing hormone (LH) and follicle-stimulating (FSH) that may arise when administered in a pulsatile regimen designed to mimic GnRH secretion. This demonstrates the intact anatomic and functional integrity of pituitary and gonads. In most cases of central hypogonadotropic hypogonadism, sporadic familial transmission has been described. This is because either anosmic or non-anosmic forms can be inherited in an autosomal dominant, autosomal recessive, or X-linked recessive manner. An oligogenic inheritance has been recognized as contributing to incomplete penetrance and variable expressivity that occurs within and across affected families (Marino et al. 2014). Table 1 shows genes that typically cause the anosmic and normosmic forms of central hypogonadotropic hypogonadism.

As mentioned above, a huge number of signals are involved in the control of GnRH neuron function and secretion. The mechanism underlying pulsatile secretion arises from a fine-tuned modulation addressed to induce a correct secretion of LH and FSH from pituitary gonadotrophs. Ovarian steroids through their nuclear receptors exert differential regulatory effects on GnRH secretion, altering both pulse frequency and amplitude. 17β-Estradiol may have both stimulatory and inhibitory effects, depending on the stage of the menstrual cycle. On the estrogen-primed endometrium, progesterone exerts an inhibitory action slowing the frequency of GnRH pulsatile secretion.

In addition to the modulatory action exerted by the ovarian steroids, a network of neurons afferent to GnRH neurons in the infundibular region and arcuate nucleus may control their pulsatile activity, whereas in humans the preoptic area mediates the surge phase. In this neural network, a relevant role is played by kisspeptin and neurokinin B neurons that are mainly present in the infundibular tract and in the preoptic area. Kisspeptin (KP) signals via a $G\alpha_{q/11}$/β-arrestin-coupled kiss1 receptor (KISS1R), whose expression on GnRH cell bodies is largely dependent on ovarian estradiol output, control the hypothalamic-pituitary-ovarian axis and are important in promoting endometrial gland development and function (Leon A). Neurokinin B is co-expressed in KP neurons (KNDy neurons) operating upstream of KP to modulate downstream GnRH pulsatility together with other peptides such as dynorphin and substance P (Skorupskaite et al. 2014). The hypothalamic neural network controlling GnRH pulse frequency and amplitude is also highly regulated by peripheral peptides from the adipose tissue and gastrointestinal tract, in particular leptin and ghrelin

Table 1 Most significant genes implicated in hypogonadotropic hypogonadism

Gene	Location	Inheritance	Phenotype
KAL1	Xp22.3	X-linked	KS ± synkinesia and renal agenesis
FGFR1	8p11.23-p11.22	Autosomal dominant	KS, IHH ± clefting
FGF8	10q24.32	Autosomal dominant	KS, IHH
FGF17	8p21.3	Autosomal dominant or autosomal recessive with oligogenicity	KS, IHH
IL17RD	3p14.3	Autosomal dominant or autosomal recessive with oligogenicity	KS ± deafness
DUSP6	12q21.33	Autosomal dominant or autosomal recessive with oligogenicity	KS, IHH
SPRY4	5q31.3	Autosomal dominant or autosomal recessive with oligogenicity	KS, IHH
FLRT3	20p12.1	Autosomal dominant or autosomal recessive with oligogenicity	KS
HS6ST1	2q14.3	Autosomal dominant or autosomal recessive with oligogenicity	KS, IHH
NELF	9q34.3	Autosomal dominant or autosomal recessive with oligogenicity	KS
WDR11	10q26.12	Autosomal dominant	KS, IHH
PROKR2	20p12.3	Autosomal dominant or autosomal recessive with oligogenicity	KS, IHH
PROK2	3p13	Autosomal recessive	KS, IHH
CHD7	8q12.1-q12.2	Autosomal dominant	CHARGE, KS, IHH
SEMA3A	7q21.11	Autosomal dominant or autosomal recessive with oligogenicity	KS
SEMA3E	7q21.11	Autosomal dominant or autosomal recessive with oligogenicity	KS
SOX10	22q13.1	Autosomal dominant or autosomal recessive with oligogenicity	KS ± deafness
FEZF1	7q31.32	Autosomal recessive	KS
TAC3	12q13.3	Autosomal recessive	IHH
TAC3R	4q24	Autosomal recessive	IHH
KISS1	1q32.1	Autosomal recessive	IHH
KISS1R	19p13.3	Autosomal recessive	IHH
GnRH1	8p21.2	Autosomal recessive	IHH
GnRHR	4q13.2	Autosomal recessive	IHH

KS Kallman syndrome, *IHH* idiopathic hypogonadotropic hypogonadism

whose changes are related to the energy stores. Leptin and ghrelin are antagonists in their effects on GnRH neurons, and changes in their function can occur linked to hypogonadotropic hypogonadism, precocious and delayed puberty, hypothalamic amenorrhea, perimenopausal transition phase, PCOS, and endometriosis (Celik et al. 2015).

Many menstrual disorders and much ovulatory dysfunction may be dependent on the dysregulation of the hypothalamic machinery that controls GnRH pulsatility (Coss 2018). Intense exercise, eating disorders, and stress can markedly affect the normal menstrual cycle. The female athlete triad is an interrelationship of menstrual dysfunction, low energy availability (with or without an eating disorder), and decreased bone mineral density (Nazem and Ackerman 2012). Energy availability, through different pathways, seems to be the key etiological factor in this condition, even if the impact of nutrient reduction (an often conscious restriction of food intake) on menstrual disorders can be modified in particular by gynecological age, psychological factors, and genetics (Williams 2017). Individuals with anorexia nervosa and amenorrhea have severe bone loss (Schorr and Miller 2017), and the restoration of a normal GnRH pulse frequency is possible only if the recovery of fat mass – determined using DXA – is over 20% of body weight (personal observation). Organic disorders of the hypothalamus can cause pituitary dysfunction, neuropsychiatric and behavioral disorders, and disturbances of autonomic and metabolic regulation. Craniopharyngiomas are rare and are partly cystic and calcified embryonic malformations of the sellar/parasellar region that have a peak incidence rate at the puberty time, altering its time of onset. However, they can arise in adults, in particular over the age of 50. Despite high survival rates, the quality of life of the patients affected is often impaired because of the sequelae caused by the anatomical proximity of the tumor to the optic nerve/chiasma and pituitary (Möller 2014). Other rare intracranial tumors (like meningiomas, ependymomas, gliomas) may affect the hypothalamic areas, causing neuroendocrine dysfunctions and menstrual cycle abnormalities (Table 2).

Table 2 Most common neuroendocrine causes of menstrual dysfunctions

Overall
Stress-induced functional hypothalamic amenorrhea
Damage from pituitary radiation therapy
Traumatic brain injury
Tumors
Craniopharyngioma
Glioma, meningioma
Pituitary macroadenomas
Angioma, plasmacytoma, colloid cysts
Lymphoma
Ependymoma
Sarcoma
Histiocytosis X
Inflammatory disease
Tuberculosis
Viral encephalitis
Vascular diseases
Aneurysm, subarachnoid hemorrhage
Arteriovenous malformation

To summarize, neuroendocrine amenorrhea often primarily affects adolescent women because of perturbations of the hypothalamic function, most frequently in response to physical, emotional, or nutritional stress and rarely because of tumors or infectious diseases.

Menstrual Disorders and Pituitary Pathologies

The anterior pituitary gland controls the secretion of the major endocrine glands, and its activity is modulated by inputs from higher brain centers to the hypothalamus that produce releasing and inhibitory neurohormones, leading to a release of pituitary and target gland hormones. Target gland hormones in turn exert a feedback action at both hypothalamus and pituitary levels, influencing metabolism and diurnal rhythms. As far as the female reproductive axis is concerned, GnRH secreted by the hypothalamus regulates the synthesis and secretion of gonadotropins, LH, and FSH, which then control steroidogenesis and gametogenesis. In women, serum LH and FSH produced by the gonadotroph cells of anterior pituitary exhibit rhythmic changes throughout the menstrual cycle that are correlated with pulse frequency of GnRH (Coss 2018). Here, dysfunctions in the LH/FSH ratio due to levels outside the normal range may be caused by drugs or exogenous hormones or linked to several endocrine diseases, even affecting other axes controlled by the pituitary gland. One paradigmatic dysfunction of the brain-hypothalamic-pituitary axis is anorexia nervosa, a psychiatric disorder characterized by altered body image, persistent food restriction, and low body weight and fat mass. In this condition, a hypogonadotropic hypogonadism is evident with relative estrogen and androgen deficiency that induces secondary amenorrhea together with growth hormone resistance, hypercortisolemia, hyponatremia, hypooxytocinemia, hypoleptinemia, and elevated plasma levels of ghrelin and peptide YY – all endocrine disturbances secondary to the low energy state of chronic starvation (Schorr and Miller 2017).

The pituitary gland is made up of an anterior lobe of epithelial origin and a posterior lobe of neural origin. Pathologies of either the anterior or posterior lobe are associated with symptoms derived from the hyper- or hypo-secretion of the different cell types involved, the growth hormone (GH), prolactin (PRL), adrenocorticotropin (ACTH), and gonadotropin (FSH/LH) secreting cells. Nonfunctioning adenomas are not identified for the dysregulated specific hormone secretion but frequently for mass effects causing headache, hypopituitarism, and visual field defects. Most of these "nonfunctional" tumors derive from the glycoprotein hormone cell lineage and express mRNA for the common α-subunit of pituitary glycoprotein hormones (LH, FSH, TSH), giving rise to biologically inactive products (Melmed 2015). Almost all anterior pituitary adenomas are benign. Locally aggressive and invasive carcinomas are extremely rare, and they are often diagnosed when distant metastases are detected. The survival of subjects affected with pituitary carcinomas is usually less than 2 years after diagnosis (Raverot et al. 2018). Senescence, characterized by a signal transduction program leading to irreversible cell-cycle arrest, seems to represent a key protective mechanism against malignancy, mostly mediated by the action

of interleukin-6 (IL-6), restraining a proliferative advantage and allowing pituitary cells to maintain their physiological functions (Sapochnik et al. 2017). The pituitary adenomas most frequently involved in the pathogenesis of secondary amenorrhea are prolactinomas which account for more than 60% of all adenomas causing galactorrhea and infertility in women (Molitch 2017). The diagnosis of prolactinoma is difficult given that hyperprolactinemia has many potential etiologies. The stress of phlebotomy may induce a rise in serum prolactin, and false elevated levels can be due to macroprolactin, also known as "big prolactin," a non-bioactive prolactin isoform usually composed of a prolactin monomer and an IgG molecule having a prolonged clearance rate similar to that of immunoglobulins. This isoform is clinically non-reactive but it interferes with immunological assays used for the detection of prolactin (Vaishya et al. 2010). The clinical expression of cell-specific prolactinomas is associated with a loss of libido, oligomenorrhea, galactorrhea, and infertility.

The diagnosis is made even more difficult because a pituitary adenoma not secreting prolactin might induce hyperprolactinemia stalk compression and obstacles to dopamine delivery. Plasma levels of prolactin are related to the size of prolactinoma evaluated on T1-weighted MR, and the size of the tumor tends to shrink with therapy given that prolactinomas are very responsive to medical therapy with dopamine agonists (bromocriptine and cabergoline). Cabergoline used in early pregnancy was not found to increase the risk of miscarriage or fetal malformations. Surgery and radiotherapy are limited to rare tumors unresponsive to dopamine agonists. One growth hormone is prolactogen; therefore, amenorrhea-galactorrhea and mild hyperprolactinemia can be due to a somatotropinoma. Somatotroph adenomas are rare, accounting for around 12% of all pituitary tumors and leading to features of acromegaly with the enlargement of bone extremities, lips, tongue, and nose and symptoms of arthritis, hypertension, headache, and hyperglycemia. Symptoms are subtle and gradual in onset and thus diagnosis can be late. The assessment of IGF-1 plasma levels represents the best screening test for acromegaly, and GH suppression with 75 gr of anhydrous glucose is used as a confirmation test. Many somatotropinomas express somatostatin receptors, mainly the sub-type 2, and are responsive to somatostatin analogs such as octreotide or lanreotide, even if large tumors require both transsphenoidal surgery and medical therapy.

Thyrotroph adenomas hypersecreting TSH are extremely rare and may be associated with mild hyperthyroidism and goiter. Corticotroph adenomas are extremely rare and produce hypercortisolism associated with disorders of the menstrual cycle, infertility, central obesity, hypertension, diabetes, and psychological disorders. A pathogenetic feature of all these tumors is their monoclonality as they appear to arise from a single cell that causes an activating mutation of the specific receptors binding the hypothalamic hormones that acquire a proliferative advantage. Their surgical resection may result in long-term remission. While 95% of pituitary adenomas arise sporadically without a known inheritable predisposing mutation, in about 5% of cases, they can arise in a familial setting, either isolated (familial isolated pituitary adenoma or FIPA) or as part of a syndrome (Pepe et al. 2019).

Whatever the case, case abnormalities in cell-cycle regulation are seen as the key event in the pathogenesis of pituitary adenomas with multiple pathways involved, due either to germline mutations or to altered gene expression, inducing isolated pituitary adenomas or syndromic diseases (Caimari and Korbonits 2016). Among the syndromic diseases, the multiple endocrine neoplasia type 1 (MEN1) is characterized by the presence of the classic triad of hyperparathyroidism (in over 90% of patients by the age of 50 years), gastroenteropancreatic neuroendocrine tumors – NETs (in approximately 60% of patients), and pituitary adenomas (in about 35% of patients), besides the possibility of association with other endocrine and non-endocrine tumor types such as bronchial and thymic NETs, facial angiofibromas, lipomas, collagenomas, adenomas of the adrenal cortex, meningiomas, breast cancer, and, rarely, pheochromocytomas (Pepe et al. 2019). MEN1 is due to germline heterozygous mutations in the *MEN1* gene which encodes menin, a scaffold protein located mostly in the nucleus and involved in several cellular processes, including transcriptional regulation, genome stability, and cell division and proliferation (Pepe et al. 2019). Rarely, some patients showing MEN1 clinical characteristics do not present *MEN1* gene mutations, a condition that has been named MEN4 (Pepe et al. 2019). These patients are affected by primary hyperthyroidism that is isolated or associated with gastroenteropancreatic NETS and pituitary adenomas.

Carney complex is a rare multiple neoplasia syndrome. This syndrome is autosomal, is dominantly inherited, and is characterized by the presence of endocrine and non-endocrine tumors, including pituitary adenomas, pigmented lesions of the skin, and cardiac and cutaneous myxomas. In Carney complex, the most common endocrine manifestation, which often occurs often in females and may be able to alter the reproductive system, is ACTH-independent Cushing syndrome due to primary pigmented nodular adrenocortical disease (Pepe et al. 2019). 75% of patients affected have thyroid follicular adenomas and elevated GH and IGF-I, often with associated hyperprolactinemia. The genetic background of Carney complex is mostly due to heterozygous inactivating mutations in the *PRKAR1A* gene coding for the regulatory subunit type 1 alpha of the protein kinase A (PKA), leading to increased cAMP-dependent PKA activity which drives tumor formation in tissues of the affected patients.

McCune-Albright syndrome is a rare disorder characterized by polyostotic fibrous dysplasia, café-au-lait skin macules, and endocrinopathies that affect the reproductive system and the ovarian function, mainly in adolescents (Sotomayor et al. 2011). It arises from post-zygotic somatic activating mutations in the *GNAS* gene, encoding the cAMP-regulating transcript α-subunit Gsα and then producing a constitutive Gs signaling which results in activation of adenylyl cyclase and dysregulated cAMP production. A pituitary involvement, mainly with GH excess, is present in about 20% of patients affected, often associated with hyperprolactinemia and menstrual dysfunction, with the age of onset being around 24 years. In patients with hereditary pheochromocytoma and paraganglioma due to germline heterozygous mutations in gene encoding dehydrogenase subunits and the SDH complex assembly factor 2 protein (*SDHAF2*), the development of pituitary tumor, mainly prolactinomas and GH-secreting adenomas, has been described. The

DICER1 syndrome, a rare autosomal dominant disorder due to germline heterozygous mutation in the *DICER1* gene, is characterized by the onset of different kinds of malignant and benign tumors, including ovarian sex cord-stromal tumors, cystic nephroma, differentiated thyroid cancer, pituitary blastoma, genitourinary embryonic rhabdomyosarcoma, and pineaoblastoma. These are disorders that may all deeply affect the reproductive system in females (Pepe et al. 2019; Caimari and Korbonits 2016).

To summarize, while most pituitary adenomas occur sporadically, some of them, about 5%, may occur in a familial setting as a result of genetic germline or somatic mutations of pituitary adenoma-predisposing genes, either isolated like FIPA or as a part of a syndrome. A modification of local expression of growth factors and reproductive hormone receptors could have a role in determining the tumor level, resistance to treatment, and a worse prognosis.

A functional anterior and posterior pituitary deficiency inducing menstrual disorders may be due to hypophysitis, an acute or chronic inflammation of the pituitary gland. Hypophysitis can be classified according to etiology, morphology, and/or histopathology. Primary hypophysitis refers to isolated inflammation of the pituitary that is not associated with drugs, infections, or other disorders, while secondary hypophysitis may be due to medications targeting cytotoxic T-lymphocyte antigen-4 (CTLA-4) or programmed cell death 1 (PD-1), rupture of Rathke's cyst, craniopharyngiomas, and hemorrhagic pituitary adenomas. Patients affected have symptoms related to mass effects from pituitary gland enlargement and pituitary dysfunction. The dominant symptom is severe headache together (less frequently) with visual symptoms due to compression of the optic and/or cranial nerves. Patients display multiple anterior pituitary hormone deficiencies often not related to the MR findings, without a clear hierarchy, as are observed in clinically nonfunctioning adenomas (Faje 2016).

Menstrual Disorders and Adrenal Pathologies

The adrenal gland integrates two embryologically distinct endocrine systems within one organ capsule, the cortex, producing steroid hormones, and the catecholamine-producing medulla. The adrenal cortex, arranged in the outermost zona glomerulosa secreting mineralocorticoids (aldosterone), the zona fasciculata producing glucocorticoids (cortisol), and the zona reticularis which produces androgens (testosterone and dehydroepiandrosterone sulfate), is mostly regulated by ACTH produced in the anterior pituitary corticotroph cells, whereas the adrenal medulla releases catecholamines in response to the activated sympathetic nervous system (SNS). An increased adrenal cortex production of DHEAS may cause hyperandrogenemia and an impaired menstrual cycle and fertility (Ross and Louw 2015).

Cushing syndrome, which may occur in young women, is an important cause of loss of menstrual cyclicity and infertility. Cushing syndrome may be ACTH-dependent, with the ectopic production of ACTH or CRH by carcinomas or neuroendocrine tumors, or ACTH-independent, caused by unilateral adenomas or carcinomas

of the adrenal gland. The hypersecretion of cortisol causes lipolysis and fat redistribution together with loss of muscle mass and bone and skin disorders. Central obesity is a paradigmatic clinical manifestation of hypercortisolism. Cortisol excess can be subtle in an early stage of the disease and its diagnosis may be missed or even confused with a metabolic syndrome. In a retrospective study performed on women affected with Cushing syndrome during the reproductive period, many of them had been diagnosed as having solely a polycystic ovary syndrome, so testing for hypercortisolism in all women with suspected PCOS is suggested (Brzana et al. 2014). A history of hirsutism with rapid onset or clinically objective elements directing a hypercortisolism, such as thin skin, proximal muscle weakness, and central obesity, should promptly activate screening aimed at excluding Cushing syndrome. To be confirmed, a diagnosis of Cushing syndrome provides the measurement of plasma ACTH that will determine if the disease is ACTH-dependent or ACTH-independent. Where ACTH values <5 pg/mL are highlighted, an ACTH-independent Cushing syndrome can be diagnosed, and an abdominal computer tomography needs to be performed. If the ACTH plasma levels are normal or elevated, a magnetic resonance imaging of the pituitary with gadolinium contrast must be programmed.

The adrenal gland is a key component of the stress system in the human body. Multiple endocrine, paracrine, and intracrine interactions between different cell types within the adrenal gland microenvironment are supported by high vascularization and the activity of the adrenal cortex extracellular matrix (Kanczkowski et al. 2017). Obesity, sepsis, metabolic syndrome, and diabetes, besides several conditions of latent stress, may alter the adrenal gland microenvironment, activating the chronic hypersecretion of glucocorticoids and catecholamines that exert different systemic actions ultimately finalized to the restoration of body homeostasis.

Not only patients with Cushing syndrome but even those suffering from Conn syndrome due to hyperaldosteronism have an increased risk of developing metabolic syndrome, depression, osteoporosis, and cardiovascular diseases. The adrenal gland has a high regenerative capacity and the ability to adapt to various physiological and pathological conditions through complex bidirectional interactions between the cortex and medulla. Females under acute or chronic psychological stress, and with chronic overproduction of glucocorticoids and catecholamines, may experience a constellation of neuroendocrine changes and may develop menstrual disorders and amenorrhea due to stress-induced suppression of gonadotropin-releasing hormone pulsatile secretion by the activated hypothalamic-pituitary-adrenal axis (Stephens et al. 2016).

ACTH release is required by the body at basal, circadian, and stress-induced levels. When the plasma cortisol levels are lower than required, ACTH rises. Therefore, in the case of adrenal enzyme deficiencies that cause impaired synthesis and reduced cortisol secretion, the chronic elevation of ACTH induces overstimulation of the adrenal cortex. This condition occurs in the case of congenital adrenal hyperplasia (CAH), a group of diseases which develop as a result of a deficiency of enzymes or cofactor proteins required for cortisol biosynthesis (Speiser and White 2003). When we consider the functional defect in the cortisol synthesis,

the negative feedback at hypothalamic and anterior pituitary levels decreases leading to an increase in plasma ACTH and, consequently, to adrenal hyperplasia. CAH is mostly caused by mutations in the *CYP21A2* gene and classified as classic and non-classic types. The classic type arises from severe impairment of the activity of the enzyme 21 hydroxylase which belongs to the cytochrome P450 superfamily encoded by the *CYP21A2* gene which can be suspected in infants born with ambiguous genitalia as simple virilizing or as the most severe salt-wasting type. In the case of non-classic congenital adrenal hyperplasia (NCCAH), genital virilization is not observed at birth, whereas premature adrenarche/pubarche and menstrual irregularities in the post-pubertal period are commonly described. NCCAH includes clinical conditions due to gene mutations or disorders in the steroid synthesis steps of the steroidogenic acute regulatory protein (StAR) that provides the transfer of cholesterol from the mitochondrial membrane to the cell. Therefore, NCCH differs significantly from CAH because it has a later and dissimilar clinical expression.

The most common forms of NCCAH are observed in 21- and 11β-hydroxysteroid dehydrogenase (OHSD) deficiencies which are manifested in hirsutism, clitoromegaly, acne, alopecia, and menstrual irregularities like oligomenorrhea and anovulation, conditions often confused with PCOS. In these cases the assay in the early morning (8 a.m.) during the follicular phase (between the third and the seventh post-menstruation days) of 17αOH progesterone levels and the determination of its levels after the i.v. administration of cortrosyn (ACTH) 250 μgr are considered the gold standards for diagnosis. A basal 17αOH progesterone concentration $>$ 2 ng/mL and plasma levels 60 minutes after intravenous ACTH $>$10 ng/mL may be in agreement with the results of genetic studies that characterize the syndrome. In the case of basal levels of 17αOH progesterone $>$10 ng/mL, a non-classic 11β-hydroxysteroid dehydrogenase deficiency may be suspected which can be confirmed by 11-deoxycortisol levels $>$18 ng mL after ACTH test and genetic studies. A non-classic 3β-hydroxysteroid dehydrogenase deficiency can be diagnosed if basal 17OH pregnenolone levels are $>$30 ng/mL together with 17OH pregnenolone/cortisol ratio $>$ 10SD and clinical symptoms of the androgen excess syndrome.

In late-onset non-classic CAH diagnosed in adults, as in the classic CAH form in the newborn, clinical genetic counseling and genetic testing for mutations in the *CYP21A2* gene on DNA from peripheral blood cells are needed. As observed above, the *CYP21A2* gene is located on the short arm of chromosome 6 (6p21.3) mapped in a duplicate locus within the human major histocompatibility complex (MHC) where it lies in close proximity to a high homologous pseudogene – *CYP21A1P* – arranged in tandem repeats with the genes encoding the fourth component of complement (*C4A* and *C4B*). The tenascin (*TNX*) and serine threonine nuclear protein kinase (*RP*) genes are also located in this region, and four genes (*CYP21A2*, *RP*, *C4*, and *TNX*) form a unit called the RCCX (Carmina et al. 2017). The RCCX modules display a high degree of sequence homology so that a misalignment of sister chromatids during mitosis can result in gene conversion where a small part of one sister chromatid is copied to the other (M New). Therefore, a sequence of the *CYP21A2* gene may be copied to the *CYP21A1P* gene and vice versa, and a large range of gene deletions can take place in the RCCX module. The patient's phenotype is usually

determined by the milder affected allele, and then at least one mildly affected allele is present in non-classic CAH, with a good correlation between the severity of the clinical disease and the mutations observed.

As far as the fertility of females affected with NCCAH is concerned, they have been shown to be more likely to became pregnant compared to females with classic CAH. The condition of chronic high androgen levels is responsible for chronic oligo-anovulation in the presence of high plasma concentrations of progesterone due to the enzyme defect which may alter the endometrial intracrine function and impair implantation. Women with CAH may have impediments to fertility for anatomical reasons as well, such as vaginal stenosis or dyspareunia which make it more difficult to have intercourse. Endogenous glucocorticoids that suppress the hypothalamic corticotrophin-releasing hormone (CRH) may restore ovulation and fertility. The bedtime administration of dexamethasone at variable doses between 0.25 and 1 mg is considered the most effective regimen for ACTH suppression. If fertility is not desired, oral contraceptives (OCs) rather than glucocorticoids are suggested for menstrual cycle management. OCs suppress ovarian androgens, ACTH, and adrenal androgens, and therefore they appear to be more effective in the treatment of androgen excess, with which the majority of CAH patients are affected.

Menstrual Disorders and Thyroid Diseases

Thyroid diseases may have adverse effects on female reproduction. Understanding thyroid physiology and the impact that thyroid hormones may have on the female reproductive machinery help to understand the still unclear relationship between thyroid function and menstrual physiology. A recent study on a longitudinal cohort of premenopausal women recorded that thyroid hormone levels within the euthyroid range were associated with several menstrual cycle function outcomes. In particular, 3,3′,5,5′-tetra-iodothyronine (T4) and 3,5,3′-tri-iodothyronine (T3) were positively related to urinary estrogens and progesterone metabolites across the menstrual cycle (Jacobson et al. 2018). The morphofunctional unit of the thyroid gland is represented by the thyroid follicle where thyroid hormones are synthesized and stored. The thyroid follicle is made up of bipolar secretory epithelial cells with their basal membrane expressing the TSH receptor and sodium/iodine symporter and the apical membrane where the thyroid peroxidase (TPO) and pendrin (involved in the active transport of iodine) are located. The follicular cells synthesize thyroglobulin (TG), a large tyrosine-rich protein that contains about 10% carbohydrate. They surround an internal cavity containing colloid where huge quantities of TG and thyroid hormones are stored. Iodide uptaken by blood is concentrated in colloid and used for the iodination of tyrosine residues present on the TG backbone so that, even with the action of TPO, the inactive precursors mono- and diiodotyrosines are produced, stored, and used for the synthesis of 3,5,3′-tri-iodothyronine (T3) and 3,3′,5,5′-tetra-iodothyronine (T4) – the two active thyroid hormones.

Plasma thyroid hormones circulate within a range controlled through a negative feedback loop on TRH at the hypothalamic level and TSH secreted by the thyrotroph cells of the anterior pituitary gland. Beyond the hypothalamic-pituitary-thyroid axis, the concentrations of thyroid hormone binding globulin (TBG), transthyretin, and albumin, in different proportions, regulate the circulating T4. TBG binds approximately 70% of the circulating T4. During pregnancy, estradiol induces a rise in liver TBG glycosylation, and the increased binding capacity of this protein can transiently decrease free T4 concentrations leading to an increase in the plasma levels of TSH and total T4. Active thyroid hormones affect all the tissues and cellular processes and are critical for normal development, growth, metabolism, adrenergic interactions, thermogenesis, and metabolic influences on the central regulation of the thyroid axis (Mullur et al. 2014). They bind specific thyroid hormone receptors (THRs) – members of the superfamily of hormone-responsive nuclear transcription factors – that are encoded by the thyroid hormone receptor α (*THRA*) and the thyroid hormone receptor β (*THRB*) genes. The *THRA* gene is located on human chromosome 17 and transcribes three THR isoforms, the THRα1, which binds T3 and may form dimers with other truncated proteins, the THRα2 and the THRα3, whose physiological importance is still not completely understood even if their heterodimerization with the full-length THR proteins seems to antagonize the T3-mediated transcriptional activation (Gauthier EMBO J). The *THRB* gene is located on human chromosome 3 and codifies for the T-3 binding isoforms THRβ1 and THRβ2 (a third isoform THRβ3 has been detected only in rats).

Therefore, the isoforms TRHα1, THRβ1, and THRβ2 are the main mediators of thyroid hormone action and have different tissue distribution as indicated by the clinical phenotype of patients with mutations and resistance to thyroid hormones (Gauthier et al. 1999). THRβ isoforms are the predominant receptors expressed in the liver and cardiac ventricles, whereas TRHα1 is preferentially expressed in the brain, heart atria, and white adipose tissue; brown adipose tissue expresses both THRα and β. Both THRα and β undergo posttranslational modification by sumoylation, which is essential for positive and negative gene regulation by thyroid hormones (Ortiga-Carvalho 2014). THRs form a heterodimer complex with the retinoid X receptor (RXR), binding to thyroid response elements (TRE) of the target cell DNA. The intracellular conversion of thyroxin to the active form – triiodothyronine – is the key intracrine mechanism of thyroid hormone action. This is provided by the iodothyronine deiodinase, deiodinases 1 (D1) and 2 (D2), activating enzymes, and deiodinase 3 (D3), an inactivating enzyme, which are all differentially expressed in mammalian tissues. D2, expressed in key thyroid-responsive tissues such as the brain, skeletal muscle, and brown fat, is the enzyme mostly responsible for the intracytoplasmatic increase in T3 which, when transferred to the nucleus, regulates gene transcription.

To summarize, if we have to consider the relationship between thyroid diseases and menstrual disorders, we cannot ignore the molecular mechanisms of thyroid hormone secretion and action modulated by the systemic hypothalamic-pituitary feedback, the plasma transport, nutrient feedback at a central and local level, the signaling pathways, and the intracrine cellular regulation (Colicchia et al. 2014).

Thyroid hormones and their receptors are present in the endometrium, and their expression changes during the menstrual cycle. TRHα1 and THRβ1 have both been described in the mid-luteal phase in glandular and luminal epithelium, showing an increase during the secretory phase and a subsequent dramatic decrease. It has also been demonstrated that transcripts in thyroid hormone synthesis and action, such as NIS, thyroglobulin, deiodinases, and thyroid peroxidase (TPO), are expressed in the endometrial epithelium (Colicchia et al. 2014). This suggests the hypothesis of a local controlled production and action of thyroid hormones at the endometrial level and emphasizes the correlations between the importance of normal thyroid function and regular menstrual flow.

Testosterone seems to be necessary for the transcriptional regulation of factors involved in THR expression and may explain the menstrual abnormalities and subfertility in women with primary hypothyroidism. Thyroid hormones have been assayed in human follicular fluid, and both granulosa and ovarian stromal cells express THRs. The expression of TRHα1, THRβ1, and THRβ2 in mature oocytes from patients subjected to in vitro fertilization suggests that the human female gamete could be directly responsive to T3, probably influencing its maturation and the secretion of hyaluronic acid which causes the pre-ovulatory cumulus expansion. However, in the mouse model, a direct thyroid hormone action on the process of cumulus expansion and meiotic maturation has been not observed, and TSH or T4 added in vitro to cultured human ovarian tissue has no effect on the development of follicles (Colicchia et al. 2014), even if they potentiate FSH-induced granulosa cell survival by inhibiting cell apoptosis and promoting cell proliferation. To summarize, thyroid hormones, which act on their specific receptors expressed by ovarian granulosa cells and oocytes at different stage of follicular maturation, seem to influence granulosa cell survival and steroidogenesis.

Both hypothyroid and hyperthyroid women have been reported to experience a greater prevalence of menstrual irregularities compared with euthyroid women (Koutras 1997). Hypothyroidism deeply alters ovarian function and ovulation. It induces a decreased metabolic clearance rate of androstenedione and estrone, reduced binding activity of SHBG, and an increasing of 17βestradiol and testosterone unbound fractions associated with increased prolactin plasma concentrations. These changes are reported to alter the length of the menstrual cycle and the amount of menstrual bleeding, such as oligomenorrhea, amenorrhea, polymenorrhea, and menorrhagia, therefore inducing anovulation and alteration in the female reproductive system (Krassas et al. 2010). In particular, hypothyroid women are more likely to experience hypomenorrhea compared with normal women, although most of the clinical reports compare women with severe hypothyroidism and myxedema to euthyroid women without taking into account the correlation with thyroid hormone plasma levels.

If we want to consider clinical studies performed as a whole, hypothyroid women experience a frequency of menstrual disturbances that is almost three times greater compared to the normal population (Kakuno et al. 2010). A separate note of interest relates to the presence in the serum of thyroid autoantibodies that may or may not be associated with reduced circulating thyroid hormone levels and seems not be related

to the development of menstrual abnormalities. The presence of an association between subclinical hypothyroidism, serum antithyroid autoantibodies, and infertility has been broadly assessed in several studies. Many authors consider subclinical hypothyroidism an infertility factor in women, a factor that is reversed by treatment with small doses of levothyroxine in order to improve the corpus luteum function. Overall, the studies correlate the plasma levels of TSH with female infertility, demonstrating that mean circulating TSH is significantly higher in infertile patients compared to control patients, even if TSH plasma levels higher than the normal range are not prevalent in infertile women (Jokar et al. 2018). Thyroid autoimmunity, which is often not diagnosed, is an endocrine disorder that is up to ten times more common in women than men. It has been related to reproductive failure, and many studies have investigated the association between thyroid autoimmunity, menstrual disturbances, and female infertility, even if the interpretation of the data is difficult due to the heterogeneity of the subject samples studied, a lack of controlled and retrospective studies, sample sizes, and the laboratory methods used.

A study conducted on a large number of women to examine the relationship between low ovarian reserve and thyroid autoimmunity failed to demonstrate a prevalence of positive thyroid autoantibodies among women with low, normal, or high anti-Mullerian hormone (AMH) levels. However, analyzing only the results obtained from women with low AMH, the authors observed a significantly higher prevalence of overt and subclinical hypothyroidism in the group with a genetic cause for low ovarian reserve (Polyzos et al. 2015). The presence of thyroid autoimmunity in women affected with PCOS has also been investigated. The studies performed revealed a higher serum evidence of thyroid antibodies, more hypoechoic areas on thyroid ultrasound, and higher mean TSH values in women with menstrual disturbances and PCOS compared to controls. This observation indicates the prevalence of autoimmune thyroiditis in women with PCOS that is threefold greater than controls, establishing a relationship between the incidence of positive auto-antibody and female infertility. Subclinical hypothyroidism and autoimmune thyroid disease have been associated with recurrent miscarriage, although the pathogenetic mechanisms and the benefit from treatment with levothyroxine are still debated.

A large number of studies over the last 20 years in the area of thyroid and pregnancy have established that the presence of thyroid autoimmunity is associated with a significant increased miscarriage risk (Lazzarin et al. 2012; Zhang et al. 2017). For this reason, the Thyroid Endocrine Society (*TES*) and the American Thyroid Association (ATA) suggested that the specific ranges for TSH in the early, middle, and late stages of pregnancy should be considered, respectively, 0–2.5 mIU/L, 0.3–3.0 mIU/L, and 0.3–3.0 mIU/L. This is a recommendation that has been adopted by the international community. A more recent meta-analysis emphasized that subclinical hypothyroidism is a risk factor for miscarriage in women before 20 weeks of pregnancy and that early treatment with levothyroxine to keep plasma TSH levels below 2.5 mIU/L can reduce the miscarriage (Zhang et al. 2017). Furthermore, a retrospective study on the association between thyroid autoantibodies with β2-glycoprotein and cardiolipin antibodies as etiological factors for recurrent miscarriage revealed that high levels of ABTPO were positively correlated with IgG

antibodies, suggesting antiphospholipid syndrome and highlighting the importance of considering the association of thyroid autoimmunity with other important autoimmune diseases in women with recurrent miscarriage (Unuane et al. 2017). In contrary fashion, other studies claim that subclinical hypothyroidism and thyroid autoimmunity are not associated with fecundity, early pregnancy loss, and live birth, so that women affected can be reassured that their chances of conceiving and achieving a live birth are likely to be unaffected by subclinical thyroid dysfunction (Plowden et al. 2016).

Women affected with hyperthyroidism present clinical symptoms of weight loss, palpitations, anxiety, increased bowel motility, and menstrual irregularities. Although menstrual cycle length, modification of the follicular and luteal phases, bleeding length and intensity, oligomenorrhea, and amenorrhea are more common in women with hyperthyroidism than in euthyroid women, there is less evidence of ovulatory dysfunction and infertility compared to what occurs in hypothyroidism, as discussed above. The most common causes of thyrotoxicosis is the Graves-Basedow disease, an autoimmune disease in which the thyroid stimulating immunoglobulin (anti-TSH receptor antibodies) leads to the unregulated stimulation of thyroid hormone production with associated orbitopathy and ophthalmopathy. Other causes of hyperthyroid function, overt or subclinical, can be an autonomous thyroid nodule and a multinodular goiter. Overall, these conditions induce thyrotoxicosis (elevated free T4 and low plasma TSH) that leads to increased serum levels of SHBG and hyper-estrogenism during all phases of the menstrual cycle and consequently to increased free estrogen levels. In hyperthyroid women, changes in androgen metabolism also occur with increased plasma levels of testosterone and androstenedione and a modified androstenedione-to-estrone ratio. The mean LH levels in both the follicular and the luteal phases of the menstrual cycle are higher, whereas the pulsatile LH and FSH secretion does not differ in hyperthyroid women compared to controls. Amenorrhea has been frequently reported as associated with hyperthyroidism, as well as various other menstrual cycle changes including oligomenorrhea, hypomenorrhea, and anovulation, and these irregularities may sometimes precede the identification of thyroid dysfunction.

To summarize, both hyper- and hypothyroidism may have adverse effects on the menstrual cycle, mainly hypomenorrhea and polymenorrhea in the case of hyperfunction and oligomenorrhea in the case of hypofunction. These endocrine disorders characteristically induce changes in SHBG and sex steroids, and the early diagnosis of their overt, subclinical, and mild expressions may reduce the incidence of menstrual abnormalities.

Conclusion

Almost all the endocrine diseases may cause irregularities of the menstrual cycle defined by modification of its length, frequency, and pattern of blood loss. In this chapter, I have mainly focused on neuroendocrine and pituitary dysfunctions and on adrenal and thyroid disorders because, when clinically symptomatic, they may be

emblematic of important changes in the dynamic activity of the endometrium. These endocrine disorders are associated with disturbances in the central regulation of the hypothalamic-pituitary axis and morphological changes within the uterus. The most frequent and important endocrine causes of menstrual abnormalities may be attributed to primary or secondary ovarian failure and PCOS. Overt and latent adrenal and thyroid diseases are associated with irregularities in the pattern of the menstrual cycle that may reflect disturbances in the local endometrial environment. A modification of the local availability of prostanoids or other angiogenic-permeability factors may activate receptor signaling and downstream modify the regulation of the gene transcription of angiogenic and anti-angiogenic factors, inducing thereby menstrual dysfunctions. The genomics and proteomics approaches will enhance the possibility of better understanding how endocrine disorders may modify the peripheral tissue metabolism of steroids. In fact, several endocrine diseases could modify the endometrial expression of enzymes that play important roles in the activation and inactivation of estrogens and androgens and their specific receptors. The identification of the intra-tissue concentrations of steroids by liquid chromatography-tandem mass spectrometry (LC-MS/MS) during endocrine disorders will be an important tool in having a better understanding of the role of cyclic steroid changes within the endometrium.

Cross-References

- ▶ Diagnostic Protocols for Infertility
- ▶ Endocrinology of Maternal-Placental Axis
- ▶ The Hypothalamus-Pituitary-Ovary Axis
- ▶ The Menstrual Cycle and Related Disorders
- ▶ The Menstrual Disorders Related to Systemic Diseases
- ▶ The Polycystic Ovary Syndrome (PCOS)

References

Barbieri RL. The endocrinology of the menstrual cycle. In: Rosenwaks Z, Wassarman P, editors. Human fertility. Methods in molecular biology (methods and protocols), vol. 1154. New York: Humana Press; 2014.

Brzana J, Yedinak CG, Hameed N, Plesiu A, McCartney S, Fleseriu M. Polycystic ovarian syndrome and Cushing's syndrome: a persistent diagnostic quandary. Eur J Obstet Gynecol Reprod Biol. 2014;175:145–8.

Caimari F, Korbonits M. Novel genetic causes of pituitary adenomas. Clin Cancer Res. 2016;22 (20):5030–42.

Carmina E, Dewailly D, Escobar-Morreale HF, Kelestimur F, Moran C, Oberfield S, Witchel SF, Azziz R. Non-classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency revisited: an update with a special focus on adolescent and adult women. Hum Reprod Update. 2017;23 (5):580–99.

Celik O, Aydin S, Celik N, Ylmaz M. Peptides: basic determinants of reproductive functions. Peptides. 2015;72:34–43.

Colicchia M, Campagnolo L, Baldini E, Ulisse S, Valensise H, Moretti C. Molecular basis of thyrotropin and thyroid hormone action during implantation and early development. Hum Reprod Update. 2014;20(6):884–904.
Coss D. Regulation of reproduction via tight control of gonadotropin hormone levels. Mol Cell Endocrinol. 2018;463:116–30.
Faje A. Hypophysitis: evaluation and management. Clin Diab Endocrinol. 2016;2:15.
Forni PE, Wray S. GnRH, anosmia and hypogonadotropic hypogonadism. Front Neuroendocrinol. 2015;36:165–77.
Fourman LT, Fazeli PK. Neuroendocrine causes of amenorrhea-an update. J Clin Endocrinol Metab. 2015;100:812–24.
Gauthier K, Chassande O, Plateroti M, Roux JP, Legrand C, Pain B, Rousset B, Weiss R, Trouillas J, Samarut J. Different functions for the thyroid hormone receptors TRalpha and TRbeta in the control of thyroid hormone production and post-natal development. EMBO J. 1999;18(3):623–31.
Gellersen B, Brosens JJ. Cyclic decidualization of the human endometrium in reproductive health and failure. Endocr Rev. 2014;35(6):851–905.
Genazzani AR, Petraglia F, Gamba O, Sgarbi L, Greco MM, Genazzani AD. Neuroendocrinology of the menstrual cycle. Ann NY Acad Sci. 1997;816:143–50.
Gray SH. Menstrual disorders. Pediatr Rev. 2013;34(6):6–18.
Jabbour HN, Kelly RW, Fraser HM, Critchley HOD. Endocrine regulation of menstruation. Endocr Rev. 2006;27:17–46.
Jacobson MH, Howards PP, Darrow LA, Meadows JW, Kesner JS, Spencer JB, Terrel ML, Marcus M. Thyroid hormones and menstrual cycle function in a longitudinal cohort of premenopausal women. Paediatr Perinat Epidemiol. 2018;32(3):225–34.
Jokar TO, Fourman LT, Lee H, Mentzinger K, Fazeli PK. Higher TSH Levels within the normal range are associated with unexplained infertility. J Clin Endocrinol Metab. 2018;103(2):632–9.
Kakuno Y, Amino N, Kanoh M, Kawai M, Fuijwara M, Kimura M, Kamitani A, Saya K, Shakuta R, Nitta s HY, Kudo T, Kubota S, Miyauchi A. Menstrual disturbances in various thyroid diseases. Endocr J. 2010;57(12):1017–22.
Kanczkowski W, Sue M, Bornstein SR. The adrenal gland microenvironment in health, disease and during regeneration. Hormones. 2017;16(3):251–65.
Koutras DA. Disturbances of menstruation in thyroid disease. Ann NY Acad Sci. 1997;816:280–4.
Krassas GE, Poppe K, Glinoer D. Thyroid function and human reproductive health. Endocr Rev. 2010;31:702–55.
Lazzarin N, Moretti C, De Felice G, Vaquero E, Manfellotto D. Further evidence on the role of thyroid autoimmunity in women with recurrent miscarriage. Int J Endocrinol. 2012;2012:1–4.
Leng G, MacGregor DJ. Models in neuroendocrinology. Math Biosci. 2018;305:29–41.
Livadas S, Chrousos GP. Control of the onset of puberty. Curr Opin Pediatr. 2016;28(4):551–8.
Lockwood CJ. Mechanisms of Normal and abnormal endometrial bleeding. Menopause. 2011;18 (4):408–11.
Maggi R, Cariboni AM, Montagnini Marelli M, Moretti RM, Andrè V, Marzagalli M, Limonta P. GnRH and GnRH receptors in the pathophysiology of the human female reproductive system. Hum Reprod Update. 2016;22(3):358–81.
Malik S, Day K, Perrault I, Charnock-Jones DS, Smith SK. Reduced levels of VEGF-A and MMP-2 and MMP-9 activity and increased TNF-alpha in menstrual endometrium and effluent in women with menorrhagia. Hum Reprod. 2006;21(8):2158–66.
Marino M, Moriondo V, Vighi E, Pignatti E, Simoni M. Central hypogonadotropic hypogonadism: genetic complexity of a complex disease. Int J Endocrinol. 2014;2014:1–13.
Maybin JA, Critchley HOD. Menstrual physiology: implications for endometrial pathology and beyond. Hum Reprod Update. 2015;21(6):748–61.
Melmed S. Pituitary tumors. Endocrinol Metab Clin N Am. 2015;44(1):1–9.
Messinis IE, Messini CI, Dafopoulos K. Novel aspects of the endocrinology of the menstrual cycle. Reprod Biomed Online. 2014;28(6):714–22.
Molitch ME. Diagnosis and treatment of pituitary adenomas: a review. JAMA. 2017;317(5):516–24.

Mullur R, Liu YY, Brent GA. Thyroid hormone regulation of metabolism. Physiol Rev. 2014;94:355–82.
Müller HL. Craniopharyngioma. Endocr Rev. 2014;35:513–43.
Nazem TG, Ackerman KE. The female athlete triad. Sports Health. 2012;4(4):302–11.
Ortiga-Carvalho TM. Thyroid hormone receptors and resistance to thyroid hormone disorders. Nat Rev Endocrinol. 2014;10(10):582–91.
Pepe S, Korbonits M, Iacovazzo D. Germline and mosaic mutations causing pituitary tumours: genetic and molecular aspects. J Endocrinol. 2019;240:R21–45.
Plowden TC, Schisterman EF, Sjaarda LA, Zarek AM, Perkins NJ, Silver R, Galai N, DeCherney A, Mumford SL. Subclinical hypothyroidism and thyroid autoimmunity are not associated with fecundity, pregnancy loss, or live birth. J Clin Endocrinol Metab. 2016;101:2358–65.
Polyzos NP, Sakkas E, Vaiarelli A, Poppe K, Camus M, Tournaye H. Thyroid autoimmunity, hypothyroidism and ovarian reserve: a cross-sectional study of 5000 women based on age-specific AMH values. Hum Reprod. 2015;30(7):1690–6.
Raverot G, Burman P, Mc Cormack A, Heaney A, Petersenn S, Popovic V, Trouillas J, Dekkers OM, On behalf of The European Society of Endocrinology. European society of endocrinology clinical practice guidelines for the management of aggressive pituitary tumours and carcinomas. Eur J Endocrinol. 2018;178(1):G1–G24.
Ross IL, Louw GJ. Embryological and molecular development of the adrenals glands. Clin Anat. 2015;28(2):235–42.
Sapochnik M, Fuertes M, Arzt E. Programmed cell senescence: role of IL-6 in the pituitary. J Mol Endocrinol. 2017;58(4):R241–53.
Schorr M, Miller KK. The endocrine manifestations of anorexia nervosa: mechanisms and management. Nat Rev Endocrinol. 2017;13(3):174–86.
Skorupskaite K, George JT, Anderson RA. The Kisspeptin-GnRH pathway in human reproductive health and diseases. Hum Reprod Update. 2014;20(4):485–500.
Sotomayor K, Iꞑiguez G, Ugarte F, Villarroel C, Lopez P, Avila A, Codner E, Cassorla F. Ovarian function in adolescent with McCune-Albright syndrome. J Pediatr Endocrinol Metab. 2011;24 (7–8):525–8.
Speiser PW, White PC. Congenital adrenal hyperplasia. N Engl J Med. 2003;349:776–88.
Stamadiates GA, Kaiser UB. Gonadotropin regulation by pulsatile GnRH: signaling and gene expression. Mol Cell Endocrinol. 2018;463:131–41.
Stephens MA, Mahon PB, McCaul ME, Wand GS. Hypothalamic-pituitary- adrenal axis response to acute psychosocial stress: effects of biological sex and circulating sex hormones. Psychoneuroendocrinology. 2016;66:47–55.
Tsutsui K, Ubuka T. How to contribute to the progress of neuroendocrinology: discovery of GnIH and progress of GnIH research. Front Endocrinol. 2018;9(662):1–16.
Tsutsui K, Osugi SYL, Ubuka T. Review: structure, function and evolution of GnIH. Gen Comp Endocrinol. 2018;1(264):48–57.
Unuane D, Velkeniers B, Bravenboer B, Drakopoulos P, Tournaye H, Parra J, De Brucker M. Impact of thyroid autoimmunity in euthyroid women on live birth rate after UI. Hum Reprod. 2017;32(4):915–22.
Vaishya R, Gupta R, Arora S. Macroprolactin; a frequent cause of misdiagnosed hyperprolactinemia in clinical practice. J Reprod Infert. 2010;11(3):161–7.
Valdes-Socin H, Almanza MR, Fernandez-Ladreda MT, Debray FG, Bours V, Beckers A. Reproduction, smell, and neurodevelopmental disorders:genetic defects in different hypogonadotropic hypogonadal syndromes. Front Endocrinol. 2014;5(109):1–8.
Williams NI. Female athlete triad: future directions for energy availability and eating disorders research and practice. Clin Sports Med. 2017;36(4):671–86.
Zhang Y, Wang H, Pan X, Teng W, Shan Z. Patients with subclinical hypothyroidism before 20 weeks of pregnancy have a higher risk of miscarriage: a systematic review and meta-analysis. PLoS One. 2017;12(4):1–13.

The Menstrual Disorders Related to Systemic Diseases

5

Larissa Milani Coutinho, Sara Clemenza, Dante Campana, and Felice Petraglia

Contents

Abstract

Women with medical problems can develop changes in their menstrual cycles and fertility related to the underlying disease process or medication in use. In most cases, the pathophysiological mechanisms involve the hypothalamic-pituitary-gonad-uterus axis. The disruption of the axis' integrity can lead to anovulation and, consequently, to periods of amenorrhea, oligomenorrhea, or irregular

L. M. Coutinho
Department of Maternal and Child Health, Universidade Federal de Juiz de Fora, Juiz de Fora, Brazil

Department of Obstetrics and Gynecology, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil
e-mail: larissamcoutinho@yahoo.com.br

S. Clemenza · D. Campana · F. Petraglia (✉)
Department of Biomedical, Experimental and Clinical Sciences, Division of Obstetrics and Gynecology, University of Florence, Florence, Italy
e-mail: felice.petraglia@unifi.it

F. Petraglia, B. C. Fauser (eds.), *Female Reproductive Dysfunction*, Endocrinology,
https://doi.org/10.1007/978-3-030-14782-2_5

episodes of metrorrhagia. On the other hand, other systemic conditions, such as hemostatic disorders and liver disease, can cause excessive menstrual bleeding not associated with anovulation.

Keywords

Menstrual disorders · Systemic disease · Amenorrhea · Oligomenorrhea · Metrorrhagia · Systemic lupus erythematosus · Celiac disease · Inflammatory bowel disease · Liver disease · Kidney disease · Hemostatic disorders · Epilepsy

Introduction

Several systemic diseases are related to the occurrence of menstrual disorders. The abnormal pattern of uterine bleeding varies from oligomenorrhea/amenorrhea to menorrhagia, depending on the underlying pathology and/or the therapeutic drug required for its management.

Systemic diseases may affect one or more levels of the hypothalamic-pituitary-gonad-uterus axis as a result of the pathology itself or through their consequences (nutritional deficiency, weight loss, psychological distress, etc.). In general, the disruption of the axis integrity impairs ovarian function and can lead to periods of amenorrhea, oligomenorrhea, or irregular episodes of metrorrhagia due to chronic anovulation. There are other systemic conditions, such as hemostatic disorders and liver disease, which cause menorrhagia, i.e., excessive menstrual bleeding not associated with anovulation and menstrual irregularity.

The most common systemic diseases related to menstrual disturbances will be discussed in the present chapter.

Autoimmune Diseases

Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is an autoimmune disease known for its female predilection and peak incidence during the reproductive years. It is a chronic and multisystem disorder, which clinical manifestations are variable and may be alternated with periods of remission. Studies have demonstrated that SLE and other autoimmune diseases, such as multiple sclerosis and rheumatoid arthritis, are closely related to high inflammatory activity and are more likely to occur in the perimenstrual phase (Steinberg and Steinberg 1985).

On the other hand, irregular menses have also been demonstrated in patients with SLE, implying a possible interference of the disease in the menstrual cycle. Oligomenorrhea is the abnormality most reported. A study showed that, among patients with SLE without exposure of alkylating agent, 54% presented oligomenorrhea. The menstrual irregularities were related to hormonal alterations, such as higher levels of prolactin, higher disease activity, and lower levels of progesterone (Pasoto et al. 2002).

Even though the direct action of the disease and the anti-ovary antibodies involvement have been described as possible causal factors for menstrual changes, fertility is probably not altered by the pathology itself. However, prolonged amenorrhea and premature ovarian failure (POF) in patients with SLE can occur by the use of immunosuppressive agents (Shabanova et al. 2008; La Barbera et al. 1988; Medeiros et al. 2009). Cyclophosphamide, notably, is related to the occurrence of ovarian failure owing to its gonadotoxic effects. A study demonstrated that the risk of POF after treatment with pulse of cyclophosphamide is as higher as greater the age at the beginning of the administration. The length of the treatment, the cumulative dose, and the grade of marrow suppression after the pulse may also have an influence (McDermott and Powell 1996).

Furthermore, high doses of corticoids prescribed for patients with SLE may cause suppression in hypothalamic-pituitary-ovary (HPO) axis, leading to reduced levels of FSH and LH, which could justify the occurrence of amenorrhea as well (Saketos et al. 1993; Silva et al. 2011).

Celiac Disease

Celiac disease is an autoimmune disorder triggered by the dietary consume of gluten. Several studies have demonstrated the occurrence of menstrual disturbs and negative reproductive outcomes in women with this enteropathy that might be justified by nutrient deficiency and autoimmune mechanisms associated with the disease (Tersigni et al. 2014).

Untreated celiac women are more likely to have menarche in an older age and earlier menopause, leading to a shorter fertile lifespan. An enhanced occurrence of secondary amenorrhea and negative reproductive outcomes have also been shown (e.g., unexplained infertility, recurrence pregnancy loss, intrauterine growth restriction, and stillbirth) (Tersigni et al. 2014; Santonicola et al. 2011).

The classic form of the disease includes villous atrophy and gastrointestinal symptoms such as chronic diarrhea, steatorrhea, abdominal distention, nauseas, and vomiting. The malabsorption leads to anemia and selective nutrient deficiencies (Rubin et al. 1960). Zinc and selenium depletion, for instance, may hamper the synthesis and secretion of gonadotropins and consequently cause a dysregulation of the HPO axis. Deficiency of folic acid, zinc, and selenium can affect pregnancy and fetal development (Bedwal and Bahuguna 1994). Weight loss can also occur and, when it is expressive, may disturb the HPO axis as well.

Patients adequately treated with a long-term gluten-free diet seem to improve the menstrual alterations and fertility (Santonicola et al. 2011).

Inflammatory Bowel Disease

The inflammatory bowel disease (IBD) comprises Crohn's disease and ulcerative colitis. These two chronic inflammatory entities have some distinct features, but many clinical and pathological aspects are shared.

Patients with IBD often report worsening of disease symptoms in perimenstrual phase. The prevalence of diarrhea is more elevated in patients with IBD than in healthy women during pre-menstrual and menstrual periods (Kane et al. 1998). Other gastrointestinal symptoms related to the disease, such as nausea, abdominal pain, flatulence, and tenesmus during menstruation, are also more frequent and more severe in these patients, when compared with controls (Lim et al. 2013). However, there is not much data about the IBD effects in the menstrual cycle. The most frequent change involved seems to be oligomenorrhea. A study that evaluated the gynecologic history of 1000 patients with IBD showed that 58% reported menstrual abnormalities (Weber et al. 1995). Probably, irregular periods may be explained by the deficient nutrition, weight loss, chronic inflammation, and stress related to the disease (Plavšić et al. 2013).

A study demonstrated that changes in menstrual function occur frequently in the year prior to IBD diagnosis. The most common abnormalities observed were alterations in the cycle length, followed by changes in the duration of the menstrual flow and in the pain pattern. There was a tendency of worsening of pain in women with previous dysmenorrhea. By longitudinal analysis, the authors suggested that menstrual cycle regularity may return to the normality over the time (Saha et al. 2014).

Kidney Disease

Impaired renal function may cause disturbances in menstruation, fertility, and sexual dysfunction, especially in women with chronic kidney disease (CKD) (Palmer and Clegg 2017; Cochrane and Regan 1997).

Menstrual disorders affect about 85% of women with CKD. Amenorrhea is the major menstrual abnormality in uremic women, although menorrhagia is also common and requires adequate control, because heavy vaginal bleeding worsens the chronic anemia of renal disease.[19] Defects in both hypothalamic pulsatile release of GnRH and pituitary cyclic release of LH and FSH seem to be involved, leading to anovulation, irregular menses, amenorrhea, and infertility. The causal factors involved in the hypothalamic defect present in women with CKD and end-stage renal disease are not clear, but hyperprolactinemia may be involved. Elevated levels of prolactin occur in around 50% of patients undergoing dialysis. The enhanced levels of prolactin in these patients are probably caused by primary pituitary abnormality, reduced hypothalamic prolactin inhibition, and impaired prolactin clearance. Dopamine agonists correct the hyperprolactinemia but do not restore normal menses, suggesting that there are other mechanisms involved. It is possible that elevated levels of endorphins, resulting from the impaired renal clearance, might decrease the release of GnRH, contributing for the inhibition of ovulation (Palmer and Clegg 2017; Holley 2004; Ahmed and Ramesh 2016).

Dialysis generally does not improve menstrual disorders, although normal menses are restored in some women. Successful transplantation is the best way for

correcting hypogonadism and all of its manifestations, including infertility (Palmer and Clegg 2017; Ahmed and Ramesh 2016).

Hemostatic Disorders

Systemic hemostatic disorders are important differential diagnosis in the management of patients with menorrhagia, although they are almost only remembered in adolescents with excessive menstrual flow. Recent studies have shown that 10–20% of women with heavy menstrual bleeding may have a systemic disturb of homeostasis, although many of which are often misdiagnosed as ovulatory dysfunctional uterine bleeding (DUB) (Fraser et al. 2005). Hemostatic disorders should be further suspected in patients with menorrhagia, without other organic causes, presenting with signs of bleeding diathesis, such as bruising or prolonged bleeding from mucosa; family history of coagulopathy; and use of medications that interfere in the coagulation or platelet function, including the nonprescription ones.

The basic hemostatic events in endometrium during menstruation are the vasoconstriction of spiral arterioles and the generation of hemostatic plug inside the superficial vessels of endometrium. The interaction of platelets with von Willebrand factor (VWF) at the endothelial surface is followed by the formation of a fibrin clot within the matrix of the platelet plug. The disruption of these mechanisms may contribute for the occurrence of heavy bleeding at menses, even though the endometrial molecular interactions involved in the control of blood loss in a normal menstrual period are not totally clear (Fraser et al. 2005).

Hemostatic disorders include inherited and acquired conditions, related to the primary hemostasis (e.g., platelet disorders) or the secondary hemostasis (e.g., coagulation factor deficiencies). The conditions related to systemic coagulopathy that may cause abnormal uterine bleeding are listed in Table 1 (Fraser et al. 2005).

The most frequent inherited bleeding disorder is von Willebrand disease (VWD), a family of clinical conditions characterized by the absolute deficiency or a qualitative defect of the namesake protein. It is present in 1% to 2% of the general population, but in women with menorrhagia, the incidence reaches 13% (Lukes et al. 2005). Because of the variable severity of the symptoms, many cases are undiagnosed. The von Willebrand factor is important in the primary hemostasis, by helping the platelet adhesiveness to the site of the injured endothelium. This protein is also required in the preservation of factor VIII in the circulation. The huge majority (78% to 93%) of women with the disease report menorrhagia. Hormones can increase the levels of VWF and normalize homeostasis. Therefore, women with the disease can present heavy menstrual bleeding during menopausal transition, when the decreasing levels of estrogen may impact on von Willebrand synthesis. Besides the menorrhagia, patients with VWD may have an enhanced risk of developing hemorrhagic cysts and endometriosis (Lukes et al. 2005; James 2006).

Other inherited bleeding disorders are rarer and little is known about their influence on menstrual cycle. A study performed by Peiyvand et al. showed that menorrhagia was related by 75% of patients with factor II deficiency and 50% of

Table 1 Conditions related to systemic coagulopathy that may cause abnormal uterine bleeding

von Willebrand disease
Coagulation factor deficiencies: VIII (hemophilia A); IX (hemophilia B); XI (hemophilia C); II; V; VII; X; XIII
Carriers of X-linked deficiencies (FVIII, FIX)
Afibrogenemia
Thrombocytopathies
Thrombocytopenia (idiopathic thrombocytopenia purpura (ITP))
Leukemias
Liver dysfunction
Vitamin K deficiency
Anticoagulant use

women affected by factor V, VII, X, afibrinogenemia and combined V + VIII deficiency (Peyvandi et al. 2002).

The platelet disorders can be divided into thrombocytopenia, defined as a numeric alteration of platelets (idiopathic thrombocytopenia purpura, leukemia), and thrombocytopathies, when there is a function disturbance. Excessive menstrual bleeding is more likely to occur in the numeric disorders of platelets and little is known about the function defects (Lukes et al. 2005).

Liver Disease

Hepatic damage may cause abnormality of the hypothalamic-pituitary-gonad-uterus axis, resulting in menstrual disorders. In women with cirrhosis, anovulation is common, which may manifest as amenorrhea, oligomenorrhea, or irregular menstrual bleeding. Menstrual abnormalities may be due either to variations in hormonal levels or to blood clotting disorders. In fact, the impaired synthesis of coagulation proteins (I, II, V, VII, IX, X, and XI) by hepatocytes, thrombocytopenia, platelet dysfunction, and hyperfibrinolysis associated with liver disease may predispose excessive bleeding (Karagiannis and Harsoulis 2005; Northup and Caldwell 2013; Marks 2013).

In cases of severe liver disorder with signs of malnutrition, women can manifest hypogonadotropic hypogonadism. In other cases, estradiol and testosterone levels are high, which can be due to decreased sex hormone binding globulin produced by the liver or to decreased metabolism of estrogens, owing to portosystemic shunting of weak androgens, which are then converted to estrogens in fat and other tissues. These increased estradiol levels could in part explain the negative effects on menstrual cycle regularity (Karagiannis and Harsoulis 2005).

Alcoholic liver disease is more severe in women than in men. Alcohol abuse causes a disturbance in the hormonal status and reproductive disorders, such as irregular menstrual cycles, anovulation, increased risk of spontaneous abortions, and early menopause. Chronic alcohol abuse is associated with hypogonadism, caused

by a reduced secretion of estrogens and gonadotropin. In the final stages of the disease, when hepatic encephalopathy occurs, the central secretion of neurotransmitters, such as norepinephrine and dopamine, is impaired, resulting in modification of the pulsatile gonadotropin secretion by the hypothalamus. This factor may be responsible for the hyperprolactinemia seen in some cases. Furthermore, long-term alcohol consumption can decrease the number and quality of oocytes (ovarian reserve) (Rachdaoui and Sarkar 2017).

In women with end-stage liver failure, successful liver transplantation and stabilization of liver function result in restoration of menstrual function, libido, and fertility (Rachdaoui and Sarkar 2017).

In Wilson's disease and hemochromatosis, the deposition of copper and iron, respectively, may also affect the pituitary and hypothalamus, resulting in secondary hypogonadism. However, cases of primary gonad involvement are also described. Reducing the excess of these metals in the body can improve menstrual irregularities (Pelusi et al. 2016).

Epilepsy

The exact prevalence of menstrual disturbance in epileptic women is not easy to establish due to a high heterogeneity among the studies available. However, it has been observed that patients with the disease are more likely to present reproductive neuroendocrine changes than general population, such as subfertility, premature menopause, PCOS, and sexual dysfunction (Bosak et al. 2018; Herzog 2006; Svalheim et al. 2003; Pennell 2009).

The central nervous system, through a number of impulses and feedback systems, is closely related to the function of the HPO axis. Therefore, epileptic discharges may impair the function of hypothalamus and pituitary, altering reproductive hormone levels. A wide range of menstrual disturbs has been reported to occur: abnormal cycle interval, oligomenorrhea, polymenorrhea, amenorrhea, increased variability of cycle interval, and menometrorrhagia. The causes are multifactorial, and the most important ones include frequency and location of seizures and the use of specific antiepileptic drugs (AED), such as carbamazepine and valproic acid (Bosak et al. 2018; Herzog 2006; Svalheim et al. 2003; Pennell 2009).

One of the most common forms of epilepsy in adults is the temporolimbic epilepsy (TLE). Temporolimbic structures, and particularly the amygdala, send direct connections to the brain structures responsible for the production and regulation of gonadotropin-releasing hormone (GnRH). Temporal lobe seizures commonly produce transient hormonal changes. Some authors have highlighted an increased variability in pulse amplitude and frequency of prolactin, FSH, and LH. An association between the cerebral hemisphere affected by the epileptic outbreak and the type of menstrual disorder manifested by the patient was also highlighted. PCOS is more common among women with left TLE and right non-TLE, while hypothalamic amenorrhea is more frequent in right TLE (Andrew et al. 2003).

In addition to the pathology itself, even drugs used to treat it have effects on the endocrine system and can cause menstrual disorders. Reduced levels of DHEAS, estradiol, thyroxin, and free thyroxin have been shown in women with epilepsy, related to the use of enzyme-inducing antiepileptic drugs, while valproate (non-enzyme-inducing) causes elevated serum concentration of testosterone, insulin resistance, and higher frequency of PCOS (Andrew et al. 2003).

It was observed that menstrual disorders are more frequent when epilepsy develops at a young age, in particular before menarche, probably owing to the precocious exposure to the epileptic discharges and the AED (Bosak et al. 2018).

Conclusion

Systemic illnesses are often associated with menstrual disorders which can sometimes be the first manifestation of the disease. Therefore, it is important to carry out an accurate medical history and diagnostic tests to exclude systemic causes. Systemic disorders clearly may exert a significant influence on neuroendocrine function and HPO axis, probably in order to prevent pregnancy in suboptimal conditions for the preservation of maternal health and/or for the survival of the fetus. The mechanisms by which systemic illnesses cause changes in hypothalamic-pituitary function are not clear in most cases, unless such illnesses cause structural lesions, such as hemochromatosis. Moreover, systemic diseases often cause significant stress to the body, either physical or psychological. Stress induces hypothalamic-pituitary-adrenal (HPA) axis activation, causing an increase in cortisol levels, and may contribute to HPO axis alterations. Finally, some drugs, like immunosuppressive agents, can contribute to menstrual disorders in women with systemic diseases.

Cross-References

- ▶ Abnormal Uterine Bleeding
- ▶ Menstrual Disorders Related to Endocrine Diseases
- ▶ The Hypothalamus-Pituitary-Ovary Axis
- ▶ The Menstrual Cycle and Related Disorders

References

Ahmed SB, Ramesh S. Sex hormones in women with kidney disease. Nephrol Dial Transplant. 2016;31(11):1787–95.

Andrew G, Herzog MD, MSc AE, Coleman MD, Alan R, Jacobs MD, Pavel Klein MB, Mark N, Friedman DO, Frank W, Drislane MD, Ransil BJ, Donald L, Schomer MD. Interictal EEG discharges, reproductive hormones, and menstrual disorders in epilepsy. Ann Neurol. 2003; 54(5):625–37.

Bedwal RS, Bahuguna A. Zinc, copper and selenium in reproduction. Experientia. 1994;50(7):626–40.

Bosak M, Słowik A, Turaj W. Menstrual disorders and their determinants among women with epilepsy. Neuropsychiatr Dis Treat. 2018;14:2657–64.
Cochrane R, Regan L. Undetected gynaecological disorders in women with kidney disease. Hum Reprod. 1997;12:667–70.
Fraser IS, Bonnar J, Peyvandi F. Requirements for research investigations to clarify the relationships and management of menstrual abnormalities in women with hemostatic disorders. Fertil Steril. 2005;84(5):1360–5.
Herzog AG. Menstrual disorders in women with epilepsy. Neurology. 2006;66(6 Suppl 3):S23–8.
Holley JL. The hypothalamic-pituitary axis in men and women with chronic kidney disease. Adv Chronic Kidney Dis. 2004;11:337–41.
James AH. Von Willebrand disease. Obstet Gynecol Surv. 2006;61(2):136–45.
Kane SV, Sable K, Hanauer SB. The menstrual cycle and its effect on inflammatory bowel disease and irritable bowel syndrome: a prevalence study. Am J Gastroenterol. 1998;93(10):1867–72.
Karagiannis A, Harsoulis F. Gonadal dysfunction in systemic diseases. Eur J Endocrinol. 2005; 152(4):501–13.
La Barbera AR, Miller MM, Ober C, Rebar RW. Autoimmune etiology in premature ovarian failure. Am J Reprod Immunol Microbiol. 1988;16(3):115–22.
Lim SM, Nam CM, Kim YN, et al. The effect of the menstrual cycle on inflammatory bowel disease: a prospective study. Gut Liver. 2013;7(1):51–7.
Lukes AS, Kadir RA, Peyvandi F, Kouides PA. Disorders of hemostasis and excessive menstrual bleeding: prevalence and clinical impact. Fertil Steril. 2005;84(5):1338–44.
Marks PW. Hematologic manifestations of liver disease. Semin Hematol. 2013;50(3):216–21.
McDermott EM, Powell RJ. Incidence of ovarian failure in systemic lupus erythematosus after treatment with pulse cyclophosphamide. Ann Rheum Dis. 1996;55(4):224–9.
Medeiros PB, Febrônio MV, Bonfá E, Borba EF, Takiuti AD, Silva CA. Menstrual and hormonal alterations in juvenile systemic lupus erythematosus. Lupus. 2009;18(1):38–43.
Northup PG, Caldwell SH. Coagulation in liver disease: a guide for the clinician. Clin Gastroenterol Hepatol. 2013;11(9):1064–74.
Palmer BF, Clegg DJ. Gonadal dysfunction in chronic kidney disease. Rev Endocr Metab Disord. 2017;18(1):117–30.
Pasoto SG, Mendonça BB, Bonfá E. Menstrual disturbances in patients with systemic lupus erythematosus without alkylating therapy: clinical, hormonal and therapeutic associations. Lupus. 2002;11(3):175–80.
Pelusi C, Gasparini DI, Bianchi N, Pasquali R. Endocrine dysfunction in hereditary hemochromatosis. J Endocrinol Investig. 2016;39(8):837–47.
Pennell PB. Hormonal aspects of epilepsy. Neurol Clin. 2009;27(4):941–65.
Peyvandi F, Lak M, Mannucci PM. Factor XI deficiency in Iranians: its clinical manifestations in comparison with those of classic hemophilia. Haematologica. 2002;87:512–4.
Plavšić I, Stimac T, Hauser G. Crohn's disease in women. Int J Women's Health. 2013;5:681–8.
Rachdaoui N, Sarkar DK. Pathophysiology of the effects of alcohol abuse on the endocrine system. Alcohol Res. 2017;38(2):255–76.
Rubin CE, Brandborg LL, Phelps PC, Taylor HC Jr. Studies of celiac disease. I. The apparent identical and specific nature of the duodenal and proximal jejunal lesion in celiac disease and idiopathic sprue. Gastroenterology. 1960;38:28–49.
Saha S, Zhao YQ, Shah SA, Esposti SD, Lidofsky S, Salih S, Bright R, Law M, Moniz H, Flowers N, Merrick M, Sands BE. Menstrual cycle changes in women with inflammatory bowel disease: a study from the ocean state Crohn's and colitis area registry. Inflamm Bowel Dis. 2014;20 (3):534–40.
Saketos M, Sharma N, Santoro NF. Suppression of the hypo-thalamic-pituitary-ovarian axis in normal women by glucocorticoids. Biol Reprod. 1993;49:1270–6.
Santonicola A, Iovino P, Cappello C, Capone P, Andreozzi P, Ciacci C. From menarche to menopause: the fertile life span of celiac women. Menopause. 2011;18(10):1125–30.
Shabanova SS, Ananieva LP, Alekberova ZS, Guzov II. Ovarian function and disease activity in patients with systemic lupus erythematosus. Clin Exp Rheumatol. 2008;26(3):436–41.

Silva CA, Deen ME, Febrônio MV, Oliveira SK, Terreri MT, Sacchetti SB, Sztajnbok FR, Marini R, Quintero MV, Bica BE, Pereira RM, Bonfá E, Ferriani VP, Robazzi TC, Magalhães CS, Hilário MO. Hormone profile in juvenile systemic lupus erythematosus with previous or current amenorrhea. Rheumatol Int. 2011;31(8):1037–43.

Steinberg AD, Steinberg BJ. Lupus disease activity associated with menstrual cycle. J Rheumatol. 1985;12(4):816.

Svalheim S, Taubøll E, Bjørnenak T, Røste LS, Mørland T, Saetre ER, Gjerstad L. Do women with epilepsy have increased frequency of menstrual disturbances? Seizure. 2003;12(8):529–33.

Tersigni C, Castellani R, de Waure C, Fattorossi A, De Spirito M, Gasbarrini A, Scambia G, Di Simone N. Celiac disease and reproductive disorders: meta-analysis of epidemiologic associations and potential pathogenic mechanisms. Hum Reprod Update. 2014;20(4):582–93.

Weber AM, Ziegler C, Belinson JL, Mitchinson AR, Widrich T, Fazio V. Gynecologic history of women with inflammatory bowel disease. Obstet Gynecol. 1995;86(5):843–7.

Hormones and Sex Behavior

6

Linda Vignozzi and Elisa Maseroli

Contents

L. Vignozzi (✉) · E. Maseroli
Andrology, Women's Endocrinology and Gender Incongruence Unit, Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Florence, Florence, Italy
e-mail: linda.vignozzi@unifi.it; elisamaseroli@gmail.com

F. Petraglia, B. C. Fauser (eds.), *Female Reproductive Dysfunction*, Endocrinology,
https://doi.org/10.1007/978-3-030-14782-2_6

Abstract

Sex steroids (in particular, testosterone and its metabolite, estradiol) have a lifelong role in establishing and maintaining gender-dimorphic behavioral aspects. In particular, prenatal and pubertal exposure to testosterone influences spatial abilities, emotion and reward processing, and vulnerability to psychiatric disorders. Women are dramatically exposed to fluctuation in estrogen and androgen levels (e.g., during the menstrual cycle and at menopausal transition), which are known to modulate many aspects of behavior, including sexuality. Indeed, preclinical and clinical studies have been constantly indicating that estrogens and androgens exert a synergic, facilitatory effect on many different aspects of the female sexual response, stimulating sexual desire through a complex network of neurotransmitters and balance between excitatory and inhibitory signals. Since physiological and pathological variations of sex hormones influence sexual function and dysfunction in women, they represent a major target as therapeutic strategies for female sexual disorders.

Keywords

Female sexual desire · Sexual hormones · Neurotransmitters · Hypoactive sexual desire disorder · Female sexual arousal disorder · Neurobiology · HSDD · Hypoandrogenism · Testosterone · Menopause

Introduction

In 1964, Young, Goy, and Phoenix reviewed current knowledge on "Hormones and Sexual Behaviour" stating that "broad relationships exist between the gonadal hormones and behaviour" (Young et al. 1964). At that time, "mechanisms of hormonal action in organizing the tissues of the central nervous system during development and in bringing behaviour to expression in the adult" (Young et al. 1964) could only be hypothesized. In the 1960s and 1970s, steroid autoradiography allowed the demonstration of nuclear sex steroid receptors in the rodent hypothalamus. Later on, membrane-associated receptors were discovered in non-neural cells and subsequently in the brain, emphasizing the physiological relevance of rapid signaling of sex steroids; in addition, the neural circuitries for sexual behavior were identified, and the role of testosterone and its metabolites, estradiol and dihydrotestosterone, in brain sexual differentiation was recognized (McEwen and Milner 2017).

Advances in our understanding of cellular and molecular actions of steroid hormones have supported the role of sex hormones in modulating not only sexual

and reproductive behavior but also cognitive abilities, emotion processing, aging, and vulnerability to stress and psychiatric disorders. Nevertheless, the sexual response is unarguably the most sex hormone-dependent female behavior.

Sex Hormones and Behavior Across the Life Span

Sexual Differentiation

Exposure to gonadal steroids and the expression of specific genes in critical stages during prenatal life and pubertal development determines sexual differences in the structure of the central nervous system (Bakker 2019). This leads to a gender dimorphism in brain morphology and cognitive, emotional, and sexual functions that exists also in adulthood.

In mammals, the first step in sex development is the establishment of genetic sex at conception, generally determined by the presence of two X chromosomes in females and one X and Y chromosome in males (known as "sex determination"). Genetic sex drives the primitive gonad to differentiate: the Y chromosome contains the *SRY* gene, which represses a negative regulator of male development inducing the formation of testes in males, whereas in typical female differentiation, the absence of the Y chromosome – and consequently of *SRY* – leads the gonad to develop into ovaries. Subsequently, non-gonadal tissues, including internal and external genitalia, will develop following the male pathway in the presence of testicular hormones [testosterone, metabolized into dihydrotestosterone (DHT), and the anti-Müllerian hormone] or the female pathway in their absence. This process is known as "sexual differentiation" and, unlike sex determination, is driven by gonadal hormones (Bakker 2019). This simple dichotomization has been challenged by recent evidence suggesting that *SRY* and other Y-linked genes have cell-autonomous effects outside of the gonads, i.e., within the brain, that the number of X chromosomes causes sex differences, and that there are female-specific effects of the inactive X chromosome (Arnold 2017). Furthermore, both histone acetylation and DNA methylation have been demonstrated to act in the development of sex differences in the brain and behavior of rodents, pointing toward the important role of epigenetic in sexual differentiation.

Animal studies have left very little doubt on the pivotal role of gonadal hormones in establishing sex differences in the brain and behavior. In rodents, brain masculinization is traditionally believed to depend on the activation of estrogen receptors (ER). According to the "aromatization hypothesis," circulating testosterone is aromatized into estrogens locally and activates ERs to masculinize the brain (Bakker et al. 2006). In the female mouse, alpha-fetoprotein (AFP) binds circulating estrogens with high affinity preventing them to cross the blood-brain barrier and thus protecting the developing brain from masculinization and defeminization by estrogens (Bakker et al. 2006). However, the aromatization hypothesis cannot fully account for sex differences in morphology of sexually dimorphic brain areas such as the hypothalamus, and androgens are likely to also play a role. This is true in

particular in primates and in humans, in which testosterone is thought to act directly upon androgen receptors (AR) for neural masculinization to occur (Bakker et al. 2006).

In male rodents, the neonatal surge of testosterone also results in lifelong changes in the synaptic pattern of the ventrolateral and ventromedial hypothalamic nucleus. These effects, known as "organizational," refer to the changes that organize the brain in either a male- or female-typical pattern and are seen as structural and, to a large extent, permanent (McEwen and Milner 2017). Organizational actions are distinguished from the so-called "activational" effects of sex hormones. Indeed, the influence of sex hormones endures later in development, going through puberty and extending to older age. In adult life, short-term changes occur in the brain depending on sex hormones fluctuations; an example is the requirement for both estrogen and progesterone to induce lordosis in female rats (Wisniewski 1998).

Gender Identity and Sexual Orientation

Although other factors, including genes and maternal determinants during gestation, have been proposed to play an important role, evidence supports a major influence of prenatal testosterone exposure in the development of sex-typed interests in childhood, as well as gender identity and sexual orientation (Roselli 2018). Clinical studies have reported that, in some XY individuals born with genital ambiguity as a result of 5α-reductase or 17β-hydroxysteroid dehydrogenase deficiency and assigned as girls at birth, male gender identity emerges in adulthood (Cohen-Kettenis 2005). On the other hand, XY children born with a complete androgen insensitivity syndrome (CAIS) due to a mutation of the AR are phenotypically female, identify as such, and are usually androphilic (Wisniewski et al. 2000). These findings indicate that androgens act directly in the developing human brain without aromatization to estrogens.

Regarding sexual orientation, animal research, mainly performed in sheep, strongly suggests that male-typical partner preferences are controlled by neurons in the preoptic area that differentiate under the influence of pre- and perinatal sex steroids (Roselli 2018). Women born with classic congenital adrenal hyperplasia (CAH), exposed to abnormally high levels of androgens prenatally, are less likely to be exclusively heterosexual than unaffected controls, although it has been argued that the sexuality of CAH women could easily been impacted by the physical and psychological consequences of living with genital anomalies (Roselli 2018).

Hormones and the Pubertal Brain

Following the prenatal period, the second wave of sex hormone-dependent neural organization is during adolescence, a phase of intense neurostructural, endocrine, emotional, and social change. During puberty, the long-term organizational effects of sex hormones intertwine with activational effects, resulting in a dramatic impact on

complex behaviors. Experiments in male hamsters castrated prior to puberty and administered testosterone after puberty resulted in abnormal sexual and reproductive behavior, highlighting the importance of gonadal steroids in modeling brain circuits in this specific time frame (Schulz et al. 2004). Animal studies have also shown that steroid hormone binding on brain structure at puberty modulates apoptosis, growth of new cells, neurotransmitter levels, expression of postsynaptic neurotransmitter receptors, dendritic branching, and spine density and connectivity, with a gender-specific pattern (Ahmed et al. 2008; Stewart and Kolb 1994).

Clinical evidence, while somewhat controversial, points for sex differences in perceptual (i.e., visuospatial), affective (i.e., mood and impulsivity), and language tasks, developing during adolescence (Bramen et al. 2012). Longitudinal neuroimaging studies evaluating regional sex differences in cortical maturation in boys and girls highlighted a delay of maturation in males compared with females in frontal areas involved in impulse control, planning, and decision-making, such as anterior cingulate (AntCC), ventromedial prefrontal cortex (vmPFC), and orbitofrontal cortex (OFC) (Raznahan et al. 2010). These discrepancies may account for the more pronounced propensity for risk-taking behaviors in adolescent males and their higher risk for later development of antisocial psychopathy relative to females (Kreiter et al. 1999). The opposite pattern of sex-biased delayed cortical maturation was observed in intraparietal sulcus (IPS) and inferior parietal lobule (IPL), crucial for visuospatial tasks, in which males are typically stronger than females (Raznahan et al. 2010). In the same study, adolescents were stratified through molecular analysis of the AR gene, showing that a more efficient AR was associated with a "masculinized" pattern of cortical maturation in both sexes, with a "dosage effect" in girls (Raznahan et al. 2010). In similar studies during puberty, cortical thickness was found to be correlated with circulating testosterone in brain regions known to be rich in AR from the animal literature (Bramen et al. 2012). Overall, these data provide a further validation for the role of androgen signaling in human cortical development.

Behavior Across the Menstrual Cycle

The enduring effects of sexual hormones fluctuations in adult life are evident in reproductive-age women. Indeed, not only functional but also microstructural changes of some brain regions, such as the hippocampus, have been demonstrated in dynamic neuroimaging studies across the menstrual cycle, with a significant positive correlation with estrogen levels (Barth et al. 2016). Such changes have been hypothesized to reflect myelination processes due to the trophic effects of estrogen on white matter (Barth et al. 2016).

In regard to cognitive functioning, the dominant theory suggests that the follicular phase (characterized by low estrogens and progesterone) is associated with a better performance on male-typical cognitive abilities, such as spatial tasks; in contrast, phases with increased estrogen and/or progesterone (e.g., late follicular or luteal) are claimed to be related with female-typical cognitive abilities, namely, verbal fluency and verbal memory (Gurvich et al. 2018). However, available evidence does not

support significant cognitive changes across the menstrual cycle, although hyperandrogenism may relate to stronger mental rotation abilities, as reported in women with polycystic ovary syndrome (PCOS) (Gurvich et al. 2018).

The cyclic actions of estradiol, testosterone, and progesterone in females significantly modulate the sexual response, with an increase in sexual desire and arousal around the time of ovulation in all vertebrates, including humans (Clayton and Vignozzi 2018) (Fig. 1). In women, it has been widely reported that sexual desire and female-initiated sexual behavior tend to peak during the fertile phase (Clayton and Vignozzi 2018). Of the three ovarian steroids, testosterone is at its highest level around the time of ovulation, whereas estradiol rises a few days earlier, peaking just before, and progesterone varies depending on the species (Pfaus and Jones 2018). Such hormonal milieu, and in particular the testosterone peak, is believed to alter the processing of sexual stimuli, leading to a shift toward an incentive value during the periovulatory phase in rats, primates, and humans (Pfaus and Jones 2018).

Furthermore, fluctuations of the perception of male attractiveness are found across the menstrual cycle, with a preference toward more masculine traits and odor cues of genetic dissimilarity in the fertile period (Feinberg et al. 2008), which

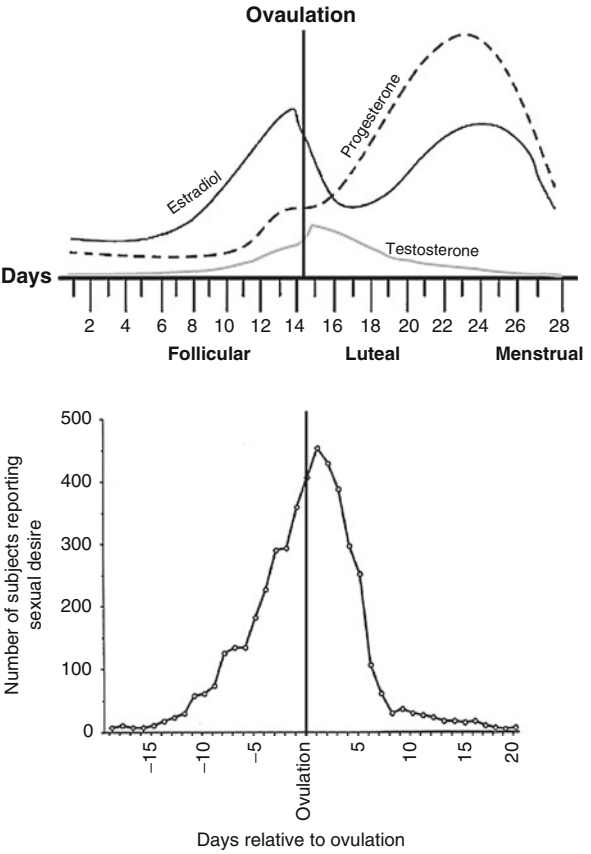


Fig. 1 Top: Schematic changes in estradiol, progesterone, and testosterone in humans across the menstrual cycle. Bottom: Expressed sexual desire in women relative to ovulation. (Reprinted from Knobil and Neill's Physiology of Reproduction, James G. Pfaus, Sherri L. Jones, Loretta M. Flanagan-Cato, Jeffrey D. Blaustein, 2287–2370, Copyright (2015), with permission from Elsevier)

could be mediated by the effect of peaking androgen levels on reward neural circuits (see section "Emotion Processing and Reward Pathways"). Such variations are not observed in women using oral contraceptives (OCs), which suppress fluctuations of ovarian steroids. Therefore, OCs may be associated with a generally weaker overall preference for cues of genetic fitness, and non-congruency of OC use between the initial phase (mate choice) and long-term relationship may have implications for relationship satisfaction (Welling et al. 2012). Finally, OCs have been reported to enhance visuospatial abilities depending on the higher androgenicity of the progestin component (Gurvich et al. 2018).

Sex Hormones, Menopause, and Aging

Human brain maintains a dramatic plasticity also during later life. Menopause is an ineluctable event for women, featuring a gradual cessation of ovarian function and the subsequent consistent decrease in ovarian steroids, in particular estrogen and progesterone. Conversely, androgen decline is considered aging- rather than menopause-related. An abrupt and even more pronounced drop in all sexual hormones, including testosterone, is observed in surgical menopause (Davison et al. 2005).

The impact of menopause on sexual and non-sexual behavior is complex, with substantial variability stemming from concurrent affective and relationship disturbances and psychosocial stress. Changes in the activation of specific brain regions, due to low sex steroids levels, are likely to be involved in the impairment of sexual desire and arousal reported in menopause (Clayton and Vignozzi 2018). A recent functional magnetic resonance imaging (fMRI) study evaluated the time course of regional brain activity induced by visual sexual stimulation in women (Kim and Jeong 2017). A significantly lower activation in post- versus premenopausal subjects was found in the thalamus, amygdala, and anterior cingulate cortex, which may represent the neurobiological correlate of menopause-related decrease in sexual arousability (Kim and Jeong 2017).

It has become increasingly evident that menopause-induced estrogen loss can exacerbate the effects of aging on cognitive functions and contribute to sex differences in brain senescence and neurodegenerative processes. It also appears that the sudden drop in estrogens with oophorectomy has more severe consequences to cognitive performance than the gradual decline seen in natural menopause (Hara et al. 2015). Neuroprotective actions of estradiol in animal models have led to clinical trials demonstrating that hormonal replacement therapy (HRT) exerts beneficial effects on many domains – in particular verbal memory and fluency – when initiated in a "critical period," soon after the menopause, whereas late initiation of HRT can have detrimental effects (Hara et al. 2015). Clinical data on the beneficial influence of testosterone therapy on cognitive measures in women is less convincing. A small but statistically significant positive effect of testosterone treatment on verbal learning and memory versus placebo has been reported in postmenopausal women not on estrogen therapy, not confirmed in other trials (Davis and Wahlin-Jacobsen 2015).

Lessons from Gender Dimorphism

Many biological differences in neuroanatomy and neurochemistry exist between males and females in adulthood. Interestingly, diffusion MRI studies have been showing that such differences reflect in functional disparities, primarily involving hemispheric specialization and brain networks regulating visuospatial skills, reward-based learning, decision-making (Feis et al. 2013), and psychiatric disorders (Elbejjani et al. 2015).

Functional Cerebral Lateralization and Spatial Abilities

Among gender disparities, males tend to exhibit more accentuated asymmetries and stronger right hemisphere dominance, while females typically show more diffuse lateralization patterns and greater left hemisphere bias (Gurvich et al. 2018). The male-typical right hemisphere dominance has been associated with stronger performance on spatial tasks. Although a causing mechanism has not yet been identified, testosterone is the most likely candidate to influence cortical dominance, acting during a critical period of the pregnancy when male fetuses produce more than 2.5 times the levels of testosterone than females (Gurvich et al. 2018).

Animal studies in the Long-Evans rat suggest that the typical cerebral pattern, namely, the male displaying a thicker right hemispheric cortex and the female revealing the reversed pattern, can be altered in both sexes by removal of the gonads at birth (Goy and McEwen 1980). In humans, functional cerebral lateralization may be studied through dichotic listening tasks, in which a right-ear advantage is thought to reflect the left hemispheric dominance for language. Based on the notion that twin girls with an opposite-sex (male) twin have a higher exposure to testosterone during fetal life, Cohen-Bendahan and colleagues investigated functional cerebral lateralization in same-sex and opposite-sex twin girls, observing that girls with a male twin had a more masculine pattern and supporting the case of an influence of prenatal testosterone on early brain organization in women (Cohen-Bendahan et al. 2004). Further support for prenatal testosterone enhancing spatial abilities comes from similar studies in females with male co-twins, exhibiting superior spatial tasks (Gurvich et al. 2018). Consistent with this view, a meta-analysis reported that women with CAH, characterized by prenatal overproduction of adrenal androgens, display advantages in spatial abilities (Puts et al. 2008).

Noteworthy, changes in spatial cognition and brain activity have been demonstrated in healthy women in intervention controlled studies using a single dose of testosterone. In a sample of women trained to navigation tasks in a virtual environment during functional MRI, the testosterone group performed significantly better on mental rotation tasks compared to the placebo group and exhibited increased activity within the medial temporal lobe (Pintzka et al. 2016). In addition, a positive correlation between testosterone load and medial temporal lobe activity was found, and a masculine 2D:4D digit ratio, a sexually dimorphic parameter reflecting fetal testosterone levels, interacted significantly with parahippocampal activity and higher performance scores (Pintzka et al. 2016).

Emotion Processing and Reward Pathways

The amygdala is a subcortical structure that has recently emerged as critical in mediating sex differences in emotional memory and sexual responses in both humans and non-human animals (Janak and Tye 2015). Indeed, the amygdala plays a key role in reward systems, a complex collection of neural pathways responsible for conditioned learning, incentive salience (i.e., motivation, wanting, and desire) and positively valenced emotions, which are deeply involved in sexuality and reproduction (Janak and Tye 2015). In particular, the amygdala is known to establish associations between environmental cues and whether or not that particular experience or stimulus (i.e., sex, food, drug of abuse) was rewarding or aversive. Interacting with the mesolimbic dopamine system, the hippocampus – critical for memory – and regions of the cerebral cortex (i.e., the orbitofrontal cortex, which provides executive control over decision-making), the amygdala exerts a major influence on choices made in the environment, for example, whether or not to seek a reward (Janak and Tye 2015). Neuroimaging studies have found that the amygdala develops structurally at different rates in human males and females and activates differently in response to emotion and sexual stimuli (Hamann et al. 2004). These discrepancies are likely to explain, at least in part, why women tend to experience greater enhancement of their memory by emotion than men, a feature that may not always be beneficial: in fact, memories of negative life events are more available for rumination in women, contributing to a greater vulnerability to depression (Hamann et al. 2004). Furthermore, functional MRI studies demonstrated that the amygdala and hypothalamus are more strongly activated in men than in women when viewing identical sexual stimuli, indicating that the amygdala may mediate the reportedly greater role of visual stimuli in male sexual behavior, paralleling prior preclinical findings (Hamann et al. 2004).

Cumulating evidence indicates that sex steroids act as pivotal neurofunctional modulators of reward circuits in the brain and that abnormal reward processing may constitute a neurobiological mechanism by which hormonal fluctuations provoke mood disorders in susceptible women (Cooke 2006). Through the investigation of neural activity during monetary reward tasks across the menstrual cycle, ovarian steroids were found to modulate reward-evoked neural function, with an augmented reactivity during the midfollicular phase, when estrogen levels are not opposed by progesterone (Dreher et al. 2007). From an evolutionary perspective, increased activity of the reward system during the follicular phase may influence basic behavioral functions of reward, representing the neurobiological substrates of menstrual cycle influence on some types of motivated behavior, e.g., stronger attraction to testosterone-dependent traits (masculine voice and virile face shape) during the most fertile phase (Dreher et al. 2007). The involvement of sex steroids in reward processing was confirmed in a more recent double-blinded placebo-controlled study, in which healthy women were randomized to receive either placebo or a gonadotropin-releasing hormone agonist (GnRHa), causing a decrease in sex steroid levels, before performing a gambling task while undergoing functional MRI (Macoveanu et al. 2016). Compared with placebo, GnRHa blunted amygdala's

response to monetary rewards, reflecting a reduced engagement in positive experiences (Macoveanu et al. 2016). Noteworthy, regional brain reactivity was positively associated with individual changes in testosterone and not in estradiol levels (Macoveanu et al. 2016). Consistent with these findings, animal research had previously demonstrated an enhanced reward dependency after testosterone administration, and in healthy young women, a single dose of testosterone was able to shift motivational balance toward a more disadvantageous pattern of decision-making compared to placebo (van Honk et al. 2004). Similar experiments with single-dose testosterone in healthy middle-aged women suggested that the underpinning mechanism could lie in a rapid functional de-coupling of the amygdala from the orbitofrontal cortex induced by testosterone, resulting in a reduction of the regulatory control over the amygdala (van Wingen et al. 2010).

Changes in neural reward processes following androgen administration have been reported also in studies investigating anabolic androgen dependence and addiction (Mhillaj et al. 2015). Preclinical evidence consistently indicates that dopaminergic pathways are necessary for such androgen-induced alterations of reward behavioral outcomes (Tobiansky et al. 2018). In fact, conditioned place preference (CPP) induced by testosterone was blocked when adult male rats were injected into the nucleus accumbens with a D1-like or D2-like dopamine receptor antagonist (Schroeder and Packard 2000).

Pathophysiology of Psychiatric and Neurological Disorders

The prevalence and clinical presentation of many psychiatric and neurological conditions are characterized by gender dimorphism (Gobinath et al. 2017). Specifically, depression, anxiety, trauma- and stress-related disorders, as well as Alzheimer's disease are more common in women than in men, whereas an opposite trend is described for autism, attention deficit-hyperactive disorder, and Parkinson's disease. In females, hormonal fluctuations seem to increase the vulnerability of experiencing mood disorders. Animal models have been highlighting the importance of estrogens and their cyclical fluctuations during estrous cycle, which seem to determine neural and behavioral effects. In particular, in rodents, densities of hippocampal dendritic spines are higher during proestrus and estrus (high estradiol levels) and lower during diestrus (low estradiol levels). Estrogens have also been described to inhibit dopamine action to increase serotonin expression, mimicking the action of atypical antipsychotics (Gobinath et al. 2017).

In women, changes in estrogens levels across the life span have also been reported to affect mental illness expression. For instance, schizophrenic symptoms are more intense during the low estradiol phase of the menstrual cycle, suggesting a protective role of estrogens. Hormonal fluctuations during pregnancy and postpartum are also related with a higher risk for psychiatric manifestations (Fisher et al. 2018). The high concentration of placental steroids during gestation is followed by low levels in the postpartum, providing an explanation for the common experience of mood changes, reported by approximately 80% of women

after childbirth, prenatal depression (12%), and postpartum depression (10–15%) (Gobinath et al. 2017).

Concerning androgens, an antidepressant and anxiolytic effect has been hypothesized. Preclinical data support a role for the *activational* effects of testosterone in reducing depressive-like behaviors in male adult animal models, to which prenatal *organizational* effects may also contribute (McHenry et al. 2014). Although results in females are less convincing, in intact adult female rodents, injections of testosterone or its metabolites – DHT and 3α-diol – reduced anxiety-like behaviors observed in the elevated plus maze and in the open field test compared to vehicle (McHenry et al. 2014). Similarly, administration of androgens to intact aged female rodents had antidepressant-like effects in the forced swim test, compared to controls (McHenry et al. 2014).

From a clinical perspective, the relationship between testosterone levels and anxiety/depressive symptoms is evident in males with hypogonadism. Despite a few inconsistent reports, the vast majority of studies, including placebo-controlled trials, support the notion that hypogonadal men are at higher risk for mood disorders and that testosterone-replacement therapy yields beneficial effects while alleviating anxiety and mitigating symptoms of depression (Zarrouf et al. 2009). Consistent with the hypothesized protective role of testosterone, depression is more prevalent in women, and the prevalence in men increases with age, as plasma testosterone drops (McHenry et al. 2014). Furthermore, compared to emotionally healthy controls, women suffering from depression express lower levels of plasma testosterone that significantly increases following psychopharmacotherapy (Kumsar et al. 2014). The relation between testosterone levels and depression in women follows a parabolic curve, with an optimum concentration and negative effects occurring in case of deviation from this range (Kumsar et al. 2014). In addition, a placebo-controlled, double-blind study reported that a single dose of testosterone (0.5 mg) influences emotional responses to pictures of fearful faces in female healthy volunteers, reducing unconscious fear but not consciously experienced anxiety, consistent with the hypothesis that testosterone's effects on emotion concern the subcortical affective pathways of the brain (van Honk et al. 2005).

The molecular mechanisms that underpin androgens' benefits on affective disorders are likely to include both rapid non-genomic actions and slower genomically mediated processes, but have not been elucidated. The MAPK-ERK (mitogen-activated protein kinase-extracellular signal-regulated kinase) signal transduction pathway and/or the activation of GABA(γ-aminobutyric acid) A receptors may be implicated (McHenry et al. 2014).

Female Sexual Behavior

In all species, sexual behavior is regulated by a complex interplay between biopsychosocial factors. However, for the purpose of this chapter, two main factors can be identified: steroid hormones (which drive sexual desire and arousal in the brain in response to incentive stimuli) and experience with sexual reward/pleasure (which

modulates the strength and trajectory of responses to sexual cues) (Pfaus et al. 2015). In humans, and in particular in women, a biopsychosocial approach that simultaneously considers not only organic (i.e., hormonal and neurovascular) but also psychological, sociocultural, and interpersonal factors is mandatory to guide clinical care and research on sexual function and well-being (Thomas and Thurston 2016).

Animal Models

A great portion of knowledge concerning the neurochemistry of sexual behavior derives from laboratory animals like rats. In all animals, sexual behavior occurs as a cascade of sequential behavioral events. Following the work of early twentieth-century ethologists and psychologists, in animal models, distinct *appetitive* and *consummatory* phases of sexual behavior were recognized (Pfaus et al. 2015). Appetitive (or "preceptive") behaviors bring an animal into contact with the potential sex partner; for example, female rats control the initiation and rate of copulation through a complex act of solicitation ("hops and darts") and runaway. These behaviors reflect the willingness of the female to initiate and engage in a sexual interaction. In contrast, consummatory (or "receptive") behaviors are performed once the animal is in direct contact with the incentive. In rats, the female holds a stationary posture called "lordosis," while the male palpates the flanks as he mounts with or without penile intromission (Pfaus et al. 2015). Lordosis is an estrogen- and progestin-dependent behavior that allows penile intromission, thanks to the arching of the back. The solicitation-mount/lordosis cycle occurs several times until the male ejaculates.

In humans, sexual desire and subjective arousal fit into an appetitive framework, whereas copulatory behavior fits into a consummatory framework. The ovariectomized female rat is an excellent model for investigating hormonal and pharmacological modulation of the female sexual response, not only because rat appetitive behaviors show several analogies with sexual interest/initiation in women but also because of the easiness of their hormonal manipulation. In an ovariectomized, estradiol benzoate-primed, Long-Evans rat model, administration of testosterone (Jones et al. 2017), testosterone plus an aromatase inhibitor (Pfaus et al. 2015), or the non-aromatizable androgen DHT (Maseroli et al. 2019) induced an enhancing effect on both appetitive and consummatory measures of female sexual behavior, strongly indicating a direct action on ARs.

The Dual Theoretical Model of Sexuality: Excitation and Inhibition

Early neurophysiologists studying fear and anxiety mechanisms proposed the notion of two separate but interactive neural systems for behavioral *excitation and inhibition*. The concept was later applied to male erectile response and finally to the overall sexual response in both sexes. Indeed, Perelman postulated a "Sexual Tipping Point®" (STP) model, in which the dynamic interplay between excitation and

inhibition generates an individual's level of sexual responsiveness, possibly varying at any given time (Pfaus 2009). Noteworthy, excitatory and inhibitory stimuli, encompassing organic, psychosocial, behavioral, and cultural issues, may be different for different individuals. The STP model stresses the *adaptive nature* of both excitatory and inhibitory processes, driving toward sex partners for reproductive or reward purposes and guarding against threatening or stressful situations, respectively (Pfaus 2009). Proper balance of excitatory and inhibitory signals is necessary for normal sexual function.

From a neurobiological perspective, the excitatory component involves the activation of neurotransmitters such as dopamine and melanocortins, which stimulate attention and desire, and norepinephrine and oxytocin, which stimulate sexual arousal (Kingsberg et al. 2015) (Fig. 2). Sexual excitation can be primed by external sexual cues or by steroid hormones – androgens, estrogens, and progestins – that, binding to their specific receptors, lead to the synthesis of different neurotransmitters in the mediobasal hypothalamus and limbic system, resulting in the brain being selectively and positively responsive to sexual incentives. Specifically, both estradiol and testosterone appear to directly or indirectly increase the release of dopamine, the most important excitatory component of behavioral reward signaling. Compelling evidence for the facilitatory role of sex steroids comes from preclinical and clinical studies showing that natural or surgically induced states of hypogonadism are associated with a loss of appetitive sexual behavior and decreased sexual responsivity, which are completely counteracted by hormonal replacement (Pfaus et al. 2015).

The most important inhibitory factors include serotonin (5-hydroxytryptamine, 5-HT), which regulates satiety; opioids, which mediate sexual rewards; and endocannabinoids, known to determine sedation (Kingsberg et al. 2015) (Fig. 2). These systems physiologically come into play at the end of the sexual response cycle,

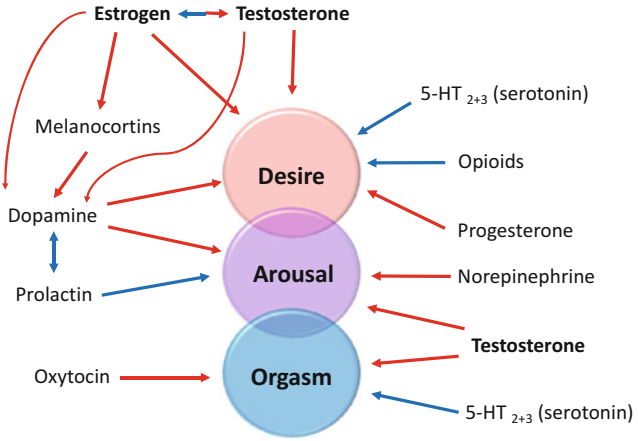


Fig. 2 Central effects of neurotransmitters and hormones on sexual functioning. (Adapted from Clayton 2007.) Red arrows indicate a stimulating effect and blue arrows an inhibitory effect

inducing a phase of "sexual satiety" or refractoriness. However, they may also be tonically activated by situational variables such as stress or by psychoactive drugs that augment their actions (e.g., selective serotonin reuptake inhibitors, SSRIs) or when sexual excitatory mechanisms are endogenously blunted. From an evolutionary point of view, sexual inhibition can be seen as an adaptive response aimed at protecting from risky or inappropriate sexual behaviors and at allowing a sufficient amount of sexual reward. Conversely, excessive central inhibition, imposed by the real or perceived aversive consequences of engaging in sexual activity or secondary to previous non-rewarding sexual experiences, increases the risk of sexual dysfunction, including inhibited arousal and desire or reduced capacity to achieve sexual satisfaction (Pfaus et al. 2015).

Physiology of Sexual Response in Women

Desire

Sexual desire is a component of procreative behavior essential for the sustenance of species. In humans, the concept encompasses physiological and behavioral aspects and can be defined as the presence of desire for, and fantasy about, sexual activity (Pfaus et al. 2015). The genesis of sexual motivation is believed to begin with the arrival of sexually stimulating sensory signals (visual, olfactory, tactile, etc.) at the hub of the telodiencephalic reproductive complex, including the medial preoptic area (mPOA) and the ventromedial hypothalamus, which have outputs to the paraventricular nucleus, supraoptic nucleus, and arcuate nucleus (Pfaus et al. 2015). In humans, motivational signals are also generated internally, through imagery or recall of past events. The mPOA plays a major role in activating the dopamine-mediated motivational system and integrating incentive signals with the reward they generate, preparing the body and autonomic systems for action (Kingsberg et al. 2015). Indeed, before sensory stimuli can result in a response or behavior, they are assessed to determine whether, according to previous experiences, they may result in beneficial or detrimental outcomes (Kingsberg et al. 2015). Such process is mainly performed in the prefrontal cortex (PFC) through a complex integration with the amygdala, a crucial site for emotional memory (see previous section "Emotion Processing and Reward Pathways").

Preclinical and clinical studies suggest that the facilitatory effects of estrogen and testosterone on sexual desire may be mediated by dopamine release in the mPOA. However, it's important to consider that, in women in particular, sexual desire does not represent an a priori functional state driven exclusively by hormones, but reflects an individual profile influenced by a myriad of variables, including mood, expectations, partner's sexual desire, overall feelings for the partner, and cultural norms (Wåhlin-Jacobsen et al. 2017). As underlined in the theoretical circular model of female sexual response, modified by Rosemary Basson, motivations for sex that go beyond sexual desire – in particular, sexual intimacy, past sexual experiences and relationship, and/or personal satisfaction – heavily influence a woman's drive toward sexual activity (Kingsberg et al. 2015). Furthermore, Basson introduced the notion

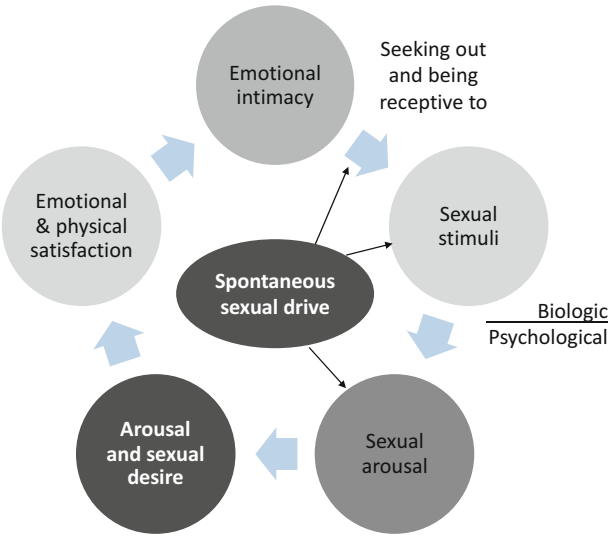


Fig. 3 Basson's circular model of female sexual response highlighting the importance of emotional intimacy as a non-sexual motivator and the spontaneous vs. responsive concept of desire. (Adapted from Thomas and Thurston 2016)

that, unlike men's, women's sexual response is less spontaneous and more responsive to emotional intimacy and subjective arousal, thus distinguishing *spontaneous* from *responsive desire* (Fig. 3).

Arousal

Sexual arousal is a physiological response to sexual stimuli that anticipates sexual activity, mediated by both central and peripheral nervous systems. Genital arousal is characterized by physiological changes in the genitals including engorgement of erectile tissue (e.g., clitoris and bulbs of vestibule), vulvovaginal lubrication (fluid transudate in the vagina and secretions of paraurethral and Bartholin's glands), and increased sensitivity. The hemodynamic mechanisms that underpin these processes are regulated by the tone of the vascular and non-vascular smooth muscle (Traish et al. 2010). Preclinical studies have constantly indicated that male and female genital contractile tissues share the same relaxant and contractile pathways and that the nitric oxide (NO)/cyclic guanosine monophosphate (cGMP) pathway plays a key role in modulating clitoral and vaginal blood flow as it does in the penile corpora cavernosa (Comeglio et al. 2016). In basal conditions, a high arterial vasomotor tone keeps genital blood flow at the minimal level through central sympathetic activation. After sexual stimulation, the decrease of central sympathetic tone and the release of vasodilator neurotransmitters induce smooth muscle relaxation with a rapid increase of blood flow to the genital tissues, which become vasocongested and tumescent (Comeglio et al. 2016).

Normal vaginal and clitoral responses during sexual arousal are dependent on the structural and functional integrity of local and systemic neural, muscular, and vascular systems. The sex steroid milieu acts as a pivotal regulator of these processes, influencing the health of the epithelium and the content and distribution of blood vessels, nonvascular smooth muscle, nerve fibers, and extracellular matrix (Traish et al. 2018) (see subsequent section "Hormones and the Female Sexual Response").

Orgasm

There have been innumerable definitions of orgasm in women. Today it is most correctly defined with the broader term "female sexual pleasure" and may be considered "a variable, transient peak sensation of intense pleasure creating an altered state of consciousness" (Meston et al. 2004). Centrally, women's orgasm entails the activation of all the major brain systems, including the brainstem, limbic system, cerebellum, and cortex, and is accompanied by the production of oxytocin and prolactin. At a peripheral level, the crucial feature of orgasm is represented by involuntary repeated pelvic muscle contractions of varying intensity and duration, often with concomitant vaginal, uterine, and anal contractions, that resolve genital vasocongestion, generally inducing satisfaction and contentment.

The stimulation of the swollen clitoris, which consists in cavernous tissue organized in corpora cavernosa essentially similar to that of the penis, is the primary anatomical source of clitoral orgasm. With regard to vaginal orgasm, the search for the "mythical" G-spot was unsuccessful in finding a unique structure as a source of female pleasure; however, recent research has highlighted the importance of the anterior vaginal wall and of the clito-urethro-vaginal (CUV) complex, a morpho-functional area which is believed to induce vaginally activated orgasm in some women when properly stimulated during penetration (Jannini et al. 2014). The anatomical structures of the CUV complex are hormone-sensitive and, hence, extremely different from subject to subject and within the same subject in relation to the different phases of life.

Female Sexual Dysfunction

Sexual dysfunction is a disturbance in sexual functioning involving one or multiple phases of the sexual response cycle or pain associated with sexual activity (McCabe et al. 2016). The term "female sexual dysfunction" (FSD) may refer to four distinct disorders recognized in the *Diagnostic and Statistical Manual of Mental Disorders* (DSM) 5: female sexual interest/arousal disorder [FSIAD, which encompasses what were previously termed hypoactive sexual desire disorder (HSDD) and female sexual arousal disorder (FSAD) in the DSM 4), female orgasmic disorder, genito-pelvic pain/penetration disorder (in which two conditions, previously termed vaginismus and dyspareunia, were merged), and substance/medication-induced sexual dysfunction (American Psychiatric Association 2013). For the diagnosis, persistency

of the dysfunction and a marked *distress* have to be present, since dysfunction does not require evaluation or treatment without distress (American Psychiatric Association 2013). Overlap of multiple diagnoses in the same patient is a common finding in clinical practice.

Hypoactive Sexual Desire Disorder

Sexual symptoms are common, with an estimated prevalence of 22–43% in women worldwide and 14% of women aged 45–64 reporting at least one sexual problem associated with significant distress (Thomas and Thurston 2016). A persistent lack of interest in sexual activity is the most frequent FSD and often affects young women, carrying a negative impact on quality of life. The abovementioned revised classification, in which desire and arousal problems had been collapsed (FSIAD), has proven highly controversial among clinicians and experts in sexual medicine, mainly due to the little empirical support for the new diagnostic categories and the practical consequences on management and treatment. Therefore, subsequent international expert panels, the International Consultation in Sexual Medicine and the International Society for Women's Sexual Health (ISSWSH) Nomenclature Committee, restored the label of HSDD, highlighting the two faces of female desire in the diagnostic criteria: decreased/absent *spontaneous desire* (i.e., sexual thoughts or fantasies), or decreased/absent *responsive desire* to erotic cues or stimulation, or inability to maintain desire or interest through sexual activity (Derogatis 2018).

According to the ISSWSH Process of Care, many psychological and medical factors may contribute to loss of sexual desire (Clayton et al. 2018). The most commonly reported psychological factors are depression and anxiety; poor self-esteem/body image; stress; history of abuse (physical, sexual, emotional); substance abuse; self-imposed pressure for sex; religious, personal, cultural, or family values; beliefs and taboos; relationship factors; lifestyle factors (e.g., fatigue, sleep deprivation); and sexual factors (e.g., inadequate stimulation) (Clayton et al. 2018). Among medical conditions associated with low sexual desire, the most important are surgically induced menopause and other disorders characterized by low androgen levels, hyperprolactinemia, thyroid disorders, metabolic disorders (diabetes mellitus, obesity, metabolic syndrome), neurological diseases, and some medications (antidepressant, antipsychotic, oral contraceptives, antiandrogens) (Clayton and Vignozzi 2018).

Female Arousal Disorder

Regarding arousal disorders, the ISSWSH recently proposed a further revision of the DSM 5 nomenclature, separating female genital arousal disorder (FGAD) from female cognitive arousal disorder (FCAD), a new category now defined as "the distressing difficulty or inability to attain or maintain adequate mental excitement associated with sexual activity [. . .] for a minimum of 6 months" (Parish et al. 2019). On the other hand, FGAD is characterized by "the distressing difficulty or inability

to attain or maintain adequate genital response, including vulvovaginal lubrication, engorgement of the genitalia, and sensitivity of the genitalia associated with sexual activity," and the causes of this disorder are identified in vascular or neurologic injury or dysfunction (Parish et al. 2019). The intention of this revision is to clarify the subtypes of arousal in order to develop better diagnostic strategies and treatment options, highlighting the importance of organic determinants of poor arousal.

In this regard, only in the past two decades, it has been hypothesized that genital arousal disorders could be related to impaired hemodynamic responses due to cardiometabolic-related perturbations in endothelial function, in women as in men (Maseroli et al. 2018a). Although the microanatomy and biochemistry of the male and female peripheral arousal response are similar, erectile dysfunction is universally considered a harbinger for cardiovascular (CV) disease, whereas the role of genital vascular impairment in the pathophysiology of FSD remains contentious. Indeed, based on limited epidemiologic and observational studies, there seems to be an association between CV risk factors (diabetes mellitus, obesity, metabolic syndrome, hypertension, dyslipidemia) and female sexual health in women (Maseroli et al. 2018a); however, this association appears milder than in men. It has been proposed that this gender discrepancy could be due not (or not only) to actual differences in CV and sexual pathophysiology (Morelli et al. 2014), but to historically different approaches toward male and female sexuality, which ultimately led to inadequate methodologic instruments to explore CV risk in patients with FSD (Maseroli et al. 2018b). In addition to altered CV integrity, risk factors for FGAD include disorders that affect the central and peripheral nervous system (multiple sclerosis, pudendal neuropathy, sacral or lumbar spinal pathology), anatomical changes associated with pelvic irradiation and/or surgery, and endocrine changes (see section "Hormones and the Female Sexual Response").

Female Orgasm Disorder

Female orgasm disorder is characterized by a persistent, or recurrent, distressing compromise of orgasm frequency, intensity, timing, and/or pleasure. Many psychological personal and relational issues may result in insufficient excitatory or increased inhibitory sexual processes within the central nervous system, resulting in orgasm disturbances. Among these, women often report past trauma or abuse history, unresolved marital conflict, ineffective sexual communication, cultural and religious prohibitions, pressure to have a sexual experience, mood disorders, and male partner sexual dysfunctions inducing inadequate arousal (and/or pain), such as erectile dysfunction or premature ejaculation (Goldstein and Komisaruk 2018).

On the other hand, medical conditions implicated in orgasm disorders include pelvic floor dysfunction; neurologic disorders involving both the peripheral (e.g., spinal cord injury, neuropathy of the perineal, pelvic or pudendal nerve) and central nervous system (e.g., multiple sclerosis, epilepsy, Parkinson's disease); genital gynecological or dermatological disorders (e.g., vaginitis, lichen sclerosus, vestibulodynia); and the use of medications such as SSRIs, antipsychotics,

antihypertensives, benzodiazepines, histamine 2 receptor antagonists, and anticonvulsants (Goldstein and Komisaruk 2018). With regard to endocrine alterations, both low testosterone and low estradiol states have been associated with orgasm dysfunction, probably acting through the same mechanisms that determine hormonal-associated disturbances in desire and arousal.

Sexual Pain Disorders

The previous edition of the DSM (4-TR) had two separate sexual pain disorders, labeled as dyspareunia (recurrent or persistent genital pain associated with sexual intercourse) and vaginismus (recurrent or persistent involuntary spasm of the musculature of the outer third of the vagina that interferes with sexual intercourse). The most recent edition combined them into a single category of genito-pelvic pain/penetration disorder (GPPPD) (American Psychiatric Association 2013). In vaginismus, adverse physical and/or psychological conditions act through a vicious cycle of fear and avoidance, in which attempted penetration causes distress and muscle tension, producing further avoidance and thus leading to fear of penile penetration. Dyspareunia, on the other hand, is often associated with organic conditions, e.g., infections (e.g., recurrent candidiasis), inflammation, neoplastic disorders (e.g., squamous cell carcinoma), neurologic alterations (e.g., postherpetic neuralgia, nerve compression), local trauma (e.g., operative delivery), iatrogenic trauma (e.g., radiation), and, most commonly, hormonal deficiencies. Indeed, dyspareunia is one of the critical symptoms of the genitourinary syndrome of menopause (GSM) (Simon et al. 2018). Interestingly, dyspareunia due to provoked vestibulodynia (pain localized to the vulvar vestibule) has been reported in young women using combined OCs. In addition to the reduction in estradiol and free androgen levels, due to the decrease in ovarian production of sex steroids and to the increase of sex hormone binding globulin (SHBG), combined OCs may contain progestogens that have anti-androgenic effects and have been reported to cause histopathologic changes in the vestibular mucosa, leading to increased vulnerability to mechanical strain and decreased pain threshold (Goldstein 2009).

Hormones and the Female Sexual Response

Estrogens and Progesterone

Estrogens and progesterone exert a pivotal role in modulating sexual behavior, mood, emotion, and cognition throughout a woman's lifespan, characterized by rising estrogen levels during puberty, cyclic fluctuation with the menstrual cycle, high levels during pregnancy, declining levels during peri-menopause, and extremely low levels in post-menopause. Estradiol positively regulates a variety of neurotransmitter systems in brain areas involved in sexual behavior (e.g., dopamine, proopiomelanocortin, and α1B-adrenergic receptors), and an estrogenic priming

seems to be necessary for progesterone-modulating effects on dopaminergic transmission (Clayton and Vignozzi 2018). In rat animal models, the lordosis reflex is dependent on estrogen, but full receptivity and full expression of appetitive behaviors depend on additional activation by progesterone (Pfaus et al. 2015).

In the genital tissue, low estrogen levels have long-established detrimental effects on vaginal epithelium, inducing vulvovaginal atrophy, with an increase in vaginal pH, a decreased content of superficial cells, and an increased proportion of parabasal cells. Low estrogen levels also reduce the volume of the vaginal muscularis layer with a considerable increase in connective tissue between muscle bundles. In animal models, ovariectomy induced dramatic impairment of the NO-dependent relaxant machinery in the clitoris and of the RhoA/ROK contractile machinery. Other preclinical studies have shown that ovariectomy impairs vaginal blood flow responses after pelvic nerve stimulation and that this response is increased after treatment with estradiol alone, testosterone alone, or estradiol plus testosterone (Simon et al. 2018).

Androgens

Androgens have been historically overlooked as pivotal regulators of female sexual and non-sexual behavior, albeit testosterone circulating at higher concentrations than estradiol during both pre- and postmenopausal years (Table 1). Common causes of low concentrations of testosterone are reported in Table 2 (Davis and Wahlin-Jacobsen 2015).

Large cross-sectional and longitudinal studies have shown consistent associations between specific androgens and self-reported measures of sexual function in premenopausal and postmenopausal women (Davis and Wahlin-Jacobsen 2015). However, in 2014 the Endocrine Society Guidelines (Wierman et al. 2014) recommended against making a clinical diagnosis of "androgen deficiency syndrome" in healthy women because of the lack of a well-defined syndrome. This has to do with several issues. First, the definition of a lower threshold for androgens that can be used to diagnose women with low sexual function or candidates for testosterone therapy is not available and is confounded by their physiological age-related decline. Second, there is a lack of standardized, accurate assays for testosterone targeted at the levels found in women. Third, complicating matters further, only 1–2% of total circulating testosterone is free or biologically available due to its high affinity SHBG, which shows important variability with elevations occurring during OC use, pregnancy,

Table 1 Mean steroid levels in women (pg/mL). (Adapted from Buster 1999)

	Reproductive age	Natural menopause	Surgical menopause
Estradiol	150	10–15	10
Testosterone	400	290	110
Androstenedione	1900	1000	700
DHEA	5000	2000	1800
DHEAS	3,000,000	1,000,000	1,000,000

DHEA dehydroepiandrosterone, *DHEAS* dehydroepiandrosterone sulfate

Table 2 Causes of low testosterone in women. (From Davis and Wahlin-Jacobsen 2015)

Spontaneous causes of androgen insufficiency	Mechanisms
Natural decline with age from mid-to-late reproductive years	Decline in production of androgens by the ovaries
Hypothalamic amenorrhea	Anovulation
Primary ovarian insufficiency	Anovulation
Hyperprolactinemia	Suppression of pituitary gonadotropins; anovulation
Adrenal insufficiency	Loss of adrenal production of pre-androgens
Panhypopituitarism	Loss of adrenal production of pre-androgens and ovarian production of androgens
Other medical conditions (e.g., chronic liver disease and HIV infection)	Increased concentrations of SHBG reduce concentrations of free testosterone
Iatrogenic causes of androgen insufficiency	*Mechanisms*
Surgical menopause at any age	Loss of ovarian production of androgens
Chemotherapy	Ovarian failure
Radiotherapy to the pelvis	Ovarian failure
Systemic glucocorticosteroid therapy	Suppression of adrenal production of pre-androgens
Drug-induced hyperprolactinemia	Suppression of pituitary gonadotropins; anovulation
Systemic hormonal contraception	Loss of ovarian production of androgens Increased concentrations of SHBG, resulting in reduced concentrations of free testosterone
Oral non-contraceptive therapy (e.g., phenobarbital, phenytoin, carbamazepine, and thyroxine)	Increased concentrations of SHBG, resulting in reduced concentrations of free testosterone

SHBG sex hormone binding globulin

hyperthyroidism, and liver disease. Finally, circulating levels do not reflect peripheral tissue exposure and sensitivity to androgens, which is thought to exhibit a dramatic interindividual variability, according to genetic differences in ARs and to the amount and activity of the 5α-reductase and aromatase enzymes in peripheral tissues (Davis and Wahlin-Jacobsen 2015).

Studies in animals and in humans consistently indicate that androgens exert facilitatory effects in all the phases of the female sexual response. As for desire, a stimulatory role on the reward system, probably mediated by dopaminergic pathways, has been identified (see previous paragraph "Emotion Processing and Reward Pathways"). Recent evidence point toward a central direct effect of androgens, independent of aromatization; however, the underpinning neurochemical mechanisms have not been clarified. The evidence that androgens are important modulators of women's sexuality stems also from studies of testosterone therapy in postmenopausal women with HSDD (Clayton and Vignozzi 2018) (see section "Outline of Hormonal Treatment Strategies for Female Sexual Dysfunction").

Furthermore, androgens are important for the maintenance of genitourinary tissue structure and function, and lack of androgenic activity can contribute to poor genital

arousal and symptoms of the GSM. Indeed, ARs have been detected throughout the genitourinary system (including the vagina, bladder, urethra, clitoris, labia, vestibule, and pelvic floor) using immunohistochemical and gene expression analyses (Traish et al. 2018). Local supplementation of androgen in postmenopausal women or systemic administration in ovariectomized animal models has been reported to induce tissue-specific responses that include cell growth, mucin production, collagen turnover, increased perfusion, and neurotransmitter synthesis (Simon et al. 2018). Furthermore, in vitro experiments in smooth muscle cells from rat clitoral biopsies demonstrated that testosterone improves the relaxation of vascular smooth muscle cells through the NO-cGMP pathway and that testosterone and estradiol are both necessary to maintain a functional contractile and relaxant machinery, which is crucial to ensure increase in blood flow and clitoral engorgement during the arousal phase (Comeglio et al. 2016).

Other Hormones

Prolactin

Prolactin is secreted by the anterior pituitary. Hyperprolactinemia is associated with hypogonadotropic hypogonadism, reduced ovarian production of sex steroids, and low sexual desire.

Thyroid Hormones

Data on the role of thyroid hormones and female sexuality are scant. However, both hypothyroidism (acting directly or through hyperprolactinemia) and hyperthyroidism (probably acting via increase of SHBG and the parallel decrease of free testosterone) have been associated with decreased libido.

Kisspeptin

Secreted by kisspeptin neurons within the hypothalamus, kisspeptin acts a as a potent activator of the hypothalamic-pituitary-gonadal axis, stimulating GnRH release and downstream gonadal hormone secretion at puberty (Morelli et al. 2008). Studies in humans and several other species have demonstrated that kisspeptin signaling effectively integrates sensory processing with limbic pathways involved in sexual arousal (Yang and Comninos 2018). Recently, kisspeptin neurons have been identified as a central regulatory hub orchestrating sexual behavior in the female mouse brain, triggering olfactory-driven mate preference via Kiss1 Receptor and eliciting copulatory behavior via NO signaling (Hellier et al. 2018).

Oxytocin and Vasopressin

These peptides are synthesized in the magnocellular neurons within the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus and released by the posterior pituitary. In rodents they modulate olfactory recognition, with complementary effects on emotions and prosocial behaviors. Specifically, oxytocin and vasopressin promote intimacy and bonding between individuals. In

humans, peripheral oxytocin has been reported to rise during sexual arousal and copulation in women, and it seems involved in trust, human-pair bonding, and selection of desirable traits in the opposite sex (Yang and Comninos 2018).

Outline of Hormonal Treatment Strategies for Female Sexual Dysfunction

Given the multifactorial nature of FSD, the first-line intervention consists in targeting modifiable risk factors identified during the assessment phase, including medications, mood disorder and psychological comorbidities, lifestyle conditions, and relationship issues. Adequate hormone replacement regimens with exogenous estrogens (in combination with progesterone in non-hysterectomized women) significantly improve natural and surgically menopause-related decline in sexual arousal and desire. Nevertheless, compelling evidence consistently demonstrates that co-therapy with estrogens and androgens offers greater therapeutic value over estrogen replacement alone. In fact, high physiologic doses of testosterone therapy improve desire, arousal, vaginal blood flow, orgasm frequency, and sexual satisfaction in surgically and naturally menopausal women, either alone or in combination with menopausal estrogen therapy (Clayton and Vignozzi 2018). In 24-week phase 3 clinical trials in postmenopausal women with HSDD, the 300 mcg/die testosterone patch was found to significantly improve sexual desire versus placebo, without concerning side effects (Somboonporn et al. 2005). Accordingly, the Endocrine Society Guidelines suggest a 3–6-month trial of testosterone treatment in postmenopausal women suffering from HSDD (Buster 1999). Nevertheless, testosterone or other androgens for HSDD in women are not currently approved in most countries, including Europe and the United States. When preparations formulated for men or by pharmacies are used off-label, measurement of testosterone levels at 3–6 months is recommended to identify supraphysiologic levels. Adequately designed follow-up studies are warranted to confirm the efficacy of testosterone treatment in premenopausal women and its long-term safety and to highlight other potential beneficial effects on cognition, cardiovascular, and general well-being (Davis and Wahlin-Jacobsen 2015).

Tibolone, a selective estrogen receptor modulator (SERM) with a weak estrogenic, progestogenic, and/or androgenic activity, has been shown to have no effect to a small benefit on overall sexual function versus controls (Clayton and Vignozzi 2018). Similarly, a recent meta-analysis showed that systemic treatment with DHEAS was not effective in improving libido and sexual function in postmenopausal women with normal adrenal function (Clayton and Vignozzi 2018).

Vaginal estrogens are an effective intervention for menopausal-related vaginal dryness and atrophy. Recently, double-blind, placebo-controlled clinical trials have demonstrated that local vaginal dehydroepiandrosterone (DHEAS) and oral ospemifene (a new SERM) are able to improve moderate to severe dyspareunia in postmenopausal women with vulvovaginal atrophy. Limited data also suggest a beneficial effect of systemic testosterone treatment on vaginal epithelial health and blood flow; further studies are needed to confirm these observations (Simon et al. 2018).

Conclusions

Species survival is dependent on successful reproduction, and nature has provided a rewarding experience to sexual act to ensure the sustenance of species. Hormones, and in particular sex steroids, are integral to satisfactory sex and not only mediate physiological and endocrine processes involved in reproduction but also act as neuromodulators within crucial brain regions to facilitate the expression of innate emotions and behaviors required for reproduction.

A significant body of research has been unraveling the key role of sex hormones in the modulation of mood states, cognition abilities, and sexual behavior in women. Estradiol and progesterone have long been known to be essential for the full expression of sexual appetitive and receptive behaviors in animal models, and natural and surgically menopause-related decline in estrogen levels has a well-established detrimental effect on sexuality. Androgens have more recently come back in the spotlight as pivotal regulators of female sexual and non-sexual behavior, and their direct stimulating effect on desire, independent of aromatization, has been suggested. Future studies are warranted to clarify the underpinning mechanisms of these actions and to develop new treatment opportunities for FSD targeting androgen signaling.

References

Ahmed EI, Zehr JL, Schulz KM, Lorenz BH, DonCarlos LL, Sisk CL. Pubertal hormones modulate the addition of new cells to sexually dimorphic brain regions. Nat Neurosci. 2008;11:995–7.

American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 5th ed. Arlington: American Psychiatric Publishing; 2013.

Arnold AP. A general theory of sexual differentiation. J Neurosci Res. 2017;95:291–300.

Bakker J. The sexual differentiation of the human brain: role of sex hormones versus sex chromosomes. Curr Top Behav Neurosci. 2019; https://doi.org/10.1007/7854_2018_70. [Epub ahead of print]

Bakker J, De Mees C, Douhard Q, Balthazart J, Gabant P, Szpirer J, Szpirer C. Alpha-fetoprotein protects the developing female mouse brain from masculinization and defeminization by estrogens. Nat Neurosci. 2006;9:220–6.

Barth C, Steele CJ, Mueller K, Rekkas VP, Arélin K, Pampel A, Burmann I, Kratzsch J, Villringer A, Sacher J. In-vivo dynamics of the human Hippocampus across the menstrual cycle. Sci Rep. 2016;6:32833.

Bramen JE, Hranilovich JA, Dahl RE, Chen J, Rosso C, Forbes EE, Dinov ID, Worthman CM, Sowell ER. Sex matters during adolescence: testosterone-related cortical thickness maturation differs between boys and girls. PLoS One. 2012;7:e33850.

Buster JE. Managing femal sexual dysfunction. In Lobo RA, ed., Treatment of the post menopausal woman, New York: Lippincott Williams and Wilkins, 1999.

Clayton AH. Epidemiology and neurobiology of female sexual dysfunction. J Sex Med. 2007;4 (Suppl 4):260–8.

Clayton AH, Vignozzi L. Pathophysiology and medical management of hypoactive sexual desire disorder. In: Goldstein I, Clayton AH, Goldstein AT, Kim NN, Kingsber SA, editors. Textbook of female sexual function and dysfunction – diagnosis and treatment. Oxford: Wiley Blackwell; 2018. p. 59–100.

Clayton AH, Goldstein I, Kim NN, Althof SE, Faubion SS, Faught BM, Parish SJ, Simon JA, Vignozzi L, Christiansen K, Davis SR, Freedman MA, Kingsberg SA, Kirana PS, Larkin L, McCabe M, Sadovsky R. The International Society for the Study of Women's Sexual Health process of care for management of hypoactive sexual desire disorder in women. Mayo Clin Proc. 2018;93:467–87.

Cohen-Bendahan CC, Buitelaar JK, van Goozen SH, Cohen-Kettenis PT. Prenatal exposure to testosterone and functional cerebral lateralization: a study in same-sex and opposite-sex twin girls. Psychoneuroendocrinology. 2004;29:911–6.

Cohen-Kettenis PT. Gender change in 46, XY persons with 5α-reductase-2 deficiency and 17β-hydroxysteroid dehydrogenase-3 deficiency. Arch Sex Behav. 2005;34:399–410.

Comeglio P, Cellai I, Filippi S, Corno C, Corcetto F, Morelli A, Maneschi E, Maseroli E, Mannucci E, Fambrini M, Maggi M, Vignozzi L. Differential effects of testosterone and estradiol on clitoral function: an experimental study in rats. J Sex Med. 2016;13:1858–71.

Cooke BM. Steroid-dependent plasticity in the medial amygdala. Neuroscience. 2006;138:997–1005.

Davis SR, Wahlin-Jacobsen S. Testosterone in women–the clinical significance. Lancet Diabetes Endocrinol. 2015;3:980–92.

Davison SL, Bell R, Donath S, Montalto JG, Davis SR. Androgen levels in adult females: changes with age, menopause, and oophorectomy. J Clin Endocrinol Metab. 2005;90:3847–53.

Derogatis LR. Nosology and epidemiology of hypoactive sexual desire disorder. In: Goldstein I, Clayton AH, Goldstein AT, Kim NN, Kingsber SA, editors. Textbook of female sexual function and dysfunction – diagnosis and treatment. Oxford: Wiley Blackwell; 2018. p. 19–24.

Dreher JC, Schmidt PJ, Kohn P, Furman D, Rubinow D, Berman KF. Menstrual cycle phase modulates reward-related neural function in women. Proc Natl Acad Sci U S A. 2007;104:2465–70.

Elbejjani M, Fuhrer R, Abrahamowicz M, Mazoyer B, Crivello F, Tzourio C, Dufouil C. Depression, depressive symptoms, and rate of hippocampal atrophy in a longitudinal cohort of older men and women. Psychol Med. 2015;45:1931–44.

Feinberg DR, DeBruine LM, Jones BC, Little AC. Correlated preferences for men's facial and vocal masculinity. Evol Hum Behav. 2008;29:233–41.

Feis DL, Brodersen KH, von Cramon DY, Luders E, Tittgemeyer M. Decoding gender dimorphism of the human brain using multimodal anatomical and diffusion MRI data. NeuroImage. 2013;70:250–7.

Fisher AD, Ristori J, Maggi M. Pharmachosexology: sex hormones and psychiatric conditions. In: Jannini EA, Siracusano A, editors. Sexual dysfunctions in mentally ill patients. Cham: Springer; 2018. p. 215–25.

Gobinath AR, Choleris E, Galea LA. Sex, hormones, and genotype interact to influence psychiatric disease, treatment, and behavioural research. J Neurosci Res. 2017;95:50–64.

Goldstein AT. Medical history, physical examination, and laboratory tests for the evaluation of dyspareunia. In: Goldstein A, Goldstein I, editors. Female sexual pain disorders: evaluation and management. Chichester: Wiley-Blackwell; 2009.

Goldstein I, Komisaruk BR. Pathophysiology and medical management of female orgasm disorder. In: Goldstein I, Clayton AH, Goldstein AT, Kim NN, Kingsber SA, editors. Textbook of female sexual function and dysfunction – diagnosis and treatment. Oxford: Wiley Blackwell; 2018. p. 221–39.

Goy RW, McEwen BS. Sexual differentiation of the brain. Cambridge, MA: MIT Press; 1980.

Gurvich C, Hoy K, Thomas N, Kulkarni J. Sex differences and the influence of sex hormones on cognition through adulthood and the aging process. Brain Sci. 2018;28:8.

Hamann S, Herman RA, Nolan CL, Wallen K. Men and women differ in amygdala response to visual sexual stimuli. Nat Neurosci. 2004;7:411–6.

Hara Y, Waters EM, McEwen BS, Morrison JH. Estrogen effects on cognitive and synaptic health over the lifecourse. Physiol Rev. 2015;95:785–807.

Hellier V, Brock O, Candlish M, Desroziers E, Aoki M, Mayer C, Piet R, Herbison A, Colledge WH, Prévot V, Boehm U, Bakker J. Female sexual behavior in mice is controlled by kisspeptin neurons. Nat Commun. 2018;9:400.

Janak PH, Tye KM. From circuits to behaviour in the amygdala. Nature. 2015;517:284–92.
Jannini EA, Buisson O, Rubio-Casillas A. Beyond the G-spot: clitourethrovaginal complex anatomy in female orgasm. Nat Rev Urol. 2014;11:531–8.
Jones SL, Ismail N, Pfaus JG. Facilitation of sexual behavior in ovariectomized rats by estradiol and testosterone: a preclinical model of androgen effects on female sexual desire. Psychoneuroendocrinology. 2017;79:122–33.
Kim G, Jeong GW. Menopause-related brain activation patterns during visual sexual arousal in menopausal women: an fMRI pilot study using time-course analysis. Neuroscience. 2017;343:449–58.
Kingsberg SA, Clayton AH, Pfaus JG. The female sexual response: current models, neurobiological underpinnings and agents currently approved or under investigation for the treatment of hypoactive sexual desire disorder. CNS Drugs. 2015;29:915–33.
Kreiter SR, Krowchuk DP, Woods CR, Sinal SH, Lawless MR, DuRant RH. Gender differences in risk behaviors among adolescents who experience date fighting. Pediatrics. 1999;104:1286–92.
Kumsar Ş, Kumsar NA, Sağlam HS, Köse O, Budak S, Adsan Ö. Testosterone levels and sexual function disorders in depressive female patients: effects of antidepressant treatment. J Sex Med. 2014;11:529–35.
Macoveanu J, Henningsson S, Pinborg A, Jensen P, Knudsen GM, Frokjaer VG, Siebner HR. Sex-steroid hormone manipulation reduces brain response to reward. Neuropsychopharmacology. 2016;41:1057–65.
Maseroli E, Scavello I, Vignozzi L. Cardiometabolic risk and female sexuality-part I. Risk factors and potential pathophysiological underpinnings for female Vasculogenic sexual dysfunction syndromes. Sex Med Rev. 2018a;6:508–24.
Maseroli E, Scavello I, Vignozzi L. Cardiometabolic risk and female sexuality-part II. Understanding (and overcoming) gender differences: the key role of an adequate methodological approach. Sex Med Rev. 2018b;6:525–34.
Maseroli E, Pfaus JG, Santangelo A, Ricca V, Maggi M, Vignozzi L. Study on the effect of non-aromatizable androgen dihydrotestosterone (DHT) on the sexual behavior of Ovariectomized female rats primed with estradiol. In: Presented as abstract at the International Society for the Study of Women's Sexual Health/International Society for Sexual Health joint meeting, Atlanta; 2019.
McCabe MP, Sharlip ID, Atalla E, Balon R, Fisher AD, Laumann E, Lee SW, Lewis R, Segraves RT. Definitions of sexual dysfunctions in women and men: a consensus statement from the fourth international consultation on sexual medicine 2015. J Sex Med. 2016;13:135–43.
McEwen BS, Milner TA. Understanding the broad influence of sex hormones and sex differences in the brain. J Neurosci Res. 2017;95:24–39.
McHenry J, Carrier N, Hull E, Kabbaj M. Sex differences in anxiety and depression: role of testosterone. Front Neuroendocrinol. 2014;35:42–57.
Meston CM, Hull E, Levin RJ, Sipski M. Disorders of orgasm in women. J Sex Med. 2004;1:66–8.
Mhillaj E, Morgese MG, Tucci P, Bove M, Schiavone S, Trabace L. Effects of anabolic-androgens on brain reward function. Front Neurosci. 2015;9:295.
Morelli A, Marini M, Mancina R, Luconi M, Vignozzi L, Fibbi B, Filippi S, Pezzatini A, Forti G, Vannelli GB, Maggi M. Sex steroids and leptin regulate the "first Kiss" (KiSS 1/G-protein-coupled receptor 54 system) in human gonadotropin-releasing-hormone-secreting neuroblasts. J Sex Med. 2008;5:1097–1113
Morelli A, Sarchielli E, Comeglio P, Filippi S, Vignozzi L, Marini M, Rastrelli G, Maneschi E, Cellai I, Persani L, Adorini L, Vannelli GB, Maggi M. Metabolic syndrome induces inflammation and impairs gonadotropin-releasing hormone neurons in the preoptic area of the hypothalamus in rabbits. Mol Cell Endocrinol. 2014;382:107–119
Parish SJ, Meston CM, Althof SE, Clayton AH, Goldstein I, Goldstein SW, Heiman JR, McCabe MP, Segraves RT, Simon JA. Toward a more evidence-based nosology and nomenclature for female sexual dysfunctions-part III. J Sex Med. 2019;16:452–62.
Pfaus JG. Pathways of sexual desire. J Sex Med. 2009;6:1506–33.

Pfaus JG, Jones SL. Central nervous system anatomy and neurochemistry of sexual desire. In: Goldstein I, Clayton AH, Goldstein AT, Kim NN, Kingsberg SA, editors. Textbook of female sexual function and dysfunction – diagnosis and treatment. Oxford: Wiley Blackwell; 2018. p. 25–52.

Pfaus JG, Jones SL, Flanagan-Cato LM, Blaustein JD. Female sexual behaviour. In: Plant TM, Zeleznik AJ, Knobil E, Neil JD, editors. Knobil and Neill's physiology of reproduction. 4th ed. Amsterdam: Elsevier; 2015. p. 2287–370.

Pintzka CW, Evensmoen HR, Lehn H, Håberg AK. Changes in spatial cognition and brain activity after a single dose of testosterone in healthy women. Behav Brain Res. 2016;298:78–90.

Puts DA, McDaniel MA, Jordan CL, Breedlove SM. Spatial ability and prenatal androgens: meta-analyses of congenital adrenal hyperplasia and digit ratio (2d:4d) studies. Arch Sex Behav. 2008;37:100–1.

Raznahan A, Lee Y, Stidd R, Long R, Greenstein D, Clasen L, Addington A, Gogtay N, Rapoport JL, Giedd JN. Longitudinally mapping the influence of sex and androgen signaling on the dynamics of human cortical maturation in adolescence. Proc Natl Acad Sci U S A. 2010;107:16988–93.

Roselli CE. Neurobiology of gender identity and sexual orientation. J Neuroendocrinol. 2018;30: e12562.

Schroeder JP, Packard MG. Role of dopamine receptor subtypes in the acquisition of a testosterone conditioned place preference in rats. Neurosci Lett. 2000;282:17–20.

Schulz KM, Richardson HN, Zehr JL, Osetek AJ, Menard TA, Sisk CL. Gonadal hormones masculinize and defeminize reproductive behaviours during puberty in the male Syrian hamster. Horm Behav. 2004;45:242–9.

Simon JA, Goldstein I, Kim NN, Davis SR, Kellogg-Spadt S, Lowenstein L, Pinkerton JV, Stuenkel CA, Traish AM, Archer DF, Bachmann G, Goldstein AT, Nappi RE, Vignozzi L. The role of androgens in the treatment of genitourinary syndrome of menopause (GSM): International Society for the Study of Women's Sexual Health (ISSWSH) expert consensus panel review. Menopause. 2018;25:837–47.

Somboonporn W, Davis S, Seif MW, Bell R. Testosterone for peri- and postmenopausal women. Cochrane Database Syst Rev. 2005;4:CD004509.

Stewart J, Kolb B. Dendritic branching in cortical pyramidal cells in response to ovariectomy in adult female rats: suppression by neonatal exposure to testosterone. Brain Res. 1994;654:149–54.

Thomas HN, Thurston RC. A biopsychosocial approach to women's sexual function and dysfunction at midlife: a narrative review. Maturitas. 2016;87:49–60.

Tobiansky DJ, Wallin-Miller KG, Floresco SB, Wood RI, Soma KK. Androgen regulation of the mesocorticolimbic system and executive function. Front Endocrinol (Lausanne). 2018;9:279.

Traish AM, Botchevar E, Kim NN. Biochemical factors modulating female genital sexual arousal physiology. J Sex Med. 2010;7:2925–46.

Traish AM, Vignozzi L, Simon JA, Goldstein I, Kim NN. Role of androgens in female genitourinary tissue structure and function: implications in the genitourinary syndrome of menopause. Sex Med Rev. 2018;6:558–71.

van Honk J, Schutter DJ, Hermans EJ, Putman P, Tuiten A, Koppeschaar H. Testosterone shifts the balance between sensitivity for punishment and reward in healthy young women. Psychoneuroendocrinology. 2004;29:937–43.

van Honk J, Peper JS, Schutter DJ. Testosterone reduces unconscious fear but not consciously experienced anxiety: implications for the disorders of fear and anxiety. Biol Psychiatry. 2005;58:218–25.

van Wingen G, Mattern C, Verkes RJ, Buitelaar J, Fernández G. Testosterone reduces amygdala-orbitofrontal cortex coupling. Psychoneuroendocrinology. 2010;35:105–13.

Wåhlin-Jacobsen S, Kristensen E, Pedersen AT, Laessøe NC, Cohen AS, Hougaard DM, Lundqvist M, Giraldi A. Androgens and psychosocial factors related to sexual dysfunctions in pre-menopausal women∗: ∗2016 ISSM female sexual dysfunction prize. J Sex Med. 2017;14:366–79.

Welling LLM, Puts DA, Roberts SC, Little AC, Burriss RP. Hormonal contraceptive use and mate retention behaviour in women and their male partners. Horm Behav. 2012;61:114–20.
Wierman ME, Arlt W, Basson R, Davis SR, Miller KK, Murad MH, Rosner W, Santoro N. Androgen therapy in women: a reappraisal: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2014;99:3489–510.
Wisniewski AB. Sexually-dimorphic patterns of cortical asymmetry, and the role for sex steroid hormones in determining cortical patterns of lateralization. Psychoneuroendocrinology. 1998;23:519–47.
Wisniewski AB, Migeon CJ, Meyer-Bahlburg HF, Gearhart JP, Berkovitz GD, Brown TR, Money J. Complete androgen insensitivity syndrome: long-term medical, surgical, and psychosexual outcome. J Clin Endocrinol Metab. 2000;85:2664–9.
Yang L, Comninos AN, Dhillo WS. Intrinsic links among sex, emotion, and reproduction. Cell Mol Life Sci. 2018;75:2197–210.
Young WC, Goy RW, Phoenix CH. Hormones and sexual behavior. Science. 1964;143:212–8.
Zarrouf FA, Artz S, Griffith J, Sirbu C, Kommor M. Testosterone and depression: systematic review and meta-analysis. J Psychiatr Pract. 2009;15:289–305.

Hormonal Contraception

7

Vincenzina Bruni, Metella Dei, and Francesca Pampaloni

Contents

Abstract

The clinical approach to hormonal contraception is based on the integration of two points of view. On the one side we have to consider the specific needs and the biological characteristics of the girl or the woman asking for contraception, according to age, period of life, and personal physical or psychological vulnerability. On the other side, it is well documented that an appropriate choice

V. Bruni (✉)
University of Florence, Florence, Italy
e-mail: vbruni.prof@gmail.com

M. Dei
Italian Society Pediatric Adolescent Gynecology, Florence, Italy
e-mail: meteldei@alice.it

F. Pampaloni
Pediatric and Adolescent Gynecology Unit Careggi Hospital Florence, Florence, Italy

F. Petraglia, B. C. Fauser (eds.), *Female Reproductive Dysfunction*, Endocrinology,
https://doi.org/10.1007/978-3-030-14782-2_7

of the formulation, maybe including therapeutic effects, facilitates the long-term adhesion to the method. This competence requires a thorough knowledge of the pharmacology of single preparations. Therefore a part of this chapter is the revision of the characteristics of various hormonal components of contraceptives in use. To follow is a synthesis of main contraindications, in agreement with current guidelines, and of therapeutic indications.

Keywords

Contraceptives · Estrogens · Progestins · Specific needs · Risks · Benefits

Introduction

The biological and psychological profile of the woman is the main object of our clinical history and it is essential for the contraceptive counselling. On the other side, especially concerning hormonal contraception, basic pharmacological information are relevant for an appropriate choice, in order to minimize side effects and to promote adhesion to treatment.

Contraception in Different Categories and Times of Life

Good consultation regarding contraception requires good knowledge of the different methods and preparations available, including their precise indications and contra-indications, and the characteristics and specific needs specific demands of the individual or couple involved. Accurate anamnesis is a key factor; it often leads to easy individuation of the best choices for the case at hand. Below is a brief survey of specific age groups and categories of girls and women who benefit from targeted counseling on the use of contraceptives.

Adolescence

This is usually the time of initiation to sexual experiences, a time of curiosity and attraction often with a partner who has had little or no personal experience. The correct choice of contraceptive is based on the information available, when possible with the help of a competent adult. Studies carried out in various countries indicate that in general, youths in this age group have poor knowledge regarding sex, sexuality, and contraception, knowledge based on fragments of information received from family, friends, web surfing, and, in the most fortunate cases, sex education at school. The young age and scarce communication within the family, especially with the mother, are additional negative factors. Progression from a stage of such limited knowledge to making a choice for oneself is also difficult, just as difficult as continuing responsible protection over time. These factors lead to limited use of adequate contraceptive

Table 1 History for evaluation of medical risk in adolescence

Family history for cardiovascular diseases dyslipidemia diabetes autoimmune disorders migraine
Personal history: migraine chronic diseases drug consumption including supplements depressive states eating disorders
Lifestyle: smoking physical activity alcohol or substance abuse attitude toward daily routines
Symptoms related to menstrual cycle: dysmenorrhea pelvic pain related to endometriosis menstrual disorders heavy menstrual bleedings premenstrual syndrome catamenial symptoms

methods, especially during the first sexual encounters. The main problems are that since subjects in this age group are very fertile and the changes of sexual partners are frequent, the young females face high risk of pregnancy, and both males and females are at high risk of contracting sexually transmitted diseases. Moreover, in particular in this age group, the lack of attention to protected sex is often a sign of personal or family problems, depression, and poor self-esteem, situations that must be faced when possible during patient consultation.

Thus, establishing a good interpersonal relationship with an adolescent is fundamental for the appropriate choice of contraceptive, and it requires more anamnestic information than is usually acquired in everyday practice (Table 1). In fact contraception counseling of adolescents is efficacious only if it is not limited to mere technical aspects or to evidencing biological risk factors: counseling must include evaluation of eventual risk related to personal and relational psychological factors (Dei and Bruni 2016; Dehlendorf et al. 2014). One of the first things to ascertain is the patient's knowledge regarding her fertility (many girls presume they are not fertile especially if they have irregular menstrual cycles) and effective contraceptive methods (we note that some adolescents use the cycle previsions of apps to calculate their "safe days" as a contraceptive method). Long-term adhesion to contraceptive use is improved by discussion of possible side effects, eventual risks and efficacy, and where to find further information should the need arise. Possible fear on the part of patients regarding weight gain and/or presumed risk of future infertility should be considered. Finally, we note that for adolescents, European and international scientific societies recommend *long-acting* (LARC) methods that eliminate the errors and interruptions of administration common with other prescriptions.

To improve adhesion to a single method, it is mandatory to explain possible side effects, eventual risks and efficacy, and where to find further information should the need arise. Possible fear on the part of patients regarding weight gain and/or presumed risk of future infertility should be considered. Finally, we note that for adolescents European and international scientific societies recommend *long-acting* (LARC) methods that eliminate the errors and interruptions of administration common with other prescriptions. Very young subjects, those who are entering a new relationship, or those who are just beginning to use a hormonal or intrauterine contraceptive should be advised to use double protection, that is, condom plus the method of choice.

Personal and family anamnesis must include the patient's weight, height, BMI, and waist measurements or waist/hip ratio in cases of suspected metabolism dysfunction. Objective clinical examination must also include the skin for eventual

signs of hyperandrogenism. Blood pressure should be measured. Other blood and routine laboratory tests are not necessary for choosing a contraceptive, although they may be useful in specific cases based on particular anamnestic elements or lack of pertinent family anamnestic data, as in the case of adoptive children. Neither is gynecological examination necessary for prescription of a contraceptive.

An appointment for contraceptive counseling is a good occasion for warning and advising young patients about the risks of smoking and substance abuse and strategies to avoid them. Other points that should be mentioned are weight control and the importance of regular physical activities.

There are no contraindications in the international guidelines regarding prescription and use of any contraceptive method in very young adolescents. These girls rarely present pathological conditions (e.g., migraine with aura, symptomatic gall bladder conditions, chronic valve disease, hereditary angioedema, certain autoimmune diseases especially those associated with antiphospholipid antibodies) in which the use of estroprogestins is contraindicated. Details about the individual menstrual cycle as well as manifestations indicating androgen excess can be used to motivate better compliance with a contraceptive that is also beneficial against invalidating symptoms, such as dysmenorrhea and heavy bleeding, or in improving skin conditions, such as acne and seborrhea. However, we have to inform the girl that it takes 4–6 months of treatment before the visible effects on the signs of hyperandrogenism become manifest.

The traditional clinical anamnesis should be accompanied by a parallel psychorelational evaluation to evidence eventual factors that might lead to generally risky behavior and, in particular, no contraceptive protection at all (Table 2). Questions about the relationship between the couple can include an evaluation of exposure to risk of contracting a sexually transmitted disease and thus raise the issue of negotiating with the partner of the eventual use of condoms or another type of contraceptive. We know well that gender asymmetry has a great impact in decisions regarding this issue. It is common in adolescent couples (12–18%) that the male plays a predominant role regarding all sexual matters, ranging from strict control of choices and coercion to acts of violence. Such situations are associated with high risk of undesired pregnancy and sexually transmitted diseases as well as sexual dysfunction (Silverman et al. 2011) because young males tend to boycott contraception. Since the adolescent female may not always recognize the difference between controlling behavior and expressions of affection, it is important to ask clear, specific questions.

Table 2 History for evaluation of behavioral risk in adolescence

Risk of exposure to sexually transmitted infections (precocious sexual debut partner turnover beginning of new relationship not exclusive couple sexual intercourses for payment dating after online contact sex in “trip” conditions)
Characteristics of relationship (time and place of intercourses dating apps used casual sex partner’s influence in contraceptive choice asymmetric relationship)
Family situation: single-parent family physical or psychical maternal disease assisted violence abuse serious economic deprivation
Personal projects and resources self-esteem body-image acceptance

Individual capacity for effectual protection of one's own health is strictly correlated with personal assertiveness and self-esteem. The consultant can explore these factors indirectly by talking about life projects, self-image, and feelings about one's body. Girls and women who have or have had eating disorders find it more difficult to accept hormone preparations and tend to interrupt contraceptive treatments; those who are overweight or present esthetic concerns have a greater tendency to practice sex without protection (Chang et al. 2015).

A good mother-daughter relationship is a protective factor in sexual behavior, as documented by studies carried out in different countries (Widman et al. 2016). Growing up in a family with a lack of parental guidance or where the parents have serious physical or mental problems leads to more solitude, also in relation to personal choices. Experiences of not being cared for as well as verbal or physical and/or sexual violence, either personal or in the family, can have repercussions on self-esteem and compromise the individual's capacity to establish friendships and gratify affective relationships, leading to difficulties in self-care and protection. It is not easy to ask direct questions regarding these issues. Consideration of the overall clinical history, with attention to particular signs of difficulty and nonverbal attitudes, can guide the physician to propose additional consulting time or referral to a psychological consultant in support of the protective choice.

Subjects with Disabilities

In recent years, the United Nations and various health agencies worldwide have called attention to the needs of the disabled in regard to reproductive health, including the need for contraceptive consultation and prescription, but to little avail. It has been estimated that approximately 3 of every 100 adults (and a slightly higher percentage of adolescents) has a physical or psychic disability. Thus, there are a significant number of fertile aged disabled persons in the world. Neither the families nor the educators nor the health services tend to pay attention to the sexuality and sexual needs of this part of the population whose need for affective relationships is grossly underestimated. Targeted sex education programs are necessary for these persons, and those who care for them need to have access to information on this topic. Finally, competent gynecological counseling is fundamental (Table 3) with discussion of the various aspects of sexuality, including the eventual need for appropriate contraceptive treatment for the disabled.

We cannot ignore the fact that sexual coercion is especially prevalent against disabled persons, who are also more frequently the victims of chronic violence within the family than healthy individuals and of sexual abuse in day-care institutions, especially those who cannot talk or have other communicative difficulties or psychic disabilities (Byrne 2018). This vulnerability is correlated to the inevitable dependence on other persons, the desire to relate, lack of knowledge about sex and sexuality, and a certain innocence (especially on online friendships) often related to difficulties in understanding the expressions and intentions of others.

Table 3 History for counseling with disabled people

Evaluation of co-characteristics of menstrual cycles, need to manage bleedings (hygiene, perimenstrual worsening of symptoms, dysmenorrhea)
Comorbidities
Current therapies (particularly antiepileptics)
In case of physical disability, evaluate skills and entity of movement
Sexual history. Possible abusive situations
Information and competence about sexuality
Parents and/or educators' opinions
In case of psychical disability, evaluate the capacity to consent to medical acts

Such situations can lead to unforeseen risks of pregnancy or contagion with sexually transmitted diseases. Menstrual anamnesis must be focused for eventual management of premenstrual symptoms (headache, epileptic crises, aggressive behaviors, psychotic disturbances, etc.) or to inhibit the menses with an extended contraceptive regime when management of menstruation is difficult (Quint and O'Brien 2016). Persons with physical and sensorial disabilities frequently present other medical conditions, in particular seizures (undiagnosed in more than 30% of cases), diabetes, overweight, cardiac dysfunction, and endocrine disturbances which are not necessarily related to the disability, but comport relatively higher risk of complications in pregnancy. It has been estimated that over 30% of disabled persons have undiagnosed epilepsy. All situations of prolonged immobility are known to be associated with increased risk of thromboembolism. Persons with spasticity at birth or from early childhood have been shown to present acquired modifications in venous capillaries of the lower limbs, which however present lower risk than such modifications in individuals with bone marrow lesions or immobility caused by acute trauma (Gaber 2005).

The best choice of contraceptive, always keeping in mind the possibility and necessity of consent of the girl and/or of the parents, is most probably a LARC. Many studies have been done on the use of intrauterine contraceptive systems with levonorgestrel even in young girls; this method allows good control of menstrual bleeding (Hillard 2012). Bone mass deficit is common in the disabled, due to limited mobility, weight problems (both over- and underweight), scoliosis, vitamin D deficit, and intake of medications that cause hypoestrogenism or directly influence bone turnover. Therefore it is important to avoid contraceptives that have a negative influence on bone mass, such as medroxyprogesterone acetate depot. Hormone preparations (estroprogestins and progestins) can be used if they are not specifically contraindicated for eventual comorbidities or possible drug interactions. Finally, before prescription one must verify that the substance will be taken regularly.

Postpartum and Breastfeeding Phase

This is a very unique time of life for women, which comports special involvement and particular vulnerability due to the physiological variations in mood, the new

tasks related to caring for the newborn, and the adaptation of the parents as a couple to this novel situation, especially with the birth of the first child. It is also a period in which undesired pregnancies may occur. Amenorrhea induced by exclusive breastfeeding, at least 6 times in 24 h, has been shown to have an efficacious contraceptive effect in the first 9 weeks postpartum, but this protection is not 100% sure. Therefore, certain protection against conception in this period requires use of a contraceptive. This is particularly important for women who have had a difficult pregnancy, for whom a new pregnancy would present medical or psychic risks. In any case, we note that the World Health Organization (WHO) advises an interval of at least 2 years between pregnancies for all women.

The choice of contraceptive must take into consideration both that the agents may be passed onto the newborn through the mother's milk and that they will have some impact on milk production in any case. Moreover in the postpartum period, women are at higher than normal risk of venous thromboembolism (VTE) venous thromboembolic disease (VTD); this risk returns to baseline levels only 6 weeks after giving birth (TepperNK et al. 2014). Non-breastfeeding women can use combined estroprogestin preparations if they do not present particular contraindications but preferably no earlier than 6 weeks after the birth. Actually, the adverse effects of these preparations have been shown to be insignificant at 41 days after birth even in women who do breastfeed, but there is a lack of follow-up studies appropriately designed to evaluate the effects over time of estrogens in the mother's milk. Progestin-only preparations (especially oral desogestrel and subcutaneous implants) are the best choice in the early phases of breastfeeding because they have no adverse effects on milk production, and the findings regarding the growth and development of the babies are reassuring. An intrauterine device (IUD) made of copper or that releases levonorgestrel can be inserted during Cesarean section or within 48 h of birth; but the expulsion rate is higher than normal in the month following the birth. A recent metanalysis showed that in any case, expulsion is more frequent in women in this period, especially with levonorgestrel-releasing IUDs (Jatloui et al. 2018). Also the risk of perforation is higher than normal during the first 6 months of breastfeeding.

Premenopause

During the final phase of fertility, women are much less prone to get pregnant, but undesired pregnancy may occur because they underestimate the possibility. Pregnancies in this period are often more risky than before for both the fetus and the mother. Therefore targeted contraception counseling is important for women in this age group (Hardman and Gebbie 2014) (Table 4).

The international guidelines do not specify contraindications for any contraceptive method solely on the basis of a woman's age. However, the prescription of hormone combinations for women over 40 requires attention to some specific items, including evaluation of cardiovascular and metabolic risk factors (based essentially on family history for cardiovascular conditions, dyslipidemia, diabetes), personal anamnesis (including eventual migraine and conditions involving chronic

Table 4 History for counseling in premenopause

Family and personal history for cardiovascular diseases or risk factors
Lifestyle (smoking, physical activity)
Pathological history (migraine, chronic diseases, depression)
Risk factors for bone mass deficiency
Symptoms related to menstrual cycle: heavy menstrual bleedings, premenstrual syndrome, menstrual disorders, diseases with catamenial exacerbation, hot flushes
Pelvic diseases (adenomyosis, fibromatosis, endometriosis)
Family history for neoplastic pathology related or not to sex hormone
Characteristics of couple relationship (risk of infections, partner's influence in contraceptive choice)
Personal acceptance of single method in relation to impact to sexuality

inflammation), gynecological and obstetric anamnesis (prolonged periods of hypoestrogenic status, preeclampsia, preterm delivery, gestational diabetes), and lifestyle (smoking, physical activity, weight). Note that smoking and episodes of migraine with or without aura are contraindications to use of estroprogestins in this age group. But also intracavital progestins can intensify migraine attacks in women with steroid hormone sensitivity. Blood pressure, BMI, and waist/hip ratio should be measured, and women should do blood chemistry tests as well as tests targeted to allow evaluation of cardiovascular risk to evidence eventual contraindications to use of preparations containing estrogens.

When anamnestic and clinical data discourage the use of estroprogestin, intrauterine contraceptives should be considered. They do not impact cardiovascular and metabolic risk; the only precaution necessary is to insert the device in appropriate clinical settings in cases of long QT. Progestin-only (oral, implant, or injection) contraception is a valid alternative for subjects who smoke, have diabetes or vascular conditions, suffer from migraine, or who are grossly overweight.

In the time preceding menopause, many women have heavy menstrual bleeding which can cause sideropenic anemia, sometimes associated with diffuse adenomyosis or fibromatosis. All hormonal contraceptives reduce menstrual bleeding, and this therapeutic effect can be the reason why women continue treatment. One should consider in particular use of a medicated IUD with levonorgestrel (release of 20 mcg/24h) that leads to 70% reduction of bleeding within 6 months. There is the possibility of occasional and sometimes prolonged spotting during the first months after insertion, especially in subjects with uterine disorders. The two natural estrogen preparations also result in noticeable reduction in bleeding, but the effect is not immediate.

Combined estrogen-progestin preparations are appropriate for women in this group who want contraceptive protection, especially those with family history of osteoporosis, have experienced periods of hypoestrogenic amenorrhea, or have been treated with drugs that have adverse effects on bone mass. We note that these preparations in particular appear to have a positive impact on the maintenance of both lumbar and femoral bone mass. However, there is no evidence that this results in reduced risk of fractures (Lopez et al. 2012).

Women in this age group frequently ask questions regarding the eventual relationship between contraceptive methods and cancer risk. Epidemiological studies have shown that both combined hormonal contraceptives and intrauterine devices reduce the risk of endometrial carcinoma; the effect is related to treatment duration and persists after suspension. Estrogen-progestin preparations and the LNG IUD (Jareid et al. 2018) have also been shown to have preventive effects on early-onset epithelial ovarian tumors; the preventive effects increase with the duration of use and persist long after suspension of treatment. Combined hormonal contraceptives also reduce the risk of carcinoma of the rectum and lymphatic and hematopoietic neoplasia, but they do cause a slight increase in the risk for carcinoma of the cervix (Iversen et al. 2017). Some recent studies have reported reduced risk for cervical neoplasia in the case of women who use a copper IUD (Cortessis et al. 2017). The findings on eventual changes in risk of developing breast carcinoma due to use of hormonal contraceptives are conflicting and do not refer specifically to perimenopausal women. The most recent studies, which include more low-dose estrogen preparations than previously, tend to indicate a current increased relative risk (albeit not elevated) especially with prolonged use. Minimal increased risk has been reported, but remains to be confirmed, also related to use of progestin-only pill (POP) and the levonorgestrel-releasing IUD. Due consideration must be given to other factors, such as lifestyle, in particular alcohol consumption and lack of physical activity which have much more negative impact on the individual risk than use of hormonal contraceptives. In any case, calm and objective presentation of the facts regarding the relationship between contraceptive use and cancer is important in helping a woman to make a pondered choice that is not based on fragmentary information and unmotivated worry.

There may be problems confirming the transition to irreversible menopause in women who are under contraceptive treatment that suppresses menstruation. Confirmation is based on FSH levels. In cases involving estrogen-progestin preparations, FSH should be measured during a pause in treatment: day 7 of the interval has been proposed, but day 14 is probably the best time. Women using the levonorgestrel IUD or progestin-only methods can do the test without interrupting treatment.

Another issue is that women in this group are still at risk of acquiring sexually transmitted infection, as documented in targeted studies (Darlympe et al. 2017). The possibility arises especially after the end of an important relationship and the beginning of a new one involving a mature partner who is considered "safe." (Individuals who use online dating services are at higher risk.) During consultation this topic should be addressed, with discretion, to uncover situations of violence and abuse which at any age involve higher risk of unwanted pregnancy and sexually transmitted infection (Maxwell et al. 2015) as well as serious repercussions on a woman's physical and psychological well-being.

It is always important to help women choose a contraceptive, and we must not forget that this applies to the disabled and those with chronic diseases. The international guidelines (WHO 2015; MMWR 2016; UK Medical Eligibility Criteria 2016) are available to everyone; they are updated regularly and include contraindications for the different methods. The methods are subdivided into specific categories of

risk: 1, no restrictions for use; 2, usually acceptable; 3, for use only in specific situations; 4, not recommended.

In any event, we recommend personalizing the choice of contraceptive, especially in the case of hormonal preparations, and we stress the importance of good pharmacological knowledge of the components in order to advise patients appropriately.

Components and Pharmacologic Characteristics of Hormonal Contraceptives

Combined Hormonal Preparations

These preparations contain two components, estrogen and progestogen. The progestogen inhibits ovulation; it causes changes in the cervical mucus, making it impenetrable, and in tubal motility; it has an antiestrogenic and secretory effect on the endometrium. The main action of estrogen is cycle regulation by proliferative action that stabilizes the endometrium and supports inhibition of ovulation.

Estrogens

The specific estrogens, synthetic or natural, used are ethinyl estradiol (EE), estradiol valerate (E2V), estradiol (E2), and, under development for contraceptive use, estetrol (Fig. 1).

EE profile. This is the estrogen mostly used in combination with hormonal contraceptives. It is more potent than estradiol due to the presence of the 17α-ethinyl group that gives good stability to the molecule. The ethinyl group prevents oxidation of the 17β-OH group, inhibiting its transformation into estrone. Cytochrome P450 3A4 oxidizes the ethinyl group leading to formation of an intermediary metabolite that is able to inhibit the cytochrome itself. This reduced inactivation comports a more pronounced effect of EE in the liver, up to 500–600 times greater than that of estradiol. Subsequently the EE molecule is hydroxylated in C2 and C4, forming catecholestrogens and EE sulfate in position 3. EE has a half-life of 24 ± 7 h and low affinity for SHBG which increases during treatment in a dose-dependent manner; 98.5% is bound with albumin with high bioavailability. EE has an important effect on the liver leading to the production of estrogen-sensitive proteins, in particular certain coagulation factors, angiotensinogen, SHBG, and CBG. The increase in SHBG that takes place during treatment with EE is considered the very expression of estrogenization in estrogen-progestogen combinations. Endometrial stability under associations containing EE is elevated; in fact type 2-17β-dehydrogenase, which stimulated by progestin causes rapid transformation of E2 into estrone, too weak to exercise good stimulation of the endometrium, has no effect on ethinyl estradiol.

About 10 years ago, formulations containing natural estrogens, such as estradiol valerate (quadriphasic association with dienogest, 26 + 2) and subsequently 17β-estradiol (monophasic association with nomegestrole acetate, 24 + 4), were added to the already available combined pills containing ethinyl estradiol. Studies are currently underway on the association of drospirenone combined with estetrol (E4), estrogen produced by the liver of the human fetus.

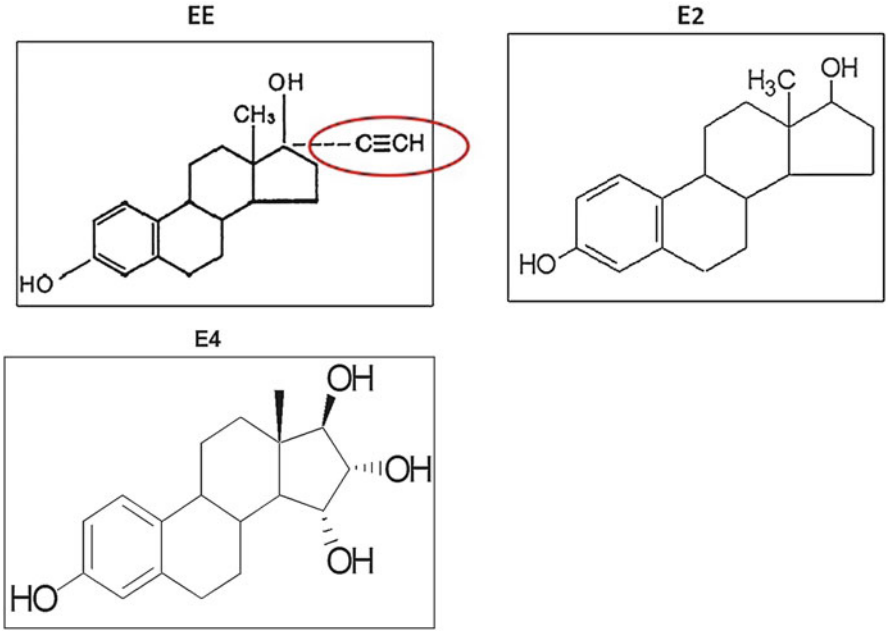


Fig. 1 Structures of ethinyl estradiol (EE), estradiol (E2), and estetrol (E4)

Table 5 Relative potency of estrogens

Estrogen	FSH	SHBG	CBG	Angiotensinogen
17β-E2	1	1	1	1
EE	120	600	500	350

E2V and E2 profile. E2 valerate (E2V) is a synthetic ester, specifically the 17-pentanoyl ester, of E2. Following absorption, E2V behaves as a prodrug, being cleaved by esterase during the initial gastrointestinal passage into E2 and valeric acid. The Cmax is 70 pg/ml, reached in 1.5–12 h after assumption of 3 mg E2V. E2V is virtually identical to E2 in pharmacokinetics and identical in pharmacodynamics and clinical effects. 1 mg of E2V is equivalent to 0.76 mg of E2. 38% of estradiol is bound to SHBG and 60% to albumin, and 2–3% circulates in free form. SHBG levels increase to 150% at the 28th day of treatment. Ninety-five percentage of estradiol is metabolized before entering the systemic circulation. Its main metabolites are estrone, estrone sulfate, and estrone glucuronide. The terminal half-life is 13–20 h due to enterohepatic recirculation and to the large circulating pool of estrogen sulfates and glucuronides. Circulating E2 has a 90-minute plasma half-life (Fruzzetti and Bitzer 2010; Trémollieres F1 2012) (Table 5).

Estetrol profile. Estetrol (E4) is synthesized by the liver of the human fetus during pregnancy only. Estetrol is present at 9 weeks of gestation with exponential increase of blood levels during pregnancy. At term the fetus produces about 3 mg/day. It is an estrogen agonist in the vagina, uterus, endometrium, vessels, bone, and brain, but

an estrogen antagonist for the breast if estradiol is present. The half-life is 30 h in women. The combined contraceptive, now being studied, with 15 mg estetrol and 3 mg drospirenone appears to give good cycle control. The preliminary data regarding impact on metabolism and coagulation are encouraging (Apter et al. 2017; Kluft et al. 2016).

Progestogens

The biological activity of synthetic progestins is mainly characterized by progestogenic activity (inhibition of ovulation, effect on cervical mucus, secretory changes in endometrium, modification of tube motility), residual androgenic activity, and the ratio between progestogen and androgenic activity (index of selectivity). The spironolactone derivative, drospirenone , also has antimineralocorticoid activity. The half-life of a progestin is an important factor in endometrial stabilization. See Table 6 for a classification of progestins.

Progesterone derivatives with progestational activity: nomegestrol acetate (NOMAc) and segesterone acetate (SGA)

NOMAc – Nomegestrol acetate is a 19-norprogesterone derivative with elevated progestational activity, noteworthy antigonadotropic activity, and 15% of antiandrogenic activity of cyproterone acetate. It has no estrogenic effects and no glucocorticoid activity. It exercises antiproliferative action on the growth of T47-D cells in the mammary gland in estrogen-free conditions and on enzymatic activity in mammary tissue (sulfatase and 17β-hydroxysteroid dehydrogenase) resulting in reduced production of estradiol (André 2005). Experiments on animals (ovariectomized rats) have shown that doses of 0.5 and 1 mg/kg/day NOMAc increase the amounts of allopregnanolone and of beta-endorphin in the hippocampus and hypothalamus, and 0.05 mg/kg/day enhances E2V action in the anterior pituitary gland and plasma (Lenzi et al. 2008). It has a half-life of more than 45 h.

SGA – Segesterone acetate is usually referred to by brand names Nestorone, Elcometrine, and Annovera. It is a norprogesterone derivative that binds selectively to progesterone receptors but not androgen receptors. It is not active when

Table 6 Classification of progestogens used in contraception

Pregnane derivatives	19 Nor testosterone derivatives	Spirolactone derivative
Pregnane derivatives acetylated	**Estranes**	**Gonanes**
Medroxyprogesterone ac. Drospirenone	Norethisterone	Levonorgestrel
Cyproterone ac.	Norethisterone ac.	*2nd generation*
Chlormadinone ac.	*1st generation*	
19 nor pregnane derivatives		
Nomegestrol ac.	Dienogest	Gestodene
Nestorone		Desogestrel
		Norgestimate
		3th generation

administered orally but is very potent when administered transvaginally, and it is highly effective at suppressing ovulation (Sitruk-Ware 2006). This synthetic progestin exhibits proliferative and protective neural effects in vitro and in vivo with no negative impact on brain function (possible neuroregenerative therapy) (Chen et al. 2018). Its half-life in the vaginal ring formulation is 4.5 h.

Progestogens with antiandrogenic activity: cyproterone acetate (CPA), drospirenone (DRSP) (see below – antimineralocorticoid activity), chlormadinone acetate (CMA), and nomegestrol acetate (NOMAc) (minor antiandrogenic activity; see above – progestational activity)

CPA – Cyproterone acetate is a powerful progestin that lowers serum concentrations of testosterone and delta-4-androstenedione by inhibiting LH. It also blocks the peripheral effects of androgens by inhibiting their binding with the receptors. In addition it inhibits 5α-reductase in the skin and the genital organs and increases the metabolic clearance of androgens; it does not bind to SHBG. It exhibits high progestogenic properties: the oral dose for endometrial transformation in woman (Kaufmann test) is 20.25 mg/cycle. The antigonadotropic effect is potent: the oral ovulation inhibitory dose is 1 mg/day. The antiestrogenic effect of CPA is weak (Koulouri and Conway 2008). CPA has a long half-life, 48 h, due to accumulation in fat tissue. This substance is not marketed in the USA. The combined formulation containing CPA and EE (35 mcg) is registered in Italy for "treatment of moderate to severe acne associated with androgen hypersensitivity (with or without seborrhea) and/or hirsutism in women of fertile age."

CMA – Chlormadinone acetate has 100% bioavailability with oral administration. Its half-life is reported to be 25–34 h after a single dose and 34–39, and even up to 89 h, after multiple doses. It accumulates in the adipose tissue, endometrium, myometrium, cervix, and tubes. It is excreted slowly; only 74% of the initial dose is eliminated after 7 days, at which time levels have stabilized. CMA suppresses prostaglandin synthesis in explants of human endometrium (Hanjalic-Beck et al. 2012). It has antiandrogenic action via receptorial competition (elevated AR affinity with doses as low as 0.5 mg) and inhibits 5α-reductase. Its antiandrogenic activity has been documented also in human and rat prostate. CMA increases allopregnanolone and beta-endorphins in selective brain areas, such as the limbic system (hippocampus and hypothalamus) and the anterior pituitary. It is hypothesized that this progestin might affect cognitive function, emotional state, and autonomic control (Pluchino et al. 2009).

Progestogen derivative with antimineralocorticoid activity: the spironolactone derivative drospirenone (DRSP)

DRSP – Drospirenone, a derivative of 17-α-spironolactone, has a pharmacological profile similar to that of natural progesterone, with clinically relevant antimineralocorticoid (Oelkers 2002) and antiandrogenic effects (30% of CPA). The antiandrogenic effect is due to its direct interference with androgen receptor, with 5α-reductase activity, and to inhibitory modulation of type 2 3β-OH steroid-dehydrogenase in the adrenal and in the ovary (Louw-du Toit et al. 2016). It modulates the mineralocorticoid receptor that regulates the renin-angiotensin-aldosterone system and, among other factors, the differentiation of preadipocytes into adipocytes blocking

adipogenesis, as has been well demonstrated in studies on 3T3-L1 adipose cells in vitro (Caprio et al. 2011). Drospirenone does not exercise estrogenic, androgenic, or glucocorticoid action. It has some interesting neuroactive properties, evidenced by the increases in allopregnanolone and β-endorphins. A reduced stimulation of mammary tissue is also documented. DRSP has a half-life of approximately 30 h; it does not bind to SHBG or CBG.

Testosterone derivatives: levonorgestrel (LNG), gestodene (GSD), desogestrel (DSG), norgestimate (NGM), norelgestromin (NGMN), and dienogest (DNG)

LNG – Levonorgestrel is a very potent second-generation progestin with elevated antigonadotropic activity (the ovulation-inhibiting dose is 50 mcg/day) (Schindler et al. 2003). There is no significant inactivation from the hepatic first-pass metabolism. It is antiestrogenic, it has 3–4% of androgenic activity, and its anabolizing activity is 20–30% that of testosterone propionate. The half-life (associated with EE) is 36 ± 13 h.

GSD – Gestodene is a third-generation progestin known for its lower androgenic activity and higher index of selectivity (progestogen/androgen activity ratio) than LNG (40 vs. 8.8) as well as for being more potent than LNG. The inhibition ovulation dose is 40 mcg/day. The half-life increases from the initial 12–18 h due to inhibition of its own catabolism (Stanczyk and Archer 2014). It is not metabolized after the first passage through the liver resulting in elevated bioavailability.

DSG – Desogestrel is a third-generation progestin, a gonan that can be considered a prodrug. After absorption in the gastrointestinal tract, it is metabolized by the liver, becoming 3-keto-desogestrel (etonogestrel). It subsequently enters into the blood stream where 96% binds to serum proteins (albumin and SHBG). DSG has a high index of selectivity, 26, compared to the 8.8 of LNG, which accounts for the scarcity of androgenic side effects, even with high doses. A minimum dose of 60 mcg/day is necessary to inhibit ovulation, and bioavailability is 84% (Grandi et al. 2014a).

NGM – Norgestimate, a progestin with weak androgenic activity, is rapidly metabolized into norelgestromin (formerly known as 17-deacetylnorgestimate or levonorgestrel-3-oxime), the main active metabolite; into levonorgestrel; and, in minimal part, into 3-keto-norgestimate (Ahire et al. 2017).

NGMN – Norelgestromin is an active metabolite of norgestimate; the oxime group in position C3 limits its capacity to bind with androgen receptors, thus minimizing androgenicity. It has elevated selectivity for progestin receptors and minimal affinity for androgen receptors (Paris et al. 2015). NGMN does not interact with SHBG.

DNG – Dienogest is a 19-nortestosterone derivative, with the 17α-ethinyl group indicative of 19-nortestosterones (as levonorgestrel) replaced with a 17α-cyanomethyl group. It is characterized by antiproliferative effect on specific cell populations and by anti-inflammatory effect with decreased expression of TNF-α and interleukin-6 with immunomodulatory action. Dienogest treatment improves progesterone resistance in endometrial tissue by increasing the relative expressions of PR-β and PR-α and decreasing the relative expressions of ERβ and ERα and exerts an inhibitory action on key genes involved in PGE2 synthesis (Tosti et al. 2017). It also suppresses aromatase and COX-2 gene expression and decreases

VEGF and NGF with anti-angiogenic and anti-neurogenic effect (Ichioka et al. 2015). Dienogest has negligible binding affinities for estrogen, glucocorticoid, and mineralocorticoid receptors and low binding affinity for androgen receptor in vitro. It exhibits antiandrogenic activity in vitro (cell cultures of prostate cancer specimens) and in vivo (seminal vesicles of gonadectomized male rats). It does not bind to SHBG, and 9% is in a biologically active free form.

Routes of Administration of Estrogen-Progestin Contraception: Oral, Vaginal, and Transdermal

See Table 7.

Vaginal Ring

The specific characteristics of the vagina, with its richly vascularized and easily permeable epithelial lining, allow rapid absorption of steroids that are in the circulatory system, avoiding intestinal absorption and initial passage through the liver. The vaginal ring or transdermal patch are the best choice for women with intolerance to lactose (contained in almost all oral formulations) or with galactosemia. Good compliance is an added advantage with these two methods because there is no daily pill or medication to remember (Table 8).

Table 7 Oral estro-progestin associations

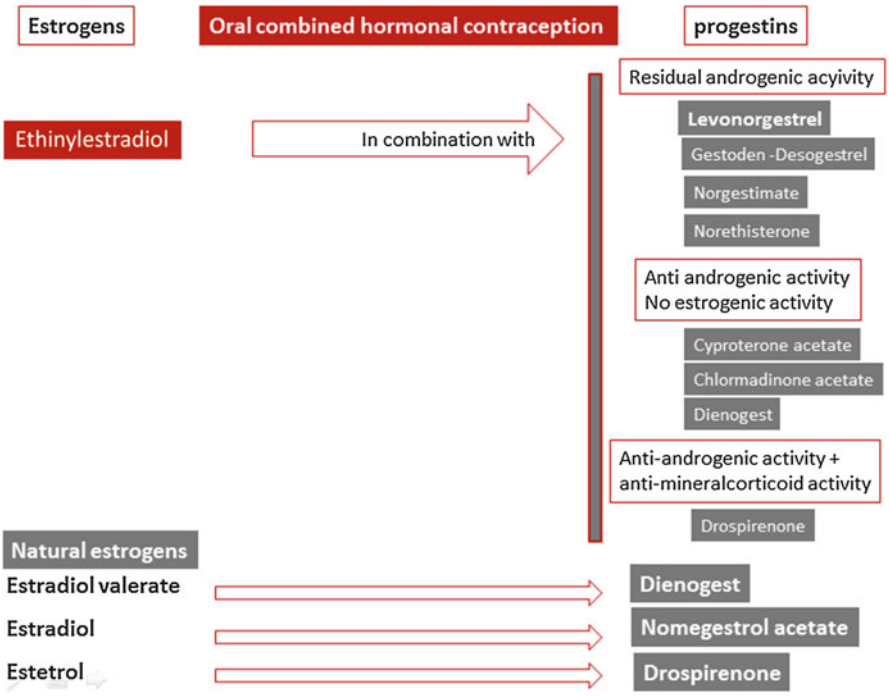


Table 8 Steroid release from combined vaginal ring

Steroid release from combined vaginal rings	
Association with Ethinyl estradiol	**Association with estradiol**
EE 30 mcg + NETA 650 mcg 850 mcg EE 20mcg + NETA 1000 mcg	E2 140 mcg+ NETA 700 mcg
	E2 200 mcg + MAP 700 mcg
	E2 150 mcg + Lng 250 mcg 180 mcg + Lng 290 mcg
	E2 300 mcg + NOMAc 700 mcg + NOMAc 900 mcg
EE 15 mcg + ENG 120 mcg	E2 300 mcg + ENG 100 mcg + ENG 125 mcg
EE 13 mcg + Segesterone acetate (Nestorone ®) 150 mcg	E2 10 – 20 – 40 mcg + Segesterone acetate (Nestorone ®) 200 mcg

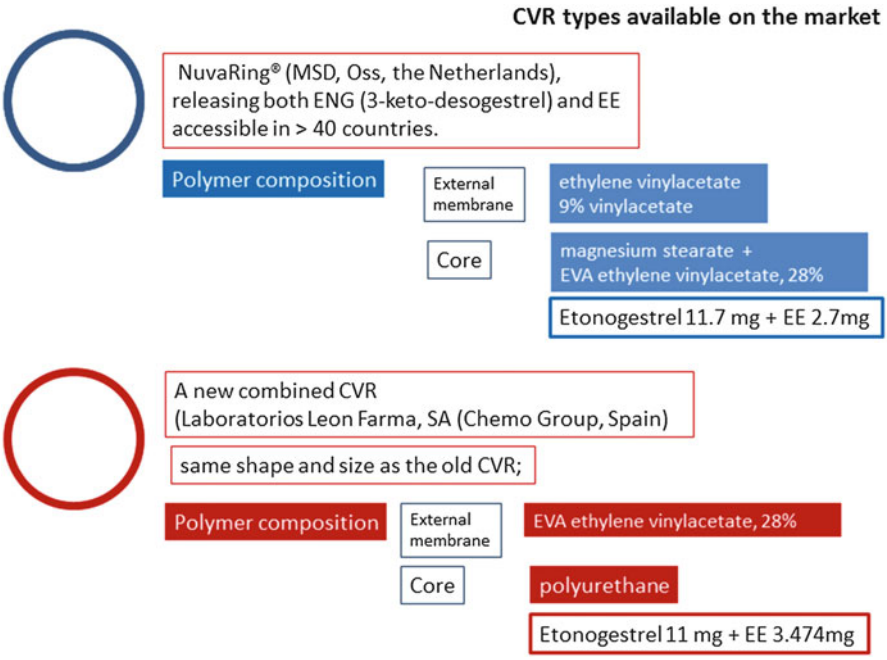
The development of a contraceptive ring started in the late 1960s, when Mishell and Lumkin presented a medroxyprogesterone acetate-releasing ring. Subsequently the first contraceptive ring marketed (NuvaRing® developed by NV Organon, Netherlands) released 120 mcg etonogestrel (ENG, 3-keto-desogestrel) and 15 mcg ethinyl estradiol (EE) per day. The steroid content was etonogestrel 11.7 mg + EE 2.7 mg. Very recently Laboratorios Leon Farma, SA (Chemo Group, Spain), has developed a ring (Ornibel®) of the same shape and size with steroid content of 11 mg etonogestrel + 3.474 mg EE (Algorta et al. 2013) (Table 9).

We note that a minor risk of bacterial vaginosis had already been seen in women using other combined hormonal contraceptives and progestin-only formulations (Riggs et al. 2007) (injection or implant). There is ample documentation of increased lactobacilli in women during use of vaginal rings with EE and ENG as well as in those with bacterial vaginosis. There is no increase in the risk of vaginal candidiasis, although leukorrhea may intensify (De Seta et al. 2012).

Transdermal Hormonal Patch

The first transdermal hormonal contraceptive was marketed in the USA in 2001 (Ortho-EVRA) and subsequently in Canada and Europe (EVRA). The US version of the patch contains 0.75 mg ethinyl estradiol and 6 mg norelgestromin.

Table 9 Combined contraceptive vaginal rings available on the market



The European/Canadian formulation contains 0.60 mg of ethinyl estradiol and 6 mg of norelgestromin (Abrams et al. 2001) and releases 33.9 mcg EE and 203 mcg norelgestromin per day. The EE exposure values are very similar to those for cycle 2 with the 35 mcg oral contraceptives. During patch use, hormone levels avoid the peaks and troughs seen with daily pill use. Although Cmax levels are higher with the pill than is currently possible with the patch, the area under the curve analysis shows 60% more estrogen exposure with patch use (Milanes-Skopp and Nelson 2009).

Progestin-Only Contraception

The choice of a progestin-only method (Table 10) depends on the specific needs specific demands, prevalently metabolic, of the user, whether adolescent or adult. The long-acting (LARC) methods requiring intake or other application less than once a month are of particular interest.

Oral Preparations

Desogestrel (DSG): Continuous daily intake of 75 mcg inhibits ovulation in 98% of cases. The secondary effects are changes in the cervical mucus which becomes impenetrable, endometrial changes which make implantation either difficult or impossible, and changes in tubal mobility similar to the effects of other POPs.

Table 10 Progestin-only contraception

SARC (short-acting reversible contraception)
Oral preparations:
Desogestrel 75 mg, norethisterone 350 μg, LNG 30 μg (continuous regimen)
Drospirenone 3 mg (24 + 4 regimen)
LARC (long-acting reversible contraception)
Monthly injections:
Medroxyprogesterone ac. depot 150 mg im
Medroxyprogesterone ac. 104 mg sc
Norethisterone enanthate 200 mg im
Implants:
Releasing levonorgestrel 150 mg
Etonogestrel 68 mg
Intrauterine systems:
Releasing levonorgestrel

Pearl index is equivalent to that of combined hormonal contraceptives (IP 0.41 for the entire population studied, including subjects with low compliance, IP 0.17 for women who are not breastfeeding) (Collaborative Study Group on the Desogestrel-containing Progestin only Pill 1998). Contraceptive protection continues for 12 h beyond the intake time in case of forgetfulness. Irregular bleeding is the main complaint, as with all long-term progestin treatments. This leads to alterations in the physiological angiogenesis of the endometrial mucosa with modifications in the spiral arteries and increases in mediators of oxidation status. About 30% of young subjects have amenorrhea or very infrequent bleeding within 12 months; 30–40% have three to five episodes of irregular bleeding per trimester; and 10% have more than two episodes a month. There are no adverse effects on bone mineral density in subjects who have reached peak bone mass, because mean blood levels of E2 are 73.5 pg/ml during treatment with 75 mg desogestrel.

Norethisterone (NET) 350 μg daily has a relatively short half-life: high concentrations are present in blood during the first 4 h after administration; the level decreases slowly over time, and levels remain considerable (5%) at 24 h. Therefore, timed daily administration is imperative. The contraceptive effect is based on the action on cervical mucus. NET may be partially metabolized into EE: aromatization of NET to EE was demonstrated in human placental microsomes, liver homogenates, human ovarian tissue, and human hepatocytes (Yamamoto et al. 1986). Daily intake of an oral contraceptive containing 1 mg NET leads to significant production of EE (20 mcg).

Levonorgestrel (LNG): The main effects of continuous daily intake of 30 mcg are seen in the cervical mucus and endometrial lining; inhibitory effect on ovulation has not been demonstrated in all users. Protection is lost if the pill is taken more than 3 h late. The most important side effect is irregular bleeding (Zigler and McNicholas 2017). No significant variations in lipid profile have been documented.

Drospirenone (DRSP): Investigations are underway on a formulation containing 4 mg drospirenone to be taken for 24 days followed by 4 days of pause to achieve

better bleeding control by inducing a "suspension flow." Ovulation inhibition is maintained despite the 4-day hormone-free period and even in the event of multiple intentional 24-hour delays in tablet intake (Duijkers et al. 2016). The efficacy against ovulation is comparable with that of continuous desogestrel use. Hemostasis is not affected (Regidor et al. 2016).

Long-acting reversible contraceptives (LARC) are now widely recommended by public health agencies and healthcare professionals because they facilitate adhesion to contraception. In addition to monthly injections, the most used methods are implants and intrauterine devices and systems.

Monthly Injections: The preparation mostly used for this method is medroxyprogesterone acetate depot, 150 mg every 3 months. This progestin has residual glucocorticoid activity which accounts partially for the possible negative vascular and metabolic impact (Glisic et al. 2018). It also has a negative effect on bone mass in young subjects. A subcutaneous formulation (104 mg) has been introduced more recently to allow possible self-administration.

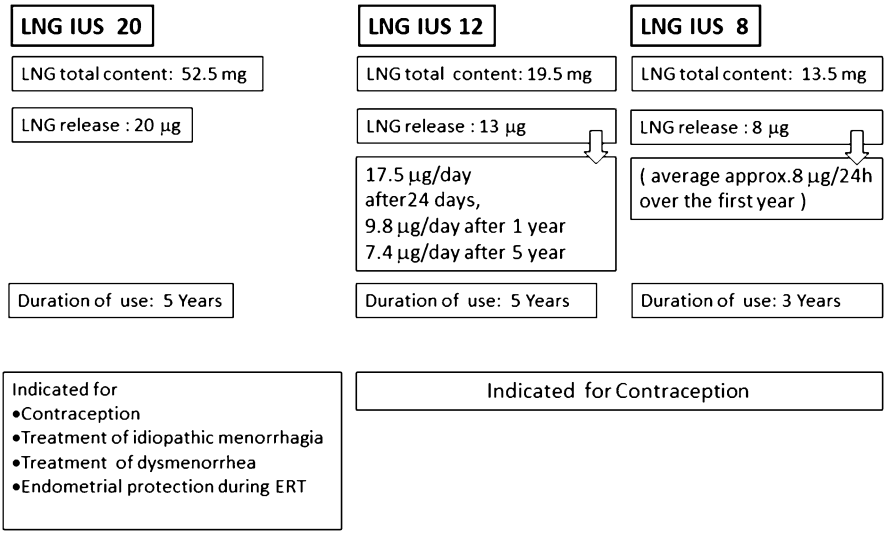
Injectable norethisterone enanthate (NETE), 200 mg every 2 months, is advised especially during puerperium or after abortion.

Implants: The most used is etonogestrel (ETG) hormonal implant, a flexible device that is inserted subdermally in the inner side of the upper non-dominant arm. The insertion point is at 8–10 cm above the medial epicondyle. It contains 68 mg ETG and releases 60–70 mcg/day during the first 5–6 weeks, 35–45 mcg at the end of the first year, 30–40 mcg at the end of the second, and 20–30 mcg at the end of the third (mean concentration 150 pg/ml). The principal mechanism of action of this contraceptive implant is the inhibition of ovulation (Graesslin and Korver 2008): the minimal inhibiting dose is 90 pg/ml. Thickening of cervical mucus and alteration of the endometrial lining are additional contraceptive effects. Pregnancy rate (0.05%) remains low in obese, overweight, and normal-weight users of the contraceptive implant, independent of body mass index (BMI) class (Xu et al. 2012). The duration of action after subdermal implantation is 3 years, but the etonogestrel implant probably continues to be effective for longer.

Levonorgestrel Intrauterine Systems: The first LNG-IUS has been available in Europe since the 1990s and in the USA since 2001. The total LNG content is 52 mg, and the daily release rate is 20 mcg for 5 years (LNG-IUS 20). Two new systems have been introduced since then: the LNG-IUS containing 13.5 mg and with 8 mcg release rate (LNG-IUS 8) and the LNG-IUS with 19.5 mg (LNG-IUS 12); the release rate of the latter is approximately 17.5 mcg/day after 24 days and decreases progressively to 9.8 mcg/day after 1 year and to 7.4 mcg/day after 5 years (Apter et al. 2014) (Table 11).

The LNG-IUS has a complex mechanism of action; it does involve some foreign body reaction, but all the systems cause evident modifications in cervical mucus that make it unfavorable for penetration by spermatozoa, and the ferning test is practically negative. The LNG-IUS 20 is the IUS that exercises the strongest progestational action on the endometrium: LNG concentrations range from 470 to 500 ng/g in the endometrial tissue, producing greater endometrial atrophy than that seen with the LNG-IUS 8, which usually leads to secretory endometrium. These characteristics evidence the particular therapeutic action of the LNG-IUS 20

Table 11 Characteristics of currently available intrauterine contraceptive systems



(in unison with its contraceptive efficacy). Its effects on the endometrium involve downregulation of E and P receptors, IGF-I mRNA suppression with increased mRNA codification for IGF-II and IGFBP-1, and increase in FAS expression with apoptosis. Reduction of mastocyte content in eutopic and ectopic endometrium and of various nerve growth factors in endometrium and myometrium has also been documented (Engemise et al. 2011; Rutanen 2000; Choi et al. 2010).

The LNG-IUS 8 has a prevalently contraceptive action, whereas its currently known principal therapeutic effect is reduction of dysmenorrhea. The LNG-IUS 20 has a minimal effect on ovulation, and the effect of the LNG-IUS 8 is irrelevant. Blood levels of estrogens are about 100 pg with both systems, but they are slightly higher with the LNG-IUS 8, which is an important factor for young women because of the problem of bone mass protection.

Risks Related to Hormonal Contraceptive Use

The contraindications to combined hormonal contraceptive use are specified in numerous guidelines (WHO 2015; MMWR 2016; UK Medical Eligibility Criteria 2016) where they are listed in different categories of risk, according to specific clinical conditions (Table 12). We will focus on few particular situations.

Bone Mass Risk

There are discordant data regarding probable negative effect of combined hormonal contraception on bone mass acquisition in subjects who are in the earliest years of post-menarche. Several prospective studies (but no randomized placebo-controlled

Table 12 Contraindication to combined hormonal contraceptive use. (From UK Medical Eligibility Criteria for Contraceptive Use 2016)

Category	Condition	
1	Postpartum >21 days in non-breastfeeding women Immediately after first- or second-trimester TOP Minor surgery without immobilization Varicose veins	Unrestricted use
2	Obesity (BMI ≥ 30–34 kg/m^2) Family history of VTE in a first-degree relative Major surgery without prolonged immobilization Superficial thrombophlebitis Sickle cell disease	Benefits > risks Broadly usable
3	Postnatal <21 days in non-breastfeeding women Ex-smoker stopped <1 year ago Obesity (BMI ≥35 kg/m^2) Family history of VTE first-degree relative age≥45 years	Risks > benefits Counsel/caution
4	Postnatal 0 to < 6 weeks in breastfeeding women Postnatal 0 to < 6 weeks in non-breastfeeding women Smokers > 15 cigarettes/day and >35 year History of cerebrovascular accident, including TIA History of VTE or current VTE (on anticoagulants) Current and history of ischemic heart disease Known thrombogenic mutation (e.g., factor V Leiden, prothrombin mutation, protein S, protein C, and ATIII° deficiencies) PA systolic ≥160 o diastolic ≥100 mmHg SLE with positive antiphospholipid antibodies Complicated valvulopathies (AF risk, history of endocarditis, pulmonary hypertension) Diabetes with retinopathy/nephropathy/ neuropathy Major surgery (>30′ duration) with prolonged immobilization Migraine with aura Impaired cardiac function and atrial fibrillation	Unacceptable risk Do not use

trials) reported that adolescent CHC users gained less spinal BMD than nonusers; a recent meta-analysis documented that CHC use is associated with significant negative changes over 24 months (Goshtazebi et al. 2019). An association with specific formulations did not emerged, and data about recovery after stopping use were not evident. We have anyway to consider that BMD is a surrogate marker for fracture risk, probably not valid for very young women (WHO 2015). However, since the mechanisms responsible for this effect are not clear, there is insufficient information to establish dosages and specific combinations to minimize the impact on bone mass.

Both oral POP and ETG implant appear to maintain levels of circulating 17β-estradiol adequate for bone mass protection. Injectable medroxyprogesterone acetate, instead, is associated in adolescent with significant BMD decrease in lumbar spine, with DMPA exposure at dose 104–150 mg IM every 12 weeks.

Depression Risk

Use of hormonal contraceptives (estro-progestins, and especially POP, including implants and the high-dose LNG-IUS) can affect mood but rarely cause true depression. There have been reports indicating that non-androgenic progestins are

less likely to have such effects. In any case it is important to take into account individual vulnerability to steroids, not always identifiable on the basis of anamnesis in younger subjects. Use of POP is certainly not the first choice for subjects who have experienced episodes of major depression (Merki-Feld et al. 2017).

Influence on Sexual Desire

Another possible, unpredictable side effect of hormonal contraceptives is reduced sexual desire. This effect is in part related to mood, and it affects about 15% of subjects who use contraceptives, prevalently non-adolescent women. In the cross-sectional analysis of 1,938 of the 9,256 participants enrolled in the Contraceptive CHOICE Project, more than 1 in 5 participants (23.9%) reported lacking interest in sex at 6 months after initiating a new contraceptive method. CHOICE participants using depot medroxyprogesterone acetate, the contraceptive ring, and implant were more likely to report a lack of interest in sex compared to copper IUD users (Boozalis et al. 2016). Subjects who start taking low-dosage estroprogestin preparations at a young age are more apt to develop acquired vestibulodynia, which is reversed after interruption of treatment (Johanesson et al. 2008).

Cardiovascular Risk

One particularly intricate issue in choosing a woman's first contraceptive is what type of hormonal contraceptive is best (COC or progesterone-only) in relation to cardiovascular risk, in particular risk of venous thromboembolic disease venous thromboembolism (VTE). Every woman is a variable with her own metabolic make-up, at each age, and she is the fundamental element in every choice. Even among healthy women of reproductive age who do not use hormonal contraceptives, there is an incidence of VTE in 1–5 individuals per 10,000 women per year, and the incidence rises with age. The risk of VTE for women using combined hormonal contraceptives, both oral and other methods, is low in absolute terms (less than 6–12 events per l0, 000 women years of exposure). The risk is even lower for women using second-generation progestin associations, which carry a risk of VTE two to three times higher than that for women who do not use a hormonal contraceptive (Lidegaard et al. 2009). The risk is related to EE dose. Reducing EE content reduces the risk. Ethinyl estradiol has a strong impact on liver proteins, and it seems to be responsible for the slight changes in procoagulation and fibrinolytic balance irrespective of the route of administration. Formulations with non-androgenic or antiandrogenic progestins and EE with relatively higher estrogen content, expressed by SHBG increase, seem to comport increased VTE risk (Raps et al. 2012). In fact SHBG levels are correlated to APC resistance. Decreased protein S and increased activated protein C (APC) resistance may explain the elevated risk of VTE during oral contraceptive use. The more androgenic progestins there are in the EP combinations, the better they are able to counteract the potent EE-induced stimulation of liver proteins and coagulation factors. As early as 1996, the Transnational Research Group on Oral Contraceptives evidenced the minor risk of VTE VTDin the combined contraceptive containing levonorgestrel plus 30 mcg EE compared to other EP associations tested (the same EE dose plus either gestodene, norgestimate, or

desogestrel). Subsequently certain important case control studies and meta-analyses (Martínez et al. 2012; Stegeman et al. 2013; Bateson et al. 2016) as well as a Cochrane review (De Bastos et al. 2014) confirmed the lower risk for levonorgestrel associations and/or second-generation progestins.

The relative VTE risk with preparations containing drospirenone, gestodene, desogestrel, or cyproterone acetate combined with 30–35 mcg EE seems to be substantially the same. Another meta-analysis shows that GSD/20EE and NRG/35 EE combinations present risks similar to those with LNG + EE 20/30 mcg. The issue regarding the relationship between VTE and COC is still controversial. A prospective controlled study (Dinger et al. 2016) (conducted in seven European countries) evaluated the incidence of cardiovascular events (VTE, arterial thromboembolism, congestive heart failure) in 59,510 women followed up for about 10 years. The study included three cohorts of patients: users of COC containing 30 mg EE + 3 mg DRSP; COC containing 30 mg EE + LNG; and COC containing EE + other progestins. The study (LASS-OC) did not evidence significant differences in events and event frequency in the three cohorts; however, the formulation containing DRSP was found to present statistically lower risk of arterial thromboembolism than the combinations with LNG and other progestins. The estrogen component usually has a positive effect on lipid asset and consequently on arterial risk, in particular when associated with a non-androgenic or antiandrogenic progestin. A review done in 2012 (Lidegaard et al. 2012) evidenced modest arterial risk for COC users, with 0–0.5 cases of myocardial infarct in 10,000 subjects: no cases with DRSP formulations; 0.58 for DSG formulations; and 0.37 for GSD formulations. There is a net increase in risk of nonfatal acute myocardial infarct (IMA) for smokers of more than 25 cigarettes per day. Age amplifies the problem to the extent that use of COC by women over 35 who smoke is considered a Category 4 risk. Regarding stroke risk, there were 0.8–1.5 cases in 10,000 in women taking 20 mg EE. A later Cochrane review (Roach et al. 2015) suggested a slight increase in risk of ischemic stroke and myocardial ischemia in women who took formulations with high doses of ethinyl estradiol. In any case, smoking, migraine, hypertension, and diabetes all enhance risk.

The first epidemiological data reported on COC with natural estrogens was related to the E2V/dienogest combination. The findings showed an absolute risk of VTE VTD of 7.2 in 10,000 women, similar to the risk reported for COCs containing LNG or other progestins (Grandi et al. 2014b). This combination was found to be metabolically neutral regarding lipid asset. The preparations with natural estrogens, also given the characteristics of the associated progestins, appear to have little impact on glucose metabolism. In fact data on insulin sensitivity are more favorable in combinations with progestins that are free of androgenic activity and/or with antiandrogenic activity. Vaginal contraceptives containing etonogestrel have been found to be advantageous (Cagnacci et al. 2009).

Cardiovascular Risk in Adolescents

Although episodes of venous thromboembolism are very rare in adolescents, the physician must pay careful attention to family history (Noboa et al. 2008) even when

the history of the subject requesting a hormonal contraception does not present any contraindications. A thrombotic episode in a first-degree relative aged 45 or under or thrombophilic diathesis in a first- or second-degree relative comports higher risk (Zoller et al. 2011), with the result that UK guidelines (UK Medical Eligibility Criteria 2016) suggest the use of progestogen-only contraception in these cases. In the absence of contraindications, special consideration should be given to low-release LNG intrauterine systems, the LNG-IUS 8 or LNG-IUS 12, which do not suppress ovulation.

Acute arterial conditions are also very rare in adolescents, but since they become more probable in subjects who take cocaine or other amphetamine derivatives, the individual's lifestyle must be appropriately investigated to evidence use of these substances. Migraine with aura, sometimes related to incomplete closure of the oval foramen, can increment arterial risk and is a contraindication to the use of COC even in this age group.

Benefits of Hormonal Contraceptive Use

Heavy Menstrual Bleeding (HMB)

Early anecdotal evidence, but only few randomized trials (Lethaby et al. 2019), strongly support the impact of COC on reducing menstrual bleeding volume and preventing the risk of secondary anemia. COC at different dosages are used in the management of dysfunctional heavy menstrual bleeding from menarche but also in adenomyosis, leiomyomas, and hemostasis disorders. Assessment of ovulatory status, structure of the endometrial cavity and myometrium, and, when indicated by the history, screening for coagulation disorders are anyway advisable in a diagnostic work-up, but are not recommended before starting a hormonal treatment in emergency. Routine ultrasound should not be obtained solely for the work-up of HMB in adolescents, as the majority of causes are nonstructural. A recent study showed that only 2 of 156 adolescent patients were found to have a structural abnormality, and ultrasound findings did not alter the management plan for any of the patients (Pecchioli et al. 2017).

Combinations with natural estrogens are generally effective after the first 2 months of treatment; the estradiol valerate-dienogest preparation has FDA approval for this specific use. Progestin-only contraception may also be a good choice because of its suppressive effect on menstrual bleeding with long-term use. The LNG-IUS is the treatment of choice for women in their reproductive years who have no identified uterine pathologies, fibroids <3 cm in diameter, or adenomyosis. Intrauterine levonorgestrel is a conservative therapeutic method for young patients with early-stage endometrioid endometrial cancer (EEC) and atypical hyperplasia (EAH). Follow-up is recommended.

Pelvic Pain Related to Endometriosis and Dysmenorrhea

Hormonal contraceptives (preferably in extended regimen) reduce endometriosis-associated dyspareunia, dysmenorrhea, and acyclic pain caused by endometriotic

implants. A progestin-only pill is theoretically preferable, because implants are estrogen sensitive, but there is evidence that an ectopic endometrium undergoes apoptotic stimulation even with estrogen-progestin combinations (Meresman et al. 2002). The LNG-IUS may be effective in cases of pain related to rectovaginal endometriosis and adenomyosis. All hormonal contraceptives have impact on primary menstrual pain and dysmenorrhea secondary to genital malformations. However, in the event of disappointing results, the prescription can be changed to an extended regimen, either fixed or flexible (i.e., interrupting treatment with a 3- or 4-day hormone-free pause after 24 days of consumption in cases of more than 3 consecutive days of bleeding or spotting).

Hyperandrogenic Status

Hormonal contraceptives are indicated for patients with moderate signs of hyperandrogenism (acne, mild hirsutism) who want contraceptive treatment. The reviews of placebo-controlled trials do not show consistent differences between COC types (Arowojolu et al. 2012), but extensive experiences indicate that combinations containing ethinyl estradiol (which increases SHBG levels) and progestins with antiandrogenic activity (cyproterone acetate, dienogest, drospirenone, etc.) achieve better results. Antiandrogenic activity is the result of various effects: interaction with androgen receptor, inhibition of peripheral 5α-reductase activity, suppression of ovarian steroidogenesis, and inhibition of adrenal 3β-OH steroid-dehydrogenase Type 2. We note that effective contraception is always required when an antiandrogen alone or a retinoid is prescribed to avoid pregnancy with possible teratogenic effects on the fetus.

Premenstrual Syndrome and Catamenial Symptoms

The term premenstrual syndrome includes an array of predictable physical, behavioral, and affective symptoms that women may experience in the luteal phase of the menstrual cycle. Approximately 20–30% of women of fertile age suffer from this syndrome, and about 6% of young women experience very severe psychological symptoms with heavy functional and social impairment, so-called premenstrual dysphoric disorders (Lete and Lapuente 2016). An integrated approach is necessary in these situations. Use of an estrogen-progestin combination is usually helpful, especially when the preparation contains a progestin with long plasma half-life and whose metabolites interact with the GABA receptors, opioid system, and serotonin pathways in the central nervous system resulting in an overall sedative and anxiolytic effect. Current guidelines indicate the combination with drospirenone as the first-choice prescription, especially for premenstrual dysphoric disorder, but also other combinations have been demonstrated to be efficacious. The use of extended regimens is an interesting option that must be considered in cases requiring control of the catamenial exacerbations of various diseases, such as headache, seizures, allergic manifestations, sleep disturbances, irritable bowel disease, and dysphoria (Nappi et al. 2016). Special attention must be given to the complete pharmacological history of these particular patients to avoid potential drug interactions.

Conclusion

Contraception is not properly a therapy, but a preventive choice. Sometimes therapeutic use may facilitate the use of an effective contraceptive. Anyway, it is important to know well pharmacological properties and specific effects of various hormonal contraceptives, as well as the characteristics of the subject we are in front of, in order to tailor the choice.

Cross-References

- ▶ Abnormal Uterine Bleeding
- ▶ Endometriosis
- ▶ The Polycystic Ovary Syndrome (PCOS)

References

Abrams LS, Skee DM, Natarajan J, Wong FA, Lasseter KC. Multiple-dose pharmacokinetics of a contraceptive patch in healthy women participants. Contraception. 2001;64(5):287–94.

Ahire D, Sinha S, Brock B, Iyer R, Mandlekar S, Subramanian M. Metabolite identification, reaction phenotyping, and retrospective drug-drug interaction predictions of 17-deacetylnorgestimate, the active component of the oral contraceptive norgestimate. Drug Metab Dispos. 2017;45:676–85.

Algorta J, Diaz M, de Benito R, Lefebvre M, Sicard E, et al. Pharmacokinetic bioequivalence, safety and acceptability of Ornibel®, a new polymer composition contraceptive vaginal ring (etonogestrel/ethinylestradiol 11.00/3.474 mg) compared with Nuvaring® (etonogestrel/ethinylestradiol 11.7/2.7 mg). Contraception. 2013;87:264–72.

André G. Actions of a 19-norprogesterone derivative on mammary gland: nomegestrol acetate. J Gynecol Obstet Biol Reprod (Paris). 2005;34(1 Pt 1):69–84.

Apter D, Gemzell-Danielsson K, Hauck B, Rosen K, Zurth C. Pharmacokinetics of two low-dose levonorgestrel-releasing intrauterine systems and effects on ovulation rate and cervical function: pooled analyses of phase II and III studies. Fertil Steril. 2014;101(6):1656–62.

Apter D, Zimmerman Y, Beekman L, Mawet M, Maillard C, et al. Estetrol combined with drospirenone: an oral contraceptive with high acceptability, user satisfaction, well-being and favourable body weight control. Eur J Contracept Reprod Health Care. 2017;22(4):260–7.

Arowojolu AO, Gallo MF, Lopez LM, Grimes DA. Combined oral contraceptive pills for treatment of acne. Cochrane Database Syst Rev. 2012;11(7):CD004425.

Bateson D, Butcher BE, Donovan C, Farrell L, Kovacs G, et al. Risk of venous thromboembolism in women taking the combined oral contraceptive: a systematic review and meta-analysis. Aust Fam Physician. 2016;45(1):59–64.

Boozalis A, Tutlam NT, Robbins CC, Peipert JF. Sexual desire and hormonal contraception. Obstet Gynecol. 2016;127(3):563–72.

Byrne G. Prevalence and psychological sequelae of sexual abuse among individuals with an intellectual disability: a review of the recent literature. J Intellect Disabil. 2018;22(3):294–310.

Cagnacci A, Ferrari S, Tirelli A, Zanin R, Volpe A. Route of administration of contraceptives containing desogestrel/etonogestrel and insulin sensitivity: a prospective randomized study. Contraception. 2009;80(1):34–9.

Caprio M, Antelmi A, Chetrite G, Muscat A, Mammi C, et al. Antiadipogenic effects of the mineralocorticoid receptor antagonist drospirenone: potential implications for the treatment of metabolic syndrome. Endocrinology. 2011;152(1):113–25.
Chang T, Davis MM, Kusunoki Y, Ela E, Hall KS, Barber JS. Sexual behavior and contraceptive use among 18 to 19 year old adolescent women by weight status: a longitudinal analysis. J Pediatr. 2015;167(3):586–92.
Chen S, Kumar N, Mao Z, Sitruk-Ware R, Diaz Brinton R. Therapeutic progestin segesterone acetate promotes neurogenesis: implications for sustaining regeneration in female brain. Menopause. 2018;25(10):1138–51.
Choi YS, Cho S, Lim KJ, Jeon YE, Yang HI, et al. Effects of LNG-IUS on nerve growth factor and its receptors expression in patients with adenomyosis. Growth Factors. 2010;28(6):452–60.
Collaborative Study Group on the Desogestrel-containing Progestin only Pill. A double-blind study comparing the contraceptive efficacy, acceptability and safety of two progestogen-only pills containing desogestrel 75 micrograms/day or levonorgestrel 30 micrograms/day. Eur J Contracept Reprod Health Care. 1998;3(4):169–78.
Cortessis VK, Barrett M, Brown Wade N, Enebish T, et al. Intrauterine device use and cervical cancer risk: a systematic review and meta-analysis. Obstet Gynecol. 2017;130(6):1226–36.
Darlympe J, Booth J, Flowers P, Lorimer K. Psychosocial factors influencing risk-taking in middle age for STIs. Sex Transm Infect. 2017;93(1):32–8.
De Bastos M, Stegeman BH, Rosendaal FR, Van Hylckama Vlieg A, Helmerhorst FM, Stijnen T, Dekkers OM. Combined oral contraceptives: venous thrombosis. Cochrane Database Syst Rev. 2014;3(3):CD010813.
De Seta F, Restaino S, De Santo D, Stabile G, Banco R, Busetti M, et al. Effects of hormonal contraception on vaginal flora. Contraception. 2012;86(5):526–9.
Dehlendorf C, Krajewski C, Borrero S. Contraceptive counseling: best practices to ensure quality communication and enable effective contraceptive use. Clin Obstet Gynecol. 2014;57(4):659–73.
Dei M, Bruni V. Guida alla ginecologia dell'infanzia e adolescenza, cap. 20 Ed. Firenze: OEO; 2016.
Dinger J, Do Minh T, Heinemann K. Impact of estrogen type on cardiovascular safety of combined oral contraceptives. Contraception. 2016;94(4):328–39.
Duijkers IJM, Hger-Mahn D, Drouin D, Colli E, Skouby S. Maintenance of ovulation inhibition with a new progestogen-only pill containing drospirenone after scheduled 24-h delays in pill intake. Contraception. 2016;93(4):303–9.
Engemise SL, Willets JM, Taylor AH, Emembolu JO, Konje JC. Changes in glandular and stromal estrogen and progesterone receptor isoform expression in eutopic and ectopic endometrium following treatment with the levonorgestrel-releasing intrauterine system. Eur J Obstet Gynecol Reprod Biol. 2011;157(1):101–6.
Fruzzetti F, Bitzer J. Review of clinical experience with estradiol in combined oral contraceptives. Contraception. 2010;81:8–15.
Gaber TA. Significant reduction of the risk of venous thromboembolism in all long-term immobile patients a few months after the onset of immobility. Med Hypotheses. 2005;64(6):1173–6.
Glisic M, Shahzad S, Tsoli S, Chadni M, et al. Association between progestin-only contraceptive use and cardiometabolic outcomes. A systematic review and meta-analysis. Eur J Prev Cardiol. 2018;25(10):1042–52.
Goshtazebi A, Subotic Brajic T, Scholes D, Lederer Goldberg TB, Berenson A, Prior JC. Adolescent use of combined hormonal contraception and peak bone mineral density accrual: a meta-analysis of international prospective controlled studies. Clin Endocrinol. 2019;90: 517–24.
Graesslin O, Korver T. The contraceptive efficacy of Implanon: a review of clinical trials and marketing experience. Eur J Contracept Reprod Health Care. 2008;13:4–12.
Grandi G, Cagnacci A, Volpe A. Pharmacokinetic evaluation of desogestrel as a female contraceptive. Expert Opin Drug Metab Toxicol. 2014a;10(1):1–10.

Grandi G, Piacenti I, Volpe A, Cagnacci A. Modification of body composition and metabolism during oral contraceptives containing non-androgenic progestins in association with estradiol or ethinyl estradiol. Gynecol Endocrinol. 2014b;30(9):676–80.

Hanjalic-Beck A, Schäfer WR, Deppert WR, Fischer L, Stein A, et al. Chlormadinone acetate suppresses prostaglandin biosynthesis in human endometrial explants. Fertil Steril. 2012;98(4):1017–22.

Hardman SM, Gebbie AE. The contraception needs of the perimenopausal woman. Best Pract Res Clin Obst Gyn. 2014;28:903–15.

Hillard PJ. Menstrual suppression with the levonorgestrel intrauterine system in girls with developmental delay. J Pediatr Adolesc Gynecol. 2012;25(5):308–13.

Ichioka M, Mita S, Shimizu Y, Imada K, Kiyono T, et al. Dienogest, a synthetic progestin, down-regulates expression of CYP19A1 and inflammatory and neuroangiogenesis factors through progesterone receptor isoforms A and B in endometriotic cells. J Steroid Biochem Mol Biol. 2015;147:103–1.

Iversen L, Sivasubramaniam S, Lee AJ, Fielding S, et al. Lifetime cancer risk and combined oral contraceptives: the Royal College of General Practitioners' Oral Contraception Study. Am J Obstet Gynecol. 2017;216(6):580 e1–9.

Jareid M, Thalabard JC, Aarflot M, Bovelstad HM, et al. Levonorgestrel-releasing Intrauterine System use is associated with a decreased risk of ovarian and endometrial cancer, without increased risk of breast cancer. Results from the NOWAC Study. Gynecol Oncol. 2018;149(1):127–32.

Jatloui TC, Whiteman MK, Jeng G, Tepper NK, et al. Intrauterine device expulsion after postpartum placement: a systematic review and meta-analysis. Contraception. 2018;132:895–905.

Johanesson U, Sahlin L, Masironi B, Hilliges M, Blomgren B. Steroid receptor expression and morphology in provoked vestibulodynia. Am J Obstet Gynecol. 2008;198(3):311.e1–6.

Kluft C, Zimmerman Y, Mawet M, Klipping C, et al. Reduced hemostatic effects with drospirenone -based oral contraceptives containing estetrol vs. ethinyl estradiol. Contraception. 2016;95(2):140–7.

Koulouri O, Conway GS. A systematic review of commonly used medical treatments for hirsutism in women. Clin Endocrinol. 2008;68(5):800–5.

Lenzi E, Pluchino N, Begliuomini S, Ninni F, Freschi L, et al. Effects of nomegestrol acetate administration on central and peripheral beta-endorphin and allopregnanolone in ovx rats. J Steroid Biochem Mol Biol. 2008;110(1–2):67–75.

Lete I, Lapuente O. Contraceptive options for women with premenstrual dysphoric disorders: current insights and a narrative review. Open Access J Contracept. 2016;7:117–25.

Lethaby A, Wise MR, Weterings MA, Bofil Rodriguez M, Brown J. Combined hormonal contraceptives for heavy menstrual bleeding. Cochrane Database Syst Rev. 2019;2:CD000154.

Lidegaard Ø, Løkkegaard E, Svendsen AL, Agger C. Hormonal contraception and risk of venous thromboembolism: national follow-up study. BMJ. 2009;339:b2890–4.

Lidegaard Ø, Løkkegaard E, Jensen A, Skovlund CW, Keiding N. Thrombotic stroke and myocardial infarction with hormonal contraception. N Engl J Med. 2012;366(24):2257–66.

Lopez JM, Chen M, Mullins S, et al. Steroidal contraceptives and bone fractures in women: evidence from observational studies. Cochrane Database Syst Rev. 2012;8:CD009849.

Louw-du Toit R, Perkins MS, Snoep JL, Storbeck KH, Africander D. Fourth-generation progestins inhibit 3β-Hydroxysteroid dehydrogenase type 2 and modulate the biosynthesis of endogenous steroids. PLoS One. 2016;11(10):e0164170.

Martínez F, Ramírez I, Pérez-Campos E, Latorre K, Lete I. Venous and pulmonary thromboembolism and combined hormonal contraceptives. Systematic review and meta-analysis. Eur J Contracept Reprod Health Care. 2012;17(1):7–29.

Maxwell L, Devries K, Zionts D, Alhusen JL, et al. Estimating the effect of intimate partner violence on women's use of contraception: a systematic review and mea-analysis. PLoS One. 2015;10(2):e0118234.

Meresman GF, Augé L, Barañao RI, Lombardi E, Tesone M, Sueldo C. Oral contraceptives suppress cell proliferation and enhance apoptosis of eutopic endometrial tissue from patients with endometriosis. Fertil Steril. 2002;77:1141–7.
Merki-Feld GS, Apter D, Bartfai G, Grandi G, Haldre K, et al. ESC expert statement on the effects on mood of the natural cycle and progestin-only contraceptives. Eur J Contracept Reprod Health Care. 2017;22(4):247–9.
Milanes-Skopp R, Nelson AL. Transdermal contraceptive patches: current status and future potential. Expert Rev Clin Pharmacol. 2009;2(6):601–7.
MMWR. US Medical eligibility criteria for contraceptive use. Recomm Rep. 2016;65:3. www.cdc.gov/MMWR
Nappi ER, Kaunitz AM, Bitzer J. Extended regimen combined oral contraception: a review of evolving concepts and acceptance by women and clinicians. Eur J Contracept Reprod Health Care. 2016;21(2):106–15.
Noboa S, Le Gal G, Lacut K, Mercier B, Leroyer C, EDITH Collaborative Study Group, et al. Family history as a risk factor for venous thromboembolism. Thromb Res. 2008;122(5):624–9.
Oelkers W. Antimineralocorticoid activity of a novel oral contraceptive containing drospirenone, a unique progestogen resembling natural progesterone. Eur J Contracept Reprod Health Care. 2002;7(Suppl 3):19–26.
Paris F, Balaguer P, Rimbault F, Gaspari L, Sultan C. Molecular actions of norgestimate: new developments. Gynecol Endocrinol. 2015;31(6):487–90.
Pecchioli Y, Oyewumi L, Allen LM, Kives S. The utility of routine ultrasound in the diagnosis and management of adolescents with abnormal uterine bleeding. J Pediatr Adolesc Gynecol. 2017;30(2):239–42.
Pluchino N, Lenzi E, Merlini S, Giannini A, Cubeddu A, et al. Selective effect of chlormadinone acetate on brain allopregnanolone and opioids content. Contraception. 2009;80(1):53–62.
Quint EH, O'Brien RF. Menstrual management for adolescents with disabilities. Pediatrics. 2016;138(1):e20160295.
Raps M, Helmerhost F, Fleischer K, Thomassen S, Rosendaal F, et al. Sex hormone binding globulin as a marker for the thrombotic risk of hormonal contraceptives. J Thromb Haemost. 2012;10(6):992–8.
Regidor PA, Colli E, Schidler AE. Drospirenone as estrogen-free pill and hemostasis: coagulatory study results comparing a novel 4 mg formulation in a 24 + 4 cycle with desogestrel 75 μg per day. Gynecol Endocrinol. 2016;32(9):749–51.
Riggs M, Klebanoff M, Nansel T, Zhang J, Schwebke J, Adrews W. Longitudinal association between hormonal contraceptives and bacterial vaginosis in women of reproductive age. Sex Transm Dis. 2007;34(12):954–9.
Roach RE, Helmerhorst FM, Lijfering WM, Stijnen T, Algra A, Dekkers OM. Combined oral contraceptives: the risk of myocardial infarction and ischemic stroke. Cochrane Database Syst Rev. 2015;8:CD011054.
Rutanen EM. Insulin-like growth factors and insulin-like growth factor binding proteins in the endometrium. Effect of intrauterine levonorgestrel delivery. Hum Reprod. 2000;15(Suppl 3):173–81.
Schindler AE, Campagnoli C, Druckmann R, Huber J, Pasqualini JT, et al. Classification and pharmacology of progestins. Maturitas. 2003;46(S1):S7–S16.
Silverman JG, Mc Cauley HL, Decker MR, Miller E, Reed E, Raj A. Coercive forms of sexual risk and associated violence perpetrated by male partners of female adolescents. Perspect Sex Reprod Health. 2011;43(1):60–5.
Sitruk-Ware R. New progestagens for contraceptive use. Hum Reprod Update. 2006;12(2):169–78.
Stanczyk FZ, Archer DF. Gestodene: a review of its pharmacology, potency and tolerability in combined contraceptive preparations. Contraception. 2014;89(4):242–52.
Stegeman BH, de Bastos M, Rosendaal FR l FR, van Hylckama Vlieg A, Helmerhorst FM, Stijnen T, Dekkers OM. Different combined oral contraceptives and the risk of venous thrombosis: systematic review and network meta-analysis. BMJ. 2013;347:f5298.

TepperNK BSL, Whiteman MK, Monsour M, et al. Postpartum venous thromboembolism: incidence and risk factors. Obstet Gynecol. 2014;123(5):987–96.
Tosti C, Biscione A, Morgante G, Bifulco G, Luisi S, Petraglia F. Hormonal therapy for endometriosis: from molecular research to bedside. Eur J Obstet Gtnecol Reprod Biol. 2017;209:61–6.
Trémollieres F1. Oral combined contraception: is there any difference between ethinyl-estradiol and estradiol? Gynecol Obstet Fertil. 2012;40(2):109–15.
UK Medical Eligibility Criteria. 2016. www.fsrh.org/ukmec
WHO. Medical eligibility criteria for contraceptive use. 2015. www.who.int/reproductivehealth/publications/MEC
Widman L, Choukas- Bradley S, Noar SM, Nesi J, HiGarrett MA. Parent- adolescent sexual communication and adolescent safer sex behavior: a meta-analysis. JAMA Pediatr. 2016;170(1):52–61.
Xu H, Wade JA, Peipert JF, Zhao Q, Madden T, Secura GM. Contraceptive failure rates of etonogestrel subdermal implants in overweight and obese women. Obstet Gynecol. 2012;120:21.
Yamamoto T, Yoshiji S, Yasuda J, Shiroshita K, Kitawaki J, Fujii M, Urabe M, et al. Aromatization of norethindrone in ethinylestradiol in human adult liver. Endocrinol Jpn. 1986;33(4):527–31.
Zigler RE, McNicholas C. Unscheduled vaginal bleeding with progestin-only contraceptive use. Am J Obstet Gynecol. 2017;216(5):443–50.
Zoller B, Li X, Sundquist J, Sundquist K. Age- and gender-specific familial risks for venous thromboembolism: a nationwide epidemiological study based on hospitalizations in Sweden. Circulation. 2011;124:1012–20.

Part II

Uterine Disorders

Endometriosis

8

Sara Clemenza, Dante Campana, Silvia Vannuccini, Larissa Milani Coutinho, and Felice Petraglia

Contents

S. Clemenza · D. Campana · F. Petraglia (✉)
Department of Biomedical, Experimental and Clinical Sciences, Division of Obstetrics and Gynecology, University of Florence, Florence, Italy
e-mail: felice.petraglia@unifi.it

S. Vannuccini
Department of Biomedical, Experimental and Clinical Sciences, Division of Obstetrics and Gynecology, University of Florence, Florence, Italy

Department of Molecular and Developmental Medicine, University of Siena, Siena, Italy

L. M. Coutinho
Department of Maternal and Child Health, Universidade Federal de Juiz de Fora, Juiz de Fora, Brazil

Department of Obstetrics and Gynecology, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

F. Petraglia, B. C. Fauser (eds.), *Female Reproductive Dysfunction*, Endocrinology,
https://doi.org/10.1007/978-3-030-14782-2_8

Abstract

Endometriosis is a chronic benign disease characterized by the presence and proliferation of endometrial tissue outside the uterine cavity. It affects approximately 6–10% of women. Typically this disease interests women in their reproductive period and affects quality of life. The pathogenesis of endometriosis has not been definitively established; the most accredited theories are retrograde menstruation, blood and lymphatic dissemination, and metaplasia of celomic epithelium and stem cell. Ectopic implants cause a local inflammatory response, accompanied by angiogenesis, adhesions, fibrosis, scarring, neuronal infiltration, and anatomical distortion. The main clinical manifestations are pain and infertility. Currently, endometriosis is classified in clinical practice in superficial or peritoneal, ovarian, and deep endometriosis. Ovary is the most affected organ. Diagnosis is based on clinical history and pelvic examination, supported by radiological investigations including transvaginal ultrasound examination that represents the first imaging approach. Endometriosis requires a lifelong management plan with the goal of maximizing the use of medical treatment and avoiding repeated surgical procedures.

Keywords

Retrograde menstruation · Pelvic pain · Dysmenorrhea · Dyspareunia · Infertility · Endometriomas · Deep endometriosis

Introduction

Endometriosis is a chronic benign disease characterized by the presence and proliferation of endometrial tissue outside the uterine cavity. The predominant location of endometriosis is the pelvic cavity, and ovary is the most common affected organ; however endometrial tissue can be also found outside of the pelvis. Typically the disease interests women in their reproductive period and affects fertility and quality of life.

Epidemiology and Risk Factors

Prevalence of endometriosis is difficult to determine because the clinical presentation is variable and the definitive diagnosis typically requires surgery. It is estimated to affect approximately 6–10% of women, while the prevalence in women

experiencing pain, infertility, or both is higher (35–50%). Endometriosis is underdiagnosed and associated with a delay from symptom onset to diagnosis ranging from 4 to 11 years (Agarwal et al. 2019). The main risk factor is represented by prolonged exposure to endogenous estrogen: nulliparity, early age at menarche, late menopause, shorter menstrual cycles, and heavy menstrual bleeding are conditions characterized by a high proliferative stimulus on the endometrium. On the contrary, multiple pregnancies, prolonged lactation, and late menarche are protective (Parazzini et al. 2017).

The first-degree relatives of affected women are at three- to ninefold higher risk of developing the disease. Twin and family studies suggest a genetic component. Genetic factors contribute about half of the variation in endometriosis risk, with an estimate of hereditability of 51%.

Polymorphisms in genes involved in detoxification processes, estrogen receptors, cytokines, immunomodulatory proteins, and factors involved in attachment and invasion have been reported in women with endometriosis (Vercellini et al. 2014).

Race may also have an influence since a higher prevalence has been reported in Caucasian and Asian women compared with black and Hispanic women. The other main risk factors are obstruction of menstrual outflow (e.g., mullerian anomalies), pigmentary traits and sun habits, and alcohol intake and environmental factors (e.g., exposure to polychlorinated biphenyls and dioxin). Diet can influence many pathogenic mechanisms involved in endometriosis such as inflammation, estrogen activity, menstrual cyclicity, and prostaglandin metabolism; however the available data on the possible protective role of vegetables and fruits and the unfavorable effects of red meat, dairy products, and unsaturated fats are inconsistent. It seems that physical activity reduces the risk of endometriosis probably due to its anti-inflammatory action (Parazzini et al. 2017).

Women with endometriosis have a two- to threefold increase in absolute risk of developing clear cell and endometrioid subtypes ovarian cancer. Other cancers such as cutaneous melanoma, brain, endocrine, thyroid, and kidney cancers have been linked to endometriosis, but further studies are needed to confirm this relationship (Wilbur et al. 2017).

Pathophysiology

The etiology of endometriosis is still unclear and no single theory explains all cases of the disease. According to the most accredited theories, endometriotic cells establishment would derive from retrograde menstruation, blood and lymphatic dissemination, and metaplasia of celomic epithelium and stem cell (Tosti et al. 2015).

Proposed by Sampson in the 1920s (Sampson 1927), the theory of retrograde menstruation supports that endometrial fragments migrate through fallopian tubes during menstruation. This phenomenon is described in about 90% of healthy women, but only some of them are affected by endometriosis, suggesting that additional factors, such as molecular defects, immunologic abnormalities, uterine hyperperistalsis, are involved. Further support for this etiology is derived from studies reporting a higher prevalence of endometriosis in women with uterine

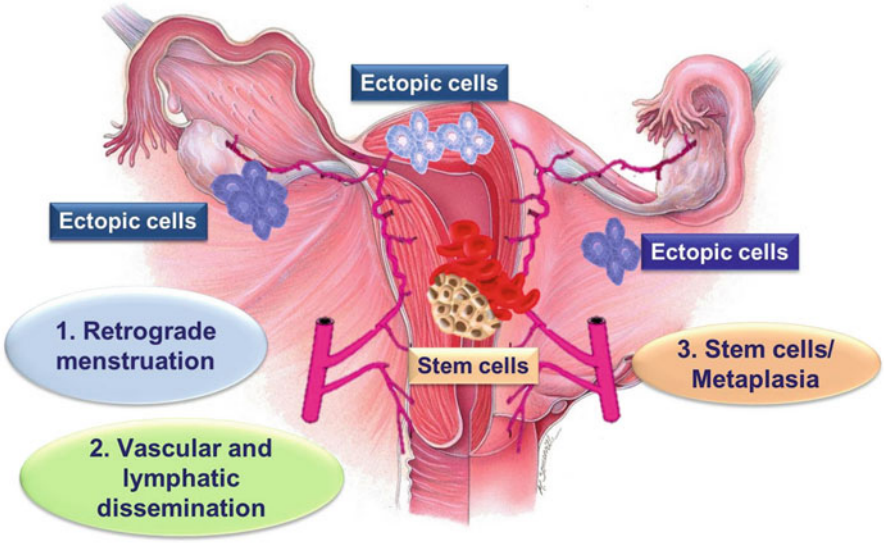


Fig. 1 Main pathogenetic theories of endometriosis

abnormalities that obstruct the menstrual flow, such as uterine septum and cervical stenosis (Dovey and Sanfilippo 2010). Furthermore, the endometrial cells may migrate from the endometrium through the lymphatic or blood circulation, similarly to the metastases of tumor cells. The strongest evidence for this theory is derived from reports of histologically proven endometriotic lesions in distant sites from the uterus to include bone, lung, and brain (Tosti et al. 2015). In 1919 Meyer (1919) hypothesized that endometriosis may develop from the metaplastic transformation of peritoneal cells, since both endometrial and peritoneal cells derive from the epithelium of the celomatic wall.

A more recent hypothesis suggests the transformation of hematopoietic, bone marrow, or mesenchymal stem cells in endometrial cells. A possible role of endometrial stem cells has also been proposed. Endometrium-derived stem/progenitor cells residing in the basalis layer and responsible for the regenerative potential of the tissue can be shed through the fallopian tube to the peritoneal cavity during menses and establish endometriotic implants (Hufnagel et al. 2015) (Fig. 1).

Immune Dysfunction and Inflammation

The immune system exerts a critical role in the development of a local immune response against endometrial cells in the peritoneal cavity. Normally, refluxed endometrial cells are cleared from the peritoneum by the immune cells, such as macrophages, natural killer (NK) cells, and lymphocytes. Impaired cellular and humoral immunity have been reported in endometriotic tissue and causes a reduced clearance of refluxed endometrial cells. The hypothesis that an altered immune

response plays a role in the pathogenesis of the disease is supported by the increased incidence of autoimmune disorders in patients with endometriosis. The main immune alterations concern natural killer cells, macrophages, neutrophils, humoral immunity, cytokines, and growth factors. Cytokines and growth factors seem to promote implantation, growth (inducing proliferation and angiogenesis), and invasion of the ectopic endometrium. The main factors involved are IL-1, IL-6, IL-8, monocyte chemotactic protein-1 (MCP-1), RANTES (regulated on activation, normal T-cell expressed and secreted), tumor necrosis factor-α (TNF-α), and vascular endothelial growth factor (VEGF) (Ahn et al. 2015).

Inflammation is associated with the overproduction of prostaglandins, cytokines, chemokines, and grow factors. The high levels of these inflammatory mediators probably enhance the adhesion of endometrial tissue fragments onto peritoneal surface; subsequently proteolytic membrane metalloproteinases, such as MMP-2, MMP-3, MMP-7, and MMP-9, increased in endometriosis, may further promote implantation of the fragments.

Oxidative stress plays a crucial role in the onset and progression of the disorder: reactive oxygen species (ROS) induce the synthesis of some members of A disintegrin and metalloproteases (ADAM) family metallopeptidase such as ADAM 17, that produces a proteolytic release of the active form of Notch proteins and its transport to the nucleus, where it induces transcriptional activation of genes related to fibrosis. Endometriosis is characterized of ADAM17/Notch signaling and a consequent increase in fibrosis (González-Foruria et al. 2017).

Apoptosis is also altered in endometriosis in favor of the survival and replenishment of ectopic tissue. Furthermore, neoangiogenesis is necessary for the development and sustenance of endometriotic lesions as peritoneal environment is poorly vascularized compared to the endometrial eutopic tissue. A fundamental role in this process seems to be played by VEGF detected in high concentration in peritoneal fluid of women with endometriosis. Neoangiogenesis is closely related with neuroangiogenesis: an overexpression of factors that provide innervation, such as nerve growth factor (NGF), has indeed been identified.

Neoangiogenesis and neuroangiogenesis contribute with inflammation to the pathophysiology of pain associated with this disorder (Tosti et al. 2015).

Hormonal Influences

One of the main pathogenetic events of endometriosis consists of an increased estrogen sensibility and of a progesterone resistance. Endometriotic implants express the aromatase and 17β17β-hydroxysteroid dehydrogenases. (17β-HSD) type 1 converting androstenedione into estrone and estrone into estradiol. Instead, endometriosis cells do not express 17β-HSD type 2 that inactivates estrogens. This combination allows the implants to be exposed to a high local concentration of bioavailable estrogens. Excess local estradiol results in proliferation of the lesions and stimulates the production of prostaglandin E_2: in this way a positive feedback mechanism between estrogen production and inflammation is established.

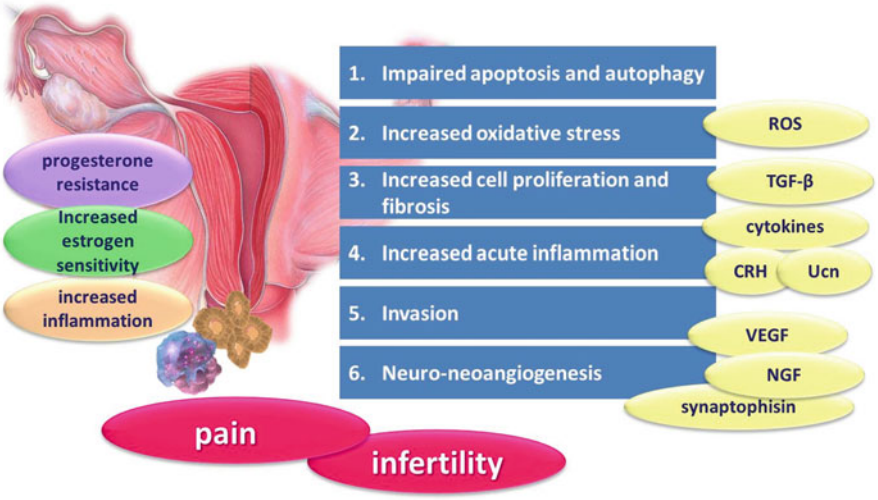


Fig. 2 Pathogenetic mediators of endometriosis

In addition to estrogen dependence, endometriosis is characterized by progesterone resistance. Endometriotic implants exhibit an overall reduction in progesterone receptors and an absence of progesterone receptor-B compared to eutopic endometrium (Clemenza et al. 2018) (Fig. 2).

Anatomic Sites

Endometriosis may develop anywhere, both in pelvic and extrapelvic sites. Ovary is the most affected organ, especially the left one, and is bilaterally involved in more than half of the cases.

Ovarian endometriosis is characterized by the presence of cysts (endometriomas, OMA) of several sizes from 5 mm to 15 cm with fibrotic walls, surrounded by duplicated ovarian parenchyma; it is internally lined by endometrium and filled with dark material composed of blood and histiocytes with hemosiderin.

Endometrioma is thought to form by an invagination of the ovarian cortex at the site of original adhesion of the ovary to the peritoneum.

The accumulation of chocolate fluid inside the cyst could be because of either metaplastic transformation of the invaginated mesothelium of the ovarian cortex, which changes into mature endometrial cells, or production of menstrual debris form the superficial ovarian implants of endometriosis which, consequent to the invagination, lines the inside of the cyst (Muzii et al. 2007).

Uterosacral ligaments, fossa ovarica, anterior and posterior cul-de-sac, and posterior broad ligament are frequently involved as deep infiltrating endometriosis (DIE). Other less frequent locations include vagina, cervix, rectovaginal septum,

and bladder. Despite the rarity of extrapelvic endometriosis, cases of endometriosis of gastrointestinal tract, urinary tract, upper and lower respiratory system, diaphragm, pleura, pericardium, brain, and abdominal scars have been reported.

Thoracic involvement is the most frequent extrapelvic location and may cause catamenial pneumothorax due to the bleeding of the pleural endometrial tissue during the menstrual cycle. Because of the wide range of its clinical expression and the symptoms similar to other pathological conditions, the diagnosis of extrapelvic endometriosis is complex and should be suspected in women with periodical symptoms (Machairiotis et al. 2013).

Classification

The classification of endometriosis is still controversial, and no single system adequately classifies endometriosis, often because only one aspect of the disease, such as anatomy, histology, surgery and fertility, is investigated. The best-known classifications are listed below:

- The current most used classification in clinical practice divides endometriosis into three categories: superficial or peritoneal (SUP), characterized by the presence of implants located on the surface of the peritoneum, ovarian (OMA) and deep endometriosis (DIE) (lesions infiltrating deeper than 5 mm under the peritoneum) (Johnson et al. 2017).
- American Fertility Society (AFS) classification: created in 1979, revised in 1985 as rAFS and modified in 1996 as revised American Society for Reproductive Medicine (rASRM) score, it is the classification most widely established throughout the world. It was a point scoring system, based on laparoscopic findings. Depending on the number, location, and size of the endometriotic lesions, the disease was classified in four stages: mild, moderate, severe, and extensive (Revised American Fertility Society classification of endometriosis 1985). Although widely used, this system has not been demonstrated to be related to symptoms frequency and severity or reproductive prognosis (American Society for Reproductive Medicine 1997).
- Adamyan classification concerns retrocervical location of endometriosis: stage I indicates retrocervical endometriosis with no vaginal involvement, stage II involves vagina, stage III involves vagina and rectum with cul-de-sac distortion, stage IV includes cul-de-sac obliteration.
- Chapron classification (2003) describes deep infiltrating endometriosis (DIE), subdividing it into anterior and posterior compartment for better surgical management;
- Enzian classification can be used for deep endometriosis. Retroperitoneal structures are divided in compartment A (rectovaginal septum and vagina), compartment B (sacrouterine ligament to the pelvic wall), and compartment C (rectum and sigmoid colon) and F (retroperitoneal distant locations). The severity of the disease is

defined for all the compartments by the invasion: grade 1 invasion<1 cm, grade 2 invasion 1–3 cm, grade 3 invasion >3 cm (Haas et al. 2013).
– Koninckx et al. created a classification based on type (subtle, typical, cystic, deep) and size of lesions.

Clinical Manifestations

Pain

Symptoms of endometriosis can range from minimal to severely debilitating. Therefore, many patients are asymptomatic and endometriotic lesions are incidental findings diagnosed during surgery for other reasons. The most common symptoms are dysmenorrhea, dyspareunia, back pain and chronic pelvic pain, but it can also occur with urinary disorders and painful defecation.

Variability in the clinical manifestation can suggest the different locations of the lesions. For example, severe dysmenorrhea is often correlated with Douglas pouch adhesions, dyspareunia with peritoneal or deeply infiltrating endometriosis, painful defecation with implants of the posterior cul-de-sac and rectovaginal septum, and urinary symptoms with bladder endometriosis. Bowel endometriosis can present with diarrhea, constipation, dyschezia, and bowel cramping.

The origin of pain remains unclear, and it has probably a multifactorial genesis: inflammation, neurogenesis, and adhesions seem to be the key mechanisms involved.

A typical feature of endometriosis is hyperalgesia, an abnormally high sensation of pain compared to the stimulus received: the local activation of nerve fibers by neurogenic factors and the direct infiltration of the peripheral nerves by endometriotic stromal cells may contribute to this phenomenon. Increased neuroendocrine (NE) cells are detected in eutopic endometrium of women with endometriosis, and the intense expression of synaptophysin (SYN) in NE cells may stimulate nerve fibers and nociceptors to induce pain signals.

Adhesions, as a consequence of the disease or as a result of previous surgery, may also contribute to pain symptoms.

Although the mentioned mechanisms are involved in clinical manifestation of endometriosis, further studies are needed to fully understand the genesis of pain in these patients.

Infertility

About a third of women with endometriosis are infertile.

It is controversial if these patients have a reduced ovarian reserve, as some authors have found low levels of circulating AMH. The genesis of infertility is also multifactorial.

Inflammation and Immune Factor

Peritoneal fluid (PF) has an important and complex role in fertility. PF of patients with endometriosis have increased concentration of cytokines, interleukins, and reactive oxygen species (ROS) which can therefore reduce fertility through several mechanisms. In particular, IL-1 and IL-6 act on mobility of sperm cells and macrophage migration inhibitory factor reduces its motility; TNF-α could cause damage to the cellular DNA of sperm; TNF-α, macrophage migration inhibitory factor, and IL-6 may prevent capacitation; TNF-α, macrophage migration inhibitory factor, IL-1, and the RANTES reduce sperm binding to the zona pellucida (de Ziegler et al. 2010). CRH and urocortin are neuropeptides produced by the endometrium and participates in the reproductive/inflammatory mechanisms of endometrial decidualization. In particular CRH via CRH receptor type 1 (CRH-R1, antalarmin) stimulates the production of IL-1 and IL-6 and interacts with prostaglandins in the decidualization process, probably initiating the local inflammation events, increased vascular permeability, remodeling of extracellular matrix, secretion of inflammatory factors, and facilitating the implantation site. The endometrium of women with endometriosis is characterized by a deranged expression of CRH and Ucn during menstrual cycle, by a reduced response to CRH and Ucn, by an impaired expression of CRH-R1 mRNA: a disrupted local CRH/Ucn/CRH-R1 pathway may also explain the reduced fertility characterizing patients with endometriosis (Novembri et al. 2011).

Mechanical Factor

Fibrosis and adhesions consequent both to the pathology itself and also to previous surgical interventions may represent a mechanical factor in achieving the pregnancy goal, especially if fallopian tubes are involved.

Iatrogenic Factor

Iatrogenic damage carried out at ovarian level following surgical interventions should not be underestimated, especially in case of repeated and bilateral interventions.

Diagnosis

Endometriosis is often underdiagnosed and diagnosed from 4 to 11 years after the onset of symptoms. The delay of diagnosis favors disease progression and adhesion formation that may compromise fertility and increase chronic pelvic pain (Agarwal et al. 2019).

The diagnosis of endometriosis is based on the history, the symptoms and signs, the physical examination, and imaging techniques although laparoscopy and histological verification are required for definitive diagnosis.

Clinical History

A detailed clinical history should be taken for all women with suspected endometriosis.

In particular it is important to investigate: age, parity, family history of endometriosis, bleeding pattern, infertility, pain (dysmenorrhea, dyspareunia, dysuria, dyschezia, chronic pelvic pain) hematochezia and/or hematuria, and previous treatment for endometriosis.

Pelvic Examination

Physical examination can identify endometriosis with high accuracy. Bimanual exploration allows to detect the presence of ovarian masses suggestive for endometriomas, palpable nodularity, and stiffened and/or thickened pelvic anatomy, especially the uterosacral ligaments, vagina, rectovaginal space, pouch of Douglas, adnexa, rectosigmoid, or posterior wall of the urinary bladder. Pelvic exam should include speculum examination in order to detect the possible presence of powder-burn lesions on the cervix or the posterior fornix of the vagina.

Ultrasound Examination

Transvaginal ultrasound examination represents the first imaging approach in the diagnosis and follow-up of endometriosis. The typical endometrioma appears as a round or ovoid unilocular cystic formation with thick, regular walls and homogeneous hypoechoic content, called "ground glass," without papillary projection, with poor peripheral vascularization and absence of .central vascularization.

Transvaginal ultrasound also allows the identification and description of other endometriotic lesions, such as adhesions and infiltrating endometrial nodules affecting the anterior, lateral, and posterior pelvic compartment.

The sliding signing maneuver, which consists in causing anterior sliding movement of the uterus with respect to the intestinal wall, exerting a pressure with the vaginal probe in the posterior fornix, allows to identify the obliteration of the Douglas pouch and adherent syndrome.

In virgin women, it is possible to use transrectal ultrasound instead of transvaginal ultrasound (Guerriero et al. 2016) (Figs. 3 and 4).

Magnetic Resonance Imaging

Magnetic resonance imaging is a noninvasive investigational tool for suspected endometriosis.

It is expensive, not universally available and lacks sensitivity; therefore MRI should be considered as a second-line technique examination after ultrasound

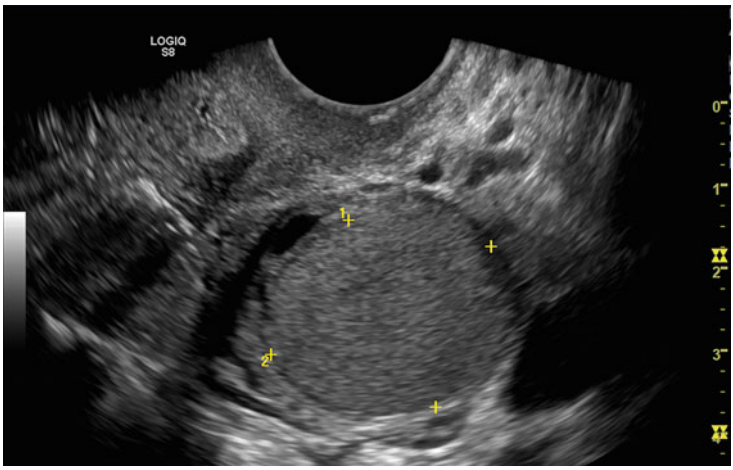


Fig. 3 Transvaginal ultrasound image of ovarian endometrioma (OMA) with the typical "ground glass" appearance

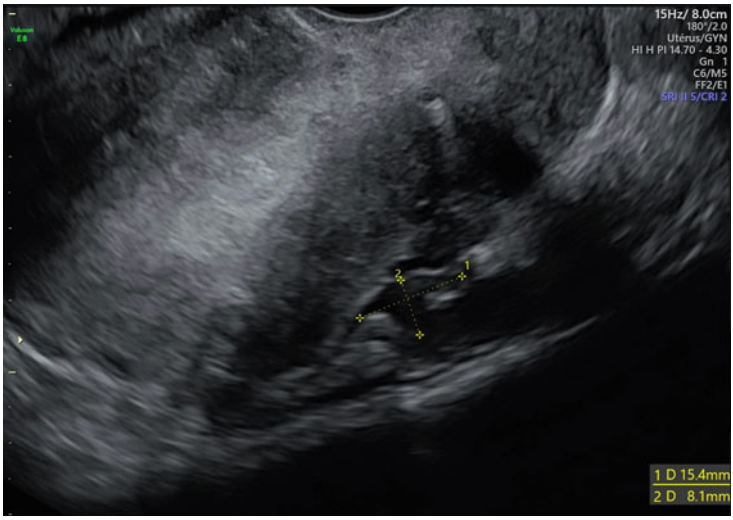


Fig. 4 Transvaginal ultrasound imagine of rectovaginal septum nodule

in the evaluation of pelvic endometriosis and before surgery for optimal preoperative staging.

Endometriosis foci have variable signal intensity on T2- and T1-weighted images, depending on the age of hemorrhage. Subacute bleeding may result in very high signal intensity on T1-weighted images and relatively low signal intensity on T2-weighted images, while fibrotic endometrial lesions present relatively low signal intensity on both T1- and T2-weighted images (Bourgioti et al. 2017).

Table 1 Differential diagnosis of endometriosis

Gynecologic	
Pelvic inflammatory disease Ovarian functional or neoplastic cysts Ectopic pregnancy Pelvic varicocele	
Non-gynecologic	
Gastrointestinal system	Appendicitis Diverticulitis Colitis Inflammatory bowel disease
Urinary system	Urinary tract infection Renal calculi Inflammatory bowel disease
Pelvic adhesion	Post-surgical Post-infectious
Musculoskeletal disorders	

Differential Diagnosis

The main diseases that may mimic the symptoms of endometriosis are shown in Table 1.

Treatment

Endometriosis is a chronic and progressive disease that requires a long-term management. In choosing the most appropriate treatment, it is important to consider the characteristics of each individual patient, in particular the age and the desire of offspring. The management of endometriosis can be both medical and surgical; however no treatment is curative as the disease tends to recur. Therapy goals are pain control and fertility improvement, maximizing the use of medical treatment and avoiding repeated surgical procedures (Practice Committee of the American Society for Reproductive Medicine 2014).

Medical Treatment

Nonsteroidal Anti-inflammatory Drugs

NSAIDs interfere with the function of the enzyme COX-1 and COX-2, inhibiting the conversion of arachidonic acid to prostaglandins, involved in the genesis of endometriosis-associated pain. NSAIDs provide effective treatment for women with pain caused by primary dysmenorrhea and appear to be the only medical option consistent with the maintenance of fertility. However women using NSAIDs must be aware that

these drugs may cause unintended effects especially in gastrointestinal, cardiovascular, and nervous systems (Clemenza et al. 2018).

Progestins

The progestins in monotherapy should be considered as a first choice treatment in the presence of algic symptoms. Progestins induce atrophy of eutopic and ectopic endometrium and have anti-inflammatory and proapoptotic properties. Their use is associated with the improvement of pain and quality of life in two thirds of patients; progesterone resistance may cause nonresponse in the remaining one third. Therefore, these compounds reduce the incidence of postoperative endometrioma recurrence.

Progestins can be administered via an oral, intramuscular, subcutaneous, or intrauterine route.

Evidences support the use of two 19-nortestosterone derivatives: norethisterone acetate (NETA) and dienogest, which have been used orally at the dose of 2.5–5.0 mg per day and 2 mg per day, respectively. NETA has stronger progestogenic effects than dienogest, but it also has androgenic activity, whereas dienogest is antiandrogenic. 150 mg medroxyprogesterone acetate (DMPA) intramuscular injections every 3–6 months may constitute a treatment alternative. The levonorgestrel intrauterine system should be considered especially in women who do not tolerate progestins used systemically (Vercellini et al. 2016).

Danazol

Danazol, derivative of 17α-ethynyl testosterone, induces the inhibition of gonadotropin release and have a strong anti-estrogenic activity. It is effective at treating endometriosis-related pain, but its use is limited by the androgenic-type adverse effects such as seborrhea, hypertrichosis, weight gain, HDL levels decrease, and LDL levels increase. Good efficacy and tolerability has been reported with danazol 200 mg per day used vaginally (Vercellini et al. 2014; Tosti et al. 2015).

Gonadotropin-Releasing Hormone (GnRH) Agonists

GnRH agonists such as Goserelin, Leuprolide, Nafarelin, Buserelin and Triptorelin are effective as progestins and estrogen-progestins in the relief of pain. These compounds bind to the GnRH receptors, initially stimulating the pituitary gland and promoting the release of hormones; subsequently the prolonged and continuous exposure to these agents cause downregulation of the pituitary-ovarian axis and hypoestrogenism. The hypoestrogenic state is responsible for significant side effects, including hot flushes, vaginal dryness, and osteopenia. In fact, GnRH agonists should be avoided in adolescents due to the negative effect they could have on bone growth (Dunselman et al. 2014).

The addition of estro-progestins add-back therapy, commonly with norethindrone, reduces these adverse effects, without reducing the efficacy of pain relief.

GnRH agonists are approved for only up to 6 months of continuous use, but the add-back therapy can permit longer-term use (Tosti et al. 2017).

GnRH Antagonist

Compared to GnRH agonists, these compounds have the advantage of causing an immediate blockage of the GnRH receptor, without the initial stimulation of the hypothalamic-pituitary-gonadal axis (flare up). Elagolix, a GnRH antagonist administered orally has recently been approved by the FDA for endometriosis treatment.

Aromatase Inhibitors

Aromatase, found in the endometriotic lesions and in the eutopic endometrium of women with endometriosis, catalyzes the conversion of testosterone and androstenedione to estradiol and estrone, respectively. Aromatase inhibitors (AIs) block estrogen synthesis both in the periphery and in the ovaries causing hypoestrogenism; therefore their use should be associated with add-back therapy. AIs should be used off-label especially in women with severe pain refractory to other medical or surgical treatment (Clemenza et al. 2018; Tosti et al. 2017) (Fig. 5).

Surgical Treatment

Compared to the past, clinicians have a much more conservative attitude, especially in young women, due to the high rate of relapse after surgery, approximately 10% per year for the first 5 years for ovarian localizations.

Before undertaking a surgical procedure, it is mandatory a correct diagnosis and radiological staging of the disease, to know in advance the possible surgical difficulties and to perform an appropriate preoperative counseling with the patient.

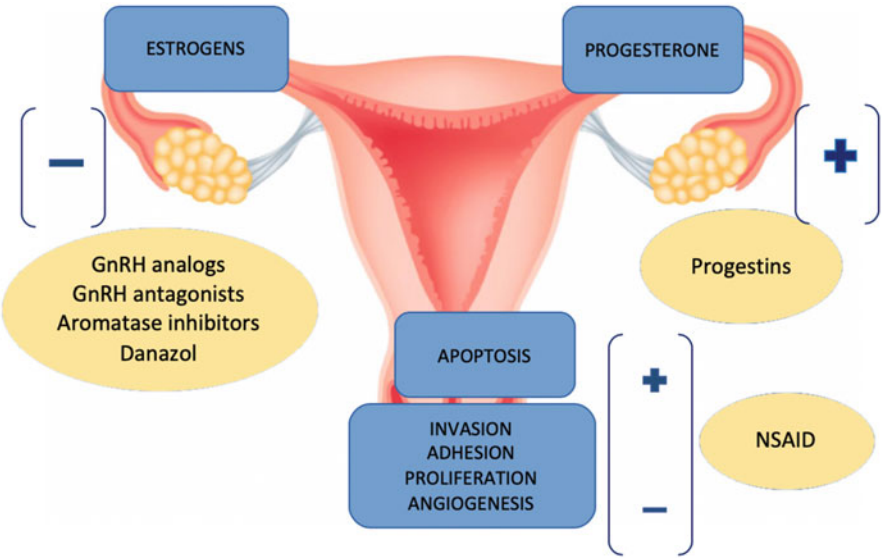


Fig. 5 Mechanisms of action of the main medical treatments of endometriosis

Surgical Treatment of Endometrioma

Ovarian cystectomy is the procedure with a lower risk of dysmenorrhea, dyspareunia, non-menstrual pelvic pain and recurrence of the endometrioma compared to drainage and electrocoagulation.

In addition, excision of the endometrioma capsule, instead of other procedures, seems to increase spontaneous pregnancy rates. However, clinicians should counsel women with endometrioma regarding the risks of reduced ovarian function after surgery and the possible loss of the ovary (Dunselman et al. 2014).

Surgical Treatment of Deep Endometriosis

Surgical treatment of deep endometriosis is technically more difficult than treatment of ovarian lesions, as sometimes it also requires a knowledge of extragenital interventions (urological, intestinal). For this reason, in selected cases of advanced pathology involving different anatomical districts, multidisciplinary approach is the most correct one.

In view of complexity of the interventions, the average young age of patients, the possible desire for offspring, the high rate of recurrence, and therefore the need to intervene again, surgery has limited indications and should be pursued as a second choice for failure of medical therapy or where there are contraindications to medical therapy.

For example, in perimenopausal women who have completed their reproductive cycle, radical bilateral hysterectomy and adnexectomy may be appropriate if the symptoms are severe enough to affect the quality of life (Dunselman et al. 2014).

A possible option in the treatment of deep endometriosis is neuroablation, in particular, uterine nerve ablation (UNA) and presacral neurectomy (PSN).

A brief reference to anatomy is fundamental to understand these surgical techniques. Pelvic organs are innervated by the sympathetic and parasympathetic system.

The corpus, cervix, and proximal fallopian tubes transmit pain through sympathetic fibers that arise from T10 to L1. These fibers partly go through the uterosacral ligaments and partly in the presacral nerves. The lateral pelvis transmits pain via nervi erigentes (pelvic splanchnic nerve) arising from S2 to S4. The presacral nerve divides into the hypogastric nerve that form the inferior hypogastric plexus, and this plexus divides into vesical, middle rectal, and uterovaginal plexuses.

These two surgical techniques, in different ways, have the goal of breaking the connection of the nerve fibers that transmit the pain.

The goal of UNA is to interrupt pain signaling through the inferior hypogastric plexus. This procedure is most commonly performed laparoscopically as a LUNA, although vaginal routes have been described. It consists in dissecting the uterosacral ligaments as close to the cervix as possible. It is a technique that can be performed by surgeons with a very short learning curve.

PSN consists in the removal of the totality of the presacral nerve fibers located in the interiliac triangle; interruption of a greater number of cervical nerve fibers, with a greater reduction of pain; it is a rather complex intervention that requires a long learning curve and burdened by a higher complication rate.

Several authors have shown that UNA was not very effective in reducing painful symptoms, so guidelines suggest avoiding this procedure, while PSN has shown a real effectiveness carried out by skilled surgeons; however it is good to know that it is a difficult procedure, potentially risk and burdened by possible post-operative complications (Proctor et al. 2005).

Conclusion

Endometriosis is a chronic, progressive, and recurrent disease that requires long-term management. Currently, the first-line drugs act by blocking ovarian function, creating a hypoestrogenic environment. The blockade of estrogen secretion and receptor activity and the activation of progesteron receptors are the main target of several current drugs. Their main limitation is the contraceptive effect for women seeking a pregnancy. Endometriosis has a highly variable phenotype and the therapeutic choices should take into account the characteristics of each individual patient, such as the age and the reproductive cycle. Endometriosis constitutes a paradigm for the new model of patient-centered medicine: a wide variety of medical treatments targeting different pathways is likely to be important to move toward personalized medicine in endometriosis. New future research about pathogenetic mechanisms of endometriosis could permit the development of ideal drugs that should relieve pain, induce regression of endometriotic lesions, and allow conception.

Cross-References

► Infertility
► The Menstrual Cycle and Related Disorders

References

Agarwal SK, Chapron C, Giudice LC, Laufer MR, Leyland N, Missmer SA, Singh SS, Taylor HS. Clinical diagnosis of endometriosis: a call to action. Am J Obstet Gynecol. 2019; pii: S0002–9378(19)30002-X.

Ahn SH, Monsanto SP, Miller C, Singh SS, Thomas R, Tayade C. Pathophysiology and Immune Dysfunction in Endometriosis. Bio Med Res Int. 2015;2015:12. Article ID 795976.

American Society for Reproductive Medicine. Revised american society for reproductive medicine classification of endometriosis: 1996. Fertil Steril. 1997;67(5):817–21.

Bourgioti C, Preza O, Panourgias E, Chatoupis K, Antoniou A, Nikolaidou ME, Moulopoulos LA. MR imaging of endometriosis: spectrum of disease. Diagn Interv Imaging. 2017;98(11):751–67.

Clemenza S, Sorbi F, Noci I, Capezzuoli T, Turrini I, Carriero C, Buffi N, Fambrini M, Petraglia F. From pathogenesis to clinical practice: emerging medical treatments for endometriosis. Best Pract Res Clin Obstet Gynaecol. 2018;51:92–101.

de Ziegler D, Borghese B, Chapron C. Endometriosis and infertility: pathophysiology and management. Lancet. 2010;376(9742):730–8.

Dovey S, Sanfilippo J. Endometriosis and the adolescent. Clin Obstet Gynecol. 2010;53(2):420–8.

Dunselman GA, Vermeulen N, Becker C, et al. ESHRE guideline: management of women with endometriosis. Hum Reprod. 2014;29:400–12.
González-Foruria I, Santulli P, Chouzenoux S, Carmona F, Chapron C, Batteux F. Dysregulation of the ADAM17/Notch signalling pathways in endometriosis: from oxidative stress to fibrosis. Mol Hum Reprod. 2017;23(7):488–99.
Guerriero S, Condous G, van den Bosch T, Valentin L, Leone FP, Van Schoubroeck D, Exacoustos C, Installé AJ, Martins WP, Abrao MS, Hudelist G, Bazot M, Alcazar JL, Gonçalves MO, Pascual MA, Ajossa S, Savelli L, Dunham R, Reid S, Menakaya U, Bourne T, Ferrero S, Leon M, Bignardi T, Holland T, Jurkovic D, Benacerraf B, Osuga Y, Somigliana E, Timmerman D. Systematic approach to sonographic evaluation of the pelvis in women with suspected endometriosis, including terms, definitions and measurements: a consensus opinion from the International Deep Endometriosis Analysis (IDEA) group. Ultrasound Obstet Gynecol. 2016;48:318–32.
Haas D, Shebl O, Shamiyeh A, Oppelt P. The rASRM score and the Enzian classification for endometriosis: their strengths and weaknesses. Acta Obstet Gynecol Scand. 2013;92(1):3–7.
Hufnagel D, Li F, Cosar E, Krikun G, Taylor HS. The role of stem cells in the etiology and pathophysiology of endometriosis. Semin Reprod Med. 2015;33(5):333–40.
Johnson NP, Hummelshoj L, Adamson GD, Keckstein J, Taylor HS, Abrao MS, Bush D, Kiesel L, Tamimi R, Sharpe-Timms KL, Rombauts L, Giudice LC. World endometriosis society consensus on the classification of endometriosis. Hum Reprod. 2017;32(2):315–24.
Machairiotis N, Stylianaki A, Dryllis G, Zarogoulidis P, Kouroutou P, Tsiamis N, Katsikogiannis N, Sarika E, Courcoutsakis N, Tsiouda T, Gschwendtner A, Zarogoulidis K, Sakkas L, Baliaka A, Machairiotis C. Extrapelvic endometriosis: a rare entity or an under diagnosed condition? Diagn Pathol. 2013;8:194.
Meyer R. Uber den Staude der Frage der adenomyosites adenomyoma in Allgemeinen und adenomyonetitis sarcomatoza. Zentralbl Gynakol. 1919;36:745–59.
Muzii L, Bellati F, Bianchi A, Palaia I, Manci N, Zullo MA, et al. Laparoscopic stripping of endometriomas: a randomized trial on different surgical techniques. Part II: pathological results. Hum Reprod Oxf Engl. 2005;20(7):1987–92.
Muzii L, Bianchi A, Bellati F, Cristi E, Pernice M, Zullo MA, Angioli R, Panici PB. Histologic analysis of endometriomas: what the surgeon needs to know. Fertil Steril. 2007;87(2):362.
Novembri R, Borges LE, Carrarelli P, Rocha AL, De Pascalis F, Florio P, Petraglia F. Impaired CRH and urocortin expression and function in eutopic endometrium of women with endometriosis. J Clin Endocrinol Metab. 2011;96(4):1145–50.
Parazzini F, Esposito G, Tozzi L, Noli S, Bianchi S. Epidemiology of endometriosis and its comorbidities. Eur J Obstet Gynecol Reprod Biol. 2017;209:3–7.
Practice Committee of the American Society for Reproductive Medicine. Treatment of pelvic pain associated with endometriosis: a committee opinion. Fertil Steril. 2014;101:927.
Proctor M, Latthe P, Farquhar C, Khan K, Johnson N. Surgical interruption of pelvic nerve pathways for primary and secondary dysmenorrhoea. Cochrane Database Syst Rev. 2005: CD001896.
Revised American Fertility Society classification of endometriosis. Fertil Steril. 1985;43(3):351–2.
Sampson J. Peritoneal endometriosis due to the menstrual dissemination of endometrial tissue into the peritoneal cavity. Am J Obstet Gynecol. 1927;14:422–69.
Tosti C, Pinzauti S, Santulli P, Chapron C, Petraglia F. Pathogenetic Mechanisms of Deep Infiltrating Endometriosis. Reprod Sci. 2015;22(9):1053–9.
Tosti C, Biscione A, Morgante G, Bifulco G, Luisi S, Petraglia F. Hormonal therapy for endometriosis: from molecular research to bedside. Eur J Obstet Gynecol Reprod Biol. 2017;209:61–6.
Vercellini P, Viganò P, Somigliana E, Fedele L. Endometriosis: pathogenesis and treatment. Nat Rev Endocrinol. 2014;10(5):261–75.
Vercellini P, Buggio L, Berlanda N, Barbara G, Somigliana E, Bosari S. Estrogen-progestins and progestins for the management of endometriosis. Fertil Steril. 2016;106(7):1552–71.
Wilbur MA, Shih IM, Segars JH, Fader AN. Cancer Implications for Patients with Endometriosis. Semin Reprod Med. 2017;35(1):110–6.

Uterine Fibroids and Adenomyosis

9

M. Gracia and F. Carmona

Contents

M. Gracia
Division of Gynecology, Institute Clinic of Gynecology, Obstetrics and Neonatology, Hospital Clinic, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Faculty of Medicine, University of Barcelona, Barcelona, Spain
e-mail: megracia@clinic.cat

F. Carmona (✉)
Department of Gynecology, Hospital Clínic of Barcelona, Barcelona, Spain
e-mail: FCARMONA@clinic.cat

F. Petraglia, B. C. Fauser (eds.), *Female Reproductive Dysfunction*, Endocrinology,
https://doi.org/10.1007/978-3-030-14782-2_9

Abstract

Uterine fibroids and adenomyosis are common gynecological conditions in patients presenting abnormal uterine bleeding (AUB), pain (dysmenorrhea and dyspareunia), and infertility. Uterine fibroids are benign myometrial tumors, and, conversely, adenomyosis is characterized by the presence of heterotopic endometrial glands and stroma within the myometrium. Although being different entities, they share symptoms and treatment options. Clinical characteristics and main diagnosis criteria for uterine fibroids and adenomyosis will be developed. Nowadays, treatment for fibroids and adenomyosis is intended to control symptoms trying to preserve future fertility up to menopausal status. In this chapter, we will review main management options currently available: surgical treatment (including radical and conservative surgery), nonsurgical options, and medical treatment. Lastly, both conditions are common among women of reproductive age, and they can alter fertility and pregnancy outcomes. Thus, in the last part, adenomyosis-related and myoma-related infertility will be reviewed, as long as reproduction implications.

Keywords

Uterine fibroids · Adenomyosis · Ultrasound diagnosis · Abnormal uterine bleeding · Pelvic pain · Infertility · Uterus-sparing surgery · Hormonal medical treatment · Hysterectomy

Introduction

Uterine fibroids are the most common benign myometrial tumors. In contrast, adenomyosis is characterized by the presence of heterotopic endometrial glands and stroma within the myometrium. Uterine fibroids and adenomyosis present similar symptoms, mainly abnormal uterine bleeding (AUB) and pelvic pain. The new PALM-COEIN Classification for Causes of Abnormal Uterine Bleeding (AUB) developed by the FIGO Menstrual Disorders Group (FMDG) has considered fibroids (AUB-L) and adenomyosis (AUB-A) structural entities causing AUB, according to the acronym PALM-COEIN [*pahm-koin*]: Polyp, Adenomyosis, Leiomyoma, Malignancy and hyperplasia, Coagulopathy, Ovulatory disorders, Endometrium, Iatrogenic, and Not classified. Since 2005, this classification contemplates up to eight types of fibroids depending on their penetrance from the endometrium to the serosa (Fig. 1) (Munro 2012).

Adenomyosis was first described in 1860 by Carl von Rokitansky, but it was not until 1972 when adenomyosis was described as a "benign invasion of endometrium in the myometrium, producing a diffusely enlarged uterus, which microscopically exhibits ectopic, non-neoplastic, endometrial glands and stroma surround by hypertrophic and hyperplastic myometrium" by Bird et al. According to the myometrial invasion extent, adenomyosis is classified as diffuse, focal, or polypoid (Garcia and Isaacson 2011).

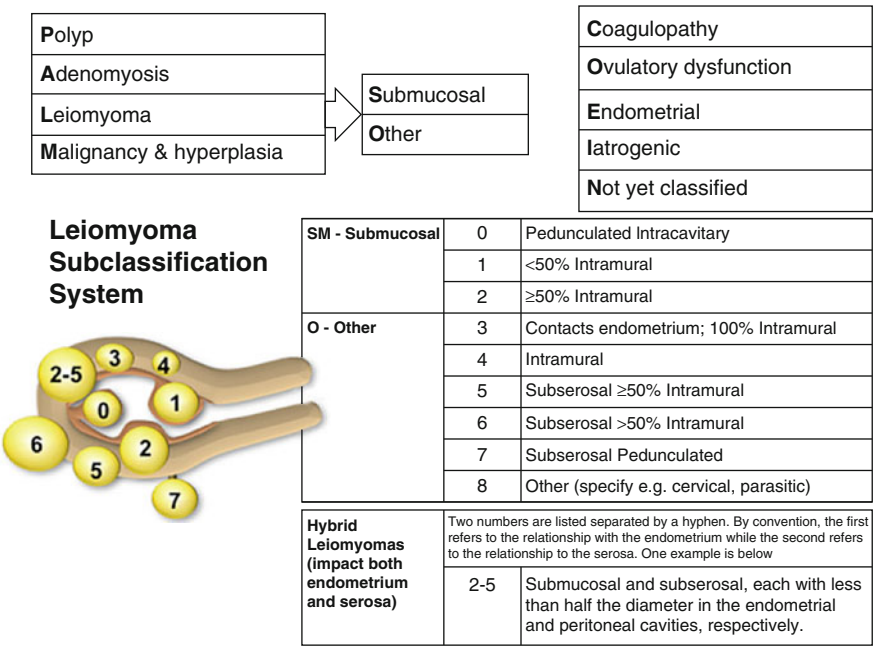


Fig. 1 FIGO classification system for AUB causes. Leiomyoma Subclassification System, with permission of Munro MG

Uterine fibroids and adenomyosis commonly coexist, and concomitant adenomyosis in hysterectomy specimens is reported in 15% to 70% of women with fibroids. Probably, the presence of concomitant adenomyosis in patients with fibroids may contribute to aggravate the symptoms (Munro et al. 2011; Taran et al. 2010).

Pelvic ultrasound remains the gold standard for diagnosis of most fibroids. In cases of adenomyosis, combined 2D-3D ultrasound has also achieved good sensitivity and specificity rates similar to MRI (magnetic resonance imaging) which was traditionally considered the technique of choice. When fibroids and adenomyosis coexist, in cases of large uterine volumes and the presence of more than four fibroids, the ultrasound accuracy decreases, and the role of MRI becomes more valuable.

Management options for uterine fibroids and adenomyosis include medical hormonal therapy and surgery. As fibroids and adenomyosis are uterine benign common diseases and present in women of reproductive age, they can alter women's fertility. Thus, the treatment is intended to control symptoms trying to preserve future fertility up to menopausal status; thus most women become asymptomatic at menopause (Shwayder and Sakhel 2014).

The following chapter aims to provide the characteristics of both entities as far as clinical aspects, diagnosis, and main treatment options.

Uterine Fibroids

Epidemiology

Uterine fibroids are the most common benign tumors in women of reproductive age. According to different series, they are present in 5–70% of women, with at least one fibroid identified in 70% of women aged 40 to 60 years (Lethaby and Vollenhoven 2015; Stewart 2001, 2015). Fibroids are also the main cause of hysterectomy with a substantial impact in terms of health and quality of life in fertile women (Borah et al. 2013).

Commonly associated risk factors include age, genetic factors, race (black women are more likely to have severe symptoms and to undergo hysterectomy, and they tend to be younger at the time of diagnosis), late menopause, early menarche, obesity, prolonged and early use of contraceptives, diets rich in red meat, alcohol, and caffeine. In contrast, progestin-only injectable contraceptives, pregnancy, and consumption of fruit, vegetables, low-fat dairy products, and tobacco would act as a protective factors (Donnez and Dolmans 2016; Stewart 2001).

Etiopathogenesis

Although some specific genetic mutations have been related to fibroid formation, the causes for the development of uterine fibroids are still unknown. Chromosomal translocations, paracentric inversions, and rearrangements are commonly seen in leiomyoma. Also, genetic mutations involving gene(s) controlling myometrial proliferation and growth factors may also play a role in fibroid formation.

Several risk factors for fibroid formation have been established. Mainly, fibroids are hormone-dependent tumors related to estrogen and progesterone environment. Fibroids originate in the smooth uterine muscle formed by a mixture of smooth muscle cells and fibroblasts. Therefore, they are classified as a clonal disease caused by a disruption in the hormonal receptors. Moreover, progesterone and progesterone receptors (PR) are required for cellular proliferation and fibroid growth. Compared to the surrounding myometrium, fibroids express elevated levels of both types of PR, PR-A and PR-B. Progesterone and its receptors therefore represent potential targets for inhibiting fibroids growth.

Fibroids also present elevated levels of extracellular matrix (ECM) components, such as collagens, fibronectin, laminins, and proteoglycans. These proteins induce integrin-Rho/p38 MAPK/ERK pathway leading to upregulation of gene expression, increasing cell proliferation, decreasing apoptosis, and resulting in an altered ECM. On the other hand, matrix metalloproteinases (MMPs), growth factors (TGF-β, activin-A, and PDGF), cytokines (TNF-α), steroid hormones (estrogen and progesterone), and microRNAs (miR-29 family, miR-200c, and miR-93/106b) have also been involved in ECM function, remodeling, and accumulation (fibrosis). Growth factors including TGF-βs (1 and 3) and activin-A may lead myofibroblast differentiation during the process of fibrosis.

Many studies have suggested the implication of miRNAs in the regulation of ECM. These miRNAs induce RNA degradation and/or directly repress protein

translation, leading to ECM accumulation and formation of uterine fibroids. In particular, uterine leiomyoma expresses lower levels of the miRNA miR-29 family, relative to myometrium. Medical treatments targeting ECM will help developing potential current and future treatment options. In fact, several compounds including gonadotropin-releasing hormone (GnRH) agonist (leuprolide acetate), GnRH antagonist (cetrorelix acetate), selective progesterone receptor modulators (ulipristal acetate and asoprisnil), and antiprogestin (mifepristone), among others, have been studied as medical treatments that target ECM in uterine leiomyoma (Bulun 2013; Donnez and Dolmans 2016; Islam et al. 2018; Stewart 2001, 2015).

Clinical Manifestations

Symptoms related to fibroids are present in 20–50% of women and include AUB, pelvic pain, compressive symptoms, or reproductive problems (Lethaby and Vollenhoven 2015; Stewart 2001, 2015). Other less common symptoms include pelvic pressure, bowel dysfunction, urinary frequency and urgency, urinary retention, low back pain, constipation, and dyspareunia. However, most of the fibroids remain asymptomatic (Lethaby and Vollenhoven 2015; Stewart 2001, 2015).

Diagnosis

Main procedures for uterine fibroid's diagnosis include anamnesis, pelvic examination, ultrasonography, and MRI. A detailed anamnesis of the menstrual bleeding pattern (usually, heavy, and prolonged) and its association (or not) with the presence of anemic syndrome and pelvic pain may orient the diagnosis. Besides, a pelvic examination is useful to determine uterine size, mobility, and shape, since women with fibroids present enlarged irregular uterine contour.

Transvaginal ultrasound is considered the test of choice for the diagnosis of uterine fibroids helping to define the size, number, and location of the fibroids. The combined 2D/3D approach allows an endocavitary study and defines the differential diagnosis with adenomyosis. Ultrasound also informs about the characteristics of the ovaries and endometrium. Hysteroscopy is considered the gold standard for the study of the endometrial cavity, and it allows the diagnosis and treatment of submucosal fibroids (FIGO 0–2).

MRI provides accurate information on the number, location, and size of the fibroids, and it has great capacity for differential diagnosis with adenomyosis in cases of big uterine volumes or fibroids (Lumsden et al. 2015; Vilos et al. 2015).

Treatment

Since most fibroids will remain asymptomatic, these will not require any treatment, just an expectant management.

The treatment of fibroids should be based on symptoms, patient's age, and desire for uterine preservation. The symptoms presented by the patient, including bleeding,

pain, compressive symptoms (vesico-rectal, voiding symptoms, ureteral compression, risk of ureterohydronephrosis), and reproductive history, will condition the management. Additionally, patient's age, future reproductive desire, and desire for uterine preservation are also taken into account. Even in asymptomatic patients, the presence of submucosal fibroids and future gestational desire is an indication for treatment. However, the size of the fibroid alone is not an indication for a treatment choice (Laughlin-Tommaso 2016). There are two main lines of fibroids treatment, medical and surgical treatment, and these will be explained below.

Medical Treatment

Hormonal medical treatment for uterine fibroids include combined oral contraceptives, levonorgestrel intrauterine device (LNG-IUD), progestins, gonadotropin-releasing hormone (GnRH) agonists, androgenic steroids, and selective progesterone receptor modulators (SPRMs) (Donnez et al. 2018; Esmya 2012; Laughlin-Tommaso 2016; Lumsden et al. 2015; Singh et al. 2018; Vilos et al. 2015).

Regarding combined oral contraceptives use, there are studies in both directions indicating that it prevents fibroid's appearance, but do not decrease the size of the fibroids. Oral contraceptives are useful for symptoms associated with fibroids such as pelvic pain, bleeding, and ovulatory alterations, but not for the control of myoma size or compressive symptomatology.

LNG-IUD is indicated for AUB, heavy menstrual bleeding (HMB) control, correcting anemia, and contraceptive effect. There is a relative contraindication in submucosal fibroids due to cavity distortion, since LNG-IUD may have higher expulsion rates in women with fibroids. In addition, progestin agents decrease the volume of bleeding due to endometrial atrophy.

GnRH agonists constitute an effective medical treatment for uterine fibroids. They are useful for suppressing blood loss (producing amenorrhea) and thus allowing the correction of the presurgical anemic syndrome. Also, they are useful for reducing the tumor size (up to 50%–70%) which may help the surgery to be performed vaginally or laparoscopically or change the type of incision in the abdominal approach. Its side effects (hypoestrogenism) limit the use to 6 months, and the uterine volume usually reaches the previous size in the year following its suspension. They produce reversible climacteric symptomatology and only in prolonged treatments >12 months increase the risk of osteoporosis.

Leuprolide acetate is the most commonly used and has been approved by the US Food and Drug Administration (FDA) for its use on preoperative treatment of anemia and fibroids in women. In the case of its use prior to myomectomy, it should be noted that it does not make surgery easier, since it hampers the cleavage plane. Thus, it is only indicated in the cases previously mentioned: to restore hematological parameters in cases of severe anemia, to enable a less invasive surgery in large volumes, and to perform pre-hysteroscopic resection of submucosal fibroids. It would be an acceptable treatment in peri-menopausal women to control symptoms and avoid surgery.

Androgenic steroids include danazol and gestrinone. They control HMB-associated anemia, but danazol does not reduce the volume of fibroids. They both

present side effects related to virilization, and limited efficacy and/or side effects limit its use.

SPRM are compounds that bind to progesterone receptors and modulate the transcription in a specific tissue form, in a positive (e.g., progesterone, pure agonist) or negative (e.g., mifepristone, pure antagonist) sense. In uterine fibroids there is an overexpression of both isoforms of PR.

- Mifepristone, RU-486, produces a decrease in the number of PG receptors in the fibroid and also in the myometrium. It reduces menstrual bleeding with amenorrhea rates in up to 70% of cases, comparable to GnRH agonists. However, its use in fibroids is not endorsed by the FDA, since high daily doses are required and have been reported cases of associated endometrial hyperplasia.
- Ulipristal acetate (UPA) at 5 mg/day use is approved by the EMA since 2012. Currently, it is approved for preoperative treatment in patients with moderate-severe symptomatic uterine fibroids, as a single treatment course in women before surgery for fibroids and as intermittent treatment for women in whom surgery is not suitable. UPA normalizes menstrual bleeding in 90% of patients and rapidly induces amenorrhea in 75% of patients in 1 week. It also reduces the size of fibroids (inducing apoptosis and inhibiting cell proliferation) in a similar way to the analogues by around 30%, maintaining this effect up to 6 months after the end of treatment, with lower side effects due to the estradiol levels in a proliferative phase. UPA returns quality of life test scores to values of healthy women, and menstruation and ovulation are resumed 1 month after the end of treatment. The physiological changes associated with progesterone receptors observed on the endometrium (Progesterone Associated Endometrial Changes, PAECs) are reversible 2 months after the end of treatment and do not translate into malignancy, nor require special assessment. These provide the first option for a long-term pharmacologic management for uterine fibroids.

Also, non-hormonal medical treatment including anti-fibrinolytic agents and NSAIDs are used, especially in pain control. However, there is less evidence supporting these medical treatments in the presence of uterine fibroids compared with AUB in normal uterus (NICE 2018).

Tranexamic acid (Amchafibrin) is an anti-fibrinolytic agent approved by the FDA in 2009 for the treatment of HMB, and it is effective in the treatment of uterine fibroid-associated HMB.

NSAIDs reduce the discomfort associated with dysmenorrhea associated or not with fibroids and decrease idiopathic AUB.

Surgical Treatment

Hysterectomy is considered the definitive treatment for symptomatic patients (with bleeding and/or pelvic discomfort) who do not wish to preserve their fertility and have been properly informed of the risks, advantages, and alternatives. Moreover, it is associated with high levels of patient's satisfaction. Fibroids are the most common indication for hysterectomy (one out of four hysterectomies are performed due to

uterine fibroids). Vaginal and laparoscopic routes, technically feasible, are the most appropriate approaches (Donnez and Dolmans 2016; Laughlin-Tommaso 2016).

Myomectomy is the treatment of choice for symptomatic women who wish to preserve their uterus by reproductive desire or by personal uterine preservation preferences. This intervention produces more blood loss than hysterectomy, the risk of 5-year recurrence is approximately of 50–60% by ultrasound. Up to 10%–25% of women who undergo myomectomy will need a hysterectomy in the next 5–10 years (especially in those with large uterine volumes and multiple fibroids). Although it is a very low risk, patients should always be informed about the potential need of performing a hysterectomy if complications occur during myomectomy (1%).

There are no specific limitations regarding the total number and size of fibroids and their surgical approach; the choice of route will depend on the circumstances of each case, the clinical judgment, and the surgeon's experience (Donnez and Dolmans 2016; Laughlin-Tommaso 2016).

Hysteroscopic myomectomy (hysteroscopic resection) is the first-line surgical treatment in symptomatic fibroids type 0–1 and in type 2 up to 4 cm and in cases of asymptomatic patients with reproductive desire (American Association of Gynecologic Laparoscopists (AAGL) 2012).

Nonsurgical Alternatives

Selective *uterine artery embolization (UAE)* is an alternative for premenopausal women with single or multiple fibroids (except subserosal pedunculated and fibroids >10 cm) that cause bleeding and in which surgery is contraindicated or rejected. This procedure is not indicated for women who wish to preserve their fertility. It requires a pre-embolization study using ultrasound, MRI-arteriography, and an endometrial biopsy. The access is made through the femoral artery, embolizing microparticles used for occlusion of both uterine arteries under anesthesia. The goal is to decrease fibroid's blood flow, causing infarction and eventual shrinking of the fibroids. It is an effective treatment: it decreases bleeding in 80–85% of the cases and in 70–80% of the patients decreases pain, although in many cases is not a definitive treatment. At 5-year follow-up, 10% of patients may require hysterectomy and 3% myomectomy, and a 2% will require a new UAE.

It can be associated with serious complications such as urgent hysterectomy after embolization (due to infection and uterine necrosis), hemorrhage, persistent pain, transitory or definitive amenorrhea, expulsion of fibroids (especially submucous fibroids), and mortality of around 0.3% (Gupta et al. 2012; Spies 2016; Zupi et al. 2016).

Magnetic resonance-guided focused ultrasound surgery (MRgFUS) is a technique that destroys the fibroids based on controlled temperature increase causing coagulative necrosis with clear-defined safety margins. Thus, fibroids lose perfusion and shrink in size, decreasing symptoms. The fibroids are not completely removed and will remain present. Image monitoring can be performed by high-resolution ultrasound (USgFUS) or by MRI (MRgFUS, magnetic resonance-guided focused ultrasound surgery). It is indicated for fibroid symptom's control in premenopausal

women with completed gestational desire. It needs a preoperative MRI study and does not require anesthesia. To note, most studies are based on treatments of up to four fibroids.

This minimally invasive therapy may be limited in cases of severe adenomyosis, ≥5 fibroids, intolerance to gadolinium, and fibroids presenting hyperintense signal in T2 and, although relatively, is not recommended in fibroids >10 cm, hypervascularized fibroids, abdominal scars, and the presence of fixed intestinal adhesions.

It can be associated with very low complications: mild pain, grades I–II skin burns, mild blood loss, or fever. No transfusions, ovarian lesions, or hysterectomies have been registered. Recurrences at 24-month follow-up are related to the treated area: if the treated area is greater than 20% of the total fibroid volume, the possibility of myomectomy is around 15% (Donnez and Dolmans 2016; Laughlin-Tommaso 2016; Zupi et al. 2016).

Laparoscopic radiofrequency volumetric thermal ablation (RFVTA) technique is performed using a laparoscopic ultrasound probe to delineate fibroids and a percutaneous radiofrequency handpiece with a deployable electrode array. The fibroids are ablated and the insertion tract coagulated on removal. It was approved in 2012 by the FDA for women who desire uterine preservation and quick recovery (less intraoperative blood loss and shorter hospital stay). We currently have less evidence, but promising results in symptom control and improving quality of life have been reported (Laughlin-Tommaso 2016). *Ultrasound-guided transvaginal radiofrequency* myolysis may be a feasible, efficacious, and safe procedure for symptomatic uterine myomas. Patients treated with this technique showed a reduction in myoma size, AUB, and a significant clinical improvement in the Uterine Fibroids Symptom and Health-related Quality-of-life Questionnaire (Jiang et al. 2014).

Laparoscopic cryomyolysis is an effective and safe alternative for symptomatic uterine myoma. It has shown to provide symptomatic relief, especially AUB, and fibroid size, at 12-month follow-up (Zupi et al. 2005).

Adenomyosis

Epidemiology

Historically, the diagnosis of adenomyosis has been made from histologic analyses; thus, the incidence of adenomyosis is not accurately established. However, it is shown to be affecting 20% of female population and being prevalent among perimenopausal women. 70%–80% of adenomyosis cases are reported in women in the fourth and fifth decades of life; 5%–25% of the cases are observed in patients <39 years; and 5%–10% occur in women >60 years. Prevalence data ranges from 5% to 70%, depending on the studies, and this wide range is due to a lack of standard diagnosis criteria (Garcia and Isaacson 2011).

Risk factors for adenomyosis include estrogen exposure and parity: age 40s and 50s, early menarche (≤10 years old), short menstrual cycles (≤24 days in length),

oral contraceptive use, elevated body mass index (BMI), tamoxifen use, multiparity, and pregnancy termination are statistically significant findings in patients with adenomyosis. The association between adenomyosis and prior surgery is still controversial (Struble et al. 2016).

Adenomyosis is an estrogen-dependent disease entailing abnormal thickening of a portion of the myometrium. Each menstrual cycle involves endometrium regeneration, and when accompanied by altered angiogenesis, both adenomyosis and endometriosis may develop. Thus, common etiology for both conditions potentially involving the same pathways is suggested. To illustrate, external adenomyosis has been associated with deep endometriosis in 49% to 60% of cases, and pelvic endometriosis is observed in 6–20% of women with adenomyosis. Moreover, concomitant endometriosis, myoma, and adenomyosis are observed in 15–25% of patients (Andres et al. 2018; Bergeron et al. 2006).

Etiopathogenesis

In the last decade, the etiopathogenesis of adenomyosis has been studied extensively, and eventually, endometriosis and adenomyosis have been considered two different entities. Both conditions present common genetic mutations and epigenetic changes in sex steroid hormone receptors and inflammatory mediators; however, a growing number of studies pointed out specific etiopathogenic pathways for adenomyosis (Vannuccini et al. 2017). Although etiopathogenesis of adenomyosis is still unknown, at least four theories have been proposed thus far.

The most common theory states that adenomyosis develops from invagination of the endometrial basalis layer into the endometrium. This deep myometrial invasion may occur due to myometrial weakness secondary to previous surgery or pregnancy. Tissue trauma disturbs myometrial-endometrial border and enables endometrial tissue to grow into the injured endometrium lining. Invagination may also come from an altered immune activity in the endometrial-myometrial interface. Supporting this, it has been reported an increase in macrophages, that once activated, leads to T and B cells to produce antibodies and cytokines that may disrupt the junction zone of the endomyometrium. Although the specific trigger for invagination is unknown; hormones such as follicle-stimulating hormone (FSH) or prolactin appear to induce adenomyosis. Prolactin may play a role stimulating migration of basalis and subsequent invasion of endometrial stroma into the myometrium. Moreover, ectopic myometrial tissue shows a higher number of estradiol receptors than the eutopic endometrium. Consistently, ectopic myometrial tissue shows a higher response to progesterone. Adenomyotic tissue contains estrogens-producer enzymes, such as aromatase and estrogen sulfatase, that stimulate further growth and expansion of the abnormal endometriotic glands and stroma into the myometrium. Adenomyosis is fostered by the increased response to estrogen.

A second theory establishes that adenomyosis derives from de novo embryologic-misplaced pluripotent mullerian cells. Ectopic and eutopic endometrium do not have the same secretory pattern: induction of apoptotic cells and *bcl-2* gene expression are

different in an ectopic and eutopic endometrium and also basic fibroblast growth factor (FGF) and angiogenic growth factor. These differences contribute to the pathogenesis of adenomyosis and support that eutopic tissue does not originate in the basal endometrium.

The two other theories are not as common. The third theory suggests that invagination of the basalis progress along the intramyometrial lymphatic system, resulting in adenomyosis, since adenomyosis has been found in specimens within intramyometrial lymphatics. Lastly, the fourth theory states that adenomyosis develops from bone marrow stem cells that are displaced through the vasculature. This theory supported that stem cells foster the development of the endometrium within the musculature of the uterus (Bergeron et al. 2006; Garcia and Isaacson 2011; Senturk and Imamoglu 2015; Struble et al. 2016).

Clinical Manifestations

Symptoms onset are usually reported in women at their 40s and 50s (80%). Among them, AUB (menorrhagia or metrorrhagia) and dysmenorrhea are common. AUB affects up to 60% of adenomyotic patients, and proposed causes of AUB include increased myometrium contractility throughout the menstrual cycle; enlarged uterus with subsequent increase of endometrial surface, vascularization. and blood flow; and overproduction of cytokines and angiogenic growth factors. Dysmenorrhea may be secondary to menorrhagia, i.e., bleeding and edema of areas of endometrial tissue, or it also may be secondary to the increased prostaglandin production in adenomyotic tissue (Garcia and Isaacson 2011; Struble et al. 2016).

Other less common symptoms reported by 7–20% of patients include dyspareunia and chronic pelvic pain. The frequency and severity of symptoms correlate with the severity and depth of adenomyosis. In contrast, one-third of the cases are asymptomatic (Garcia and Isaacson 2011; Struble et al. 2016).

Common signs suggesting the presence of adenomyosis include diffuse uterine enlargement, tender uterus, associated uterine abnormalities, abnormalities at hysteroscopy, and, especially in women <39 years old, infertility. Uterine enlargement is reported on 30% of adenomyosis patients during physical exam. Proliferation of the ectopic endometrial tissue causes hyperplasia and hypertrophy of smooth muscle cell, and these lead to a globular uterus (Garcia and Isaacson 2011; Struble et al. 2016).

Up to 80% of patients with adenomyosis exhibit associated uterine pathology. The most frequent is leiomyomata (35–55%), followed by endometrial polyps (2.3%), hyperplasia without atypia (7.0%) and with atypia (3.5%), and adenocarcinoma (1.4%) (Garcia and Isaacson 2011; Struble et al. 2016).

Diagnosis

The diagnosis of adenomyosis is usually made by histological analysis of hysterectomy specimens. Microscopically, histological findings denote foci of endometrial

glands and stroma tissue deep inside the myometrium. The distance from endomyometrial junction considered for adenomyosis diagnosis ranges between 0.5 and 2 low-power fields or minimal invasion extension from 1 to 4 mm. Overall, most of the studies consider a cutoff value of 2.5 mm for minimal depth of invasion definition. Another common criterion indicates involvement deeper than 25% of myometrial thickness (especially in postmenopausal woman). Although histological confirmation is definitive for diagnosis, it has not been developed a uniform criteria based on it. Apart from hysterectomy specimens' analysis, a histologic diagnosis can also be obtained from hysteroscopic or laparoscopic myometrial biopsies, with findings indicating irregular endometrium with pitting endometrial defects, abnormalities in vascularization, and cystic hemorrhagic lesions (Andres et al. 2018; Dueholm 2006).

There is no consensus regarding the most suitable preoperative imaging tools for adenomyosis diagnosis, albeit MRI and transvaginal ultrasound (TVUS) are the most commonly used for guiding adenomyosis diagnosis. Ultrasound examination of the myometrium may be done by transabdominal (TAS) or transvaginal (TVUS) means; however, there is a lack of standardized terms for normal or pathological myometrium or uterine masses description. In an attempt to describe the sonographic features of the myometrium using ultrasound, the MUSA (Morphological Uterus Sonographic Assessment) group agreed a consensus on terms, definitions, and measurements to describe sonographic features of myometrium and uterine masses (Van Den Bosch et al. 2015).

At ultrasound, findings for adenomyosis include globular uterus, myometrial cysts (1 to 7 mm round anechoic areas), areas of heterogeneous myometrium, asymmetrical myometrial wall, hypoechoic linear striations, echogenic subendometrial lines, and irregular and interrupted endometrial-myometrial junction (Andres et al. 2018; Garcia and Isaacson 2011; Van Den Bosch et al. 2015). Since a high number of non-specific changes are observed in the myometrium with adenomyosis, strict criteria for diagnosis are needed.

Both 2D TVUS and 3D TVUS are effective methods for diagnosis of adenomyosis with overall sensitivity of 84% and 89% and overall specificity of 64% and 56%, respectively. TVUS with color Doppler can also help to differentiate myomas from adenomyosis and presents a diagnostic accuracy for adenomyosis of 93.8%. Although less common, TVUS elastography has been also used in the diagnosis of adenomyosis in patients subjected to hysterectomy (Andres et al. 2018; Exacoustos et al. 2011). Diagnosis through TVUS is preferred (ahead of transabdominal ultrasound or MRI) in women with suspicion of adenomyosis (NICE 2018).

Main evidences for adenomyosis at MRI include large, regular asymmetric uterus (without leiomyomas), thickened junctional zone (from 8 to 12 mm), or an abnormal ratio of junctional zone to myometrial thickness greater than 40%, abnormal myometrial signal intensity, and myometrial foci of high-signal intensity on T1-weighted images.

Without concomitant leiomyomas, MRI and TVUS show similar diagnostic accuracy. TVUS exhibits 53%–89% sensitivity and 50%–99% specificity for the

diagnosis of adenomyosis, and MRI shows 67% sensitivity and specificity of 82% when myomas are present. In contrast, MRI demonstrates sensitivity of 87% and specificity of 100% when myomas are absent (Andres et al. 2018).

Treatment

Adenomyosis treatment must be individualized based on type of adenomyosis, symptomatology, desire to fertility, comorbidities, etc. The definitive therapy for uterine adenomyosis is hysterectomy, although this option does not contemplate women wishing to preserve their uterus. At present, medical treatment or minimally invasive conservative surgical techniques are preferred. Nevertheless, evidence for conservative surgical is still scarce and benefits debatable, although may be of use in patients unable to receive long-term medical treatment. Studies evaluating outcomes of conservative surgery on fertility and symptom management are limited and involve a reduced number of women and short follow-up periods. Notwithstanding, they have reported that treatment is efficient for symptom management. Regarding the outcomes of conservative surgery on fertility, focal adenomyosis reports higher pregnancy outcome after surgery when compared to diffuse adenomyosis. However, further prospective controlled trials are required to investigate the benefits of conservative surgery over medical treatment on fertility outcomes. To note, half of adenomyosis patients have concomitant endometriosis and/or myomas, and these may interfere with the results of conservative surgery (Grimbizis et al. 2014; Younes and Tulandi 2018).

Surgical Treatment

Main uterine-sparing surgery techniques used for adenomyosis treatment include hysteroscopic treatment, endometrial ablation/resection, and cytoreductive surgery. Not all types of adenomyosis can be treated by hysteroscopy, and the procedure and effect are only described in case reports. Endometrial ablation/resection is commonly used when medical treatment does not allow symptom control, although it is not indicated in women wishing to conceive. It is a second-line choice of procedure and commonly used together with LNG-IUD in women who do not desire pregnancy. Cytoreductive surgery is mainly used in diffuse adenomyosis and in cases of localized adenomyosis can remove the lesion completely (Dueholm 2018).

Nonsurgical Alternatives

Techniques for induction of necrosis in adenomyosis include uterine artery embolization (UAE) and high-intensity focused ultrasound (HIFU). UAE is an effective treatment for adenomyosis symptoms (AUB and uterine size). However, it is relatively contraindicated in women wishing to conceive after surgery and presents risk of reoperation. HIFU is a method that delivers targeted ultrasonic energy and induces coagulation tissue necrosis. There are very few studies in adenomyosis, and main drawbacks include compromised fertility and high costs.

Medical Treatment

Medical therapy should be the first-line treatment for adenomyosis. Overall, these are effective, especially in women with moderate uterus enlargement. Main options include oral contraceptives (OC), progestins, and LNG-IUD.

OC and progestins inhibit estrogen and estradiol production, respectively, and cause decidualization and atrophy of the endometrium. Also, OC provide good symptom control in two-thirds of women (Dueholm 2018; Pontis et al. 2016).

LNG-IUD causes decidualization of the endometrium and decreased bleeding acting through different levels: it downregulates estrogen receptor (ER), alters the expression of steroid receptor coregulators, reduces the expression of COX-2, decreases levels of vascular endothelial growth factor (VEGF), increases the expression of adrenomedullin, and reduces lymphangiogenesis. Its efficacy has been demonstrated in many clinical trials, has fewer side effects than other treatments, and is cost-effective (Dueholm 2018).

Danazol is an anti-gonadotropic agent that inhibits DNA synthesis and induces apoptosis. It has been widely used for endometriosis and adenomyosis; however, its safety profile includes a number of adverse effects (i.e., weight gain, muscle cramps, reduced breast size, acne, hirsutism, oily skin, decreased high-density lipoprotein levels, increased liver enzymes, hot flashes, mood changes, depression, and deepening of the voice) (Dueholm 2018; Pontis et al. 2016).

GnRH agonists suppress ovarian function. They are effective in the treatment of pain, although long-term treatment is not recommended due to hypogonadic side effects such as vasomotor syndrome, genital atrophy, and mood instability, among others. GnRH agonists have been used in several trials along with surgery (add-back therapy) (Dueholm 2018; Pontis et al. 2016).

Aromatase is an enzyme responsible for a key step in the synthesis of estrogens from androgens. It mediates the conversion of androstenedione and testosterone to estrone and estradiol, respectively. In a randomized trial, aromatase inhibitors (AI) were as efficient as GnRH agonists (Dueholm 2018); however, the European Society of Human Reproduction and Embryology (ESHRE) only recommends its use when other treatment options have failed, due to its severe side effects (Dunselman et al. 2014).

Selective estrogen receptor modulators (SERM) are novel synthetic estrogen receptor (ER) ligands associated with controversial effects. Currently, available data evaluating SERM on adenomyosis are still scarce. Thus, considering its restricted use in adenomyosis, their use is not indicated.

SPRM are new agents that may act on the progesterone receptor as weak agonist if progesterone is present or as a progestagenic agent in some tissues, e.g., the endometrium. Currently, two SPRM drugs are approved for gynecological treatment: mifepristone and UPA. In a study with premenopausal women with adenomyosis and symptomatic uterine myomas, standard UPA treatment rapidly controlled bleeding and improved quality of life, along with a good safety profile (Donnez et al. 2012; Gracia et al. 2018; Pontis et al. 2016). Other SPRMs under study, such as asoprisnil and telapristone, have also been reported to relieve pain in adenomyosis patients. Nevertheless, SPRMs need to be investigated in depth, and their long-term effects in patients with adenomyosis must be evaluated in detail.

Finally, novel agents such as angiogenesis inhibitors, oxytocin receptor modulators, demethylating agents, anti-platelet therapy, and valproic acid are actually under study. Overall, they seem to have a role in pain control, uterine size reduction, suppressed myometrial infiltration, and contractibility control. However, caution should be exercised because these investigations are still preliminary and further investigations are required.

Adenomyosis, Uterine Fibroids, and Reproduction

Pathophysiology of Infertility in Adenomyosis

Around 70%–80% of adenomyosis cases are reported in women in their 40s and 50s with symptoms of AUB and pain. However, younger patients suffering adenomyosis usually present adenomyosis-associated infertility. Overall, 5%–25% of adenomyosis cases are observed in women of childbearing potential (<39 years). In addition, in western countries women have children at older ages; thus, the relation between adenomyosis and infertility is even more significant nowadays (Garcia and Isaacson 2011).

Although the pathophysiology of infertility in adenomyosis is not well-known, several correlations between adenomyosis and infertility have been proposed. In adenomyosis, a thickening of the junctional zone accompanied by muscular changes (hyperplasia and fibrosis) is commonly observed. This abnormal thickening may alter shape, elasticity, and turgor of the uterus and, consistently, its function. Previous studies reported a thinner junctional zone in fertile women when compared to infertile women (Dueholm 2017; Soave et al. 2018). As a consequence of myometrial thickening, abnormal uterine contractibility may occur. This abnormal contractibility may alter both sperm migration and embryo implantation, leading to infertility. In women with diffuse adenomyosis, dysperistalsis occurs; also, a thickened junctional zone is related to dysperistalsis. Moreover, adenomyosis may cause anatomical distortion of the uterine cavity, especially on the posterior wall of the uterus. Several anatomical uterine alterations may involve blocking of the ostium of the uterine tube, leading to sperm migration and embryo transportation impairment. It has been reported that up to 33% of women with adenomyosis show moderate to severe distortion on the endometrial cavity highlighting the possible correlation between adenomyosis and infertility (Dueholm 2017; Soave et al. 2018).

At molecular level, fertile women express antioxidant enzymes, such as superoxide dismutase (SOD) or nitric oxide synthase (NOS) during the early and mid-proliferative phases at low levels, and these increase in the midsecretory phases, reaching a peak in the late secretory phase. Patients with adenomyosis overexpress these enzymes. Thus, this overexpression could play a role in embryo implantation and development, accounting for early miscarriages (Dueholm 2017; Soave et al. 2018). Also, increased expression of cytochrome P450 and aromatase is observed in patients with adenomyosis.

Adenomyosis-Related Infertility

Currently, no studies have examined natural conception on women with adenomyosis (Dueholm 2017). Moreover, evidence is not strong enough to support that adenomyosis increases the risk of obstetric complications, albeit it has been reported an increased risk for preterm birth and preterm rupture of membranes in adenomyosis patients (Soave et al. 2018).

Currently, main treatments for adenomyosis include medical treatment, minimally invasive conservative surgery, or combined therapy. However, studies evaluating the impact of these treatments on fertility outcomes are still limited. Among medical treatments, GnRH agonists may diminish or reduce NOS expression, reducing free radical burden in the endometrium. They may also reduce the expression of P450 aromatase, increasing uterine receptivity. Several cases of spontaneous pregnancy and delivery have been reported after short-term treatment with GnRH agonists in infertile women with adenomyosis (Soave et al. 2018). Also, pretreatment with GnRH agonists before in vitro fertilization (IVF) is associated with significantly higher pregnancy rates, implantation, and clinical pregnancy in adenomyosis patients (Dueholm 2017). In contrast, none of the other medical treatments available for adenomyosis are helpful in adenomyosis-related infertility.

Conservative cytoreductive uterine-sparring techniques such as myometrial reduction are a good option for preserving fertility, and several cases of pregnancy have been reported after this procedure. However, it may reduce the size of the uterus and predispose toward miscarriage or premature delivery. It is noted that some conservative surgery techniques modify the anatomy of the uterus, and these alterations may contribute to reduce the postoperative pregnancy rate. Also, surgery may induce uterine scars, weaken the uterine wall, and subsequently increase the risk of uterine rupture during pregnancy (Soave et al. 2018).

Finally, the advantages of combined therapy (surgery and medical treatment) on fertility outcomes are still controversial. Although successful pregnancies have been reported after combined therapy, no significant differences were found between patients treated with conservative surgery alone when compared to combined therapy (Dueholm 2017; Soave et al. 2018).

Pathophysiology of Uterine Fibroid-Related Infertility

Several mechanisms of action have been proposed for explaining the impact of myomas on fertility. As in adenomyosis, changes in the anatomy of the endometrium may lead to obstruction of the fallopian tubes or endometrial cavity distortion. In the same line, functional changes such as increased uterine contractility may alter sperm progression. Also, abnormal blood supply due to fibroids may alter fertility, and chronic inflammation may play a role in altering myometrial environment hindering implantation. Histological alterations, such as elongation/distortion of the glands, hyperplasia, polyposis, and ectasia may also impair implantation. Finally, an abnormal hormonal environment and fibroids' paracrine effects on the endometrium may also impact on fertility. Cells involved in endometrial decidualization, such as

macrophages and uterine natural killer cells, are also reduced in women with uterine fibroids, negatively affecting implantation. Molecular mechanisms underlying the effect for fibroids on fertility are unclear; however, uterine fibroids reduce endometrial HOXA-10 and HOXA-11 expression, which are factors that mediate stromal cell proliferation during implantation. Also, cytokines involved in implantation and early embryonic development are reduced in women with submucosal fibroids (Purohit and Vigneswaran 2016; Zepiridis et al. 2016).

Uterine Fibroid-Related Infertility

When considering the type of fibroid and its impact on infertility, subserosal fibroids do not seem to have a significant effect on reproductive outcome. Thus, there is no evidence of benefit on fertility when myomectomy is performed to remove subserosal fibroids (Purohit and Vigneswaran 2016; Zepiridis et al. 2016).

In contrast, the effect of intramural fibroids on fertility is controversial, and it is still a matter of debate. Intramural fibroids have been associated to a negative effect on implantation, pregnancy/life birth, and spontaneous miscarriage (Parazzini et al. 2016; Purohit and Vigneswaran 2016; Zepiridis et al. 2016). Sunkara et al. reported a significant decrease in pregnancy rates and in the live birth in women presenting intramural fibroids undergoing IVF treatment (Sunkara et al. 2010). Additionally, it should be taken into account that number and size of intramural fibroids also play a role on infertility. When considering fibroids' size, evidences point out that intramural fibroids $\geq$4 cm may also negatively influence fertility (Parazzini et al. 2016; Purohit and Vigneswaran 2016; Zepiridis et al. 2016).

Regarding submucous fibroids, these have been clearly associated to a detrimental effect on implantation, pregnancy/life birth, and spontaneous miscarriage. Hence, benefit on fertility should be expected when fibroids are removed. In this line, evidence reported higher spontaneous pregnancy rates on women who undergo myomectomy versus non-myomectomized patients at 12 months after surgery (Purohit and Vigneswaran 2016; Zepiridis et al. 2016). When patients underwent a myomectomy prior to IVF, higher pregnancy rates were observed compared to non-myomectomized group (Raffi et al. 2012). Additionally, surgery is a potential source of damage and must be taken into account. Surgery not only involves morbidity risk but also implies myometrial trauma, suturing and scar healing, and potential development of defective functional myometrium and adhesions. Also, intrauterine adhesions correlate with a decrease in reproductive outcomes. Due to the increased uterine rupture risk involved in surgery, it is recommended to postpone future conception to 6 months after myomectomy.

During pregnancy, uterine fibroids have been associated with an increased risk of spontaneous miscarriage rates, complications, and adverse pregnancy outcomes. Fibroids adjacent to the placenta are prone to bleeding in early pregnancy and spontaneous miscarriage. In contrast, no difference is observed in preterm delivery rates among women with fibroids (Lumsden et al. 2015). Fibroid treatment may improve pregnancy outcome, although large randomized clinical trials are needed to support limited data available (Parazzini et al. 2016). Also, timely treatment is

crucial, since women who receive treatment early are most likely to have successful outcomes compared to women with long-duration infertility. In these women, prognosis is worse irrespective of treatment (Purohit and Vigneswaran 2016).

Following myomectomy is reported a decrease in the miscarriage rate. Moreover, in women who underwent myomectomy for submucosal myomas, clinical pregnancy, live birth, and spontaneous abortion rates normalize over time, compared to infertile women without fibroids (Parazzini et al. 2016).

After MRgFUS Surgery a high rate of pregnancies and deliveries have been reported; however, data are still preliminary. To note, the miscarriage rate reported after the procedure was 28%. UAE is not a treatment of first choice for women with infertility, since an increased risk of negative pregnancy outcome may be present following the procedure; however, it has not been adequately studied (Parazzini et al. 2016; Purohit and Vigneswaran 2016).

UPA is a novel and effective treatment used for fibroid reduction that shows feasible and safe conception, but also high miscarriage rates. Thus, the presence of fibroids impacts on pregnancy rates, since pregnancy rates after myomectomy are higher than after reductive therapies (Purohit and Vigneswaran 2016).

Conclusions

Uterine fibroids and adenomyosis are benign pathologies highly prevalent in women of reproductive age that commonly coexist. Both entities share symptomatology, being two of the main causes of AUB. In contrast, many women remain asymptomatic. TVUS is the gold standard for diagnosis, and treatment is aimed to achieve symptom control preserving future fertility. Fibroids and adenomyosis are also the main causes of hysterectomy, the most common gynecological surgical procedure. However, the first-line treatment is mainly based on hormonal medical therapy. Future trend is based on less-invasive procedures, aimed to achieve effective symptom control presenting fewer side effects, and involving minimal morbidity and low recurrence rates.

Overall, the impact of uterine fibroids and adenomyosis on infertility and pregnancy outcomes is still not completely understood, and large, well-controlled, and properly designed studies are still needed. Although early and patient-tailored treatments may improve pregnancy outcome in patients with uterine fibroids and adenomyosis, further investigation is needed to determine comparative efficacy of different treatments, as well as their safety and effectiveness.

Cross-References

- ▶ Abnormal Uterine Bleeding
- ▶ Endometriosis
- ▶ Hormonal Contraception
- ▶ Infertility

References

American Association of Gynecologic Laparoscopists (AAGL). Advancing minimally invasive gynecology worldwide. AAGL Practice report: Practice guidelines for the diagnosis and Management of Submucous Leiomyomas. J Minim Invasive Gynecol. 2012;19(2):152–71.

Andres MP, Borrelli GM, Ribeiro J, Baracat EC, Abrão MS, Kho RM. Transvaginal ultrasound for the diagnosis of Adenomyosis: systematic review and meta-analysis. J Minim Invasive Gynecol. 2018;25(2):257–64.

Bergeron C, Amant F, Ferenczy A. Pathology and physiopathology of adenomyosis. Best Pract Res Clin Obstet Gynaecol. 2006;20(4):511–21.

Borah BJ, Nicholson WK, Bradley L, Stewart EA. The impact of uterine leiomyomas: a national survey of affected women. Am J Obstet Gynecol. 2013;209(4):319.e1–20.

Bulun SE. Uterine Fibroids. N Engl J Med. 2013;369(14):1344–55.

Donnez J, Dolmans MM. Uterine fibroid management: from the present to the future. Hum Reprod Update. 2016;22(6):665–86.

Donnez J, Tatarchuk TF, Bouchard P, Puscasiu L, Zakharenko NF, Ivanova T, et al. Ulipristal acetate versus placebo for fibroid treatment before surgery. N Engl J Med. 2012;366(5):409–20.

Donnez J, Courtoy GE, Donnez O, Dolmans MM. Ulipristal acetate for the management of large uterine fibroids associated with heavy bleeding: a review. Reprod Biomed Online. 2018;37(2):216–23.

Dueholm M. Transvaginal ultrasound for diagnosis of adenomyosis: a review. Best Pract Res Clin Obstet Gynaecol. 2006;20(4):569–82.

Dueholm M. Uterine adenomyosis and infertility, review of reproductive outcome after in vitro fertilization and surgery. Acta Obstet Gynecol Scand. 2017;96(6):715–26.

Dueholm M. Minimally invasive treatment of adenomyosis. Best Pract Res Clin Obstet Gynaecol. 2018;51:119–37.

Dunselman GAJ, Vermeulen N, Becker C, Calhaz-Jorge C, D'Hooghe T, De Bie B, et al. ESHRE guideline: management of women with endometriosis. Hum Reprod. 2014;29(3):400–12.

Esmya. Summary of Product Characteristics. 2012. last Updated 2018[Internet]. Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages%252Fmedicines%252Fhuman%252Fmedicines%252F002041%252Fhuman_med_001542.jsp&mid=wc0b01ac058001d124.

Exacoustos C, Brienza L, Di Giovanni A, Szabolcs B, Romanini ME, Zupi E, et al. Adenomyosis: three-dimensional sonographic findings of the junctional zone and correlation with histology. Ultrasound Obstet Gynecol. 2011;37(4):471–9.

Garcia L, Isaacson K. Adenomyosis: review of the literature. J Minim Invasive Gynecol. 2011;18(4):428–37.

Gracia M, Alcalà M, Ferreri J, Rius M, Ros C, Saco MA, et al. Ulipristal acetate improves Clinical symptoms in women with Adenomyosis and uterine Myomas. J Minim Invasive Gynecol. 2018;25(7):1274–80.

Grimbizis GF, Mikos T, Tarlatzis B. Uterus-sparing operative treatment for adenomyosis. Fertil Steril. 2014;101(2):472–487.e8.

Gupta JK, Sinha A, Lumsden MA, Hickey M. Uterine artery embolization for symptomatic uterine fibroids. Cochrane Database Syst Rev. 2012;5:CD005073. https://doi.org/10.1002/14651858.CD005073.pub3.

Islam MS, Ciavattini A, Petraglia F, Castellucci M, Ciarmela P. Extracellular matrix in uterine leiomyoma pathogenesis: a potential target for future therapeutics. Hum Reprod Update. 2018;24(1):59–85.

Jiang X, Thapa A, Lu J, Bhujohory VS, Liu Y, Qiao S. Ultrasound-guided transvaginal radio-frequency myolysis for symptomatic uterine myomas. Eur J Obstet Gynecol Reprod Biol. 2014;177:38–43.

Laughlin-Tommaso SK. Alternatives to hysterectomy: management of uterine Fibroids. Obstet Gynecol Clin N Am. 2016;43(3):397–413.

Lethaby A, Vollenhoven B. Fibroids (uterine myomatosis, leiomyomas). BMJ Clin Evid. 2015;2015:814.

Lumsden MA, Hamoodi I, Gupta J, Hickey M. Fibroids: diagnosis and management. BMJ. 2015;351:1–10.
Munro MG. Classification of menstrual bleeding disorders. Rev Endocr Metab Disord. 2012;13(4):225–34.
Munro MG, Critchley HOD, Fraser IS. The FIGO classification of causes of abnormal uterine bleeding in the reproductive years. Fertil Steril. 2011;95(7):2204–2208.e3.
NICE. Heavy menstrual bleeding: assessment and management. NICE guideline. 2018. Available from: https://www.nice.org.uk/guidance/ng88/resources/heavy-menstrual-bleeding-assessment-and-management-pdf-1837701412549.
Parazzini F, Tozzi L, Bianchi S. Pregnancy outcome and uterine fibroids. Best Pract Res Clin Obstet Gynaecol. 2016;34:74–84.
Pontis A, D'Alterio MN, Pirarba S, de Angelis C, Tinelli R, Angioni S. Adenomyosis: a systematic review of medical treatment. Gynecol Endocrinol. 2016;32(9):696–700.
Purohit P, Vigneswaran K. Fibroids and Infertility. Curr Obstet Gynecol Rep. 2016;5:81–8.
Raffi F, Metwally M, Amer S. The impact of excision of ovarian endometrioma on ovarian reserve: a systematic review and meta-analysis. J Clin Endocrinol Metab. 2012;97(9):3146–54.
Senturk LM, Imamoglu M. Adenomyosis: what is new? Womens Heal. 2015;11(5):717–24.
Shwayder J, Sakhel K. Imaging for uterine Myomas and Adenomyosis. J Minim Invasive Gynecol. 2014;21(3):362–76.
Singh SS, Belland L, Leyland N, von Riedemann S, Murji A. The past, present, and future of selective progesterone receptor modulators in the management of uterine fibroids. Am J Obstet Gynecol. 2018;218(6):563–572.e1.
Soave I, Wenger JM, Pluchino N, Marci R. Treatment options and reproductive outcome for adenomyosis-associated infertility. Curr Med Res Opin. 2018;34(5):839–49.
Spies JB. Current role of uterine artery embolization in the management of uterine fibroids. Clin Obstet Gynecol. 2016;59(1):93–102.
Stewart EA. Uterine fibroids. Lancet. 2001;357(9252):293–8.
Stewart EA. Clinical practice. Uterine Fibroids. N Engl J Med. 2015;372(17):1646–55.
Struble J, Reid S, Bedaiwy MA. Adenomyosis: a Clinical review of a challenging gynecologic condition. J Minim Invasive Gynecol. 2016;23(2):164–85.
Sunkara SK, Khairy M, El-Toukhy T, Khalaf Y, Coomarasamy A. The effect of intramural fibroids without uterine cavity involvement on the outcome of IVF treatment: a systematic review and meta-analysis. Hum Reprod. 2010;25(2):418–29.
Taran FA, Weaver AL, Coddington CC, Stewart EA. Characteristics indicating adenomyosis coexisting with leiomyomas: a case-control study. Hum Reprod. 2010;25(5):1177–82.
Van Den Bosch T, Dueholm M, Leone FPG, Valentin L, Rasmussen CK, Votino A, et al. Terms, definitions and measurements to describe sonographic features of myometrium and uterine masses: a consensus opinion from the morphological uterus sonographic assessment (MUSA) group. Ultrasound Obstet Gynecol. 2015;46(3):284–98.
Vannuccini S, Tosti C, Carmona F, Huang SJ, Chapron C, Guo SW, et al. Pathogenesis of adenomyosis: an update on molecular mechanisms. Reprod Biomed Online. 2017;35(5):592–601.
Vilos GA, Allaire C, Laberge P-Y, Leyland N, Vilos AG, Murji A, et al. The Management of Uterine Leiomyomas. J Obstet Gynaecol Can. 2015;37(2):157–78.
Younes G, Tulandi T. Conservative surgery for Adenomyosis and results: a systematic review. J Minim Invasive Gynecol. 2018;25(2):265–76.
Zepiridis LI, Grimbizis GF, Tarlatzis BC. Infertility and uterine fibroids. Best Pract Res Clin Obstet Gynaecol. 2016;34:66–73.
Zupi E, Marconi D, Sbracia M, Exacoustos C, Piredda A, Sorrenti G, et al. Directed laparoscopic cryomyolysis for symptomatic leiomyomata: one-year follow up. J Minim Invasive Gynecol. 2005;12(4):343–6.
Zupi E, Centini G, Sabbioni L, Lazzeri L, Argay IM, Petraglia F. Nonsurgical alternatives for uterine Fibroids. Best Pract Res Clin Obstet Gynaecol. 2016;34:122–31.

Abnormal Uterine Bleeding

10

Rohan R. Chodankar and Hilary O. D. Critchley

Contents

R. R. Chodankar · H. O. D. Critchley (✉)
MRC Centre for Reproductive Health, The Queen's Medical Research Institute, The University of Edinburgh, Edinburgh, UK
e-mail: Rohan.chodankar@ed.ac.uk; Hilary.Critchley@ed.ac.uk

F. Petraglia, B. C. Fauser (eds.), *Female Reproductive Dysfunction*, Endocrinology,
https://doi.org/10.1007/978-3-030-14782-2_10

Abstract

Abnormal uterine bleeding (AUB) is a chronic debilitating condition affecting women worldwide with a significant negative impact on their quality of life. Management choices are determined in partnership with an individual woman's need for uterine and fertility preservation. Although a diverse range of medical and surgical treatment choices are available, a tailored and phenotype-dependent approach to management still remains lacking. AUB, therefore, represents an area of unmet clinical need where future research is needed to optimize information underpinning management choices for women.

Keywords

Abnormal uterine bleeding (AUB) · Heavy menstrual bleeding (HMB) · PALM-COEIN · Hysterectomy · Fibroids · Leiomyomas · Menstrual cycle · Menstruation

Introduction

Abnormal uterine bleeding (AUB) encompasses any symptomatic variation from a normal menstrual cycle. A menstrual cycle is defined as being "normal" based on four factors described in Table 1 (Fraser et al. 2007a, 2011; Munro et al. 2018). AUB affects women globally and has a negative impact on their quality of life, in some instances, being chronic and debilitating. It also represents a significant proportion of healthcare demand in both primary and secondary care settings.

In the United Kingdom (UK), evidence from a recent national audit by the Royal College of Obstetricians and Gynaecologists (RCOG) suggests that nearly a third of women are unsatisfied with their current management choices and have to opt for potentially fertility ending invasive surgical options (RCOG 2014a). The lack of clinically useable biomarker(s) further compounds the problem (Chodankar and Critchley 2018). In an era of medicine where care is delivered based on different patient phenotypes, AUB is an area where a greater focus to ensure tailored care is needed.

Table 1 Parameters of the normal menstrual cycle. (Adapted from (Munro et al. 2018))

Parameter	Normal limits (5th to 95th centile)
Frequency of menses (days)	24–38 days
Regularity (cycle length)	Cycle length is the number of days from the first day of bleeding in one menstrual cycle to the first day of bleeding in the next. No more than 7–9 days difference between the shortest to longest cycles: ≤7 to 9 days
Duration (days of bleeding in a single menstrual period)	≤8 days
Volume (monthly blood loss)	Clinical definition is subjective and defined as a volume of menstrual blood loss that does not interfere with a woman's physical, social, emotional, and/or material quality of life

Definition and Classification

The Menstrual Disorders Working Group within the International Federation of Gynecology and Obstetrics (FIGO), subsequently the Menstrual Disorders Committee (MDC), was responsible for two classification systems currently in use (Fraser et al. 2011; Munro et al. 2018).

- Terminology and Definitions (FIGO-AUB System 1)
- Classification of Causes of AUB in the Reproductive Years, the PALM-COEIN system (FIGO-AUB System 2)

These were initially published in 2007 (Fraser et al. 2007a; Fraser et al. 2007b) and 2011 (Fraser et al. 2011; Munro et al. 2011) and revised in 2018 (Munro et al. 2018).

Acute Non-gestational AUB

Acute non-gestational AUB is an episode of uterine bleeding in a woman of reproductive age that is of sufficient quantity to require immediate intervention to prevent further blood loss (Munro et al. 2018).

Chronic Non-gestational AUB

Chronic non-gestational AUB in the reproductive years is defined as bleeding from the uterine corpus that is abnormal in duration, volume, frequency, and/or regularity and has been present for the majority of the preceding six months (Munro et al. 2018).

Intermenstrual Bleeding

When AUB occurs between well-defined cyclical menses, the symptom is called intermenstrual bleeding (IMB).

These may be further subdivided as:

- Cyclic mid-cycle IMB – Small quantity of frank vaginal bleeding or discharge around mid-cycle. This may be physiological due to the nadir in circulating estradiol levels at the time of ovulation (Speroff and Fritz 2005).
- Cyclic pre- or postmenstrual IMB – Cyclical IMB that predictably occurs either early in the cycle (follicular phase) or late (luteal phase) and typically presents as very light vaginal bleeding for one or more days.
- Acyclic IMB – When IMB is not cyclical or predictable.

The FIGO MDC recommends that terms such as dysfunctional uterine bleeding (DUB), menorrhagia, metrorrhagia, oligomenorrhea, polymenorrhea, hypomenorrhea, hypermenorrhea, or combinations thereof should no longer be used (Woolcock et al. 2008).

Heavy Menstrual Bleeding (HMB)

The National Institute for Care and Excellence (NICE) defines HMB as excessive menstrual blood loss (MBL) that interferes with the physical, social, emotional, and/or material quality of life (NICE 2007, 2018). It represents a definition of AUB that is woman centered, is subjective, and steers away from the classic objective criteria of blood loss >80 mL/cycle.

PALM-COEIN Classification: Causes of AUB

As per the FIGO MDC, there are nine main categories which represent the causes of non-gestational AUB, with an aligned shorthand nomenclature.

P	Polyp (AUB-P)
A	Adenomyosis (AUB-A)
L	Leiomyoma (AUB-L)
M	Malignancy and hyperplasia (AUB-M)

C	Coagulopathy (AUB-C)
O	Ovulatory dysfunction (AUB-O)
E	Endometrial (AUB-E)
I	Iatrogenic (AUB-I)
N	Not otherwise classified (AUB-N)

In general, the components of the PALM group are discrete (structural) entities that may be identified visually with imaging techniques and/or histopathology, whereas the COEIN group is related to entities that are not defined by imaging or histopathology (non-structural) and usually ascertained with a comprehensive history. See Fig. 1.

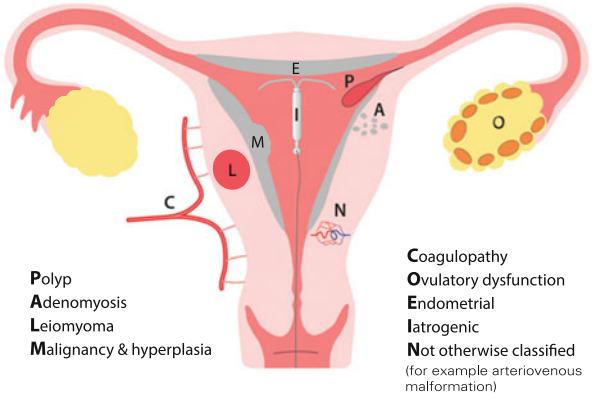


Fig. 1 PALM-COEIN classification of AUB

The 2018 update suggests the FIGO MDC is currently working on subclassification systems for adenomyosis and endometrial polyps. There is consideration for subclassification systems for AUB-C, AUB-O, AUB-E, and AUB-I, but these initiatives are still in the very early stages of development.

Polyps (AUB-P)

Endometrial polyps are epithelial proliferations arising from the endometrial stroma and glands and may contribute to AUB (Lieng et al. 2009). Polyps may be diagnosed by clinical examination, ultrasonography (US, sometimes accompanied with saline infusion of the uterine cavity), hysteroscopy, or histopathology. Polyps may be endometrial or endocervical.

Adenomyosis (AUB-A)

Adenomyosis is defined as the presence of ectopic endometrial glands and stroma in the myometrium. The genesis of the condition still remains unclear, as does its association with AUB. Traditionally, the diagnosis of adenomyosis was made in retrospect, after histopathological analysis following hysterectomy for AUB. Currently, there are defined sonographic criteria and magnetic resonance imaging (MRI) criteria for diagnosis (Champaneria et al. 2010; Van den Bosch et al. 2015). Despite this, the prevalence remains unclear, and estimates suggest a 5–70% occurrence in histological diagnosis in hysterectomy specimens (Abbott 2017). Newer modalities such as elastography (ultrasound mode) have shown promise in reaching a diagnosis (Liu et al. 2018). Currently there is limited evidence to guide the management of women with adenomyosis, either medically or surgically.

Leiomyomas (AUB-L)

Leiomyomas (fibroid, myoma) are extremely common; the estimated cumulative incidence by age 50 is >80% for black women and nearly 70% for white women (Baird et al. 2003). Fibroids may be asymptomatic, and most often contribute to AUB, when submucous (Stewart 2001; Jacobson and Enzer 1956). The FIGO leiomyoma system is extensive and so far, the only subclassification to be ratified by the FIGO MDC.

Malignancy (AUB-M)

There are pre-existing classification systems by the World Health Organization (WHO) and FIGO for gynecological malignancies, and the PALM-COEIN system aims to complement these systems, rather than replace them (Pecorelli et al. 1999;

Zalewski et al. 2015). Although relatively uncommon, atypical endometrial hyperplasia (endometrial intraepithelial neoplasia or EIN) and malignancy are important potential causes of, or findings associated with, AUB and must be considered in nearly all women of reproductive age. Risk factors that may increase the risk of endometrial hyperplasia and neoplasia in premenopausal women have been defined by the RCOG (RCOG 2014b). A raised body mass index (BMI) is a risk factor that particularly requires recognition. Cervical cancer may present as persistent IMB or post-coital bleeding (PCB).

An important consideration is leiomyosarcoma (LMS), which is a sarcoma arising in a uterine fibroid and may present with AUB and increasing fibroid growth. The incidence of uterine sarcoma is a topic of current interest and good quality data are required. These tumors are aggressive and have a poor prognosis and a high recurrence rate following treatment. Age and perimenopausal status are important considerations. Recent data highlights an increased incidence of unexpected uterine sarcomas for women undergoing hysterectomy for benign indications, including fibroids (Brohl et al. 2015; Sizzi et al. 2018; Multinu et al. 2019). This risk increases with age, and the risk is higher in women >45 years (Brohl et al. 2015; Multinu et al. 2019). A recent RCOG publication provides further information on this issue (https://www.rcog.org.uk/globalassets/documents/guidelines/consent-advice/consent-advice-no-13-morcellation-myomectomy-hysterectomy.pdf). As the evidence base concerning risk of LMS in women with uterine fibroids builds, important information will be available to clinicians to inform management discussions. Symptomatic postmenopausal women with uterine fibroids represent a particularly high-risk group (Chen et al. 2018).

At the present time, there is no laboratory test, e.g., a tumor maker or an imaging study (ultrasound, MRI, CT scan), that can reliably diagnose uterine LMS preoperatively (Roberts et al. 2018). There is a need for more studies in this important clinical area.

Recent evidence also highlights the importance of performing an endometrial sampling in women with AUB with suspected benign disease. Although the sensitivity of diagnosing uterine sarcomas is low, it may be able to reduce the risk of unexpected non-benign histology in women post hysterectomy (Multinu et al. 2019). In addition, younger women who are obese are also at a risk of endometrial cancer and a such should be considered for endometrial sampling (Thomas et al. 2009).

Coagulopathy (AUB-C)

Underlying bleeding disorders may contribute to AUB and may be discovered in adult life, rather than immediately after menarche. They are reported to affect 12–14% of the women presenting with HMB to the gynecology services, most commonly von Willebrand disease (Shankar et al. 2004). A simple set of screening questions may allow identification of women at high risk, such that an appropriate onward referral to a hematologist can be undertaken. They may be identified in 90% of women using this approach (Kouides et al. 2005). See Table 2.

Table 2 Detection of bleeding disorders. (Adapted from (Kouides et al. 2005))

Structured history – positive screen if
(a) Excessive menstrual bleeding since menarche or
(b) History of one of the following: postpartum hemorrhage, surgery-related bleeding, or bleeding associated with dental work or
(c) History of two or more of the following: bruising greater than 5 cm once or twice/month, epistaxis once or twice/month, frequent gum bleeding, family history of bleeding symptoms

Ovulatory Disorders (AUB-O)

Anovulation results in unopposed estrogen excess exposure upon the endometrium, a persistent proliferative endometrium, which in turn manifests as AUB often in association with infrequent cycles. AUB ranging from amenorrhea to light sporadic bleeding to extreme blood loss requiring transfusion or surgery may occur. Anovulation is common at menarche and during the perimenopause transition due to change in the hormonal milieu (lack of exposure of the estrogen-primed endometrium to cyclic progesterone). Anovulation may also be associated with other conditions such as hypothyroidism, hyperprolactinemia, extremes of weight (including sudden changes in weight), mental stress, and excessive exercise to state a few.

Endometrial (AUB-E)

AUB that occurs in the context of a structurally normal uterus with regular menstrual cycles in the absence of a bleeding disorder is likely to represent a primary endometrial disorder. Classically this may have been defined as "dysfunctional uterine bleeding (DUB; may previously have included disorders of coagulopathy and ovulation)," a term the FIGO MDC recommends should no longer be used. The exact etiology remains poorly understood, although defective local hemostasis may contribute (Maybin and Critchley 2015; Critchley and Maybin 2011; Reavey et al. 2018). There are no validated tests currently available for clinical use to diagnose AUB-E, which in essence should be a diagnosis of exclusion.

Iatrogenic (AUB-I)

AUB-I comprises of problems related to the medications in four major categories.

- Use of exogenous sex steroids, including levonorgestrel-releasing intrauterine systems (LNG-IUS); long-acting progestin preparations, e.g., etonogestrel implants; and gonadotropin-releasing hormone agonists and antagonists. These drugs alter the prevailing endocrine (Guttinger and Critchley 2007) environment and often contribute to unscheduled or breakthrough bleeding. Up to one in five

women using progestin only contraception may develop AUB-I (Abdel-Aleem et al. 2013). Hormonal polytherapy may also be contributory.
- Use of medications that alter drug bioavailability by altering hepatic enzyme metabolism. Examples include anti-epileptic or antituberculous drugs, which may alter the circulating level of sex steroids.
- Use of anticoagulants such as warfarin, unfractionated heparin and low-molecular-weight heparin with impaired formation of an adequate "plug" or clot within the vascular lumen.
- Tricyclic antidepressants (e.g., amitriptyline and nortriptyline) and phenothiazines impact dopamine metabolism and result in hyperprolactinemia with subsequent anovulatory dysfunction.

Not Otherwise Classified (AUB-N)

Occasionally AUB may be associated with rare conditions, and potentially some may only be defined by cellular or molecular studies. One in five women in the UK undergoes a caesarean section (CS); isthmoceles are uterine defects in CS scar that may contribute to AUB (Morris 1995; Borges et al. 2010). Uterine arteriovenous malformations may also be responsible (Stoffel et al. 2009; Hoffman et al. 1997).

Management of AUB

History

A thorough structured history and clinical examination, including an assessment of body mass index (BMI), are paramount when managing AUB. Often these basic measures may point to AUB etiology. NICE guidance does not routinely recommend a physical examination prior to commencing pharmacological treatment (except LNG-IUS) in women with regular HMB in the absence of associated symptoms (Table 3) or in women who are considered low risk for a structural and or histological abnormality (NICE 2018). Table 3 summarizes the relevant history in women with AUB.

Investigations

All women with AUB should be offered a full blood count and treatment of anemia if indicated with oral or parenteral iron. In the UK, a thyroid profile, female hormone profile (follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol, progesterone), ferritin level, and coagulation profile are not routinely recommended (NICE 2018). If a structural abnormality (fibroids, adenomyosis) is suspected, an ultrasound scan is recommended. When appropriate an MRI may be organized for gynecological imaging. If the history suggests a submucous fibroid, polyp, or other

Table 3 Relevant history in women with AUB. (Adapted from (Chodankar and Critchley 2019))

History in women with AUB		
Menstrual history • Menstrual diary • Duration of AUB symptoms • Impact on the quality of life (QoL) • Intermenstrual or postcoital bleeding • Age at menarche (if relevant) • Previous treatments for AUB	*Associated symptoms* • Dysmenorrhea • Chronic pelvic pain • Abnormal vaginal discharge • Pressure symptoms (pelvic heaviness, constipation, urinary frequency)	*Sexual and reproductive history* • Parity and mode of birth • Need for uterine or fertility preservation • Need for contraception • History or high risk of sexually transmitted infections (STIs) • Cervical smear history
Personal history • Smoking, alcohol, or recreational drug abuse • Prescription or OTC (over the counter) medications, including any supplements (iron, vitamins) • Systemic disorders • Routine medications • Psychological history: recent stress, anxiety, or traumatic event • Recent weight gain or loss • Coagulopathy screen (Table 2)	*Family history* • Venous thromboembolic events (VTE) • Gynecological or Gastrointestinal cancers • Bleeding diathesis	

endometrial pathology, NICE recommends that an outpatient vaginoscopic hysteroscopy should be used as a first-line investigation (NICE 2018). In certain units this may be used in a "see and treat" fashion, e.g., polypectomy. NICE also recommends that a blind endometrial biopsy should not be performed, although logistically this currently remains a challenging step for managing AUB in the UK.

One of the most important investigations in women with AUB is endometrial sampling which helps to identify endometrial hyperplasia and neoplasia. NICE suggests considering a hysteroscopy and endometrial biopsy for women who are at high risk of endometrial pathology, such as women with persistent intermenstrual or persistent irregular bleeding, women with infrequent heavy bleeding who are obese or have polycystic ovary syndrome (PCOS), women taking tamoxifen, and women for whom treatment for HMB has been unsuccessful (NICE 2018). Arbitrary age cutoffs have been used in the past (45 years) to recommend endometrial sampling.

Women with a raised BMI, diabetes, hypertension, PCOS, age >45 years, nulliparity, late menopause, unopposed estrogen exposure, tamoxifen use, and family history of breast, colon, and endometrial cancer, e.g., Lynch syndrome, are at a high risk of AUB-M, and endometrial sampling should be considered irrespective of age (RCOG 2014b; Stoffel et al. 2009).

Treatment of AUB

Management of women with AUB/HMB is complex with an interplay of several factors, where a major consideration will be desire for preservation of fertility/uterus. Thus management options may include conservative treatment (wait and watch), medical or surgical (excisional and non-excisional) therapies, depending on the patient's age, parity, cause of bleeding, coexisting morbidities including BMI, the desire for fertility or uterine preservation, impact on the quality of life, and patient preference (Chodankar et al. 2018).

Non-pharmacological Treatment

Often women with AUB seek reassurance rather than a defined treatment option. Exclusion of sinister pathology, e.g., atypical endometrial hyperplasia or neoplasia, a period of surveillance with imaging and a symptom diary may be adequate in carefully selected women. Management of AUB in a large proportion of women may be indicated to improve their quality of life; as in these instances, the appropriate implementation of risk versus benefit should be the woman's choice.

Opportunities to promote a healthy lifestyle (diet, exercise, smoking cessation) should be taken when available. Loss of weight and attaining a healthy BMI may result in resumption of ovulation and help treat AUB-O.

Pharmacological Treatment

Pharmacological treatment is summarized in Table 4.

Women using progestogen-only methods should be counseled about the risk of irregular and often unpredictable spotting or bleeding. This may be seen in up to one in five (20%) users. Management of unscheduled bleeding on hormonal contraception should be managed as per the Faculty of Sexual and Reproductive Healthcare (FSRH) guidelines (FSRH 2015).

Non-excisional Treatment

Non-excisional treatment of AUB is summarized in Table 5 (Chodankar and Allison 2018; Daniels et al. 2012; Fergusson et al. 2013; Garcia and Isaacson 2011).

Excisional Treatment

A brief summary of excisional treatments for AUB is presented in Table 6.

Table 4 Medical management of AUB

Drug	Dose	Comments
Nonhormonal treatment		
Tranexamic acid	1 g TDS orally between D1 and D4 of the cycle. Up to 4 g per day	Antifibrinolytic effect. Useful in women with AUB who are actively trying to achieve pregnancy
Mefenamic acid (NSAIDs)	250–500 mg TDS orally between D1 and D5 of the cycle	Anti-inflammatory effect. May help in management of AUB with significant dysmenorrhea. May inhibit ovulation in women attempting to conceive
Hormonal treatment (oral)		
Combined oral contraceptive pill (COCP)	Monocyclic or Tricyclic fashion	UKMEC criteria should be used to assess suitability (http://www.fsrh.org/ukmec/)
Progestogen-only pill (POP)	OD	UKMEC criteria should be used to assess suitability (http://www.fsrh.org/ukmec/)
Norethisterone (NET)	5 mg BD-TDS from D5 to D26 of the cycle	Progestogens used only in the luteal phase offer poor AUB control. NET is partly metabolized to ethinyl estradiol after oral administration. High-dose NET therapy should be used with caution in women at risk of VTE (Mansour 2012)
Depot medroxyprogesterone acetate (MPA)	10–20 mg BD (up to TDS) from D5 to D26 of the cycle	Progestogens used only in the luteal phase offer poor AUB control
Hormonal treatment (injectable)		
Depot medroxyprogesterone acetate (DMPA)	150 mg IM every 12 weeks	Long-term use associated with reversible decrease in bone mineral density. Exercise caution in the young. Delay in resumption of ovulation may be noted on stopping the drug
Medroxyprogesterone acetate (DMPA)	104 mg SC every 13 weeks	As above. Subcutaneous use may have higher acceptability in the younger age group and in women with bleeding diathesis
Hormonal treatment (devices)		
Etonogestrel implant	68 mg implant. 3-year use	Should be inserted and removed only by healthcare professionals who have completed training for the use of the device
Levonorgestrel-releasing intrauterine system (LNG-IUS)	52 mg device. 5-year use	Should be trialed for at least 6 cycles or 6 months, prior to discontinuation

(continued)

Table 4 (continued)

Drug	Dose	Comments
Hormonal treatment (other)		
Gonadotropin-releasing hormone analogues (GnRH-A)	Can be administered as IM, SC, or nasal sprays	Induces a profound hypogonadal state and a state of reversible menopause. Associated with loss of trabecular bone with more than six months of use. Side effects may be minimized with the use of add back HRT. May be used as respite measure in women with debilitating AUB, pending investigations or surgery
Ulipristal acetate (UPA)	5 mg OD orally for treatment courses of up to three months each. Repeated intermittent treatment has been studied up to four intermittent courses (eMC 2018)	For management of AUB in association with fibroids. Revised EMA and MHRA guidance should be used to guide UPA use (EMA 2018; MHRA 2018)

OD, Once a day; BD, Two times a day; TDS, Three times a day; g, Gram; mg, Milligram; D, Day; IM, Intramuscular; SC, Subcutaneous; UKMEC, UK Medical Eligibility Criteria for Contraceptive Use; MHRA, Medicines and Healthcare products Regulatory Agency

Table 5 Surgical management of AUB (non-excisional)

AUB	Second-generation endometrial ablation Transcervical resection of the endometrium (TCRE)
Fibroids	Uterine artery embolization Magnetic resonance-guided focused ultrasound (MRgFUS) Radiofrequency volumetric thermal ablation (RFVTA)[a] Laparoscopic and vaginal uterine artery ligation[a] Laparoscopic thermomyolysis[a] Laparoscopic cryomyolysis[a]
Adenomyosis	Uterine artery embolization[a] Magnetic resonance-guided focused[a] ultrasound (MRgFUS)[a] Myometrial electrocoagulation[a] Global endometrial ablation[a]

[a]Limited evidence

Conclusion

The FIGO-AUB systems are designed to help research, education, and clinical care by aiming to unify AUB terminology, suggest possible etiology in women with AUB, and guide investigations and management choices. Women with AUB have a variety of treatment options available to them, but a tailored approach, ideally targeted to the underlying cause, and joint decision-making are crucial to manage

Table 6 Surgical management of AUB (excisional)

Polyp (Lieng et al. 2010)	Transcervical resection of polyp (TCRP) Polyp avulsion Blind endometrial curettage
Adenomyosis (Garcia and Isaacson 2011)	Adenomyoma excision[a] Myometrial reduction or wedge resection[a] Hysterectomy
Leiomyoma	Transcervical resection of fibroid (TCRF) Myomectomy Hysterectomy
Malignancy	Often a combination of radical excisional surgery +/− radiotherapy+/− chemotherapy or palliation

[a]Limited evidence

expectations and avoid potentially fertility-ending surgical choices. Although significant advances in menstrual physiology and dysfunction have been made, much more research is required to understand the complexities of AUB.

Cross-References

- ▶ Endometriosis
- ▶ Menstrual Disorders Related to Endocrine Diseases
- ▶ The Menstrual Cycle and Related Disorders
- ▶ The Menstrual Disorders Related to Systemic Diseases
- ▶ The Polycystic Ovary Syndrome (PCOS)
- ▶ Uterine Fibroids and Adenomyosis

Conflict of Interest Rohan Chodankar is supported as a Clinical Research Fellow by Bayer AG and has no other conflict of interest. HODC has clinical research support for laboratory consumables and staff from Bayer AG and provides consultancy advice (but with no personal remuneration) for Bayer AG, PregLem SA, Gedeon Richter, Vifor Pharma UK Ltd, AbbVie Inc., Myovant Sciences GmbH. HODC receives royalties from UpToDate for an article on abnormal uterine bleeding.

References

Abbott JA. Adenomyosis and abnormal uterine bleeding (AUB-A)-pathogenesis, diagnosis, and management. Best Pract Res Clin Obstet Gynaecol. 2017;40:68–81.

Abdel-Aleem H, d'Arcangues C, Vogelsong KM, Gaffield ML, Gulmezoglu AM. Treatment of vaginal bleeding irregularities induced by progestin only contraceptives. Cochrane Database Syst Rev. 2013;7:CD003449.

Baird DD, Dunson DB, Hill MC, Cousins D, Schectman JM. High cumulative incidence of uterine leiomyoma in black and white women: ultrasound evidence. Am J Obstet Gynecol. 2003;188(1):100–7.

Borges LM, Scapinelli A, de Baptista Depes D, Lippi UG, Coelho Lopes RG. Findings in patients with postmenstrual spotting with prior cesarean section. J Minim Invasive Gynecol. 2010;17(3):361–4.

Brohl AS, Li L, Andikyan V, Obican SG, Cioffi A, Hao K, et al. Age-stratified risk of unexpected uterine sarcoma following surgery for presumed benign leiomyoma. Oncologist. 2015;20(4):433–9.

Champaneria R, Abedin P, Daniels J, Balogun M, Khan KS. Ultrasound scan and magnetic resonance imaging for the diagnosis of adenomyosis: systematic review comparing test accuracy. Acta Obstet Gynecol Scand. 2010;89(11):1374–84.

Chen I, Firth B, Hopkins L, Bougie O, Xie RH, Singh S. Clinical characteristics differentiating uterine sarcoma and fibroids. J Soc Laparoendosc Surg. 2018;22(1):e.2017.00066.

Chodankar R, Allison J. New horizons in fibroid management. J Curr Obsatet Gynaecol Rep. 2018;7(2):106–15.

Chodankar R, Critchley HOD. Biomarkers in abnormal uterine bleeding. Biol Reprod. 2018. https://doi.org/10.1093/biolre/ioy231.

Chodankar R, Critchley HO. Abnormal uterine bleeding (including PALM COEIN classification). Obstet Gynaecol Reprod Med. 2019;29:98.. (in press)

Chodankar R, Harpur A, Mahmood T. Heavy menstrual bleeding. Obstet Gynaecol Reprod Med. 2018;28(7):196–202.

Critchley HO, Maybin JA. Molecular and cellular causes of abnormal uterine bleeding of endometrial origin. Semin Reprod Med. 2011;29(5):400–9.

Daniels JP, Middleton LJ, Champaneria R, Khan KS, Cooper K, Mol BW, et al. Second generation endometrial ablation techniques for heavy menstrual bleeding: network meta-analysis. BMJ. 2012;344:e2564.

EMA. Esmya: new measures to minimise risk of rare but serious liver injury; 2018. https://www.ema.europa.eu/documents/referral/esmya-article-20-procedure-esmya-new-measures-minimise-risk-rare-serious-liver-injury_en-0.pdf

eMC. Esmya 5 mg tablets (ulipristal acetate) electronic Medicines Compendium (eMC); 2018. http://www.medicines.org.uk/emc/medicine/26068

Fergusson RJ, Lethaby A, Shepperd S, Farquhar C. Endometrial resection and ablation versus hysterectomy for heavy menstrual bleeding. Cochrane Database Syst Rev. 2013;11:CD000329.

Fraser IS, Critchley HO, Munro MG, Broder M. Can we achieve international agreement on terminologies and definitions used to describe abnormalities of menstrual bleeding? Hum Reprod. 2007a;22(3):635–43.

Fraser IS, Critchley HO, Munro MG, Broder M. A process designed to lead to international agreement on terminologies and definitions used to describe abnormalities of menstrual bleeding. Fertil Steril. 2007b;87(3):466–76.

Fraser IS, Critchley HO, Broder M, Munro MG. The FIGO recommendations on terminologies and definitions for normal and abnormal uterine bleeding. Semin Reprod Med. 2011;29(5):383–90.

FSRH. Clinical guidance: problematic bleeding with hormonal contraception: faculty of sexual & reproductive healthcare (FSRH); 2015. https://www.fsrh.org/documents/ceuguidanceproblematicbleedinghormonalcontraception/

Garcia L, Isaacson K. Adenomyosis: review of the literature. J Minim Invasive Gynecol. 2011;18(4):428–37.

Guttinger A, Critchley HO. Endometrial effects of intrauterine levonorgestrel. Contraception. 2007;75(6 Suppl):S93–8.

Hoffman MK, Meilstrup JW, Shackelford DP, Kaminski PF. Arteriovenous malformations of the uterus: an uncommon cause of vaginal bleeding. Obstet Gynecol Surv. 1997;52(12):736–40.

Jacobson FJ, Enzer N. Uterine myomas and the endometrium; study of the mechanism of bleeding. Obstet Gynecol. 1956;7(2):206–10.

Kouides PA, Conard J, Peyvandi F, Lukes A, Kadir R. Hemostasis and menstruation: appropriate investigation for underlying disorders of hemostasis in women with excessive menstrual bleeding. Fertil Steril. 2005;84(5):1345–51.

Lieng M, Istre O, Sandvik L, Qvigstad E. Prevalence, 1-year regression rate, and clinical significance of asymptomatic endometrial polyps: cross-sectional study. J Minim Invasive Gynecol. 2009;16(4):465–71.

Lieng M, Istre O, Qvigstad E. Treatment of endometrial polyps: a systematic review. Acta Obstet Gynecol Scand. 2010;89(8):992–1002.

Liu X, Ding D, Ren Y, Guo SW. Transvaginal Elastosonography as an Imaging Technique for Diagnosing Adenomyosis. Reprod Sci. 2018;25(4):498–514.

Mansour D. Safer prescribing of therapeutic norethisterone for women at risk of venous thromboembolism. J Fam Plann Reprod Health Care. 2012;38(3):148–9.

Maybin JA, Critchley HO. Menstrual physiology: implications for endometrial pathology and beyond. Hum Reprod Update. 2015;21(6):748–61.

MHRA. Esmya (ulipristil acetate) for symptoms of uterine fibroids: restrictions to use and requirement to check liver function before, during and after treatment: medicines and healthcare products regulatory agency (MHRA); 2018. https://www.gov.uk/drug-safety-update/esmya-ulipristal-acetate-and-risk-of-serious-liver-injury-new-restrictions-to-use-and-requirements-for-liver-function-monitoring-before-during-and-after-treatment

Morris H. Surgical pathology of the lower uterine segment caesarean section scar: is the scar a source of clinical symptoms? Int J Gynecol Pathol. 1995;14(1):16–20.

Multinu F, Casarin J, Tortorella L, Huang Y, Weaver A, Angioni S, et al. Incidence of sarcoma in patients undergoing hysterectomy for benign indications: a population-based study. Am J Obstet Gynecol. 2019;220(2):179 e1–e10.

Munro MG, Critchley HO, Broder MS, Fraser IS (2011), FIGO classification system (PALM–COEIN) for causes of abnormal uterine bleeding in nongravid women of reproductive age. Int J Gynaecol Obstet. 2011;113:3–13. https://doi.org/10.1016/j.ijgo.2010.11.011

Munro MG, Critchley HOD, Fraser IS, Committee FMD. The two FIGO systems for normal and abnormal uterine bleeding symptoms and classification of causes of abnormal uterine bleeding in the reproductive years: 2018 revisions. Int J Gynaecol Obstet. 2018;143(3):393–408.

NICE. Clinical Guideline 44; Heavy menstrual bleeding: National Institute for Health and Clinical Excellence (NICE); 2007. http://www.nice.org.uk/nicemedia/pdf/CG44FullGuideline.pdf

NICE. NG88: heavy menstrual bleeding: assessment and management: National Institute for Health and Clinical Excellence (NICE); 2018. https://www.nice.org.uk/guidance/ng88

Pecorelli S, Benedet JL, Creasman WT, Shepherd JH. FIGO staging of gynecologic cancer. 1994–1997 FIGO committee on gynecologic oncology. International Federation of Gynecology and Obstetrics. Int J Gynaecol Obstet. 1999;65(3):243–9.

RCOG. National heavy menstrual bleeding audit – final report. London: RCOG; 2014a. https://www.rcog.org.uk/globalassets/documents/guidelines/research%2D%2Daudit/national_hmb_audit_final_report_july_2014.pdf

RCOG. Advice for Heavy Menstrual Bleeding (HMB) Services and Commissioners; 2014b. https://www.rcog.org.uk/globalassets/documents/guidelines/research%2D%2Daudit/advice-for-hmb-services-booklet.pdf

Reavey JJ, Maybin JA, Critchley HO. Physiology of menstruation. In: Kadir RA, James PD, Lee CA, editors. Inherited bleeding disorders in women. 2nd ed. Hoboken: Wiley Blackwell; 2018. p. 29–44.

Roberts ME, Aynardi JT, Chu CS. Uterine leiomyosarcoma: a review of the literature and update on management options. Gynecol Oncol. 2018;151(3):562–72.

Shankar M, Lee CA, Sabin CA, Economides DL, Kadir RA. von Willebrand disease in women with menorrhagia: a systematic review. BJOG. 2004;111(7):734–40.

Sizzi O, Manganaro L, Rossetti A, Saldari M, Florio G, Loddo A, et al. Assessing the risk of laparoscopic morcellation of occult uterine sarcomas during hysterectomy and myomectomy: literature review and the ISGE recommendations. Eur J Obstet Gynecol Reprod Biol. 2018;220:30–8.

Speroff L, Fritz MA. Clinical gynecologic endocrinology and fertility. 7th ed. Philadelphia: Lippincott Williams & Wilkins; 2005.

Stewart EA. Uterine fibroids. Lancet. 2001;357(9252):293–8.
Stoffel E, Mukherjee B, Raymond VM, Tayob N, Kastrinos F, Sparr J, et al. Calculation of risk of colorectal and endometrial cancer among patients with Lynch syndrome. Gastroenterology. 2009;137(5):1621–7.
Thomas CC, Wingo PA, Dolan MS, Lee NC, Richardson LC. Endometrial cancer risk among younger, overweight women. Obstet Gynecol. 2009;114(1):22–7.
Van den Bosch T, Dueholm M, Leone FP, Valentin L, Rasmussen CK, Votino A, et al. Terms, definitions and measurements to describe sonographic features of myometrium and uterine masses: a consensus opinion from the Morphological Uterus Sonographic Assessment (MUSA) group. Ultrasound Obstet Gynecol. 2015;46(3):284–98.
Woolcock JG, Critchley HO, Munro MG, Broder MS, Fraser IS. Review of the confusion in current and historical terminology and definitions for disturbances of menstrual bleeding. Fertil Steril. 2008;90(6):2269–80.
Zalewski K, Misiek M, Gozdz S, Bidzinski M. The new FIGO staging system for ovarian, fallopian tube and primary peritoneal cancer – 2014 update. Oncol Clin Pract. 2015;11(3):129–34.

Part III

Infertility

Infertility

11

Epidemiology and Etiology

Antonio La Marca and Elisa Mastellari

Contents

Abstract

Infertility is a disease characterized by the failure to establish a clinical pregnancy after 12 months of regular and unprotected sexual intercourse. It is estimated to affect between 8% and 12% of reproductive-aged couples worldwide and, since reproduction represents a biological and psychological requirement, the presence of infertility is a disease which generates disability as an impairment of function.

In infertile couples, a female factor is present in about 65% of cases. Recent definitions related to female infertility and its causes are reported in this chapter. The most frequent causes of infertility are advanced maternal age, tubal damages due to infections, polycystic ovary syndrome, and endometriosis. However, any

A. La Marca (✉) · E. Mastellari
Mother-Infant Department, Obstetrics and Gynecology, University of Modena and Reggio Emilia, University Hospital of Modena, Modena, Italy
e-mail: antlamarca@libero.it; elisa.mastellari@libero.it

F. Petraglia, B. C. Fauser (eds.), *Female Reproductive Dysfunction*, Endocrinology,
https://doi.org/10.1007/978-3-030-14782-2_11

cause that impairs reproductive organs anatomy, ovarian reserve, or hypothalamic–pituitary–ovarian axis can cause infertility.

However, in 15–30% of patients, infertility remains unexplained.

Couples (2–3%) are able to conceive but experience *a recurrent pregnancy loss*, a condition that impairs fecundity as well as chances of childbearing, although this condition is not properly included in the infertility definition.

Keywords

Infertility · Epidemiology · Etiology · Age · Hypothalamus · Pituitary gland · Ovary

Introduction

Infertility is a major health problem, as it involves millions of people around the world, worsening the life quality and having not only medical but also psychological and social implications. The first step in understanding and treating this pathology is to define its nature and its causes according to definitions shared by the scientific community.

Infertility: Definitions and Epidemiology

In order to harmonize communication among the medical and scientific communities, a 2017 consensus (Zegers-Hochschild et al. 2017) has elaborated the latest international glossary on infertility and fertility care to be used in reporting fertility issues.

Based on this glossary, *fertility* is defined as the capacity to establish a clinical pregnancy and *fecundity* is defined as the capacity to have a live birth.

Infertility (or *subfertility*) is a disease characterized by the failure to establish a clinical pregnancy after 12 months of regular, unprotected sexual intercourse or due to an impairment of a person's capacity to reproduce either as an individual or with his/her partner (Zegers-Hochschild et al. 2017). Primary infertility defines an infertile woman who has never been diagnosed with a clinical pregnancy or an infertile man who has never initiated a clinical pregnancy. If a previous pregnancy occurred in patient's history, infertility is defined as secondary. While the definition of infertility can be referred to a certain time period, *sterility* is defined as a permanent state of infertility.

Infertility is estimated to affect between 8% and 12% of reproductive-aged couples worldwide (Vander and Wyns 2018; Ombelet et al. 2008) and, since reproduction represents a biological and psychological requirement, the presence of infertility is a disease which generates disability as an impairment of function (Zegers-Hochschild et al. 2017).

Time of unwanted non-conception is a key factor to determine which couples are infertile. Indeed, physiological probability of conception after a single intercourse during the fertile window of the menstrual cycle (*fecundability*) is estimated to be 25%, even if this probability is variable within the population and particularly decreases with increasing age of the female partner. Eighty percent of the pregnancies occur in the first six cycles with regular intercourse in the fertile window of the menstrual cycle and 90% of couples will conceive spontaneously in 12 months. After 12 unsuccessful cycles, 10% of the couples are defined as infertile, even if is reported that spontaneous live birth rates among them will reach nearly 55% in the next 36 months. After 48 months, 5% of the couples are definitively infertile with a nearly zero chance of becoming spontaneously pregnant (Vander and Wyns 2018; Gnoth et al. 2005).

The major factors affecting the spontaneous probability of conception are age of the female partner and disease-related infertility (Gnoth et al. 2005).

Maternal age is related to a physiological decrease in fertility, as well as to an increase in pregnancy complications. The decline of female fertility starts at around 25–30 years of age. Especially in Western societies, this aspect of human biology conflicts with social, economic, and cultural expectations of an increasing number of women who decide to continue schooling and to acquire a profession before having children, with a significant postponement of childbearing, often after 35 years (Eijkemans et al. 2014). Therefore, it would be important to make women aware that delaying childbearing increases the risk of infertility and that the assisted reproductive technologies can not completely balance the fertility decline associated with advancing age (Maheshwari et al. 2008).

On the other hand, disease-related infertility can affect male or female partner regardless of age.

Female infertility has been recently defined as infertility caused primarily by female factors encompassing: ovulatory disturbances; diminished ovarian reserve; anatomical, endocrine, genetic, functional, or immunological abnormalities of the reproductive system; chronic illness and sexual conditions incompatible with coitus. In infertile couples, a female factor is present in about 65% of cases (Zegers-Hochschild et al. 2017; Gelbaya et al. 2014).

Male infertility is, instead, an infertility caused primarily by male factors encompassing: abnormal semen parameters or function; anatomical, endocrine, genetic, functional, or immunological abnormalities of the reproductive system; chronic illness; and sexual conditions incompatible with the ability to deposit semen in the vagina (Zegers-Hochschild et al. 2017).A male factor is found to be solely responsible in about 20–30% of infertility cases and it is a contributory factor, added to a female factor, in a further 25% of couples. However, these percentages seem to be higher in continents like Africa and lower in others, like America, Australia, and Europe (Vander and Wyns 2018; Gelbaya et al. 2014).

In some cases, however, infertility is unexplained: it is estimated that a standard fertility evaluation will fail to identify an abnormality in approximately 15–30% of infertile couples (Gelbaya et al. 2014).

Finally, 2–3% of couples are able to conceive but experience *a recurrent pregnancy loss*, a condition that impairs fecundity of a couple and its chances of childbearing, although this condition is not properly included in the infertility definition.

Etiology of Female Infertility

Reduced Ovarian Reserve

Reproductive activity in women depends on ovarian function. In particular, preantral follicles present in the ovaries are the biological structures responsible for hormonal production and the release of a mature oocyte (ovulation) every month during reproductive age: a small number of these follicles develop in every menstrual cycle starting from the pool of primordial follicles contained in the ovarian cortex. The number of primordial follicles stored in the ovaries is determined at birth and progressively decreases during life. The number of primordial follicles contained in both ovaries at a given moment of life is called "*ovarian reserve*" as it represents the functional reserve of the reproductive female organs. The reduction and depletion of this precious reserve compromise female reproductive potential. It can occur physiologically or it can be due to pathological causes and can express itself clinically with a continuum of severity ranging from menstrual abnormalities to infertility to premature ovarian insufficiency.

Age and Physiological Ovarian Reserve Reduction

The female reproductive potential is confined to a biological time that begins with menarche (average age: 12–13 years old in Western countries) and ends with menopause (average age of 51 years old in Western countries), representing the moment of life in which the ovaries exhaust their function. However, population studies (Hull et al. 1985) have shown that the decline of female fertility begins at the age of 25, increases between 35 and 37 years, and becomes predominant over 40 years: spontaneous fertility loss, defined as the maternal age to the last pregnancy, occurs at an average age of 41 years (O'Connor et al. 1998), about 10 years before menopause. The age-dependent loss of fertility is primarily determined by the continuous depletion of ovarian reserve. At the fourth month of fetal life 6–7 million of primordial follicles are stored in the ovaries. This number rapidly decreases due to apoptosis in the second half of fetal life and at birth only 1–2 million primordial follicles remain in the ovarian cortex. After birth, the loss of follicles becomes slow and when the menarche occurs there are still about 300,000–400,000 primordial follicles present (te Velde and Pearson 2002). During the reproductive age, there is a continuous and gradual decline of this number, with an acceleration around 37–38 years, and an exhaustion of the pool (less than 1000 follicles) when the menopause occurs (Faddy et al. 1992).

In addition, it is well established that oocyte and follicle quality also deteriorate with advancing reproductive age. In particular, an increased number of oocyte

chromosomal abnormalities is reported (Jones 2008): the presence of aneuploidy in the mature oocyte is reported to involve 5% of oocytes at 30 years old, but 50% of oocytes over 45 years old. It is not clear if this depends on an increase in meiotic segregation errors in normal disomic oocytes, perhaps from altered/reduced production of proteins that stabilize the meiotic spindle or if it is due to the presence of a pool of aneuploid oocytes formed during fetal life which would be recruited for ovulation at later stages of life than those genetically more stable. Ovarian aging also involves a morphological and functional alteration of granulosa cells that should support oocyte maturation. In vitro granulosa cells from older women shows reduced production of steroids and glycoproteins, increased number of altered mitochondria with reduced production of antioxidant enzymes and ATP, reduced mitotic process, increased apoptosis, and reduced in vivo production of IGF-1 (Klein et al. 1996; Tatone et al. 2006). Furthermore, the follicular microenvironment also shows age-related changes such as a reduction in perifollicular blood flow with a lower oxygen supply and free radicals' accumulation.

Age-related impairment of the ovarian reserve results not only in reduced fertility but also in failure to implant the embryo, early pregnancy loss, or fetal chromosomal abnormalities, conditions that are known to be increased in women with a more advanced reproductive age.

Although the age-related decline of the ovarian follicular pool affects all women, it does not occur in all women in the same way and the ovarian reserve may present an extremely variable range of values in women with similar age (Wallace and Kelsey 2010).

Some evidences, like the positive correlation of the age at the beginning of menopause between mother and daughter or between sisters, suggest that ovarian reserve and its evolution have a genetically determined component. However, it is probably a multifactorial component which involves genes that are not completely known and whose expression can also be influenced by environmental factors.

Environmental Factors

Exposure to some environmental substances is associated with a nonphysiological ovarian reserve and fertility reduction.

Cigarette smoking could have negative effect on fertility in both male and female. The proportion of women who smoke between 25 and 44 years of age is estimated around 20.7% in the United States. Association between cigarette smoking and infertility is strongly supported by literature, even if a specific causality has not been defined yet (Practice Committee of the American Society for Reproductive Medicine 2018). In particular, smoking is associated with an increased risk of conception delay over 1 year, and this effect seems to be related to the numbers of cigarettes smoked in a dose-dependent manner. The mean biological mechanism seems to be an accelerated follicular depletion due to chromosomal and DNA damage by harmful substances contained in smoke, like heavy metals, polycyclic hydrocarbons, nitrosamines, and aromatic amines (Vander and Wyns 2018; Practice Committee of the American Society for Reproductive Medicine 2018). As reported in literature, women who smoke have significantly higher basal follicle stimulating

hormone (FSH) levels, lower concentration of antimullerian hormone (AMH, a sieric marker of ovarian reserve), and undergo menopause 1–4 years earlier than nonsmokers.

Endocrine Disrupting Compounds (EDCs) are other substances that can affect fertility. These are exogenous chemicals that contaminate food and environment, and interfere with any aspect of hormone activity in the body. The main EDCs are bisphenol A (used in the manufacture of plastics and resins), phthalates and their esters (plasticizers to provide flexibility to materials), the pesticide atrazine, the polychlorinated biphenyls, and DDT/DDE insecticide (Vander and Wyns 2018). Animal studies suggest a negative influence of EDC on follicular number, ovulation, meiosis, and embryo implantation even if data on humans are scarce.

Iatrogenic Causes

Cancer treatments like radiation therapy or chemotherapy may affect the fragile ovarian follicles cells when they are administered to women in fertile age. The effects on ovarian reserve depend on type of agent (alkylating agents and procarbazine seem to be more frequently associated with ovarian damage), duration of therapy, and total cumulative dose administered. However, the level of patient's ovarian reserve before treatment seems to play a role as well, since it is reported that younger patients are more likely to have some follicles survive after therapy (The American College of Obstetricians and Gynecologists 2014).

Surgery on the ovaries can impair ovarian reserve in fertile-age patients. This is certainly true for surgery that removes ovarian tissue, like bilateral oophorectomy (when necessary in gynecological cancer patients) or single oophorectomy. However, there has been growing evidence suggesting a decline in ovarian reserve also as a result of more conservative procedures, namely, ovarian cystectomy, since many studies show a postoperative decline in circulating antimüllerian hormone (AMH). The ovarian damage seems to be related to the surgical technique, the experience of the surgeon, and the nature, size, and localization of the cysts. In particular, the risk appears increased for bilateral lesions, cysts of big size, and endometriotic cysts (Mohamed et al. 2016; Raffi et al. 2012).

Premature Ovarian Insufficiency

Premature ovarian insufficiency (POI) is a relatively rare condition, with a prevalence of 1% in general population (European Society for Human Reproduction and Embryology (ESHRE) Guideline Group on POI 2016). It is defined as amenorrhea due to the loss of ovarian function before 40 years of age in the presence of an elevated serum FSH measured (>25 IU/l) on two separate occasions. It includes premature menopause (menopause before 40 years of age) and primary amenorrhea (absence of spontaneous menarche) (Nguyen et al. 2017). POI is characterized by a decrease in the number of follicles in the ovaries and represents a relevant health issue because the resulting low estrogen levels are correlated with menopausal symptoms, cardiovascular morbidity, osteoporosis, earlier cognitive decline, and premature mortality; furthermore, as it occurs early in life, patients experience an infertility condition as well as, often, significant psychosocial issues (European

Society for Human Reproduction and Embryology (ESHRE) Guideline Group on POI 2016). The occurrence of a spontaneous pregnancy is described in literature with a probability between 3% and 10% in POI patients, as a consequence of an intermittent but unpredictable resumption of ovarian function (The American College of Obstetricians and Gynecologists 2014). It represents however, a very small chance.

POI's etiology is various and includes:

- Autoimmune diseases: responsible for 20% of POI; this association is described with several autoimmune conditions, but the most clinically important autoimmune conditions associated with POI are thyroid autoimmunity and Addison's disease; the last one can occur before the diagnosis of POI but also many years after.
- Genetic diseases: responsible for 10% of cases of POI; among these, the most frequent is Turner Syndrome, due to a karyotype abnormality of the X chromosome pair and the presence of Fragile X pre-mutation (FMR1). Moreover, several other mutations and genetic syndromes associated with POI development have recently been highlighted although they occur rarely, such as the GALT gene mutation in Galactosemia (Huhtaniemi et al. 2018) and many others. A particular genetic condition, mutations of BRCA 1 and 2 genes, make patients at genetically determined high risk of developing early ovarian or breast cancer and of getting POIs for the treatments they must undergo to prevent it (prophylactic bilateral oophorectomy) or treat it (chemotherapy).
- Infections due to human immunodeficiency virus (HIV), tuberculosis, malaria, herpes zoster, cytomegalovirus.
- Environmental factors and iatrogenic treatments: how these factors can impair ovarian reserve to cause POI in some cases has been described above.

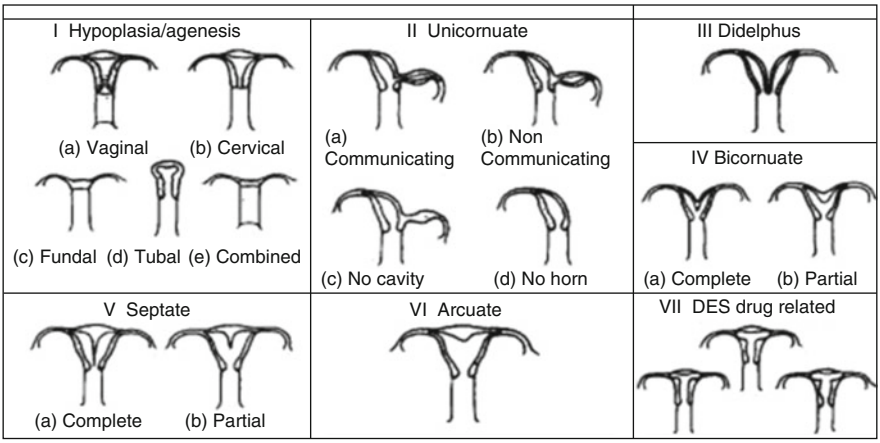
However, a large part of POI remains unexplained (idiopathic POI) (Nguyen et al. 2017).

Uterine Anomalies

Congenital Uterine Anomalies

Congenital uterine anomalies are deviations from normal anatomy due to abnormal development of the two paramesonephric (mullerian) ducts prior to the 20th embryonic week. Different types and severity of malformations can occur depending on the phase in which development and fusion processes of the two ducts are impaired. These malformations are collectively referred to as "Mullerian anomalies" and they recur in 6% of the general population.

Variations in uterine anomalies have been described by different classification systems. The American Fertility Society classification (1988) has been the reference for these pathologies for many years (American Fertility Society 1988, Fig. 1). According to this classification, relevant congenital mullerian tract anomalies



The American Fertility Society classification of adnexal adhesions, distal tubal occlusion secondary to tubal ligation, tubal pregnancies, Mullerian anomalies and intrauterine adhesions. Fertil Steril, 1988; 49: 944

Fig. 1 AFS Mullerian anomalies classification (American Fertility Society 1988)

include uterine aplasia, unicornuate, didelphys, bicornuate, and septate uteri. Uterine aplasia obviously causes amenorrhea and infertility, while the other anomalies are described to be associated with recurrent pregnancy loss, having women with this pathology an increased incidence (16%) of uterine anomalies.

Although it is still controversial, septate uterus – among the less severe and more common anomalies – is suggested in literature to have a negative impact on fertility. In fact, most women with a septate uterus have efficient reproductive function, even if low grade evidence shows that hysteroscopic septum incision is associated with improved clinical pregnancy rates in women with infertility (Practice Committee of the American Society for Reproductive Medicine 2016). The evaluation of this particular issue is also made more complex by a not uniform definition of septate configuration. Actually, new classification criteria were proposed in 2013 by the European Society of Human Reproduction and Embryology– European Society for Gynecological Endoscopy (ESHRE–ESGE) in order to include uteri that had remained unclassified with the previous classification and with the aim of making the uterus anomalies diagnosis less subjective than in the original AFS classification (Grimbizis et al. 2013, Fig. 2). The criteria of ESHRE–ESGE classification for septate uterus are more objectifiable but seem to include in this category a larger number of uteri without a specific correlation with their pathological implications (Ludwin and Ludwin 2015).

Acquired Uterine Anomalies

Subfertility and pregnancy loss had also been associated with acquired uterine abnormalities that distort the uterine cavity such as intrauterine adhesions, submucosal leiomyomas, and endometrial polyps.

Endometrial polyps seem to cause infertility due to decreased embryonic implantation potential and have, in fact, been associated with decreased mid-secretory

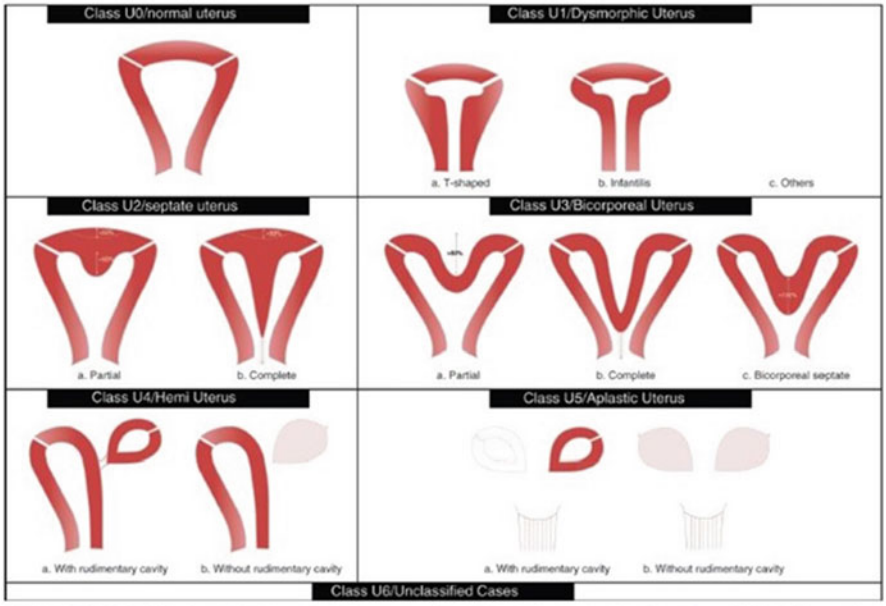


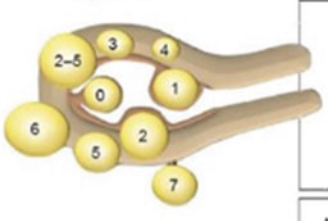
Fig. 2 ESHRE/ESGE 2013 classification of uterine anomalies (Class U2: internal indentation >50% of the uterine wall thickness and external contour straight or with indentation <50%; Class U3: external indentation >50% of the uterine wall thickness)

concentrations of IGFBP-1, TNF alpha, and osteopontin as markers of implantation, which were shown to be reversed following surgical polypectomy (Vander and Wyns 2018)

Uterine fibroids or Leiomyomas are the commonest benign tumors in the female reproductive tract, with a reported prevalence of about 25% in the world female population, with more prevalence in black women and an increased incidence with age up to menopause. These are probably underestimated data as uterine fibroids are symptomatic with menorrhagia, pelvic pain, or dyspareunia only in 20–50% of cases and prevalence reaches 50% of cases after autopsy.

At present, leiomyomas are classified on the basis of the PALM-COEIN classification system (Munro et al. 2011, Fig. 3) according to their anatomical location, and even though their role on female infertility is not completely understood, evidence to date suggests that the anatomic location may be related to reproductive outcomes. In particular, submucosal fibroids negatively affect fertility, when compared to women without fibroids. Intramural fibroids above a certain size (>4 cm) may negatively influence fertility, while the presence of subserosal myomas seems to have no effect on fertility.

Leyomiomas are present in 5–10% of infertile patients, but it is estimated that they are the only cause of infertility in no more than 3% of cases (Zepiridis et al. 2016).



SM – Submucosal	0	Pedunculated intracavitary
	1	<50% intramural
	2	≥50% intramural
O – Other	3	Contacts endometrium; 100% intramural
	4	Intramural
	5	Subserosal ≥50% intramural
	6	Subserosal <50% intramural
	7	Subserosal pedunculated
	8	Other (specify eg, cervical, parasitic)
Hybrid leiomyomas (impact both endometrium and serosa)	Two numbers are listed separated by a hypen. By convention, the first refers to the relationship with the endometrium, while the second refers to the relationship to the serosa. One example is given below	
	2–5	Submucosal and subserosal, each with less than half the diameter in the endometerial and peritoneal cavities, respectively

FIGO leyomioma subclassification (within the PALM-COEIN system for causes of abnormal uterine bleeding).Int J Gynaecol Obstet.2011;113(1):3-13.

Fig. 3 FIGO leiomyoma subclassification (within the PALM-COEIN system for causes of abnormal uterine bleeding). (Munro et al. 2011)

Several possible mechanisms have been reported on how leiomyomas may affect fertility such as anatomical distortion of endometrial cavity, abnormal uterine contractility, reduced blood supply to the endometrium, and altered endometrial receptivity (Vander and Wyns 2018).

Tubal Factors

The fallopian tubes play an important role in female fertility as they are responsible for the uptake of the oocyte, transport, and capacitation of the spermatozoa and transport of the embryo once fertilization has happened within them. Tubal diseases, i.e., tubal blockage or functional alterations, account for 25–35% of female factor infertility, representing this one of the more frequent female infertility etiologies (The Practice Committee of the American Society for Reproductive Medicine 2015).

Several factors can affect the tubal function.

Congenital malformations occur rarely, are due to abnormal development of mullerian ducts and are often associated with uterine abnormalities. Possible anomalies are mono or bilateral agenesia (absence of the tube), aplasia (lack of development), hypoplasia (incomplete development), or atresia (incomplete canalization) of a portion of the tube.

Ectopic pregnancy (EP) or extrauterine pregnancy refers to the implantation of a developing blastocyst that occurs outside the endometrial cavity of the uterus. Clinical signs and symptoms include abdominal pain, vaginal bleeding, and delay of an expected menses, with classic presentation that occurs at around 6–8 weeks of gestation. Most EPs are life-threatening situations for the mother and can represent a hospital emergency because of bleeding risk.

Today, EP accounts for approximately 2% of all recognized pregnancies, and a little major incidence (2–11%) is reported in pregnancies resulting from IVF (Marion and Meeks 2012). The majority of ectopic pregnancies implants in the ampullary region of the fallopian tube followed by the isthmus, the fimbriae, and the cornual portion; these constitute more than 95% of all EP.

Disruption of tubal anatomy is consistently associated with EP and infection is the most likely predisposing factor. Other factors include congenital anomalies, endometriosis, and surgery.

Always EP impairs tubal integrity, depending on the severity, on the timing of the diagnosis (greater damage if late diagnosis) and on the kind of therapy performed (administration of methotrexate, salpingostomy, or salpingectomy).

Therefore, having an EP is associated with future infertility due to tubal factor. For women who become pregnant, 80% of subsequent pregnancies are intrauterine, but up to 20% have another EP. Also, in this case, the choice of therapy modulates overall risks that, for example, are reported to be greater with salpingostomy.

Pelvic infections represent the most frequent but also preventable cause of tubal impairment. Different germs can ascend from the lower genital tract and thereby affect the uterus, fallopian tubes, and ovaries, resulting in pelvic inflammatory disease (PID). PID induces a proinflammatory immune response in the human fallopian tubes epithelia and subsequently can lead to tubal obstruction or damage of fallopian tube cilia with adverse reproductive health outcomes, including ectopic pregnancy and infertility (Vander and Wyns 2018; Tsevat et al. 2017).

Most cases of PID and tubal factor infertility are attributable to untreated sexually transmitted diseases. Of more than 32 known sexually transmitted pathogens, the most frequently involved in PID and tubal infections are *Chlamydia trachomatis* and *Neisseria gonorrhoeae*.

C. trachomatis is the most common pathogen in the United States as it affects nearly 1.5 million people annually. The infection has higher prevalence in young women (16–24 years) and the incidence may be greater in some geographical areas (such as Africa). Unfortunately, *C. trachomatis* infections are asymptomatic in up to 70% of women and often go unnoticed, untreated, and underreported (Tsevat et al. 2017).

N. gonorrhoeae is still the second most common sexually transmitted pathogen in the United States and, in this case also, infections are often asymptomatic among women, most often young women (Tsevat et al. 2017). Other sexually transmitted organisms, including *Mycoplasma genitalium*, *Trichomonas vaginalis*, and other microorganisms within the vaginal microbiome, may be involved in the PID and infertility etiology. The risk that *C. trachomatis* or *N. gonorrhoeae* infection evolves into PID is not quantifiable. However, it is estimated that 10% women who have had a PID become sterile, and the risk increases in case of repeated episodes.

Finally, tubal ciliary disfunction can be (rarely) due to a primary disorder of ciliary structure and function: *Primary Ciliary Dyskinesia* (PCD). This pathology will also impair tubal transport and predispose to ectopic pregnancy and subfertility. Certain geographically isolated communities or ethnic groups may have a higher PDC prevalence due to consanguinity, such as the Volendam population in the

Netherlands, the British Asian population, and the Amish and Mennonite communities in the United States (Vander and Wyns 2018).

Endocrine-Ovulatory Factors

The female reproductive physiology is regulated by a complex interaction of neuroendocrine and endocrine signaling between the hypothalamus, the pituitary gland, and the ovaries, which form the hypothalamic–pituitary–ovarian axis. At the level of the hypothalamus, gonadotropin releasing hormone (GnRH) pulses activate the pituitary release of the two gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH). FSH and LH act on the ovaries to stimulate follicular growth and result in the production of estradiol and, following ovulation, progesterone. Estradiol, together with ovarian inhibin B, acts primarily in a negative feedback loop on the hypothalamus suppressing the release of FSH. When estradiol reaches a sustained threshold in concentration and duration, for at least 24–48 h, it provides a positive feedback that increases the frequency and decreases the amplitude of GnRH pulses, thereby activating increased pituitary release of LH and causing LH surge and ovulation.

The menstrual cycle is a reproductive vital sign and provides insight into hormonal imbalance as well as pregnancy. The significance of estrogen, however, extends beyond fertility and plays a role on tissues and organs throughout the body (Shufelt et al. 2017).

Hypothalamic–pituitary–ovarian axis alterations due to various pathologies show different clinical manifestations but, at any level, interfere with female fertility.

Hypothalamic Amenorrhea

The hypothalamic alterations capable of influencing the pituitary-ovarian axis activity are expressed with a clinical spectrum of manifestations depending on the severity of the hormonal production impairment, from the only deficiency of the luteal phase with cycles <21 days (polymenorrhagia), to oligomenorrhea, up to amenorrhea which is the most common manifestation.

Amenorrhea is defined as lack of menstrual bleeding in reproductive-age women and can be classified as primary (absence of menarche) or secondary (which occurs after menarche).

Hypothalamic amenorrhea is due to hypogonadotropic hypogonadism, which leads to insufficient gonadal stimulation by Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH), due to insufficient/absent pulsatile secretion of hypothalamic Gonadotropin-Releasing Hormone (GnRH) (Vander and Wyns 2018). This results in estrogen deficiency (with related systemic compliances) and anovulation.

Various etiological factors may be responsible for a hypothalamic amenorrhea.

In some cases, hypothalamic amenorrhea is associated with anosmia: this association identifies the *Kallman syndrome*, a particular condition due to the failure of migration of the GnRH secretory neurons from the olfactory bulb where they

originate to the hypothalamus (Vander and Wyns 2018). Recently, some genetic mutations have been identified that can explain part of these syndromic cases (for example, mutation in gene KAL-I e II, or PROK2).

However, many patients present isolated and normosmic hypothalamic amenorrhea, which is considered *idiopathic.*

Hypothalamic amenorrhea can be due to *organic pathologies* that create anatomical damage to the hypothalamus and to its connection with the pituitary gland.

- Endocranial tumors (like craniopharyngiomas) can often compress the hypothalamic-pituitary connection with various degrees of hypopituitarism (75% of cases present GnRH deficiency, 40% gonadotropin deficiency, 25% hypothyroidism, and 10% diabetes insipidus)
- Infectious diseases such as encephalitis or tertiary syphilis
- Systemic diseases (X histiocytosis, neurosarcoidosis, and Wegner granulomatosis)
- Cranial trauma with the application of anteroposterior force which stretches pituitary stalk
- Hypovolemic shock with consequent ischemic phenomena (sometimes contributing to trauma)

However, the most frequent type of hypothalamic amenorrhea is *functional hypothalamic amenorrhea* (Shufelt et al. 2017). It affects 2.5–3% of reproductive-age women and is characterized by suppression of gonadotropin-releasing hormone (GnRH) due to functional neuroendocrine disorder in the absence of any organic disorders (Tranoulis et al. 2018)

The cause of this disorder is related to psychological stress, excessive physical exercise, rapid or excessive weight loss, eating disorders (in particular anorexia nervosa), or a combination of these factors (Shufelt et al. 2017).

As a matter of fact, the GnRH secretion (and therefore the reproductive function) is influenced by various substances involved in the energy homeostasis regulation and/or in the adaptive response to stress. The main known substances and neuroendocrine networks involved in functional hypothalamic amenorrhea are the following: activation of the hypothalamic-pituitary-adrenal axis with production of CRH and cortisol, which reduces the pulsatile GnRH secretion in stressful conditions; reduced levels of leptin, a hormone normally produced by adipose tissue in the case of energy abundance, able to reduce appetite and food intake, to stimulate energy expenditure, and indirectly (through central GABA and kisspeptin neurons) to increase the GnRH pulsatility, this effect being lacking in case of caloric deprivation with little leptin produced; increased levels of ghrelin, a orexigenic enterokine produced by the stomach, which can decrease both GnRH secretion and pulsatility in the hypothalamus, possibly through NPY neurons (Goldsammler et al. 2018).

Causes of functional hypothalamic amenorrhea are described as reversible. However, also in the case of restoration of a correct dietary and life style, not all patients recover ovulation and menstruation, and this seems to depend, among other factors, on the period of estrogen deprivation duration (possible reproductive organ tissues atrophy) and on the degree of metabolic impairment.

The most difficult resolution appears to be in cases associated with anorexia nervosa, where the psychical component and the activation of stress response pathway seem to play a greater role than the simple caloric intake regulation (Gwirtsma et al. 1989).

Hypopituitarism

Hypopituitarism is defined as a deficiency of one or more pituitary hormones due to a decline in function of the pituitary gland and/or hypothalamus (Heidelbaugh 2016).

The causes of hypopituitarism secondary to deficiency of hypothalamic function have been discussed above.

Hypopituitarism due to a primitive impairment of the pituitary gland is a rare condition and is usually caused by an anatomical damage of the gland, although idiopathic cases exist.

Pathologies that can directly damage the pituitary gland are:

- Pituitary adenomas (one of the most frequent causes) or intracranial tumors (like craniopharyngiomas)
- Congenital defects (septo-optic dysplasia, cleft lip and palate, holoprosencephaly, empty sella syndrome, pituitary aplasia, or hypoplasia)
- Ischemic necrosis of the pituitary gland, which could occur following trauma, thrombosis, or vascular aneurysms especially of the internal carotid artery, hemorrhagic infarction, shock with hypoperfusion (this is the case of Sheehan syndrome in the postpartum that can develop into a rapid onset form with risk of coma and death, or slow-onset form with chronic onset that begin with missed lactation or not resumed menstruation after pregnancy) or in patients with diabetes mellitus or sickle cell anemia
- Inflammatory processes (meningitis, abscesses, and sarcoidosis)
- Autoimmune processes (lymphocyte hypophysitis)
- Systemic diseases: hemochromatosis, histiocytosis
- Latrogenic procedures such as irradiation or surgical ablation

The clinical manifestations of hypopituitarism result from the degree of the specific hormone deficiency. In fact, one or more of the hormones usually produced by pituitary gland could be involved (gonadotrophins, GH, TSH, and ACTH).

If gonadotrophins production is deficient, hypogonadotropic hypogonadism occurs with low levels of Luteinizing Hormone (LH), Follicle-Stimulating Hormone (FSH) and Estradiol despite a normal hypothalamic GnRH production.

Hyperprolactinemia

Prolactin is a hormone synthesized by the pituitary gland and regulated by the inhibition of hypothalamic dopamine. Basal levels fluctuate with sleep, physical exercise, and menstrual cycle (increase in luteal phase) (Capozzi et al. 2015).

Physiologically, prolactin levels increase during pregnancy, contributing to the development of mammary gland, while after delivery and the decrease in circulating estrogens, it stimulates breast milk production. At the same time, it inhibits ovarian

function in this phase. Furthermore, in all mammals, PRL increases in conditions of psycho-physical stress, reducing ovarian function and therefore fertility in these conditions, for which PRL is also considered one of the major physiological fertility control systems. This is probably due to a reduction in GnRH pulsatility with reduced LH production and preovulatory peak inhibition, but also due to a direct reduction of the ovary response to gonadotropins and therefore steroidogenesis.

Hyperprolactinemia is more frequent in women of reproductive age with disorders of the menstrual cycle, while it is rare among asymptomatic women with infertility only. Depending on the prolactin levels, clinical manifestation could be deficiency of the luteal phase, oligomenorrhea or amenorrhea, and infertility. Galactorrhea is present in 30–50% cases.

Possible causes are (Capozzi et al. 2015):

- Psycho-physical stress
- Drugs like tricyclic antidepressants, antidopaminergic drugs, opioids, H2 antagonists
- Prolactinomas (prolactin-secreting pituitary adenomas)
- Diseases that damage the pituitary stalk, with loss of dopamine control on prolactin production
- Primitive hyperthyroidism can cause hyperprolactinemia because with low levels of thyroid hormone, negative feedback on hypothalamus is lost, which increases TRH production: TRH stimulates pituitary to secrete TSH but is also a releasing factor for prolactinotropic cells.
- Hepatic failure, chronic renal insufficiency, paraneoplastic syndrome

Thyroid Dysfunction

Thyroid function plays an important role in a woman's reproductive life and several studies attest to the correlation between thyroid disorders and alterations of menstrual cycles, infertility, and increased morbidity during pregnancy

Hypothyroidism is a common condition of thyroid hormone deficiency. The prevalence of overt hypothyroidism in the general population varies between 0–3% and 3–7% in the USA and between 0–2% and 5–3% in Europe.

Hypothyroidism can be classified as primary (due to thyroid hormone deficiency), secondary (due to TSH deficiency), tertiary (due to thyrotropin-releasing hormone deficiency) even if secondary and tertiary hypothyroidism together represent 1% of cases only.

Clinical primary hypothyroidism is defined as thyroid-stimulating hormone (TSH) concentrations above the reference range and free thyroxine concentrations below the reference range.

Subclinical hypothyroidism, which is commonly regarded as a sign of early thyroid failure, is defined by TSH concentrations above the reference range and free thyroxine concentrations within the normal range (Chaker et al. 2017).

Clinical manifestations of hypothyroidism range from life threatening (in the case of myxedema coma) to no signs or symptoms. The most common symptoms in adults are fatigue, lethargy, cold intolerance, weight gain, constipation, change in voice, and dry skin.

Most common causes of primary hypothyroidism are iodine deficiency, autoimmune thyroiditis, iatrogenic causes (drugs including amiodarone, lithium and INF, surgical removal or radiation therapy). Secondary and tertiary hypothyroidism are due to more rare pathologies including pituitary adenomas, brain tumors in the hypothalamic region, drugs (lithium, dopamine), radiation, surgery, genetic causes, and hypovolemic shock (Sheehan syndrome in postpartum).

Not only clinical but also subclinical hypothyroidism has been found to be associated with adverse reproductive outcomes, such as infertility, miscarriage, preterm birth, and complications of pregnancy (Seungdamrong 2016).

Mechanisms that correlate hypothyroidism and infertility could be the lack of the thyroid hormones effect on their receptors on granulosa cells (synergism with FSH and LH) and, in primary hypothyroidism, the presence of hyperprolactinemia induced by hypothalamic TRH.

Hyperthyroidism is a clinical syndrome characterized by an excess of thyroid hormones, which clinically presents weight loss, profuse sweating, tachycardia/tachypnea, hyperactivity-irritability, heat intolerance, and concentration disorders. Basedow's disease is the most common cause.

These patients often present hypomenorrhea (less abundant menstrual cycles, whose mechanism is not clear) but appear to be normo-ovulatory and the impact of hyperthyroidism on fertility is not yet clearly defined.

Polycystic Ovary Syndrome (PCOS)

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder affecting reproductive-aged women, with a prevalence of between 8% and 13% depending on the population studied and definitions used (Vander and Wyns 2018; Teede et al. 2010).

PCOS is classically described by the Rotterdam criteria (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2004) as a syndrome consisting of two of the following three criteria, i.e., infrequent or absent ovulation (oligomenorrhea), a typical morphological pattern of the ovaries by ultrasound assessment and hyperandrogenism. It is a complex condition, with heterogeneous manifestations that could involve reproductive, metabolic, and psychological aspects (Vander and Wyns 2018; Teede et al. 2010).

In these women, Infertility is primary due to an ovulatory menstrual cycle.

The exact pathophysiology of PCOS is complex and remains largely unclear; however, an underlying hormonal imbalance created by a combination of increased androgens and/or insulin is often present.

Hyperandrogenism is a well-established contributor to PCOS etiology, detected in around 60–80% of cases and expressed clinically with acne, hirsutism, and alopecia. Mechanism leading to hyperandrogenism is unclear but the presence of hypothalamic pituitary abnormalities with an increased pulsatility of LH is known, with hyperstimulation of ovarian theca cells that become hyperplastic, increase production of ovarian androgens (testosterone and androstenedione) and surround ovarian follicles whose maturation is arrested.

Insulin resistance is a pathophysiological contributor in around 50–80% of women with PCOS and contributes to metabolic but also to reproductive features through augmenting LH and androgen production and increasing free androgens by reducing sex hormone binding globulin (SHBG). (Teede et al. 2010)

Obesity is often associated with PCOS and exacerbates metabolic and ovulatory dysfunction, while a correct life-style with weight loss has been found to restore ovulation and reduce hyperandrogenism. Also, genetic and environmental factors are probably involved (Vander and Wyns 2018).

Adrenal causes of hyperandrogenism (such as tumors or 21-alpha-hydroxylase deficiency) must be excluded, even if they are rare.

Obesity

Obesity represents a growing public health problem whose prevalence is increasing worldwide.

It is estimated to affect approximately 39% of the population over the age of 20.

Besides being a risk factor for multiple metabolic disorders, obesity could affect female and male reproduction. Women who are overweight are less likely to ovulate and to conceive spontaneously even after infertility care. Upon conception, they also have an increased risk of miscarriage and are predisposed to an adverse pregnancy outcome (Vander and Wyns 2018). A direct correlation has been demonstrated between a higher body mass index (BMI) and a poorer fertility prognosis (Talmor and Dunphy 2014).

Although the etiopathogenetic mechanisms are not fully explained, it is known that obesity influences the hypothalamic-pituitary-ovary axis at different levels. In literature, changes in leptin, adiponectin, and ghrelin levels due to metabolic abundance, increase in estradiol and testosterone levels due to increased peripheral aromatase activity, and due to hyperinsulinemia, with stimulation of ovarian androgen production, decrease of SHBG, and hypersecretion of luteinizing hormone (LH) are described. The final result is an impaired hypothalamus-pituitary activity and altered folliculogenesis. Then, also a low quality of the oocytes produced is described. Moreover, chronic inflammation typical of obese patients has been hypothesized to directly affect ovarian function by increased macrophage infiltration in the ovaries through pro-inflammatory molecules like advanced glycation end products (AGEs) and monocyte chemotactic protein-1 (MCP-1) (Goldsammler et al. 2018; Talmor and Dunphy 2014).

Endometriosis

Endometriosis is a gynecologic disorder that is estimated to affect 5–10% of fertile-age women. It is characterized by the presence of endometrial-like tissue outside the uterus, which causes a chronic inflammatory reaction in consequence of its hormone-dependent activity. The lesions can potentially involve all pelvic structures (ovaries, tubes, peritoneum, uterus supporting structures, bowel, ureters, and bladder) and sometimes extra-pelvic ones (Ozkan et al. 2008; Kock et al. 2012).

This condition often causes disabling pain symptoms, predominantly dysmenorrhea, dyspareunia, and chronic pelvic pain (Dunselman et al. 2014), but it is asymptomatic in 20–25% of cases.

Furthermore, endometriosis can impair fertility: 30–50% of women with endometriosis are suffering from infertility (The Practice Committee of the American Society for Reproductive Medicine 2012), the prevalence of endometriosis is markedly higher in infertile patients (25–50%) than in the general population, and endometriosis represents 10% of fertility treatments indications (Somigliana et al. 2017).

Pathogenetic mechanisms that could link endometriosis and infertility are chronic inflammation of the pelvis, pelvic adhesions, and distortion of the pelvic anatomy (in particular of the tubes), adenomyosis, damage of endometriotic cysts on ovarian tissue, dyspareunia, iatrogenic damage on ovarian tissue in case of surgery for removal of endometriomas. All these factors together lead to reduced tubal function, impaired folliculogenesis and oocyte quality, alteration of the uterine microenvironment with reduced endometrial receptivity, and obstacle to spermatozoa progression (Somigliana et al. 2017).

However, many patients with endometriosis are able to achieve spontaneous conception. For this reason, although the relationship between endometriosis and infertility is supported by substantial evidence, a precise causal relationship is not determinable in all cases and the mechanisms of infertility related to endometriosis have not yet been fully understood (Kock et al. 2012).

Cystic Fibrosis

Cystic Fibrosis (CF) is a genetic disease characterized by abnormal mucus secretion due to mutations in the CF Transmembrane Conductance Regulator (CFTR) gene. It is a rare disease, but is more common in northern European whites (approximately 1 in 2500 individuals). Life expectancy of cystic fibrosis (CF) patients continues to increase, therefore more and more attention is given to the effects of CF on both male and female fertility.

Less is known about how CF affects female fertility, but it seems to be due to the secretion of a thick mucus by the epithelial cells, predominantly in the cervix, with sperm penetration impairment. The effect on the epithelium of uterine cavity and the fallopian tube seems to be less significant (Vander and Wyns 2018).

However, not all CFTR mutations appear to cause infertility and, unlike affected men, who have significant anatomical abnormalities of the reproductive tract, most women with CF have anatomically normal reproductive tracts and up to half may be able to conceive spontaneously (Ahmad et al. 2013).

Unexplained Infertility

Unexplained infertility can be diagnosed when known causes of infertility have been excluded using complete standard fertility investigations. It is estimated that a

standard fertility evaluation will fail to identify an abnormality in approximately 15–30% of infertile couples (Gelbaya et al. 2014).

Many couples with a provisional diagnosis of unexplained infertility will subsequently conceive spontaneously: these couples might be reassured that even after 12 months of unsuccessful attempts, 50% will conceive in the following 12 months and another 12% in the subsequent year. In the patient with unexplained infertility, the two most important prognostic factors are the age of the woman and the duration of infertility; the higher they are, the more the chances of spontaneous pregnancy are reduced (Gelbaya et al. 2014).

Recurrent Miscarriage

Recurrent spontaneous abortion/miscarriage is defined as the spontaneous loss of two or more clinical pregnancies prior to 22 completed weeks of gestational age, that is, before the fetus has reached viability (Vander and Wyns 2018). Miscarriage occurs in up to 15–20% of apparently normal couples and becomes recurrent in 2–3% of these couples.

Recurrent miscarriage is one of the most frustrating and difficult areas in reproductive medicine, since the etiology is often unknown and evidence-based diagnostic and treatment strategies are scarce (Garrido-Gimenez and Alijotas-Reig 2015).

General etiological categories of recurrent miscarriage include the followings (Garrido-Gimenez and Alijotas-Reig 2015; Rai and Regan 2006):

- *Genetic factors:* These are the most common cause of early spontaneous miscarriage (50–60%) and could be due to parental chromosomal rearrangements (2–4% of cases; often balanced translocations) or more frequently to fetal aneuploidy (present in at least 50% of sporadic miscarriages and up to 57% of the recurrent one).
- *Endocrine dysfunction* may account for 15–20% of all cases of recurrent miscarriage; poorly controlled diabetes mellitus, significant thyroid dysfunction, and hyperprolactinemia have been associated with recurrent miscarriage; polycystic ovarian syndrome has been associated with an increased rate of miscarriage if insulin resistance is present; the role of thyroid autoimmunity (with autoantibodies, including cases that are euthyroid) is debated.
- *Anatomical factors*: Congenital uterine malformations are detected in approximately 10–15% of women with recurrent miscarriage, septate uterus, due to vascular insufficiency in the septum, being the most common (35%); submucous leiomyomas that deform the endometrial cavity and adenomyosis can also cause recurrent miscarriage; cervical insufficiency is a recognized cause of late recurrent miscarriage.
- *Immune factors*: Antiphospholipid syndrome (APS) refers to the association of antiphospholipid antibodies with venous and/or arterial thrombosis, and with embryo–fetal morbidity; it is the most important treatable cause of recurrent miscarriage (5–15% of women with recurrent miscarriage) and is associated with other obstetric complications such as preterm delivery, intrauterine growth restriction, and

early pre-eclampsia, all due to placental injury. The obstetric manifestations, preceding thrombotic ones, can be the first to introduce the syndrome.

- *Inherited thrombophilic disorders:* There is a large and contradictory literature on the association between maternal inherited thrombophilia and recurrent miscarriage. However, current studies have generally confirmed an association particularly for late fetal loss; the presumed mechanism seems to be the thrombosis of the uteroplacental circulation. The most prevalent polymorphisms are the heterozygous variant of factor V Leiden, the abnormal heterozygous prothrombin gene, and the homozygosity for methylenetetrahydrofolate reductase (MTHFR).

However, no cause can be found in up to 50% of cases (Garrido-Gimenez and Alijotas-Reig 2015).

Nevertheless, the prognosis for a successful future pregnancy is generally good: a woman who has had three miscarriages still has an approximately 40% chance of having a successful pregnancy the fourth time, and the overall live birth rates after normal diagnostic evaluations for recurrent miscarriage is around 77%.

Conclusions

Infertility is a disease characterized by the failure to establish a clinical pregnancy after 12 months of regular and unprotected sexual intercourse. It affects between 8% and 12% of reproductive-aged couples, worsens life quality, and has not only medical but also psychological and social implications. The major factors affecting the spontaneous probability of conception are age of the female partner and disease-related infertility.

The postponement of childbearing for socioeconomic reasons exposes women to a greater risk of infertility due to a physiological reduction of the ovarian reserve with age. On the other hand, a series of nonphysiological conditions may compromise female fertility: environmental factors like cigarette smoking or endocrine disrupting compounds (EDCs), surgical interventions on the ovaries but also genetic conditions can decrease ovarian reserve until premature ovarian failure; then anatomical alterations of the reproductive organs (in particular of the uterus and tubes) can occur; in particular, for tubal damage the role of pelvic infections is to be emphasized. Endometriosis, hypothyroidism, obesity, lifestyle, and cystic fibrosis are other factors for which a correlation with a decreased fertility is reported.

Cross-References

- ▶ Diagnostic Protocols for Infertility
- ▶ Menstrual Disorders Related to Endocrine Diseases
- ▶ The Menstrual Cycle and Related Disorders
- ▶ The Polycystic Ovary Syndrome (PCOS)
- ▶ Uterine Fibroids and Adenomyosis

References

Ahmad A, Ahmed A, Patrizio P. Cystic fibrosis and fertility. Curr Opin Obstet Gynecol. 2013;25(3):167–72.

American Fertility Society. The American Fertility Society classifications of adnexal adhesions, distal tubal occlusion, tubal occlusion secondary to tubal ligation, tubal pregnancies, müllerian anomalies and intrauterine adhesions. Fertil Steril. 1988;49(6):944–5.

Capozzi A, Scambia G, Pontecorvi A, Lello S. Hyperprolactinemia: pathophysiology and therapeutic approach. Gynecol Endocrinol. 2015;31(7):506–10.

Chaker L, Bianco AC, Jonklaas J, Peeters RP. Hypothyroidism. Lancet. 2017;390(10101):1550–62.

Dunselman GA, Vermeulen N, Becker C, Calhaz-Jorge C, D'Hooghe T, De Bie B, Heikinheimo O, Horne AW, Kiesel L, Nap A, Prentice A, Saridogan E, Soriano D, Nelen W, European Society of Human Reproduction and Embryology. ESHRE guideline: management of women with endometriosis. Hum Reprod. 2014;29(3):400–12.

Eijkemans MJ, van Poppel F, Habbema DF, Smith KR, Leridon H, te Velde ER. Too old to have children? Lessons from natural fertility populations. Hum Reprod. 2014;29(6):1304–12.

European Society for Human Reproduction and Embryology (ESHRE) Guideline Group on POI. ESHRE Guideline: management of women with premature ovarian insufficiency. Hum Reprod. 2016;31:926–37.

Faddy MJ, Gosden RG, Gougeon A, Richardson SJ, Nelson JF. Accelerated disappearance of ovarian follicles in mid-life: implications for forecasting menopause. Hum Reprod. 1992;7:1342–6.

Garrido-Gimenez C, Alijotas-Reig J. Recurrent miscarriage: causes, evaluation and management. Postgrad Med J. 2015;91:151–62.

Gelbaya TA, Potdar N, Jeve YB, Nardo LG. Definition and epidemiology of unexplained infertility. Obstet Gynecol Surv. 2014;69(2):109–15.

Gnoth C, Godehardt E, Frank-Herrmann P, Friol K, Tigges J, Freund G. Definition and prevalence of subfertility and infertility. Hum Reprod. 2005;20(5):1144–7.

Goldsammler M, Merhi Z, Buyuk E. Role of hormonal and inflammatory alterations in obesity-related reproductive hypothalamic-pituitary-ovarian axis. Reprod Biol Endocrinol. 2018;16(1):45.

Grimbizis GF, Gordts S, Di Spiezio Sardo A, Brucker S, De Angelis C, Gergolet M, Li T-C, Tanos V, Brölmann H, Gianaroli L, Campo R. The ESHRE–ESGE consensus on the classification of female genital tract congenital anomalies. Gynecol Surg. 2013;10:199–212.

Gwirtsma HE, et al. Central and peripheral ACTH and cortisol levels in anorexia nervosa and bulimia. Arch Gen Psychiatry. 1989;78(1):61–9.

Heidelbaugh JJ. Endocrinology update: hypopituitarism. FP Essent. 2016;451:25–30.

Huhtaniemi I, Hovatta O, La Marca A, Livera G, Monniaux G, Persani L, Heddar A, Jarzabek K, Laisk-Podar T, Salumets A, Tapanainen JS, Veitia RA, Visser JA, Wieacker P, Wolczynski S, Misrahi M. Advances in the molecular pathophysiology, genetics, and treatment of primary ovarian insufficiency. Trends Endocrinol Metab. 2018;29(6):400–19.

Hull MGR, Glazener CMA, Kelly NJ, Conway DI, Foster PA, Hinton RA, Coulson C, Lambert PA, Watt EM, Desai KM. Population study of causes, treatment, andoutcome of infertility. Br Med J (Clin Res Ed). 1985;291:1693–7.

Jones KT. Meiosis in oocytes: predisposition to aneuploidy and its increased incidence with age. Hum Reprod Update. 2008;14:143–58.

Klein NA, Battaglia DE, Miller PB, Branigan EF, Giudice LC, Soules MR. Ovarian follicular development and the follicular fluid hormones and growth factors in normal women of advanced reproductive age. J Clin Endocrinol Metab. 1996;81:1946–51.

Kock J, Rowan K, Rombatus L, Yazdani A, Chapman M, Johnson N. Endometriosis and infertility – a consensus statement from ACCEPT (Australasian CREI Consensus Expert Panel on Trial evidence). Aust N Z J Obstet Gynaecol. 2012;52:513–22.

Ludwin A, Ludwin I. Comparison of the ESHRE–ESGE and ASRM classifications of müllerian duct anomalies in everyday practice. Hum Reprod. 2015;30(3):569–80.

Maheshwari A, Porter M, Shetty A, Bhattacharya S. Women's awareness and perceptions of delay in childbearing. Fertil Steril. 2008;90:1036–42.
Marion LL, Meeks GR. Ectopic pregnancy: history, incidence, epidemiology, and risk factors. Clin Obstet Gynecol. 2012;55(2):376–86.
Mohamed AA, Al-Hussaini TK, Fathalla MM, El Shamy TT, Abdelaal II, Amer SA. The impact of excision of benign nonendometriotic ovarian cysts on ovarian reserve: a systematic review. Am J Obstet Gynecol. 2016;215(2):169–76.
Munro MG, Critchley HO, Broder MS, Fraser IS, for the FIGO Working Group on Menstrual Disorders. FIGO classification system (PALM-COEIN) for causes of abnormal uterine bleeding in nongravid women of reproductive age. Int J Gynecol Obstet. 2011;113:3–13.
Nguyen HH, Milat F, Vincent A. Premature ovarian insufficiency in general practice: meeting the needs of women. AFP Focus. 2017;46(6):360–6.
O'Connor KA, Holman DJ, Wood JW. Declining fecundity and ovarian ageing in natural fertility populations. Maturitas. 1998;30:127–36.
Ombelet W, Cooke I, Dyer S, Serour G, Devroey P. Infertility and the provision of infertility medical services in developing countries. Hum Reprod Update. 2008;14(6):605–21.
Ozkan S, Murk W, Arici A. Endometriosis and infertility: epidemiology and evidence-based treatments. Ann N Y Acad Sci. 2008;1127:92–100.
Practice Committee of the American Society for Reproductive Medicine. Uterine septum: a guideline. Fertil Steril. 2016;106(3):0015–282.
Practice Committee of the American Society for Reproductive Medicine. Smoking and infertility: a committee opinion. Fertil Steril. 2018;110(4):0015–282.
Raffi F, et al. The impact of excision of ovarian endometrioma on ovarian reserve: a systematic review and meta-analysis. J Clin Endocrinol Metab. 2012;97(9):3146–54.
Rai R, Regan L. Recurrent miscarriage. Lancet. 2006;368:601–11.
Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril. 2004;81(1):19–25.
Seungdamrong A. The impact and management of subclinical hypothyroidism for improving reproductive outcomes such as fertility and miscarriage. Semin Reprod Med. 2016;34(6):331–6.
Shufelt CL, Torbati T, Dutra E. Hypothalamic amenorrhea and the long-term health consequences. Semin Reprod Med. 2017;35:256–62.
Somigliana E, Vigano P, Benaglia L, Busnelli A, Berlanda N, Vercellini P. Management of endometriosis in the infertile patient. Semin Reprod Med. 2017;35(1):31–7.
Talmor A, Dunphy B. Female obesity and infertility. Best Pract Res Clin Obstet Gynaecol. 2014;29(4):498–506.
Tatone C, Carbone MC, Falone S, Aimola P, Giardinelli A, Caserta D, Marci R, Pandolfi A, Ragnelli AM, Amicarelli F. Age-dependent changes in the expression of superoxide dismutases and catalase are associated with ultrastructural modifications in human granulosa cells. Mol Hum Reprod. 2006;12(11):655–60.
te Velde ER, Pearson PL. The variability of female reproductive ageing. Hum Reprod Update. 2002;8:141–54.
Teede H, Deeks A, Moran LR. Polycystic ovary syndrome: a complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan. BMC Med. 2010;8:41.
The American College of Obstetricians and Gynecologists. Primary ovarian insufficiency in Adolescents and young women. Committee Opinion n 605. Obstet Gynecol. 2014;124(1):193–7.
The Practice Committee of the American Society for Reproductive Medicine. Endometriosis and infertility: a committee opinion. Fertil Steril. 2012;98(3):591–8.
The Practice Committee of the American Society for Reproductive Medicine. Role of tubal surgery in the era of assisted reproductive technology: a committee opinion. Fertil Steril. 2015;103(6): e37–43.

Tranoulis A, Laios A, Pampanos A, Yannoukakos D, Loutradis D, Michala L. Efficacy and safety of pulsatile gonadotropin-releasing hormone therapy among patients with idiopathic and functional hypothalamic amenorrhea: a systematic review of the literature and a meta-analysis. Fertil Steril. 2018;109(4):0015–282.

Tsevat DG, Wiesenfeld HC, Parks C, Peipert JF. Sexually transmitted diseases and infertility. Am J Obstet Gynecol. 2017;216(1):1–9.

Vander BM, Wyns C. Fertility and infertility: definition and epidemiology. Clin Biochem. 2018;62:2–10. https://doi.org/10.1016/j.clinbiochem.2018.03.012.

Wallace WH, Kelsey TW. Human ovarian reserve from conception to the menopause. PLoS One. 2010;5(1):e8772.

Zegers-Hochschild F, Adamson GD, Dyer S, Racowsky C, de Mouzon J, Sokol R, Rienzi L, Sunde A, Schmidt L, Cooke ID, Simpson JL, van der Poel S. The international glossary on infertility and fertility care. Fertil Steril. 2017;108(3):393–406.

Zepiridis LI, Grimbizis GF, Tarlatzis BC. Infertility and uterine fibroids. Best Pract Res Clin Obstet Gynaecol. 2016;34:66–73.

Diagnostic Protocols for Infertility

12

Fernando M. Reis and Francisco A. N. Pereira

Contents

Abstract

Infertility is a rare example of two-person diagnosis, which is defined by the failure of a couple to conceive after 12 months of unprotected, regular intercourse. Thus, the couple must be evaluated together. A meticulous, systematic investigation should be carried out in order to establish the cause or causes of the couple's infertility and therefore allow the definition of the best treatment strategy. The main syndromic/anatomic categories of infertility are tubal, uterine, ovarian, and male factors. A well-balanced choose of the diagnostic tests is desirable to permit the best syndromic and, whenever possible, etiological diagnosis while avoiding unnecessary invasiveness.

F. M. Reis (✉) · F. A. N. Pereira
Division of Human Reproduction, Hospital das Clínicas, and Department of Obstetrics and Gynecology, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil
e-mail: reis.ufmg@gmail.com; fassisnp@gmail.com

F. Petraglia, B. C. Fauser (eds.), *Female Reproductive Dysfunction*, Endocrinology,
https://doi.org/10.1007/978-3-030-14782-2_12

Keywords

Infertility · Diagnostic tools · Hysterosalpingography · Hysterosonography · Laparoscopy · Transvaginal ultrasound · Hysteroscopy · Anovulation · Ovarian reserve · Seminal analysis

Introduction

Infertility is defined as the failure of getting pregnant after 12 months of regular unprotected intercourse. Infertility can be primary, if the couple has never been pregnant, or secondary, if they had been pregnant before (Practice Committee of the American Society for Reproductive Medicine 2015; National Institute for Health and Care Excellence (NICE) 2013).

The duration of infertility is the time interval between the discontinuation of the contraceptive method previously used, i.e., the moment at which the couple was actually exposed to the possibility of pregnancy, until the date of the pregnancy confirmation. The monthly conception rate in fertile couples is around 20%. Approximately 84% of young couples conceive within 1 year, 92% until the end of the second year, and 93% up to the end of the third year (Practice Committee of the American Society for Reproductive Medicine 2015; American College of Obstetricians and Gynecologists Committee on Gynecologic Practice and Practice Committee 2014).

Female fertility begins to decline several years before menopause, while regular menstrual cycles still occur. Although there is no definition of advanced reproductive age, the prevalence of infertility is 6% from 20 to 24 years of age, 9% from 25 to 29 years, 15% from 30 to 34 years, 30% from 35 to 39 years, and 64% from 40 to 44 years. At the same time, the rate of spontaneous abortion per clinical pregnancy increases with age, being 14% for women under 35 years, 19% between 35 and 37 years, 25% between 38 and 40 years, and 40% after 40 years (NICE 2013; American College of Obstetricians and Gynecologists Committee on Gynecologic Practice and Practice Committee 2014).

The causes of infertility vary in proportion according to the study population. There are situations with female and male factors, isolated or combined, and situations with no apparent cause. In a British primary care population-based study, 25% of the infertility cases were related to ovulatory disorders, 18% to tubal factor, and 25% to male factor, 7% had more than one diagnosis, and 38% were classified as unexplained infertility (Wilkes et al. 2009).

The fundamental principle for the assessment of the infertile couple is to evaluate the man and the woman independently of the presence of an evident illness in one of them (Practice Committee of the American Society for Reproductive Medicine 2015). Fertility assessment is indicated to every couple after a year or more of pregnancy attempt. Early evaluation is indicated to women over 35 years or with a history of oligomenorrhea/amenorrhea, known tubal disease or endometriosis, or if the partner is known to be subfertile.

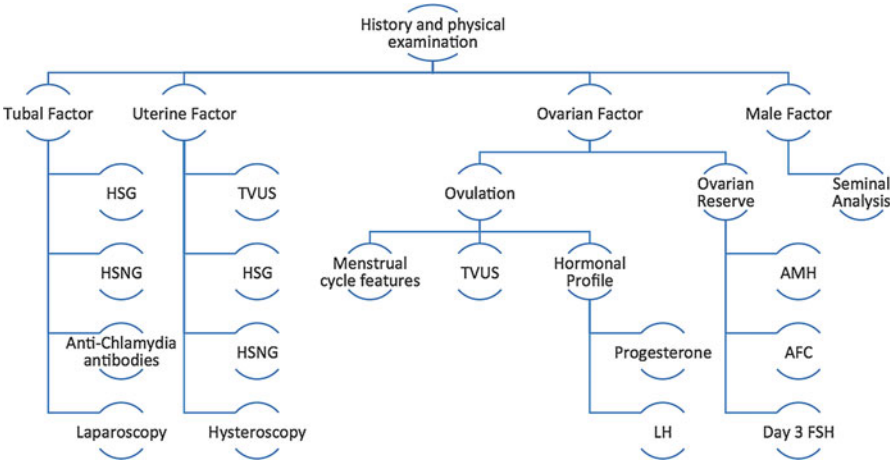


Fig. 1 Diagnostic evaluation of the infertile couple. *HSG* hysterosalpingography, *HSNG* hysterosonography, *TVUS* transvaginal ultrasonography, *LH* luteinizing hormone, *AMH* anti-Müllerian hormone, *AFC* antral follicle count, *FSH* follicle-stimulating hormone

Preconception counseling, a complete clinical history, and physical examination of the woman are mandatory. Physical examination of the man is only indicated when the semen is altered. An adequate initial evaluation should include tubal, uterine, ovarian, and seminal tests starting with the less invasive and expensive, as outlined in Fig. 1 (Practice Committee of the American Society for Reproductive Medicine 2015; NICE 2013).

Investigation of the Woman

The history and physical examination of the woman is the first part of the investigation. Some aspects are especially important because they may suggest changes or diseases related to infertility (Table 1).

Tubal Factor

Relevant tubal diseases include obstruction and adhesions due to infections, endometriosis, or previous surgeries. Pelvic inflammatory disease (PID) may cause chronic pelvic pain, ectopic gestation, or tubal infertility as the sequelae. Among the etiological agents of PID, *Chlamydia trachomatis* is more prevalent in developed countries, being present in 11% of the sexually active population under 19 years of age. The most common etiological agent of PID in developing countries is *Neisseria gonorrhoeae*. Other agents such as *Mycoplasma genitalium* and *Trichomonas vaginalis* may also be associated with infertility (Practice Committee of the

Table 1 Association of female history findings with potential causes of infertility

Clinical history finding	Possible association with
Irregular menstrual cycles, less than 25 or greater than 35 days	Anovulation
Obesity	Anovulation
Signs of hyperandrogenism (acne, hirsutism)	Anovulation
Excessive exercise	Anovulation
Signs and symptoms of hypothyroidism	Anovulation
Use of antidopaminergic drugs	Hyperprolactinemia
Galactorrhea	Hyperprolactinemia
Age greater than 35 years	Low ovarian reserve
Short menstrual cycles	Low ovarian reserve
Poor response to ovulation induction	Low ovarian reserve
Hot flashes, vaginal dryness	Primary ovarian insufficiency
Increased menstrual flow	Uterine factor (polyps, fibroids, etc.)
Reduced menstrual flow after uterine surgeries or curettage	Uterine factor (uterine synechiae)
Palpable abdominal masses	Uterine factor (fibroids)
Sexually transmitted diseases, pelvic inflammatory disease, appendicitis	Tubal factor
Pelvic surgery	Tubal factor
Severe, progressive dysmenorrhea	Endometriosis
Dyspareunia	Endometriosis
Pain on pelvic palpation	Endometriosis, pelvic inflammatory disease
Use of alcohol, tobacco, and illicit drugs	Reduction of natural fertility
Low frequency of intercourse	Female or male sexual disorders (e.g., vaginismus, impotence, etc.)

American Society for Reproductive Medicine 2015; NICE 2013; Tsevat et al. 2017; Gorwitz et al. 2017; Lenz and Dillard 2018).

The following tests are used to assess tubal factor infertility:

Hysterosalpingography (HSG)

HSG is a contrast-enhanced X-ray of the uterus and fallopian tubes, usually performed shortly after the end of the menstrual flow. It is the initial test of choice for evaluation of tubal diseases (Practice Committee of the American Society for Reproductive Medicine 2015). It should always be performed in the first phase of the menstrual cycle to avoid the possibility of gestation and also to avoid that the endometrial thickening that occurs in the ovulatory or luteal phase disrupts the interpretation of the images (NICE 2013).

HSG has approximately 65% sensitivity and 83% specificity to detect tubal pathology in infertile women. Considering a prevalence of tubal factor infertility of 14% (data from developed countries), it is estimated that when HSG detects the presence of tubal obstruction, this will be confirmed by laparoscopy in only 38% of the cases. On the contrary, when the HSG evidences tubal patency, this will be confirmed by laparoscopy in 94% of the cases (Practice Committee of the American Society for Reproductive Medicine 2015; NICE 2013).

Hysterosonography (HSNG)

Tubal patency can also be assessed through hysterosonography, which is an ultrasonography enhanced by liquid infusion into the uterine cavity. The use of saline solution only permits the visualization of free liquid at the cul de sac, with the inferring that at least one of the tubes is patent. There are special solutions with stabilized microbubbles that make them visible while passing through the tubes, allowing a more specific diagnosis of unilateral or bilateral tubal patency or occlusion. It should also be carried out in the first phase of the menstrual cycle.

Comparative studies showed good diagnostic performance of HSNG in relation to HSG and laparoscopy, with sensitivity varying from 75% to 96% and specificity from 67% to 100% (Luciano et al. 2014). HSNG is well tolerated and may be a feasible alternative to HSG for tubal assessment in expert hands.

Anti-Chlamydia Antibodies

Anti-*Chlamydia* antibody test has modest sensitivity (40–50%) and positive predictive value (60%) but high negative predictive value (80–90%) for tubal obstruction (Practice Committee of the American Society for Reproductive Medicine 2015). It should be emphasized, however, that these values were calculated in countries where *Chlamydia* is the main cause of tubal factor infertility, and should not be extrapolated to countries where *N. gonorrhoeae* is the most common etiological agent (Rantsi et al. 2018).

Laparoscopy

Laparoscopy is recommended to confirm the diagnosis of tubal abnormalities and is considered the gold standard in tube-peritoneal investigation. Laparoscopy may be indicated when there is evidence or strong suspicion of endometriosis, pelvic/adnexa adhesions, or tubal disease by clinical history, HSG, or HSNG. Laparoscopy has the advantage of being able to perform the see-and-treat approach. When performing laparoscopy, the surgeon must be prepared to treat the identified changes immediately.

The finding of unilateral or bilateral tubal obstruction by laparoscopy has a greater impact on spontaneous pregnancy rates than the same findings in HSG. Unilateral tubal obstruction at HSG does not affect spontaneous pregnancy rate compared with no obstruction (fecundity ratio 0.80, 95% CI 0.39 to 1.60), whereas bilateral tubal obstruction at HSG significantly decreases spontaneous pregnancy rates (fecundity ratio 0.49, 95% CI 0.26 to 0.95). However, when the diagnosis is made by laparoscopy, the impact on pregnancy rates is much higher, with fecundity ratios of 0.51 (95% CI 0.20 to 1.30) and 0.15 (95% CI 0.04 to 0.63) for unilateral and bilateral tubal obstruction, respectively (Mol et al. 1999).

The recommendations of the Royal College of Obstetricians and Gynaecologists for tubal factor evaluation are (NICE 2013):

- Women with no history of pelvic infections, surgeries, ectopic pregnancies, or endometriosis should be submitted to HSG for initial evaluation.
- When available, HSNG can be used in initial screening for tubal disease.
- Women with suspected pelvic diseases should undergo laparoscopy with chromopertubation, so that the tubal and pelvic evaluation can be done at the same time.

It is important to note that HSG, HSNG, and even laparoscopy basically evaluate aspects such as tubal patency and motility. It is not possible to evaluate other specialized tubal functions necessary for the occurrence of natural gestation, such as the capacity of oocyte uptake and the intratubal conditions necessary for the transport and maintenance of gametes and embryos.

In the face of suspected tubal factor, the cost-benefit of laparoscopy should be assessed. In some situations such as patients over 35 years or patients with other infertility factors, going straight to in vitro fertilization without a laparoscopy may be a better option.

Uterine Factor

The uterine disorders that may be associated with infertility are fibroids, polyps, synechiae, adenomyosis, Müllerian alterations, and sequelae of previous uterine surgeries. In women who undergo assisted reproduction, the presence of submucosal or intramural uterine fibroids that distort the uterine cavity is associated with reduced chances of clinical pregnancy or live birth (Whynott et al. 2017).

In addition to history and physical examination, the investigation of these uterine infertility factors can be done through the following methods:

Transvaginal Ultrasonography (TVUS)

The diagnostic performance of TVUS varies according to the operator skills and imaging resolution, but in general it has good accuracy in the evaluation of uterine fibroids, having less accuracy in the identification of uterine cavity lesions (Soares et al. 2000).

Hysterosalpingography

HSG defines the size and shape of the uterine cavity and may reveal Müllerian abnormalities such as unicornuate, septate, or bicornuate uterus, as well as acquired abnormalities such as endometrial polyps, submucosal fibroids, and synechiae. It has relatively low sensitivity (50%) and positive predictive value (30%) for the diagnosis of submucosal polyps and fibroids in asymptomatic infertile women (Soares et al. 2000).

Hysterosonography

HSNG consists of sterile saline infusion into the uterine cavity under the continuous monitoring of TVUS. HSNG has excellent performance in the identification of uterine cavity lesions, reaching sensitivity, specificity, and positive and negative predictive values close to hysteroscopy (Practice Committee of the American Society for Reproductive Medicine 2015; Soares et al. 2000; Bittencourt et al. 2017; Calles-Sastre et al. 2018).

Hysteroscopy

Hysteroscopy is superior to TVUS, HSG, and HSNG to diagnose intrauterine lesions since it allows the direct visualization of the uterine cavity and the detailed study of

the intracavitary lesions, followed by biopsy and even the excision of small lesions on an outpatient basis, without the need for anesthesia.

Current evidence suggests that hysteroscopy should be performed in selected cases where intrauterine lesions are suspected based on symptoms or imaging findings (Practice Committee of the American Society for Reproductive Medicine 2015). A systematic review with meta-analysis found moderate-quality evidence that performing hysteroscopy routinely before in vitro fertilization treatment may increase the pregnancy rate but only very low-quality evidence that such procedure increases the live birth rate (Di Spiezio Sardo et al. 2016), which was subsequently refuted by a randomized controlled trial (Smit et al. 2016). Another systematic review concluded that removing endometrial polyps before intrauterine insemination may improve the clinical pregnancy rate, whereas the benefit of removing submucosal fibroids in women with otherwise unexplained infertility remains uncertain (Bosteels et al. 2018).

Ovarian Factor

The ovarian factor has two aspects that must be evaluated: ovulation and ovarian reserve.

Ovulation

There are direct and indirect, real-time, and retrospective methods to detect ovulation (Practice Committee of the American Society for Reproductive Medicine 2015; NICE 2013). No method is perfect, but an accurate retrospective method like menstrual cycle pattern or serum progesterone level may be sufficient to diagnose or rule out ovulatory disorders, whereas a real-time method like TVUS is useful to guide infertility treatments such as timed intercourse and intrauterine insemination.

- Menstrual cycle pattern: Women with regular cycles (between 25 and 35 days) with normal flow, who usually present with premenstrual symptoms, are more than 95% likely to be ovulating normally.
- TVUS: The visualization of the growth and rupture of the dominant follicle, the aspect of the endometrium, the presence of cervical mucus, and the formation of the corpus luteum are indicative of ovulation (Practice Committee of the American Society for Reproductive Medicine 2015).
- Hormonal measurements: Serial measurements of LH in blood and/or urine allow the identification of its peak which is a sign of imminent ovulation. However, a serum progesterone level above 3 ng/ml in the mid-luteal phase is the best retrospective method to confirm ovulation.

The World Health Organization (WHO) classifies ovulation disorders into:

- Group 1: Hypothalamic-pituitary failure – characterized by pituitary deficiency in producing FSH and LH leading to failure of follicular development and hypoestrogenism. It occurs in 10% of cases of anovulation.

- Group 2: Hypothalamic-pituitary dysfunction – characterized by a dysfunction in the hypothalamic-pituitary-ovarian axis. The levels of estrogen and gonadotropins are normal, but anovulation occurs, with oligomenorrhea/amenorrhea. Most cases are related to polycystic ovarian syndrome (PCOS). This group is responsible for 85% of cases of ovulation disorders.
- Group 3: Primary ovarian insufficiency, with low estrogen production by the ovaries and the consequent elevation of serum gonadotropin levels. It occurs in 4% to 5% of cases of anovulation.

Some etiologies of chronic anovulation like hyperprolactinemia, chronic stress, extenuating exercise, and nutritional disorders may manifest as WHO Group 1 or Group 2 depending on the severity of the disease. Serum prolactin levels should be assessed in women with symptoms of anovulation and galactorrhea or pituitary adenoma (NICE 2013). Thyroid dysfunction can also lead to menstrual and/or ovulatory disorders. However, women with infertility problems have the same risk as the general population of the same age of having thyroid disease; therefore, the evaluation of thyroid function should be reserved for women with symptoms of thyroid dysfunction (NICE 2013).

Ovarian Reserve

The functional ovarian reserve depends on the number of primordial follicles in the ovary, which declines with aging until complete exhaustion at the time of menopause. In the absence of direct methods to count the reserve of primordial follicles in vivo, surrogate methods based on the imaging or hormonal activity of small antral follicles are used in clinical practice, as listed in Table 2. The best predictors of follicular reserve are patient age, antral follicle count, and anti-Müllerian hormone levels (NICE 2013; Wilkes et al. 2009).

Overall, ovarian reserve tests are not useful to predict the chance of natural conception (Steiner et al. 2017) and have a low predictive value for the chance of

Table 2 Parameters for evaluation of the ovarian reserve and expected results after in vitro fertilization

	Results	
Parameters	Favorable	Unfavorable
Age (years)	<35	>35
FSH day 3 (UI/L)	<10	≥10
Estradiol day 3 (pg/ml)	<75	≥75
AMH (pmol/l)	15.7–45.8	<15.7
Inhibin B day 3 (pg/ml)	>45	≤45
Antral follicle count	≥5	<5
Ovarian volume (cm^3)	≥3	<3
Anterior cycle of ovarian stimulation	Delivery	No pregnancy

FSH follicle-stimulating hormone
AMH anti-Müllerian hormone.

pregnancy in assisted reproductive therapies (Tal et al. 2015). The best performance of these tests is in predicting the ovarian response to ovulation induction, which may be particularly useful for patients with low or high response.

The evaluation of the ovarian reserve may be relevant in the following situations: women over 35 years; family history of primary ovarian insufficiency; patients with a single ovary or previous history of ovarian surgery, chemotherapy, or pelvic radiotherapy; patients with unexplained infertility; with previous history of poor response to stimulation with gonadotropins; or patients who will undergo assisted reproduction techniques (Practice Committee of the American Society for Reproductive Medicine 2015). Poor ovarian response is defined as less than four oocytes retrieved after ovarian stimulation and is usually due to low follicular reserve, although rare cases of gonadotropin resistance due to molecular defects with apparently normal follicles have been described (Practice Committee of the American Society for Reproductive Medicine 2015; Boudjenah et al. 2012).

Cervical Factor

Abnormalities in the production of cervical mucus or in the interaction of mucus and spermatozoa are rarely identified as isolated causes of infertility. The best technique and time to evaluate mucus-sperm interactions are unknown, the results are subjective and poorly reproducible, and therefore the test has little clinical utility (Practice Committee of the American Society for Reproductive Medicine 2015).

Infectious Factor

Chlamydia infection can often be unrecognized and therefore not properly treated (Gorwitz et al. 2017). Invasive testing can reactivate a *Chlamydia* infection or cause a cervical infection to spread to the upper genital tract. Cases of acute iatrogenic PID are described in 4% of patients submitted to HSG and in up to 10% of patients who had previous tubal disease.

Pretreatment with antibiotics (doxycycline or azithromycin) is effective in reducing these risks. Treatment is therefore recommended prior to invasive examinations in women who have been screened positive for *Chlamydia* or who have not been screened.

Viruses such as HIV, Hepatitis B, and Hepatitis C do not cause infertility directly, but their screening is advised during the infertility workup for adequate pregestational counseling, as well as for measures to reduce the risk of vertical transmission (NICE 2013).

The presence of bacterial vaginosis in infertile patients was associated with a higher prevalence of tubal factor infertility and also with an increased risk of spontaneous abortion, but did not impact the clinical pregnancy or live birth rates (Haahr et al. 2019).

Investigation of the Man

The couple with infertility should have their male partner evaluated in parallel to the female assessment. The tests can be performed earlier if the man has a known risk factor for infertility, such as bilateral cryptorchidism (NICE 2013). Seminal analysis is the initial test to evaluate male infertility. The current trend is to initiate male evaluation by the reproductive history and a seminal analysis. If this analysis is normal, it is not necessary to repeat the examination. In the presence of abnormalities, it is recommended to order new semen analysis with at least 2–3 months of interval, as this is the time of the complete spermatogenesis cycle. In case of disparity between the first two tests, a third one may be requested in order to establish the mean or trend of the samples.

The WHO defines male factor as the presence of one or more abnormalities in the seminal analysis or the presence of inadequate sexual function or ejaculatory dysfunction. It should be remembered that men with previous children may have acquired a new factor of infertility and should be evaluated in the same way as men who have never had children.

Seminal Analysis

Seminal analysis is the evaluation of objective characteristics in a sperm sample, compared to reference values defined by the WHO. It is not a direct evaluation of the male's fertility, which can only be confirmed by the partner's gestation.

The current reference values for seminal analysis were defined by the WHO in 2010 based on a study that included more than 4,500 men from 14 countries (Table 3). In this study, parameters were identified for men whose partners became pregnant within 12 months after discontinuation of the contraceptive method, and the threshold of altered results was established as the fifth centile of the study sample (Cooper et al. 2010). Therefore, normal values do not ensure fertility, nor do altered values imply the impossibility of achieving a gestation.

In the case of seminal analysis alterations, the partner should be evaluated by an andrologist to reach specific diagnosis and plan possible treatments. If there is

Table 3 Lower reference limits (percentile 5 and their 95% confidence intervals) according to the World Health Organization (Cooper et al. 2010)

Parameters	Lower reference limit
Seminal volume (ml)	1.5 (1.4–1.7)
Total sperm number (10^6 per ejaculate)	39 (33–46)
Sperm concentration (10^6 per ml)	15 (12–16)
Total motility (%)	40 (38–42)
Progressive motility (%)	32 (31–34)
Vitality (%)	58 (55–63)
Sperm morphology (%)	4 (3–4)

nonobstructive azoospermia, i.e., absence of spermatozoa in the ejaculate due to impaired sperm production rather than transit, the patient should undergo a detailed evaluation that includes history, physical examination, hormonal assays, genetic study of the Y chromosome, and karyotype (NICE 2013).

Unfortunately most cases of male factor infertility do not have effective treatments, and the couple will need to be submitted to assisted reproduction techniques such as intrauterine insemination or more commonly in vitro fertilization with intracytoplasmic sperm injection.

Conclusions

The investigation of the major causes of infertility can be carried out by the primary care physician. As a large proportion of the male partners show some alterations in the seminal analysis, the couple must be investigated together. The main diagnostic tools are history and physical examination of the woman, HSG, TVUS, and seminal analysis, for the initial evaluation of the ovarian, tubal, uterine, and male factors, respectively. In secondary and tertiary care settings, other diagnostic methods with greater complexity and/or invasiveness may clarify etiological factors and help in defining the best treatment strategies.

Cross-References

- ▶ Endometriosis
- ▶ Infertility
- ▶ Premature Ovarian Insufficiency
- ▶ The Menstrual Cycle and Related Disorders
- ▶ The Polycystic Ovary Syndrome (PCOS)

References

American College of Obstetricians and Gynecologists Committee on Gynecologic Practice and Practice Committee. Female age-related fertility decline. Committee opinion no. 589. Fertil Steril. 2014;101(3):633–4.

Bittencourt CA, Dos Santos Simões R, Bernardo WM, Fuchs LFP, Soares Júnior JM, Pastore AR, et al. Accuracy of saline contrast sonohysterography in detection of endometrial polyps and submucosal leiomyomas in women of reproductive age with abnormal uterine bleeding: systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2017;50(1):32–9.

Bosteels J, van Wessel S, Weyers S, Broekmans FJ, D'Hooghe TM, Bongers MY, et al. Hysteroscopy for treating subfertility associated with suspected major uterine cavity abnormalities. Cochrane Database Syst Rev. 2018;12:CD009461.

Boudjenah R, Molina-Gomes D, Torre A, Bergere M, Bailly M, Boitrelle F, et al. Genetic polymorphisms influence the ovarian response to rFSH stimulation in patients undergoing in vitro fertilization programs with ICSI. PLoS One. 2012;7(6):e38700.

Calles-Sastre L, Engels-Calvo V, Ríos-Vallejo M, Serrano-González L, García-Espantaleón M, Royuela A, et al. Prospective study of concordance between Hysterosalpingo-contrast Sonography and hysteroscopy for evaluation of the uterine cavity in patients undergoing infertility studies. J Ultrasound Med. 2018;37(6):1431–7.

Cooper TG, Noonan E, von Eckardstein S, Auger J, Baker HW, Behre HM, et al. World Health Organization reference values for human semen characteristics. Hum Reprod Update. 2010;16(3):231–45.

Di Spiezio Sardo A, Di Carlo C, Minozzi S, et al. Efficacy of hysteroscopy in improving reproductive outcomes of infertile couples: a systematic review and meta-analysis. Hum Reprod Update. 2016;22(4):479–96.

Gorwitz RJ, Wiesenfeld HC, Chen PL, Hammond KR, Sereday KA, Haggerty CL, et al. Population-attributable fraction of tubal factor infertility associated with chlamydia. Am J Obstet Gynecol. 2017;217(3):336.e1–336.e16.

Haahr T, Zacho J, Bräuner M, Shathmigha K, Skov Jensen J, Humaidan P. Reproductive outcome of patients undergoing in vitro fertilisation treatment and diagnosed with bacterial vaginosis or abnormal vaginal microbiota: a systematic PRISMA review and meta-analysis. BJOG. 2019;126(2):200–7.

Lenz JD, Dillard JP. Pathogenesis of *Neisseria gonorrhoeae* and the host defense in ascending infections of human fallopian tube. Front Immunol. 2018;9:2710.

Luciano DE, Exacoustos C, Luciano AA. Contrast ultrasonography for tubal patency. J Minim Invasive Gynecol. 2014;21(6):994–8.

Mol BW, Collins JA, Burrows EA, van der Veen F, Bossuyt PM. Comparison of hysterosalpingography and laparoscopy in predicting fertility outcome. Hum Reprod. 1999;14(5):1237–42.

National Institute for Health and Care Excellence (NICE): Fertility problems: assessment and treatment. Clinical guideline [CG156] Published date: February 2013 Last updated: September 2017. Available at https://www.nice.org.uk/guidance/cg156

Practice Committee of the American Society for Reproductive Medicine. Diagnostic evaluation of the infertile female: a committee opinion. Fertil Steril. 2015;103(6):e44–50.

Rantsi T, Öhman H, Puolakkainen M, Bloigu A, Paavonen J, Surcel HM, et al. Predicting tubal factor infertility by using markers of humoral and cell-mediated immune response against chlamydia trachomatis. Am J Reprod Immunol. 2018;80(5):e13051.

Smit JG, Kasius JC, Eijkemans MJC, et al. Hysteroscopy before in-vitro fertilisation (inSIGHT): a multicentre, randomised controlled trial. Lancet. 2016;387:2622–9.

Soares SR, Barbosa dos Reis MM, Camargos AF. Diagnostic accuracy of sonohysterography, transvaginal sonography, and hysterosalpingography in patients with uterine cavity diseases. Fertil Steril. 2000;73(2):406–11.

Steiner AZ, Pritchard D, Stanczyk FZ, Kesner JS, Meadows JW, Herring AH, et al. Association between biomarkers of ovarian reserve and infertility among older women of reproductive age. JAMA. 2017;318(14):1367–76.

Tal R, Tal O, Seifer BJ, Seifer DB. Antimüllerian hormone as predictor of implantation and clinical pregnancy after assisted conception: a systematic review and meta-analysis. Fertil Steril. 2015;103(1):119–30.e3.

Tsevat DG, Wiesenfeld HC, Parks C, Peipert JF. Sexually transmitted diseases and infertility. Am J Obstet Gynecol. 2017;216(1):1–9.

Whynott RM, Vaught KCC, Segars JH. The effect of uterine fibroids on infertility: a systematic review. Semin Reprod Med. 2017;35(6):523–32.

Wilkes S, Chinn DJ, Murdoch A, Rubin G. Epidemiology and management of infertility: a population-based study in UK primary care. Fam Pract. 2009 Aug;26(4):269–74.

Hormonal Treatments in the Infertile Women

13

Konstantinos Dafopoulos and Basil C. Tarlatzis

Contents

Abstract

Ovulatory dysfunction may affect up to 40% of infertile women, and its treatment of is ovulation induction aiming at the selection of a single follicle.

Anovulatory infertility includes hypogonadotropic hypogonadism, normogonadotropic anovulation (mainly women with the polycystic ovary syndrome, PCOS), and hypergonadotropic anovulation. Ovulation induction in hypogonadotropic hypogonadism may be the exogenous administration of both FSH and LH, or in cases with intact pituitary, the exogenous GnRH administration in a pulsatile way.

K. Dafopoulos
Department of Obstetrics and Gynecology, School of Medicine, University of Thessaly, Larissa, Greece
e-mail: kdafop@yahoo.com; kdafop@med.uth.gr

B. C. Tarlatzis (✉)
Department of Obstetrics and Gynecology, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece
e-mail: basil.tarlatzis@gmail.com

F. Petraglia, B. C. Fauser (eds.), *Female Reproductive Dysfunction*, Endocrinology,
https://doi.org/10.1007/978-3-030-14782-2_13

In women with PCOS, clomiphene citrate has been considered as the first-line pharmacological ovulation induction treatment, while recent data have shown that letrozole is superior to clomiphene. Metformin may have a role in combination to clomiphene. The second-line treatment includes the low-dose gonadotropins protocols and laparoscopic ovarian drilling in selected cases, while the third-line treatment is IVF.

Keywords

Ovulation induction · Hypogonadotropic hypogonadism · PCOS

Introduction

Ovulatory dysfunction may account for approximately 15% of infertility and may be diagnosed in up to 40% of infertile women (Practice Committee of the American Society for Reproductive Medicine 2015). The basis for the treatment of anovulatory infertility is ovulation induction. The goal of ovulation induction is the selection of a single follicle and ultimately mono-ovulation, with normal ovarian steroidogenesis resulting in proper endometrial preparation for successful implantation following ovulation.

Anovulatory infertility, according to the World Health Organization (WHO), was classified into three groups, based on gonadotropin and estrogen blood levels. Although this classification is simplified, it is still valid, and ovulation induction may apply in patients with the diagnosis of hypogonadotropic hypogonadism (WHO Group 1) and normogonadotropic anovulation (WHO group 2). The vast majority of WHO group 2 patients are women with the polycystic ovary syndrome (PCOS) (Broekmans et al. 2006; ESHRE Capri Workshop Group 2012). For WHO group 3 women with hypergonadotropic anovulation, the only realistic option to conceive is oocyte donation. WHO group 1 patients clinically have amenorrhea, while the gonadotropins and estradiol levels in circulation are low, due to reduced secretion of gonadotropins by the pituitary gland that results in quiescence of the ovaries. PCOS patients represent a heterogeneous group with four phenotypes according to the diagnostic criteria of the 2003 Rotterdam Consensus Meeting (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2004).

In the present chapter, the ovulation induction treatments for hypogonadotropic hypogonadism and PCOS patients will be reviewed.

Hypogonadotropic Hypogonadism

In these patients, there are two options to induce ovulation: firstly, by exogenous administration of both FSH and LH, which are absent, and secondly, in cases with intact pituitary, by exogenous GnRH administration in a pulsatile way, mimicking the physiological secretion of endogenous GnRH.

Gonadotropins

Both FSH and LH administration are needed in these patients because their endogenous production is deficient, due to hypothalamic pituitary failure. If only FSH is given, then follicular growth is stimulated, but the secretion of estradiol is severely compromised, since LH is necessary for thecal androgen production and conversion to estradiol (E2) in the granulosa cells (Schoot et al. 1992).

Gonadotropins, either urinary or recombinant, may be used for ovulation induction in these patients. Regarding the dose of LH needed, it has been found to be 75 IU when it is given with 150 IU of recombinant FSH (The European Recombinant Human LH Study Group 1998; Burgues and The Spanish Collaborative Group on Female Hypogonadotrophic Hypogonadism 2001). From the urinary menopausal gonadotropins (HMG), those containing 75 IU FSH and 75 IU LH per ampoule are appropriate.

In these patients, some critical issues should be addressed to ensure monoovulation, thus avoiding multiple follicular development and, consequently, the risk of ovarian hyperstimulation syndrome (OHSS) and multiple pregnancies.

These issues include the starting dose of FSH and the increments, the monitoring of treatment, the criteria for HCG administration, and the luteal phase support.

Physiologically, the antral follicles in these women may be stimulated and start growing once FSH and LH are present in blood circulation. However, gonadotropin stimulation should start with low doses, and further increments should also be low, aiming at identifying the FSH threshold, i.e., the FSH dose that induces monofollicular development. Once this is accomplished, then no further increases in exogenous gonadotropins are needed. Monitoring of treatment includes serum E2 measurements and transvaginal ultrasound scans of the ovaries.

The protocol for ovulation induction that is still used in clinical practice has been described many years ago (Brown et al. 1969). This is a "step-up" regimen, with a usual starting dose of HMG being 150 IU/day that is administered for $\geq$5 days; if there is no increase in serum E2 levels, the dose is increased by 33% every 5 days. Although there are no relevant data, it is very important to have a baseline serum E2 measurement to interpret the stimulatory effect during the first 5 days. If there is an increase of E2 levels although they are still very low, it may be worthwhile to continue with the same dose for more days until follicle selection is evident. Ultrasonographically, the follicle(s) selected and destined to ovulate is(are) the one (s) measuring at least 10 mm, and gonadotropic stimulation continues until the day of HCG administration. To induce final oocyte maturation and follicle rupture, urinary HCG (5000 IU i.m.) or recombinant HCG (250 μg s.c.) are given. In some studies, a starting dose of 75 IU of HMG has been used. In the most recent study, the threshold HMG dose was $\leq$75 IU in about 20% of patients, while 19% of patients required doses higher than 150 IU/day. The age and BMI of the hypogonadotropic women that required HMG doses >150 IU/day were higher than the patients with HMG threshold less than 150 IU/day (White et al. 2018).

There is no consensus on the criteria for HCG administration. In earlier clinical practice without ultrasonographic monitoring, they have been defined as a serum E2

level measurement around 2000 pmol/l (Bergquist et al. 1983). Others using ultrasound monitoring suggested that HCG should be administered when the leading follicle is >16 mm, with no more than three follicles >14 mm and the serum E2 levels between 500 and 3000 pmol/l (Tadokoro et al. 1997). However, with both practices, there is a significant risk for multiple pregnancies, despite the goal of ovulation induction to select a single follicle. More recently, it was proposed that HCG should be given when the leading follicle is 18 mm, with no more than three follicles ≥15 mm (White et al. 2018).

It has been shown that the low serum LH levels, due to central insufficiency, during the course of ovarian stimulation are further suppressed, suggesting that the negative feedback mechanism in some of these patients is active (Burgues and The Spanish Collaborative Group on Female Hypogonadotrophic Hypogonadism 2001). Therefore, the severe LH insufficiency results finally in a significant luteal phase defect that should be corrected with exogenous HCG administration. For luteal phase support, a regimen of exogenous HCG at a dose of 2500 IU, 3 days and 6 days after the HCG ovulation trigger, seems appropriate (White et al. 2018).

Treatment in this group of patients is very efficient with high cumulative pregnancy rates of about 90% after six treatment cycles, with the majority of pregnancies occurring within the first three cycles (White et al. 2018).

Earlier studies have reported a range of multiple pregnancies between 19% and 30% (Rabau et al. 1967; Thompson and Hansen 1970; Dale et al. 1989; Yildirim et al. 2000). However, there were significant drawbacks in the methodology of these studies, since only in few, the starting dose of HMG was less than 150 IU, some of them included mixed cases of hypogonadotropic hypogonadism and other anovulatory patients, and also the amount of HMG increment varied significantly between these studies. A recent study reported a low rate of multiple pregnancies (5%), and all these cases were twins (White et al. 2018). All reports have shown that the rate of OHSS is very low (0–1.6%).

GnRH Pump

In hypogonadotropic hypogonadism cases with intact pituitary, i.e., hypothalamic dysfunction, GnRH infusions may be given in a pulsatile way via a mini-pump, mimicking the hypothalamic stimulation of the pituitary, and this results in an almost physiological ovulatory menstrual cycle. The advantages of this method include the low rate of multiple pregnancies and no risk of OHSS. The GnRH pump is placed intravenously (i.v.) or subcutaneously (s.c.) and is usually programmed to deliver 1 pulse of GnRH every 90 min. The GnRH dose is 5–10 μg/pulse i.v. and 15–20 μg/pulse s.c. Lower doses of GnRH should be given during the first treatment cycle, and if needed, they should be adapted according to ovarian response. Regarding the efficacy to induce ovulation of the i.v. versus the s.c. route, studies have shown contradictory results as yet (Leyendecker and Wildt 1983; Christin-Maitre et al. 2007; Christou et al. 2017). However, the s.c. route is more convenient than the i.v. route, which may be further complicated by phlebitis in 1.5% of cases (Christin-Maitre et al. 2007).

Treatment monitoring and dose adaptation is performed by serial measurements of serum FSH, LH, estradiol, and progesterone levels, combined with ultrasonographic measurements of follicle(s). Ovulation is detected by the disappearance of the dominant follicle with serum progesterone elevation (≥5 ng/mL) 7 days after the presumed date of ovulation.

Many studies have shown that HCG is not required for dominant follicle rupture, while the pulsatile GnRH is given in a steady way. However, others proposed to trigger ovulation with HCG administration when the dominant follicle reaches 18–20 mm in diameter. Although the only advantage of HCG may be the more accurate prediction of ovulation, it has been suggested that HCG triggering and higher GnRH dose administration may be associated with higher rates of multiple pregnancies (Braat et al. 1989).

During the luteal phase, some have suggested to program the GnRH pulse frequency of the mini-pump at lower values (every 4 h), resembling the GnRH pulses during the luteal phase of the normal menstrual cycle. In case that the treatment is successful with pregnancy, the GnRH pump should be discontinued. Other investigators proposed that, after the detection of ovulation, the pump should be withdrawn and the luteal phase be supported with three intramuscular (i.m.) injections of HCG, at the dose of 1500 IU each, every 3 days (Christou et al. 2017; Dumont et al. 2016).

The disadvantages of the GnRH pump include the need for the pump to be connected to the body all day for a considerable number of days, the necessity to refill the pump at frequent intervals, and the possible skin reactions at the site of injection.

Generally, the ovulation rates with GnRH pump are high, and the cumulative pregnancy rate after six cycles may be excellent, above 90% (Filicori et al. 1994; Homburg et al. 1989; Martin et al. 1993).

Polycystic Ovary Syndrome (PCOS)

The PCOS prevalence is estimated to be 6% according to the NIH diagnostic criteria and 10% according to the Rotterdam and AE-PCOS criteria (Bozdag et al. 2016). About 50% of PCOS women may be overweight and obese. In these patients, first-line treatment is weight loss by diet, exercise, behavioral modification, pharmacological interventions, or bariatric surgery (Messinis et al. 2015; Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2008). However, it is still unclear whether weight loss prior to ovulation induction is indeed beneficial, since one RCT (Mutsaerts et al. 2016) showed that this intervention was associated with significantly lower live birth rates as compared to immediate ovulation induction, while another one (Legro et al. 2016) exactly the opposite.

In the present chapter, the ovulation induction treatments for PCOS patients will be presented. In general, clomiphene citrate has been considered as the first-line pharmacological infertility therapy (Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2008), while recent data have suggested that letrozole is superior to clomiphene (Franik et al. 2018). Metformin may have a role in

combination to clomiphene. The second-line treatment includes the low-dose gonadotropin protocols and laparoscopic ovarian drilling. The third-line treatment is IVF, which is offered when the previous ovulation induction methods have failed.

Clomiphene Citrate

Clomiphene citrate (CC) is a selective estrogen receptor modulator (SERM) that has been used for ovulation induction since 1961. It is a racemic mixture of two isomers, zuclomiphene and enclomiphene with the first being more biologically active, and has a half-life of 5–7 days. The mechanism of CC action is the blocking of the estrogen receptors in the hypothalamic-pituitary compartment of the reproductive axis; as a result, the estrogen negative feedback effect centrally is interrupted increasing the secretion of gonadotropins from the anterior pituitary (Fig. 1). Therefore, during the administration of CC, the blood levels of endogenous FSH increase, mimicking the window of FSH, which is the determinant of follicle selection.

Once the follicle(s) is(are) selected, the estradiol levels in blood increase, and a midcycle LH surge occurs invariably. This means that in PCOS patients treated with CC, HCG administration for follicle rupture is not necessary.

The doses of CC for ovulation induction in PCOS are 50–150 mg/day for 5 days starting on days 2–5 of the menses, which may be spontaneous or induced by progestin withdrawal. The starting day of treatment, whether on cycle day 2–5, does not influence the results, and there are no advantages in using a daily dose of more than 150 mg, since it does not increase the ovulation rate and follicular recruitment (Dickey et al. 1997).

Before starting ovulation induction, a pregnancy test is often performed together with a baseline ultrasound to evaluate ovarian and endometrial morphology. The simplest method to monitor whether the dose of CC is ovulatory is to measure progesterone levels in blood on day 21 and, if low, again on day 28 of the cycle when treatment started on day 2. High progesterone levels indicate ovulation, but when they are low, during the next cycle, the CC dose should be increased (up to a maximum dose of 150 mg daily). However, since with CC ovulation induction the multiple pregnancy rate is significantly higher than in spontaneous conceptions,

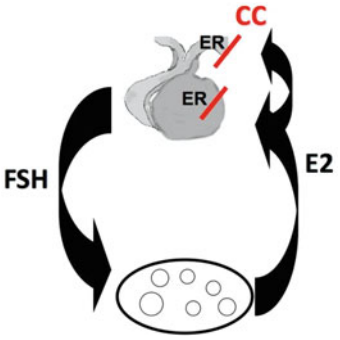


Fig. 1 Administration of clomiphene citrate (CC) for 5 days, blocks estrogen receptors (ER) at the level of the pituitary and hypothalamus. As a result, estrogen negative feedback centrally is interrupted, and FSH secretion increases from the anterior pituitary leading to multiple follicular growth

monitoring with ultrasound to detect multifollicular development may give additional information about the risk of multiple pregnancies. Ultrasound monitoring has not been suggested as mandatory to improve the outcome, but in many centers, it is commonly used to monitor the first cycle and facilitate dose adjustments in subsequent cycles (Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2008).

The ovulation rate with 50 mg is 46%, with additional increases of 21% and 8% with the doses of 100 mg and 150 mg daily, respectively (Rostami-Hodjegan et al. 2004). Approximately, from 100 PCOS patients treated with CC, 75 will have ovulation, and 35–40 will become pregnant. The miscarriage and the multiple pregnancy rates are about 20% and 8%, respectively (van Santbrink et al. 2005). Therefore, it is expected that the probability the PCOS patient treated with CC to have a singleton live birth is approximately 25% (Homburg 2008). OHSS has rarely been reported following ovulation induction with CC (Brown and Farquhar 2016).

The term "CC resistance" means that despite the administration of maximal doses of CC, there is no ovulation, while the term "clomiphene failure" means that despite ovulation, no pregnancy is achieved. CC resistance is associated with amenorrhea, high BMI, as well as high serum androgen, insulin, and LH levels. The reasons for the discrepancy between the ovulation (about 75%) and pregnancy (35–40%) rates have been suggested to be the antiestrogenic effects of CC that may inhibit proper endometrial thickening, pinopode formation, and finally implantation (Homburg 2008). The most significant parameters to predict live birth following CC treatment are age, BMI, free androgen index (FAI: testosterone × 100/SHBG), and history of amenorrhea (Imani et al. 2002a).

Today, treatment with CC is limited to six ovulatory cycles, and CC resistance or failure requires a change of treatment with the second-line therapy by low-dose FSH ovulation induction or laparoscopic ovarian drilling (LOD) (Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2008). Approximately 75% of pregnancies occur within the first three cycles, and the cumulative live birth rate is 50–60% after six cycles (Kousta et al. 1997).

Some side effects of CC like hot flushes and blurred vision, although infrequent, may be burdensome.

Another SERM, chemically similar to CC, is tamoxifen, with primary indication for the adjuvant treatment of breast cancer. It has also been used for ovulation induction, administered in the same way to CC but much less frequently than CC. It has the same ovulation, clinical pregnancy, miscarriage, and live birth rates with CC (Brown and Farquhar 2016). It has been suggested that tamoxifen does not have the negative adverse effect of CC on the endometrium and substitution of CC with tamoxifen (20 mg for every 50 mg of CC) may avoid this problem (Homburg 2008).

Metformin, an oral biguanide, is an insulin sensitizer that may reduce hyperinsulinemia in PCOS patients. Although metformin is inferior compared to CC as an ovulation induction agent (Legro et al. 2007), it may have a role in ovulation induction, since when metformin is added to CC in CC-resistant patients, it may increase ovulation and pregnancy rates (George et al. 2003). A network meta-analysis comparing the combination of CC and metformin versus gonadotropins

showed that they are equal in terms of pregnancy achievement (Wang et al. 2017). Hence, it has been suggested that before turning to the costlier FSH therapy, this combination may be tried (Homburg 2008).

Letrozole

Aromatase inhibitors were introduced for ovulation induction in 2001. The third-generation aromatase inhibitor, letrozole, is a nonsteroidal agent that blocks the action of the enzyme aromatase and inhibits estrogen synthesis from the ovarian follicles, the peripheral conversion of androgens, also locally in the brain. Indeed, the blood estrogen levels are suppressed by at least 97–99% (Pavone and Bulun 2013). As a result, the hypothalamic-pituitary axis is released from the estrogenic negative feedback effect, and gonadotropin secretion increases from the pituitary with subsequent stimulation of ovarian follicles (Fig. 2). Therefore, letrozole has the same end result as CC, which is the increase of endogenous FSH, and is also administered in a similar way, at the dose of 2.5–7.5 mg/day for 5 days starting on day 2–5 of spontaneous or progestin-induced menses.

Theoretically, since letrozole has a different mode of action than CC, it may have some advantages, such as no effect on estrogen receptors, the cervical mucus, or the endometrium. The estrogen negative feedback effect is intact, and, during the rise of blood estrogen levels from the leading follicle, FSH is expected to be suppressed, similarly to the normal menstrual cycle, reducing therefore the selection of multiple follicles and multiple pregnancies. Letrozole has a shorter half-life (45 h) than CC, and the side effects are minor, such as mild headache and muscle or joint pain.

The superiority of letrozole over CC, as a first-line ovulation induction treatment, has been shown in many RCTs. A Cochrane meta-analysis (Franik et al. 2018) of 42 RCTs (7935 women) compared letrozole to CC followed by timed intercourse. Live birth rates were higher with letrozole (31.7% vs. 21.4%, OR 1.68, 95%

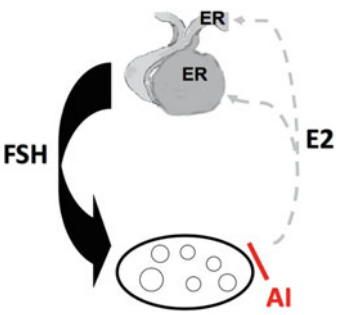


Fig. 2 With the administration of an aromatase inhibitor (AI) for 5 days, the ovarian estradiol secretion is suppressed. Therefore, the estrogen negative feedback at the pituitary and hypothalamus is attenuated, and FSH secretion from the anterior pituitary increases, resulting in stimulation of multiple ovarian follicle growth

CI 1.42–1.99). There was a higher clinical pregnancy rate with letrozole (35.9% vs. 26.4%, OR 1.56, 95% CI 1.37–1.78). There were no differences between letrozole and CC in the miscarriage rate (21.3% vs. 20%, OR 0.94, 95% CI 0.70–1.26), multiple pregnancy rate (1.2% vs. 1.8%, OR 0.69, 95% CI 0.41–1.16), and OHSS rate (0.4% vs. 0.4%, risk difference −0.00, 95% CI −0.01–0.00).

Recently, an international evidence-based guideline recommended that letrozole and not CC should be the first-line ovulation induction treatment (Teede et al. 2018).

In an earlier unpublished report (Biljan et al. 2005), letrozole was suggested as a potential teratogenic agent with subsequent concerns for utilization as an ovulation induction drug. Although numerous other studies (Wang et al. 2017) showed that letrozole is safe, its usage for ovulation induction is still off label. However, in many countries, letrozole use for ovulation induction is allowed, and women should be informed about the reassuring evidence, possible concerns, and side effects.

Gonadotropins

Gonadotropins are the second-line pharmacological treatment for ovulation induction in PCOS women, in whom the first-line oral agents have failed. Classically, with CC for first-line treatment, the gonadotropins are the next step in cases of clomiphene resistance or failure. Treatment with exogenous gonadotropins aims to increase the FSH levels in circulation above a threshold that will select, ideally, one follicle and maintain its growth to preovulatory size.

Initially, the "conventional protocol" with high starting dose of 150 IU daily was used, but it was associated with multiple follicular development. An early study showed that with this high HMG dose protocol, the pregnancy, abortion, multiple pregnancy, mild OHSS, and severe OHSS rates were 65.9%, 24.1%, 36.3%, 7.8%, and 3.9%, respectively (Wang and Gemzell 1980). Therefore, protocols with lower starting doses, i.e., the low-dose "step-up" and "step-down," were developed and replaced the original conventional protocol, aiming to reduce the risk of excessive ovarian stimulation.

Low-dose step-up protocol: This protocol is based on the concept that the gradual increase of exogenous FSH will identify the FSH threshold for follicular selection and development. Therefore, the aim of the low-dose step-up protocol is monoovulation, which means ovulation of a single follicle, with subsequent elimination of OHSS and multiple pregnancies.

With this protocol, a starting dose of 50–75 IU of gonadotropins (HMG or recombinant FSH) is given for 7 days, and an ultrasound is performed to find whether there is follicle selection, which is defined as a follicle of 10 mm size. If there is no follicle selection on ultrasound after 1 week, the gonadotropin dose is increased by 50% of the previous dose, and after 1 week the follicle selection is investigated ultrasonographically, etc. Once a follicle above 10 mm is found, the same FSH dose is maintained until the leading follicle reaches preovulatory size and then HCG is given for follicle rupture.

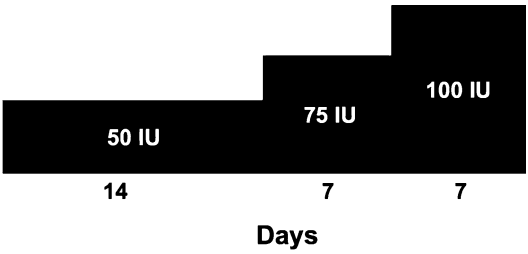


Fig. 3 The chronic low-dose protocol. In this example, a starting dose of 50 IU/day of FSH was administered for 14 days, and an ultrasound was performed to detect a follicle ≥10 mm. Since there was no follicle selection on ultrasound, the FSH dose was increased to 75 IU/day, and after 7 days the ultrasound was repeated showing no follicle above 10 mm; therefore the dose of daily FSH increased to 100 IU. After 1 week, a follicle above 10 mm was found, and the same dose of 100 IU daily was continued until the criteria of HCG administration were met

A modification of this regimen is the chronic low-dose protocol, which may further reduce the risk of multiple follicular development (Fig. 3). With this approach, a small starting dose of 37.5–75 IU of gonadotropin is given for 14 days. If after 2 weeks there is no follicle selection on ultrasound, the gonadotropin dose is increased by 50% of the previous dose, and after 1 week the ultrasound is repeated to investigate whether a follicle is selected, etc. During the interval of 14 days, most women (>90%) will respond to a dose of 75 IU FSH. It has been suggested to withhold HCG administration and cancel the cycle, when more than two follicles 16 mm or more than one follicle 16 mm and two additional follicles 14 mm are found, in women with PCOS younger than 38 years without any other infertility factors (Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2008). Generally, with the step-up approach, it is prudent to use small starting and incremental dose rises in the first treatment cycle, which may be adjusted for further cycles. This means that, since the threshold dose has been identified in the first cycle, the same dose or a dose slightly less may be used as the starting dose in subsequent cycles.

Step-down protocol: This protocol has been developed, based on the physiology of the normal menstrual cycle taking into account the crucial role of the FSH window for follicle selection. A high dose of FSH is given initially to overcome the FSH threshold, and, when a follicle is selected, the FSH dose is gradually reduced. The starting dose of FSH is 150 IU for 5 days and, if no follicle ≥10 mm is found ultrasonographically, the dose is increased by 37.5 IU every 3 days. Once a selected follicle is detected, the FSH daily dose is reduced by 37.5 IU every 3 days, until the criteria for HCG administration are reached (van Santbrink and Fauser 1997). The largest randomized trial comparing the step-down protocol with the low-dose step-up protocol showed similar pregnancy rates and shorter duration of FSH administration, but the low- dose step-up protocol had a lower rate of overstimulation, higher rate of monofollicular cycles, and a higher ovulation rate (Christin-Maitre et al. 2003). Therefore, the low-dose step-up protocol is considered to be safer and more efficient than the step-down protocol and is preferred by the majority of clinicians.

Sequential step-up and step-down protocol: With this regimen, the classic step-up protocol is applied, and, when the leading follicle reaches 14 mm in diameter, the FSH threshold dose is reduced by half. The results have been found to be similar to the low-dose step-up protocol (Hugues et al. 1996).

It has been suggested that the duration of gonadotropin therapy should not exceed six ovulatory cycles (Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2008).

Gonadotropins show great heterogeneity, which is physiologically relevant, and the commercial products used in ovulation induction and ART comprise a mixture of isohormones. A recent Cochrane review compared the effectiveness and safety of gonadotropins as a second-line treatment for ovulation induction in women with PCOS; the meta-analysis included 15 trials (2387 women). Recombinant FSH (recFSH) was compared with urinary gonadotropins, HMG, and FSH-HP, and it was found that there were no differences in clinical pregnancy, live birth, multiple pregnancy, miscarriage, and OHSS rates (Weiss et al. 2019).

The results with the low-dose step-up protocol and a starting dose of 75 IU are good, and a review of 1391 cycles in 717 patients showed mono-ovulation rate 69%, clinical pregnancy rate 40%, fecundity rate 20%, OHSS rate 0.14%, and multiple pregnancies 5.7% (Homburg and Howles 1999). Also, with a lower starting FSH dose of 52.5 IU daily, the results are similar to the starting dose of 75 IU (White et al. 1996).

A more recent study in 364 PCOS patients, with a starting FSH dose of 52.5 IU daily, showed a high proportion of ovulatory cycles (83%) with a percentage of unifollicular ovulatory cycles of 77%. Forty-nine percent of patients had at least one pregnancy, while the rate of multiple pregnancies (all twins) was small (0.01% of cycles and 4% of all pregnancies) (White et al. 2018).

A prospective study of 240 normogonadotropic anovulatory infertile women that were treated with CC as the first-line and gonadotropins as the second-line protocol showed a high cumulative singleton live birth rate of 71% after 24 months (Eijkemans et al. 2003).

There are characteristics of PCOS women that are associated with the outcome of ovulation induction with gonadotropins and may be used as clinical predictors. A meta-analysis showed that obesity correlated positively with the total amount of FSH consumption, cycle cancellation, and miscarriage rate and negatively with ovulation rate. Also, insulin resistance was positively associated with the total amount of FSH administered and negatively with pregnancy rate (Mulders et al. 2003). Hence, a model has been developed to predict the individual FSH response dose for ovulation induction, including body mass index, response to CC, baseline FSH level, and free insulin growth factor I (IGF-I) or insulin-to-glucose ratio (Imani et al. 2002b). Nevertheless, these prediction models need to be validated in large RCTs.

Although gonadotropins are generally considered as the second-line pharmacological treatment for ovulation induction in PCOS women, it has been suggested that they may be considered as first-line therapy. A prospective randomized, multicenter study (Homburg et al. 2012) showed that after three cycles with either CC (starting

doses 50–150 mg) or recombinant FSH (low-dose step-up protocol starting with 50 IU and weekly increments of 25 IU), ovulation induction with this low-dose FSH protocol was superior to CC, since the pregnancies and live births were achieved more effectively and faster with FSH than with CC. Indeed, in the first cycle, the pregnancy rate with FSH was twice that with CC (30% vs. 14.6%). Furthermore, the pregnancy and live birth rates per woman were significantly higher with FSH (58% and 52%) than with CC (44% and 39%, respectively). Similarly, within three cycles of ovulation induction, the cumulative pregnancy and live birth rates were significantly higher with FSH than CC (52.1% vs. 41.2% and 47.4% vs. 36.9%, respectively). However, there are important differences in cost and convenience in favor of CC that may be taken into account if FSH is chosen as the first-line treatment.

After six ovulatory cycles with exogenous gonadotropins that have failed to result in pregnancy, the third-line treatment is ovarian stimulation and IVF (Franik et al. 2018). It is obvious that in PCOS women who have associated pathologies, such as tubal damage, severe endometriosis, severe male factor infertility, etc., IVF becomes the first-line treatment (Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2008). In those cases, it is recommended to use a GnRH antagonist protocol, since it is associated with a significantly lower risk for OHSS. In addition, it allows to use a GnRH agonist to trigger final oocyte maturation, which, combined with a freeze-all strategy, can practically eliminate the occurrence of severe OHSS (Tarlatzis et al. 2017).

Conclusion

Ovulation induction is the treatment of anovulatory infertility and aims at the selection of a single follicle, reducing the risks of multiple pregnancies and ovarian hyperstimulation syndrome. Ovulation induction is feasible in cases with hypogonadotropic hypogonadism and PCOS. In hypogonadotropic hypogonadism, the exogenous administration of both FSH and LH, or in cases with intact pituitary, the exogenous GnRH administration in a pulsatile way, may be used. In women with PCOS, it seems that letrozole is superior to clomiphene citrate as the first-line ovulation induction treatment. The second-line treatment includes the low-dose gonadotropins protocols and laparoscopic ovarian drilling in selected cases, while the third-line treatment is IVF.

Cross-References

► The Hypothalamus-Pituitary-Ovary Axis
► The Menstrual Cycle and Related Disorders
► The Polycystic Ovary Syndrome (PCOS)

References

Bergquist C, Nillius SJ, Wide L. Human gonadotropin therapy. I. Serum estradiol and progesterone patterns during conceptual cycles. Fertil Steril. 1983;39:761–5.

Biljan M, Hemmings R, Brassard N. The outcome of 150 babies following the treatment with letrozole or letrozole and gonadotropins. Fertil Steril. 2005;84(Suppl 1):O-231. Abstract 1033.

Bozdag G, Mumusoglu S, Zengin D, Karabulut E, Yildiz BO. The prevalence and phenotypic features of polycystic ovary syndrome: a systematic review and meta-analysis. Hum Reprod. 2016;31:2841–55.

Braat DD, Ayalon D, Blunt SM, Bogchelman D, Coelingh Bennink HJ, Handelsman DJ, Heineman MJ, Lappohn RE, Lorijn RH, Rolland R, et al. Pregnancy outcome in luteinizing hormone-releasing hormone induced cycles: a multicentre study. Gynecol Endocrinol. 1989; 3:35–44.

Broekmans FJ, Knauff EA, Valkenburg O, Laven JS, Eijkemans MJ, Fauser BC. PCOS according to the Rotterdam consensus criteria: change in prevalence among WHO-II anovulation and association with metabolic factors. BJOG. 2006;113:1210–7.

Brown J, Farquhar C. Clomiphene and other antioestrogens for ovulation induction in polycystic ovarian syndrome. Cochrane Database Syst Rev. 2016;12:CD002249.

Brown JB, Evans JH, Adey FD, Taft HP, Townsend L. Factors involved in the induction of fertile ovulation with human gonadotrophins. J Obstet Gynaecol Br Commonw. 1969;76:289–307.

Burgues S, The Spanish Collaborative Group on Female Hypogonadotrophic Hypogonadism. The effectiveness and safety of recombinant human LH to support follicular development induced by recombinant human FSH in WHO group I anovulation: evidence from a multicentre study in Spain. Hum Reprod. 2001;16:2525–32.

Christin-Maitre S, Hugues JN, Recombinant FSH Study Group. A comparative randomized multicentric study comparing the step-up versus step-down protocol in polycystic ovary syndrome. Hum Reprod. 2003;18:1626–31.

Christin-Maitre S, de Crécy M, Groupe Français des pompes à GnRH. Pregnancy outcomes following pulsatile GnRH treatment: results of a large multicenter retrospective study. J Gynecol Obstet Biol Reprod (Paris). 2007;36:8–12.

Christou F, Pitteloud N, Gomez F. The induction of ovulation by pulsatile administration of GnRH: an appropriate method in hypothalamic amenorrhea. Gynecol Endocrinol. 2017;33:598–601.

Dale PO, Tanbo T, Henriksen T, Magnus O, Abyholm T. Gonadotropin therapy of female infertility. Acta Endocrinol (Copenh). 1989;120:395–9.

Dickey RP, Taylor SN, Curole DN, Rye PH, Lu PY, Pyrzak R. Relationship of clomiphene dose and patient weight to successful treatment. Hum Reprod. 1997;12:449–53.

Dumont A, Dewailly D, Plouvier P, Catteau-Jonard S, Robin G. Comparison between pulsatile GnRH therapy and gonadotropins for ovulation induction in women with both functional hypothalamic amenorrhea and polycystic ovarian morphology. Gynecol Endocrinol. 2016;32: 999–1004.

Eijkemans MJ, Imani B, Mulders AG, Habbema JD, Fauser BC. High singleton live birth rate following classical ovulation induction in normogonadotrophic anovulatory infertility (WHO 2). Hum Reprod. 2003;18:2357–62.

ESHRE Capri Workshop Group. Health and fertility in World Health Organization group 2 anovulatory women. Hum Reprod Update. 2012;18:586–99.

Filicori M, Flamigni C, Dellai P, Cognigni G, Michelacci L, Arnone R, Sambataro M, Falbo A. Treatment of anovulation with pulsatile gonadotropin-releasing hormone: prognostic factors and clinical results in 600 cycles. J Clin Endocrinol Metab. 1994;79:1215–20.

Franik S, Eltrop SM, Kremer JA, Kiesel L, Farquhar C. Aromatase inhibitors (letrozole) for subfertile women with polycystic ovary syndrome. Cochrane Database Syst Rev. 2018;5: CD010287.

George SS, George K, Irwin C, Job V, Selvakumar R, Jeyaseelan V, Seshadri MS. Sequential treatment of metformin and clomiphene citrate in clomiphene-resistant women with polycystic ovary syndrome: a randomized, controlled trial. Hum Reprod. 2003;18:299–304.

Homburg R. Polycystic ovary syndrome. Best Pract Res Clin Obstet Gynaecol. 2008;22:261–74.
Homburg R, Howles CM. Low-dose FSH therapy for anovulatory infertility associated with polycystic ovary syndrome: rationale, results, reflections and refinements. Hum Reprod Update. 1999;5:493–9.
Homburg R, Eshel A, Armar NA, Tucker M, Mason PW, Adams J, Kilborn J, Sutherland IA, Jacobs HS. One hundred pregnancies after treatment with pulsatile luteinising hormone releasing hormone to induce ovulation. Br Med J. 1989;298:809–12.
Homburg R, Hendriks ML, König TE, Anderson RA, Balen AH, Brincat M, Child T, Davies M, D'Hooghe T, Martinez A, Rajkhowa M, Rueda-Saenz R, Hompes P, Lambalk CB. Clomifene citrate or low-dose FSH for the first-line treatment of infertile women with anovulation associated with polycystic ovary syndrome: a prospective randomized multinational study. Hum Reprod. 2012;27:468–73.
Hugues JN, Cédrin-Durnerin I, Avril C, Bulwa S, Hervé F, Uzan M. Sequential step-up and step-down dose regimen: an alternative method for ovulation induction with follicle-stimulating hormone in polycystic ovarian syndrome. Hum Reprod. 1996;11:2581–4.
Imani B, Eijkemans MJ, te Velde ER, Habbema JD, Fauser BC. A nomogram to predict the probability of live birth after clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility. Fertil Steril. 2002a;77:91–7.
Imani B, Eijkemans MJ, Faessen GH, Bouchard P, Giudice LC, Fauser BC. Prediction of the individual follicle-stimulating hormone threshold for gonadotropin induction of ovulation in normogonadotropic anovulatory infertility: an approach to increase safety and efficiency. Fertil Steril. 2002b;77:83–90.
Kousta E, White DM, Franks S. Modern use of clomiphene citrate in induction of ovulation. Hum Reprod Update. 1997;3(4):359–65.
Legro RS, Barnhart HX, Schlaff WD, Carr BR, Diamond MP, Carson SA, Steinkampf MP, Coutifaris C, McGovern PG, Cataldo NA, Gosman GG, Nestler JE, Giudice LC, Leppert PC, Myers ER, Cooperative Multicenter Reproductive Medicine Network. Clomiphene, metformin, or both for infertility in the polycystic ovary syndrome. N Engl J Med. 2007;356:551–66.
Legro RS, Dodson WC, Kunselman AR, Stetter CM, Kris-Etherton PM, Williams NI, Gnatuk CL, Estes SJ, Allison KC, Sarwer DB, Diamond MP, Schlaff WD, Casson PR, Christman GM, Barnhart KT, Bates GW, Usadi R, Lucidi S, Baker V, Zhang H, Eisenberg E, Coutifaris C, Dokras A. Benefit of delayed fertility therapy with preconception weight loss over immediate therapy in obese women with PCOS. J Clin Endocrinol Metab. 2016;101:2658–66.
Leyendecker G, Wildt L. Induction of ovulation with chronic intermittent (pulsatile) administration of Gn-RH in women with hypothalamic amenorrhoea. J Reprod Fertil. 1983;69:397–409.
Martin KA, Hall JE, Adams JM, Crowley WF Jr. Comparison of exogenous gonadotropins and pulsatile gonadotropin-releasing hormone for induction of ovulation in hypogonadotropic amenorrhea. J Clin Endocrinol Metab. 1993;77:125–9.
Messinis IE, Messini CI, Anifandis G, Dafopoulos K. Polycystic ovaries and obesity. Best Pract Res Clin Obstet Gynaecol. 2015;29:479–88.
Mulders AG, Laven JS, Eijkemans MJ, Hughes EG, Fauser BC. Patient predictors for outcome of gonadotrophin ovulation induction in women with normogonadotrophic anovulatory infertility: a meta-analysis. Hum Reprod Update. 2003;9:429–49.
Mutsaerts MA, van Oers AM, Groen H, Burggraaff JM, Kuchenbecker WK, Perquin DA, Koks CA, van Golde R, Kaaijk EM, Schierbeek JM, Oosterhuis GJ, Broekmans FJ, Bemelmans WJ, Lambalk CB, Verberg MF, van der Veen F, Klijn NF, Mercelina PE, van Kasteren YM, Nap AW, Brinkhuis EA, Vogel NE, Mulder RJ, Gondrie ET, de Bruin JP, Sikkema JM, de Greef MH, ter Bogt NC, Land JA, Mol BW, Hoek A. Randomized trial of a lifestyle program in obese infertile women. N Engl J Med. 2016;374:1942–53.
Pavone ME, Bulun SE. Clinical review: the use of aromatase inhibitors for ovulation induction and superovulation. J Clin Endocrinol Metab. 2013;98:1838–44.
Practice Committee of the American Society for Reproductive Medicine. Diagnostic evaluation of the infertile female: a committee opinion. Fertil Steril. 2015;103:44–50.

Rabau E, David A, Serr DM, Mashiach S, Lunenfeld B. Human menopausal gonadotropins for anovulation and sterility. Results of 7 years of treatment. Am J Obstet Gynecol. 1967;98:92–8.

Rostami-Hodjegan A, Lennard MS, Tucker GT, Ledger WL. Monitoring plasma concentrations to individualize treatment with clomiphene citrate. Fertil Steril. 2004;81:1187–93.

Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod. 2004;19(1):41–7.

Schoot DC, Coelingh Bennink HJ, Mannaerts BM, Lamberts SW, Bouchard P, Fauser BC. Human recombinant follicle-stimulating hormone induces growth of preovulatory follicles without concomitant increase in androgen and estrogen biosynthesis in a woman with isolated gonadotropin deficiency. J Clin Endocrinol Metab. 1992;74:1471–3.

Tadokoro N, Vollenhoven B, Clark S, Baker G, Kovacs G, Burger H, Healy D. Cumulative pregnancy rates in couples with anovulatory infertility compared with unexplained infertility in an ovulation induction programme. Hum Reprod. 1997;12:1939–44.

Tarlatzis BC, Bosdou J, Kolibianakis EM. Elimination of OHSS by GnRH agonist and freezing embryos. In: Rizk B, Gerris J, editors. Complications and outcomes of assisted reproduction. Cambridge: Cambridge University Press; 2017. p. 149–63.

Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, Piltonen T, Norman RJ, International PCOS Network. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Fertil Steril. 2018; 110:364–79.

The European Recombinant Human LH Study Group. Recombinant human luteinizing hormone (LH) to support recombinant human follicle-stimulating hormone (FSH)-induced follicular development in LH- and FSH-deficient anovulatory women: a dose-finding study. J Clin Endocrinol Metab. 1998;83:1507–14.

Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Consensus on infertility treatment related to polycystic ovary syndrome. Hum Reprod. 2008;23:462–77.

Thompson CR, Hansen LM. Pergonal (menotropins): a summary of clinical experience in the induction of ovulation and pregnancy. Fertil Steril. 1970;21:844–53.

van Santbrink EJ, Fauser BC. Urinary follicle-stimulating hormone for normogonadotropic clomiphene-resistant anovulatory infertility: prospective, randomized comparison between low dose step-up and step-down dose regimens. J Clin Endocrinol Metab. 1997;82:597–602.

van Santbrink EJ, Eijkemans MJ, Laven JS, Fauser BC. Patient-tailored conventional ovulation induction algorithms in anovulatory infertility. Trends Endocrinol Metab. 2005;16:381–9.

Wang CF, Gemzell C. The use of human gonadotropins for the induction of ovulation in women with polycystic ovarian disease. Fertil Steril. 1980;33:479–86.

Wang R, Kim BV, van Wely M, Johnson NP, Costello MF, Zhang H, Ng EH, Legro RS, Bhattacharya S, Norman RJ, Mol BW. Treatment strategies for women with WHO group II anovulation: systematic review and network meta-analysis. BMJ. 2017;356:j138.

Weiss NS, Kostova E, Nahuis M, Mol BWJ, van der Veen F, van Wely M. Gonadotrophins for ovulation induction in women with polycystic ovary syndrome. Cochrane Database Syst Rev. 2019;1:CD010290.

White DM, Polson DW, Kiddy D, Sagle P, Watson H, Gilling-Smith C, Hamilton-Fairley D, Franks S. Induction of ovulation with low-dose gonadotropins in polycystic ovary syndrome: an analysis of 109 pregnancies in 225 women. J Clin Endocrinol Metab. 1996;81:3821–4.

White DM, Hardy K, Lovelock S, Franks S. Low-dose gonadotropin induction of ovulation in anovulatory women: still needed in the age of IVF. Reproduction. 2018;156:1–10.

Yildirim M, Noyan V, Tiras MB. Estrogen-progestagen pre-treatment before HMG induction in hypogonadotropic patients. Int J Gynaecol Obstet. 2000;71(3):249–50.

Assisted Reproductive Technologies

14

Maria Elisabetta Coccia, Francesca Rizzello, and Giulia Orlandi

Contents

M. E. Coccia (✉) · G. Orlandi
Azienda Ospedaliero-Universitaria Careggi Ostetricia e ginecologia, Florence, Italy

Department of Clinical and Experimental Biomedical Sciences, University of Florence, Florence, Italy
e-mail: mariaelisabetta.coccia@unifi.it; giulietta.orlandi@gmail.com

F. Rizzello
Azienda Ospedaliero-Universitaria Careggi Ostetricia e ginecologia, Florence, Italy

Assisted Reproduction Center, Careggi University Hospital, Florence, Italy
e-mail: francesca.rizzello@gmail.com

F. Petraglia, B. C. Fauser (eds.), *Female Reproductive Dysfunction*, Endocrinology,
https://doi.org/10.1007/978-3-030-14782-2_14

Abstract

Since the first successful in vitro fertilization pregnancy (IVF), the progress of assisted reproduction has been surprising. With developments such as intracytoplasmic sperm injection (ICSI), egg and sperm donation, preimplantation diagnosis, and aneuploidy screening, there are many more couples who can benefit from assisted reproductive technologies (ART).

Endocrine dysfunctions represent the indication for treatments in 5–19.8%, and probably these figures are underestimated. The evaluation and treatment of women with poor ovarian reserve and polycystic ovarian (PCO) syndrome in ART remain a challenge. Numerous protocols and procedures have been proposed, but none has yet reached sufficient evidence.

In this chapter, the most used techniques of assisted reproduction and newer developments are discussed, as well as the possible complications associated with ART. Furthermore, the strategies employed during the procedure in patients with reduced ovarian reserve and PCO have been deepened.

Keywords

Assisted reproductive technologies · IVF · ICSI · Poor responder · PCO

Introduction

In 1978 the collaboration between Patrick Steptoe and Robert Edwards led to the birth of Louise Brown, the first baby to be born as a result of in vitro fertilization (IVF). This meaningful event revolutionized treatment options for couples with infertility.

Afterward, the introduction of assisted reproductive technology (ART) with fertilization of human oocytes outside of the body, culturing of the embryo in a laboratory, and its subsequent transfer to the uterus has led to the birth of tens of thousands of children worldwide. It has been estimated that more than seven million babies have been born worldwide since the first IVF baby, and around 1.5 million ART cycles are now reported each year worldwide. Data are probably underestimated, as registry figures are thought to represent around 70% of all ART treatments (ESHRE 2018).

ARTs comprise all treatments which include the handling of eggs and sperm and/or embryos. Some examples of ART are IVF, intracytoplasmic sperm injection (ICSI), gamete intrafallopian transfer, pronuclear stage tubal transfer, tubal embryo transfer, and zygote intrafallopian transfer (ASRM n.d.). Intrauterine insemination (IUI), IVF, and ICSI are the most common techniques.

The intent of this chapter is to provide an overview of the most used techniques of assisted reproduction and the corresponding indications. Subsequently, the strategies employed during the procedure in patients with endocrinological disorders (mainly reduced ovarian reserve and polycystic ovaries) have been deepened.

Intrauterine Insemination (IUI)

Although IUI has not been classified as an ART, it is widely used an empirical treatment, for a broad range of subfertility indications. According to the European Society of Human Reproduction and Embryology (ESHRE), it represents a "mild" ART procedure (ESHRE 2018).

IUI has evolved through innovations such as sperm preparation, monitoring for preovulatory timing, and induction of ovulation with human chorionic gonadotropin (hCG). Furthermore, IUI has been combined with ovarian stimulation using clomiphene citrate (CC) or gonadotropins.

This simple and noninvasive technique has several advantages: it can be performed without expensive infrastructure, has a good couple compliance (low dropout rate) and a very low risk for complications such as ovarian hyperstimulation syndrome (OHSS).

After the ESHRE workshop in 2009, IUI was considered a poor substitute for IVF treatment as it was associated with modest pregnancy rate (12% per cycle in treatment with ovarian stimulation) and a significant rate of multiple pregnancies (13% multiple births). Cycles with mild stimulation (1–2 follicles) might reduce the costs and multiple birth rates but would involve more cycles of treatment (ESHRE Capri Workshop Group 2009).

IUI is indicated for a broad range of pathologic conditions. The most obvious condition is male infertility with donor sperm. Other indications are unexplained infertility, mild endometriosis, and mild male factor infertility (NICE 2013).

IUI Technique

The IUI treatment aims to increase the chance that the maximum number of healthy sperms reaches the oocyte. Thus, the rationale is to bypass a possible cervical factor. However, the postcoital test is not a recommended routine test anymore (The Practice Committee of the American Society for Reproductive Medicine 2015). Prior to perform IUI, tubal patency and semen quality evaluation are recommended (ESHRE 2018).

There is general consensus that the chances of pregnancy are higher if IUI is performed after mild ovarian stimulation and the maturation of a maximum of two or three follicles. In the majority of the published studies, the insemination is done 32–36 h following hCG administration.

Prior to IUI, the seminal fluid should be processed by centrifuging spermatozoa through a culture medium or density gradients followed by re-suspension in suitable culture media. Procedure of IUI by unprocessed semen was associated with increased risk of pelvic infection (Boomsma et al. 2007). Moreover, unprocessed seminal fluid might induce uterine contractions through prostaglandins contained in the seminal plasma.

For the insemination sample, the recommended lower limit ranges from 3 to 5–10 million motile sperm (ESHRE Capri Workshop Group 2009).

Artificial inseminations can be done intravaginally, intracervically, pericervically using a cap, intrauterine (IUI), transcervical intrafallopian, or directly intraperitoneal. IUI is by far the most common method.

The majority of pregnancies occur during the first three or four IUI cycles (Ombelet et al. 2008). A double insemination did not prove more advantageous than a single procedure (ESHRE Capri Workshop Group 2009). Obviously, evaluating the duration of an IUI program, the age of the woman and her comorbidities should be considered, to guarantee timely reassignment to second-level treatments if indicated.

In Vitro Fertilization (IVF)/Intracytoplasmic Sperm Injection (ICSI)

Both IVF and ICSI involve ovarian stimulation, oocyte retrieval, and fertilization outside of the body. IVF involves combining an egg with sperm in a laboratory dish; the resulting embryo is then transferred into the woman's uterus. ICSI is a micromanipulation procedure in which a single sperm is injected directly into an egg (Fig. 1). Controlled ovarian stimulation (COS) and ultrasound monitoring (with or without estradiol levels) constitute part of IVF treatment (NICE 2013).

Daily doses of gonadotropins (follicle-stimulating hormone (FSH), luteinizing hormone (LH), human menopausal gonadotropin (hMG), corifollitropin alfa, follitropin delta) are used to induce multifollicular response in the ovaries. Although the number of eggs retrieved seems to depend on the starting/total doses of gonadotropins, individual woman's response differs. An individualized starting dose of gonadotropins is recommended, based on factors that predict ovarian response such as age, BMI, presence of polycystic ovaries, and ovarian reserve (NICE 2013).

Monitoring follicular growth usually involves a combination of hormonal assays and ultrasonic measurements of follicle size. It plays an important role in the prediction of ovarian responsiveness to exogenous gonadotropins (predictive of

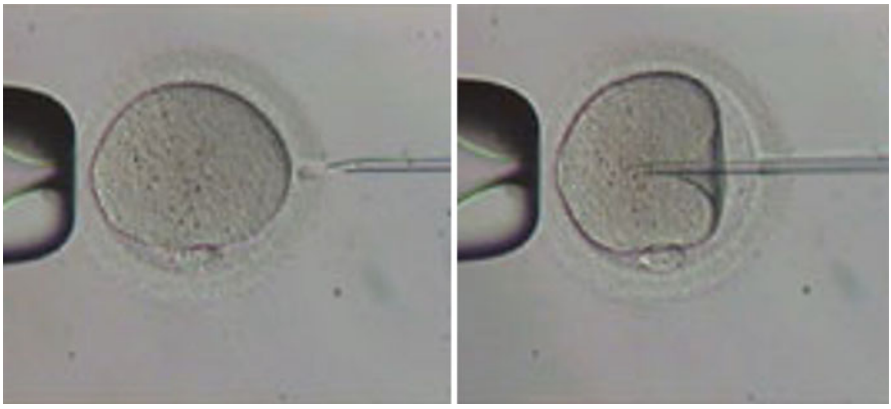


Fig. 1 Intracytoplasmic sperm injection (ICSI). A single spermatozoa is injected into each oocyte using fine micro-manipulation equipment

oocytes competence), estimation of the appropriate time to trigger the final oocyte maturation before ovum pickup, and assessment of OHSS risk.

IVF/ICSI Indications

IVF indications include doubtful tubal patency, advanced-stage endometriosis resulting in tubal disease or dysfunction, moderate alterations of semen characteristics, unexplained infertility, failure of several previous cycles of ovulation induction or IUI, and circumstances that require preimplantation screening/diagnosis to prevent genetically inherited diseases. Furthermore, IVF must be offered as a first-line treatment in women of advanced maternal age, irrespective of the cause of infertility (NICE 2013).

Originally developed for severe sperm abnormalities, ICSI was implemented in ART and applied in other conditions, such as after failed or low fertilization in previous attempts. According to *Good Clinical Treatment in Assisted Reproduction* of the ESHRE, ICSI should not represent the most suitable treatment for female conditions as poor ovarian response or previous implantation failures (Good Clinical Treatment in Assisted Reproduction 2008).

In regard to male factor, in patients with normal seminal parameters or mild male factor infertility, a number of studies demonstrated equal or higher fertilization rates with ICSI compared with IVF (Plachot et al. 2002), but once fertilization is achieved, the pregnancy rate is not enhanced if compared to IVF (NICE 2013). In patients with severe male factor infertility, the ICSI procedure results in a much higher fertilization rate and, therefore, a more beneficial outcome.

Total motile sperm concentration (TMC), calculated by multiplying the volume, the concentration (million sperm/ml), and the percentage of progressive motile spermatozoa, has been adopted as criterium to decide between IUI, conventional IVF, and ICSI. In cases of post-processing TMC $<1 \times 10^6$, IUI has no benefit, and IVF, ICSI, or a combination of both, i.e., a split setup, may be the initial suggestion to the couple (van Weert et al. 2004).

Certainly, when less than 0.5×10^6 progressively motile spermatozoa are obtained after seminal fluid processing or sperms are recovered surgically from the testis or epididymis, ICSI should be performed. In remaining cases, current strategies for choosing between IVF and ICSI are either formulated using preset cutoff values or based on the assumption that ICSI is the more robust insemination technique (Tournaye 2012).

Actually, the prevalence of complete fertilization failure after standard IVF is reported to be as high as 50%, while ICSI cycles with complete fertilization failure are less than 3% of started cycles (Tournaye 2012). Total fertilization failure during conventional IVF might be related to either oocyte, sperm, or laboratory factors. Although these events might have negative consequences, with a severe distressing impact on the couple, the aim of reproductive medicine should always be the adoption of the simplest, least expensive, and invasive procedure with the greatest chance of pregnancies and long-term prognosis of healthy children.

An alternative strategy for choosing between ICSI and IVF, in cases of moderate male factor infertility, is constituted by a combination of both, i.e., a split IVF-ICSI approach, in which sibling oocytes are either inseminated conventionally or micro-injected. Although there is still poor evidence, this method may prevent complete fertilization failure in one out of four cycles (Tournaye 2012).

Nevertheless, the crucial difference between IVF and ICSI is that, during ICSI, only mature oocytes are injected, while immature oocytes are set apart in order to complete maturation. As oocyte maturation is unpredictable, most oocytes are injected irrespective of the time when they mature. Thus, the timing of ICSI may not always be optimal, especially since research indicates that expulsion of the polar body alone is not enough to determine maturity and that the amount of time between polar body extrusion and time of insemination influences fertilization rates (Balakier et al. 2004).

Egg and Sperm Donation

Egg donation (ED) is a fertility treatment for women unable to produce their own eggs, at high risk of transmitting a genetic disease or with previous several unsuccessful IVF cycles.

Egg donation, like sperm donation, has considerably increased in recent years. From the 18th ESHRE report on ART, publishing data for 2014, a total of 56,516 cycles with ED were performed in a population of ~208 million inhabitants. These data showed a definite growing trend, 22,911 cycles more than 2012 and 15,272 more than 2013 (De Geyter et al. 2018).

Oocyte donation is associated with the highest pregnancy rate among ARTs (almost 50%) with lower delivery rates (33% with fresh eggs, 25% after frozen embryo transfers, and 21% after embryo transfers from frozen eggs) (ESHRE 2017).

Donated eggs are fertilized with partner's sperm as in a conventional IVF/ICSI treatment cycle, and one (or two) embryo(s) are transferred. Women undergoing egg donor replacement cycles need an adequate hormonal preparation of the endometrium to optimize chances of pregnancy. Many drugs and various modes of administration have been tried in order to optimize implantation rates and consequently improve the success rates of the embryo transfer procedures. Obviously, success of oocyte donation is influenced by further factors, including donor's age, quality and number of transferred embryos, and recipient's age. Donor age is clearly the most important prognostic factor (Faddy et al. 2011).

Medical and obstetrical complications are significantly increased in pregnancies obtained with oocyte donation, especially in women older than 45 years. Hypertensive disorders of pregnancy and gestational diabetes are the most frequent obstetric complications. Previous studies observed that out of 45 heterologous pregnancies of healthy women aged 50–63 years, 35% experienced pregnancy-induced hypertension, 20% developed gestational diabetes, and 78% underwent a cesarean section (Paulson et al. 2002).

Sperm donation, or therapeutic donor insemination (TDI), is an option when the male partner has severe abnormalities such as azoospermia, severe oligospermia, or other significant sperm or seminal fluid abnormalities, genetic defects, or ineradicable sexually transmissible infections. TDI is associated with a series of pregnancy complications such as preterm birth, low birth weight, preeclampsia, and increased cesarean delivery rate, but these correlations were not found in all studies that correlated sperm donation versus non-donor sperm cycles (Adams et al. 2017; Bartal et al. 2018).

Preimplantation Genetic Screening/Diagnosis (PGS/D)

Previous studies have shown that approximately 25% of oocytes in women in their early 30s and more than 75% among women older than 40 years are chromosomally abnormal (Fragouli et al. 2011). Embryonic chromosomal abnormalities could significantly decrease the proportion of successful pregnancies in women or result in early miscarriage, late abortion, or the delivery of children with chromosomal abnormalities.

Preimplantation genetic screening/diagnosis (PGS/D) is used for early identification of genetic defects of embryo. PGD aims to test the embryo for known conditions before implantation. On the other hand, PGS refers to screening embryo for aneuploidy. PGD is indicated in couples at risk of transmitting genetic abnormalities to their offspring while PGS in couples with advanced maternal age, a medical history of recurrent first-trimester pregnancy losses, or recurrent implantation failure in prior IVF/ICSI cycles.

The timing of biopsy includes polar body (PB), cleavage-stage embryos (blastomere), morula-stage embryos, and blastocyst-stage embryos (trophectoderm biopsy). PB biopsy involves the removal and subsequent analysis of the first and second PB before embryo cleavage. It avoids the removal of cells from the embryo but allows the examination only for maternal chromosomes or genes. Moreover, the small amount of genetic material might lead to loss of one allele during polymerase chain reaction (PCR) amplification of DNA (allelic dropout).

A blastomere biopsy is based on the removal of one or two blastomeres from a six- to eight-cell embryo. In this case both maternal and paternal genetic contributions to the embryo can be analyzed, but the small quantity of the genetic material represents a potential cause for misdiagnoses. Unfortunately, the removal of cells at the cleavage stage seems to slow the development of the embryo and decrease implantation and pregnancy rates. Moreover, mosaic rates have been estimated 60% at the cleavage stage.

Development of sequential culture media allowed the culture of embryos to the blastocyst stage. Then, trophectoderm biopsy was introduced in clinical practice, allowing multiple cells to be biopsied. The biopsy of trophectoderm cells and blastocyst stage transfer showed an improvement in the accuracy of PGD/PGS. However, a rate approximately 20% of mosaicism has been described at the blastocyst stage (Sullivan-Pyke and Dokras 2018).

Techniques used for genetic and chromosomal analysis comprise polymerase chain reaction (PCR); fluorescence in situ hybridization (FISH); microarray technologies, including single-nucleotide polymorphism (SNP) microarrays and array comparative genomic hybridization (aCGH); and next-generation sequencing (NGS). Platforms based on NGS are being increasingly used in PGD/PGS because of high accuracy and high throughput (Sullivan-Pyke and Dokras 2018).

A number of studies demonstrated the safety and efficacy of PGD in IVF/ICSI cycles as well as increased implantation, pregnancy, and live birth rates. The transfer of euploid blastocysts confirmed by aCGH after day 3 biopsy has shown higher implantation (52.8% vs. 27.6%) and live birth rates per transfer (64.7% vs. 27.4%) when compared with untested blastocysts (Rubio et al. 2017). Blastocyst-stage embryo biopsy analyzed with rapid qPCR, and subsequent single-embryo transfer resulted in higher ongoing pregnancy rates (55.0% vs. 41.8%) and lower miscarriage rates (10.5% vs. 24.8%) versus single-embryo transfer without PGS (Forman et al. 2012). Furthermore, data on impact of blastomere biopsy on growth parameters, birth weight, hospitalizations, or congenital malformations in children followed up to 2 years of age are reassuring (Desmyttere et al. 2012). It is important to highlight that couples should be counselled that PGD/PGS do not replace routine prenatal screening methods as these procedures have an intrinsic error rate.

ART and Female Endocrine Dysfunction

An adequate endocrine environment is imperative to maintain the reproductive function in women. Female endocrine dysfunctions affecting fertility include a heterogeneous group of disorders. ART plays an important role when the first-line treatment does not achieve the correction of the disorder. According to the Italian National Registry of ART (2016), endocrine dysfunction represents the indication for treatments in 19.8% of IUI cycles and 5.5% of ICSI/IVF (Italian National Registry of ART 2016) (Figs. 2 and 3).

These figures probably underestimate the real impact of endocrine disorders in infertile couples. Indeed, other indications as "endometriosis" also include endocrine dysfunction (3.3% in I level ARTs and 4.5% in the II level ARTs). Moreover, patients with multiple factors might include endocrine dysfunctions.

The World Health Organization (WHO) defined three categories of anovulatory disorders (Table 1):

- WHO class 1 – hypogonadotropic hypogonadal (HA) anovulation is the least common, occurring in 5–10% of cases, for example, hypothalamic amenorrhea from functional aetiologies such as excessive exercise or low body weight.
- WHO class 2 – normogonadotropic normoestrogenic anovulation is the most common, accounting for 70–85% of cases, for example, polycystic ovary syndrome (PCOS).
- WHO class 3 – Hypergonadotropic hypoestrogenic anovulation occurs in 10–30%, for example, with primary gonadal failure (POF) or gonadal dysgenesis.

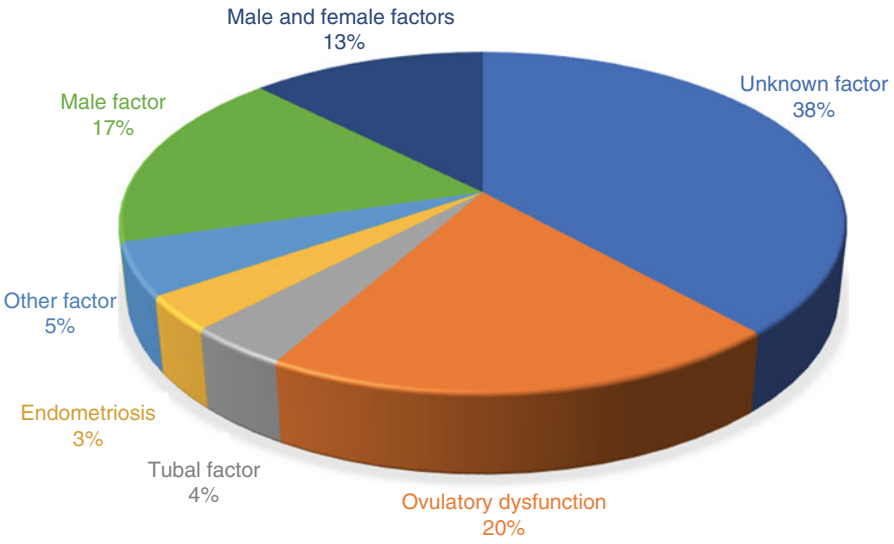


Fig. 2 Distribution of couples treated with IUI without gamete donation, according to the causes of infertility. 2016. 13,281 cycles

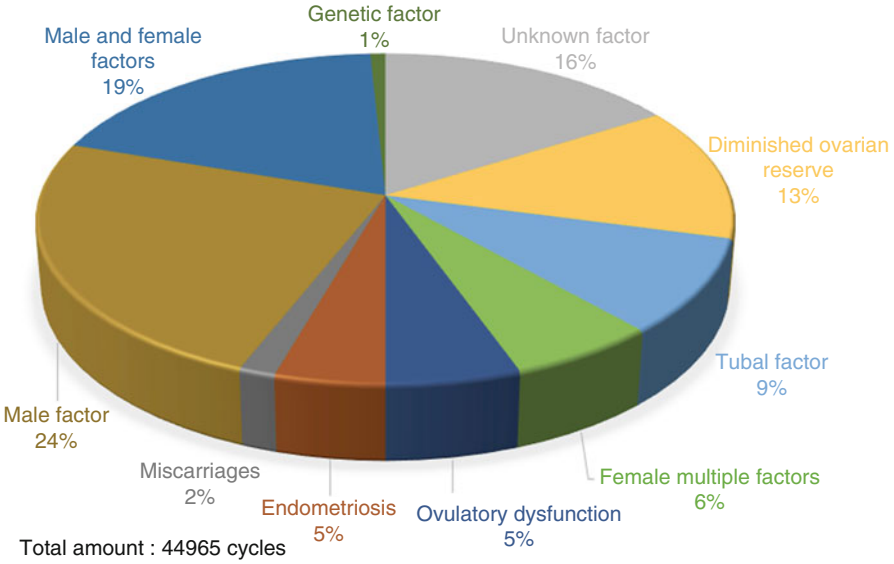


Fig. 3 Distribution of couples treated with IVF/ICSI, without gamete donation, according to the causes of infertility. 2016. 44,965 cycles

Table 1 Anovulatory disorders classified by the World Health Organization (WHO)

Main groups	Subdivision	Treatment
1. Hypothalamic dysfunction	1.a Clomiphene-resistant (hypogonadotropic, hypo-estrogenic)	Pulsatile GnRH
	1.b Clomiphene-responsive (normogonadotropicanovulation)	Gonadotropins
		Clomiphene
2. Ovarian dysfunction (PCOS)	2.a Lean patients	Clomiphene
	2.b Obese patients	Weight reduction Metformin Clomiphene
	2.c Clomiphene-resistant patients	Metformin-clomiphene Ovariandrilling HMG
3. Hyperprolactinemia		Cabergoline
4. Ovarian failure		Hormonal replacement therapy

The four most common ovulatory disorders include PCOS, poor ovarian reserve (POR), HA, and hyperprolactinemia.

POR and polycystic ovary (PCO) represent two indications to ART of particular interest, both for the frequency with which they are found and for the complexity of the management.

Poor Ovarian Reserve (POR)

Definition and Diagnosis

The standard definition of POR (or poor ovarian responder) remains uncertain, and consequently the proposed protocols adopted to manage poor responders are very difficult to compare. In a systematic review, Polyzos et al. found 41 different definitions among 47 randomized trials (Polyzos and Devroey 2011).

The Bologna criteria developed under the auspices of ESHRE in 2011 represent the first attempt to build an international consensus in the definition of POR (Ferraretti et al. 2011).

According to the Bologna criteria, at least two of the following features are required to define a POR:

1. Advanced maternal age ($\geq$40 years) or any other POR risk factor (genetic or acquired conditions, pelvic infections, ovarian endometriomas, and patients who have undergone ovarian surgery for ovarian cyst, chemotherapy, shortening of the menstrual cycle)
2. A previous cycle with POR ($\leq$3 oocytes with a conventional stimulation protocol)

3. A low ovarian reserve test in terms of anti-mullerian hormone (AMH) (<0.5–1.1 ng/ml) (<3.6–7.8 pmol/l) and antral follicle count (AFC) (<5–7 follicles)

However, two cycles with three oocytes or less after maximal stimulation are enough to classify a patient as a poor responder even in the absence of the other two criteria (Ferraretti et al. 2011).

Despite the efforts to optimize the definition of this subgroup of patients, the Bologna criteria were criticized for the heterogeneity of patients included by this definition.

Recently, the Patient-Oriented Strategies Encompassing IndividualizeD Oocyte Number (POSEIDON) group suggested a more detailed novel stratification of women with low ovarian response to stimulation. Four groups of "low prognosis patients" in ART were identified on the basis of age and the expected aneuploidy rate, biomarkers of ovarian reserve (AFC and AMH), and ovarian response in previous stimulation cycle (Table 2). The new classification introduces a new concept: the ability to achieve an adequate number of oocytes in order to give the patient the higher chance to obtain at least one euploid embryo (Humaidan et al. 2016).

The chance of pregnancy after IVF is highly dependent upon the number and quality of retrieved oocytes, as both factors determine the number of good-quality embryos.

Recently, the cumulative live birth rate (CLBR) has been suggested as indicator of quality and success in IVF. It incorporates the totality of live birth episodes following successive treatments of fresh as well as thawed frozen embryo transfer. CLBR per oocyte retrieval is a more meaningful indicator for both clinicians' and patients' perspective: the outcome of the whole IVF-ICSI cycle (including cryopreservation) allows better evaluations between different centers with different strategies for freezing and extended culture of embryos. Moreover, CLBR would be more appropriate for making economic and political decisions (Maheshwari et al. 2015).

In recent literature, several authors tried to define the ideal number of oocytes to optimize live birth rates in fresh embryo transfer cycles. Generally, a number of 10–15 oocytes has been considered adequate in order to give the patients the maximum chance of pregnancy after a fresh embryo transfer cycle: 13 according to Van der Gaast et al. (2006), 10 according to Verberg et al. (2007), 18 according to Fatemi et al. (2011), and 15 for Sunkara et al. (2011).

Table 2 Four groups of women with low prognosis in ART according to the POSEIDON's stratification. *AFC* antral follicle count, *AMH* anti-Müllerian hormone (Ferraretti et al. 2011)

Group 1: patients younger than 35 with adequate ovarian reserve (AFC ≥5, AMH ≥ 1.2 ng/ml) and with unexpected poor/suboptimal ovarian response Subgroup 1a:<4 oocytes[a] Subgroup 1b: 4–9 oocytes[a]	Group 2: patients older than 35 with adequate ovarian reserve (AFC ≥5, AMH ≥1.2 ng/ml) and with unexpected poor/suboptimal ovarian response Subgroup 2a:<4 oocytes[a] Subgroup 2b: 4–9 oocytes[a]
Group 3: patients younger than 35 with poor ovarian reserve (AFC <5, AMH <1.2 ng/ml)	Group 4: patients older than 35 with poor ovarian reserve (AFC <5, AMH <1.2 ng/ml)

[a]After standard ovarian stimulation

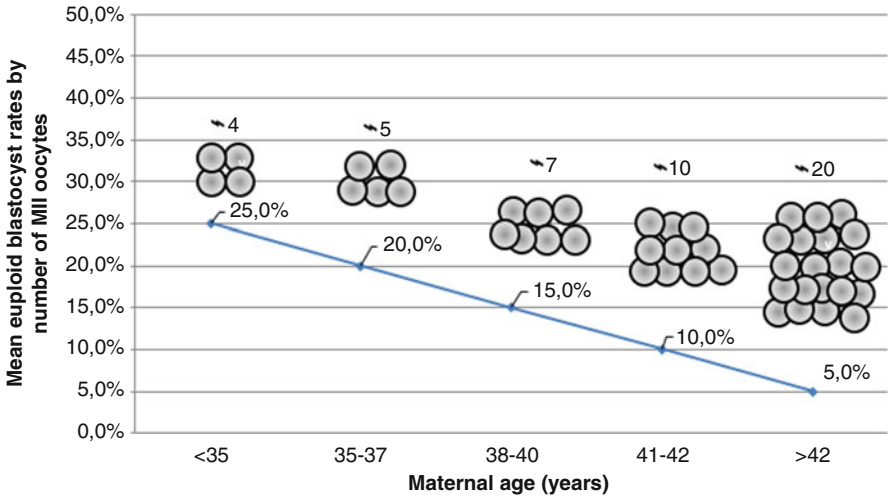


Fig. 4 Estimated number of MII oocytes needed to achieve an euploid blastocyst during IVF-ICSI cycles. (Modified from Vaiarelli et al. (2018))

Nevertheless, the prediction of a live birth cannot be based only on the oocyte yield. Indeed, the aneuploidy chromosomal constitution is strongly related to the woman's age, ranging from 25% to 30% in women younger than 35 to over 90% in women older than 42, and represents a critical element affecting embryo reproductive competence (Vaiarelli et al. 2018). Thus, women with the same number of retrieved oocytes might have opposite clinical outcomes according to the age-dependent blastocyst aneuploidy rate. Though, implantation rate of a euploid blastocyst is independent from the woman's age (Fig. 4).

In this context, the number of oocytes retrieved after COS greatly influences the clinical outcome. Thus, the optimization of the strategies of COS based on the ovarian reserve of each single patient is essential in order to maximize the age-related chances to obtain at least one euploid blastocyst.

Prevalence

Due to the lack of definition, it is difficult to determine the prevalence of POR condition. Its reported frequency in IVF cycles varies from 9% to 30% in different studies (Ferraretti et al. 2011; Keay et al. 1997). According to data published on the Italian Registry, couples treated for POR constituted the 13.1% (ART registry 2016).

Management

The management of patients with low AFC and/or AMH is still a debated issue in reproductive medicine. Certainly IVF-ICSI compared with IUI showed superior

pregnancy rates in the setting of patients with poor ovarian response after COS (Reichman et al. 2013).

IVF Protocols

The obvious and most used approach for women with POR is to increase the daily dose of gonadotropin. Actually, higher gonadotropin dosages can increase the number of transferable embryos and, therefore, cumulative pregnancy chances. The National Institute for Health and Care Excellence (NICE) guideline on fertility recommends not to use a dosage of FSH of more than 450 IU/day for COS in poor responder patients (NICE n.d.). A recent open-label, multicenter randomized controlled trial (RCT) recommended using a standard dose of 150 IU/day in women scheduled for IVF/ICSI with a predicted poor response. Five hundred eleven women were randomized in the study, 234 with an AFC $\leq$ 7 and 277 with an AFC 8–10. The cumulative live birth rate for increased versus standard dosing was 42.4% (106/250) versus 44.8% (117/261), respectively [relative risk (RR): 0.95 (95%CI, 0.78–1.15), $P = 0.58$]. The authors concluded that an increased dose strategy, despite significant higher costs, doesn't improve live birth rates. Thus, they recommended using a standard dose of 150 IU/day in these women (van Tilborg et al. 2017). Unfortunately, the authors adopted a different definition of poor responders, the study permitted small dose adjustments between cycles, and the AFC might have suffered from interobserver variation.

Among the various protocols and strategies toward optimization of management for poor responders, there is no concrete evidence on the advantage of any stimulation protocol over another. Examples of recommended protocols for poor responder patients include:

- Low-dose (or "mild") protocol
- Low-dose clomiphene/gonadotropin protocol
- Augmented natural cycle protocol
- Delayed start antagonist protocol
- Flare-up agonist protocol
- Microdose flare GnRH agonist protocol

Mild COS protocols using low doses of gonadotropins have been implemented in clinical practice, demonstrating significant advantages, including cost-effectiveness and patient-friendly regimens. This protocol optimizes the balance between outcomes and risks of treatment, although the expected number of retrieved oocytes is low, usually ranging from two to seven (Verberg et al. 2009).

Mild stimulation is based on the following evidences:

- Because of low-dose stimulation, only the healthier follicles with more competent egg(s) are encouraged to develop (Verberg et al. 2009).
- The physiologic hormonal follicular milieu might be altered when the follicles are exposed to a high dose of gonadotropins (von Wolff et al. 2014).

- Follicular AMH has been shown to be significantly higher in natural cycles compared with that with ovarian stimulation (von Wolff et al. 2014).
- A RCT found that the number of euploid embryos with conventional IVF was no higher than that with mild stimulation IVF (Baart et al. 2007).
- Supraphysiologic levels of serum E_2 could affect implantation (Fauser and Devroey 2003).

The low-dose gonadotropin protocol involves initiating 150 IU of gonadotropins daily on day 2 for 9 days. GnRH antagonist is administered when the lead follicle reaches ≥12 mm in diameter, and the ovulation trigger is suggested when the lead follicle is 16–17 mm (Gonda et al. 2018).

According to Siristatidis et al., although convincing scientific evidences, mild ovarian stimulation was shown inferior to conventional regimes when applied to poor responders undergoing IVF/ICSI, in terms of retrieved oocytes (Siristatidis et al. 2017).

Low-dose clomiphene/gonadotropin protocols may be a good option for patients who have previously responded to clomiphene, but did not have a successful cycle. This protocol is characterized by the administration of clomiphene citrate 100 mg/day for 5 days beginning on day 2. A 2016 meta-analysis suggested that mild COS protocol with CC may obtain equal pregnancy outcome in POR patients undergoing IVF treatment compared with conventional COS protocol (Song et al. 2016).

In augmented natural cycles, patients are monitored for estradiol production >20 pg/ml and/or the presence of 3–4 mm-sized basal antral follicles. Once these conditions are satisfied, ovarian stimulation is initiated with a low-dose combination of HP-hMG and rFSH (e.g., 75 IU/day of each) and continued for approximately 6 days. When the lead follicle reaches ≥12 mm, GnRH antagonist is added. Ovulation is triggered with hCG 10,000 IU or leuprolide (Gonda et al. 2018).

Delayed-start antagonist protocol is based on the observation that endogenous FSH may stimulate larger follicles in the prior luteal phase and subsequently lead to a size discrepancy in the cohort of developing follicles. According to this protocol, a GnRH antagonist is administered from day 2 and continued for 7 days, then ovarian stimulation is started with gonadotropins, and GnRH antagonist is added again if the ultrasound monitoring showed at least one follicle with diameter ≥ 14 mm and continued until the trigger day (Davar et al. 2018).

The flare-up protocols are based on the 24-h-long surge in endogenous FSH and LH released when administering GnRH agonists in the early follicular phase. GnRH agonists are initiated in the follicular phase of a stimulation cycle before commencing gonadotropin injections.

The very low-dose, "microdose" GnRH agonist flare protocol represents an attempt to decrease the suppressive effects of GnRH agonists during a flare protocol. Daily GnRH agonists are administered at the dose of 20–50 μg twice and continued until the day of hCG administration. After 2 days (on the fourth day of menstruation), the patients received rFSH 300 IU/day (Davar et al. 2018).

Synchronizing Early Follicle Development

Ovarian follicles mature over a period of approximately 2–4 months. COS can only support the cohort of follicle responsive to the stimulation without generating de novo follicles. The synchronization of earlier follicle wave with oral estrogens, contraceptive pill, or progestins, prior to traditional COS, has been suggested as a possible strategy to increase the number of responsive follicles, particularly for poor responders (McGee and Hsueh 2000).

In some patients, diminished ovarian reserve was due to an androgen deficiency state. In these women, androgen supplementation via testosterone or dehydroepiandrosterone (DHEA) may help to stimulate early follicle development and improve functional ovarian reserve (Gleicher et al. 2010).

DHEA is produced in the zona reticularis of the adrenal cortex and by ovarian theca cells. It promotes follicular development and granulosa cell proliferation and can also enhance the level of follicular insulin-like growth factor-1 (IGF-1), which promotes folliculogenesis by enhancing the effect of gonadotropin and reducing follicular arrest.

After the beginning of androgen supplementation, the follicles require 6–8 weeks to achieve synchronization and become mature enough to respond to COS. Among androgenic supplements, DHEA is the preferred method over testosterone as it is metabolized by organs as needed, whereas testosterone overflows the body with a fixed level. However, it is important to note that conclusive clinical evidence of the influence of DHEA or testosterone is limited and the use of androgen supplementation is still considered experimental. Furthermore, patients offered these supplements should be informed about the potential side effects such as acne, oily skin, deepening of the voice, hirsutism, and hair loss (Li et al. 2015).

Owen et al. (1991) observed that growth hormone (GH) co-treatment improved the ovarian response to COS in poor responders. This conclusion was supported by studies demonstrating that GH, either directly or indirectly via insulin-like growth factor 1 (IGF-1), regulates oocyte maturation by increasing the sensitivity of the ovaries to gonadotropins and promoting early follicle development. Clinical studies demonstrated contrasting effects of GH on oocyte and embryo-related outcomes; moreover studies are few in number and included small sample size. A Cochrane review on GH as adjuvant in poor responders concluded that, although the use of GH in poor responders showed a significant improvement in live birth rates, it was unable to identify which subgroup of poor responders might benefit from this co-treatment. The result needs to be interpreted with caution; the included trials were few in number and with small sample size. More recently, a study evaluating the effects of GH supplementation on oocyte maturation in vivo in aged and young mice and its effect on mitochondrial function observed a potential role of GH in improving mitochondrial function in oocytes from aged mice (Hou et al. 2019). Further research is, however, necessary to fully define GH role in IVF treatment.

Polycystic Ovary Syndrome (PCOS)

Definition and Diagnosis

According to the "Rotterdam criteria," diagnosis of PCOS requires two of the following criteria (Table 3): oligo- and/or anovulation (having an interval of >35 days between menstrual periods and/or amenorrhea, described as the absence of vaginal bleeding for at least 6 months), clinical and/or biochemical signs of hyperandrogenism (evaluated by modified Ferriman-Gallwey (mF-G) scoring method), and PCO at ultrasound (12 or more follicles in either ovary measuring 2–9 mm in diameter and/or increased ovarian volume) (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2004; Fig. 5).

Certainly, the diagnosis of PCOS is only confirmed when other conditions are excluded (e.g., oligo−/anovulation disorders and/or hyperandrogenism, such as thyroid disease, nonclassic congenital adrenal hyperplasia (NCCAH), hyperprolactinemia, and androgen-secreting tumors).

Table 3 Rotterdam criteria for diagnosis of PCOS. Total T: total testosterone. DHEA-S: deidroepiandrosterone solfato

Diagnosis confirmed by 2 of 3 criteria after exclusion of other etiologies:
1. Oligo and/or anovulation
2. Biochemical and/or clinical signs of hyperandrogenism Biochemical: Total T > 70 ng/dL, androstenedione >245 ng/dL, DHEA-S > 248 ug/dL Clinical: severe cystic acne, progressive hirsutism, acanthosis nigricans
3. Polycystic ovaries evaluated in ultrasound: ≥ 12 follicles (2–9 mm diameter) in each ovary or ovarian volume > 10 cc

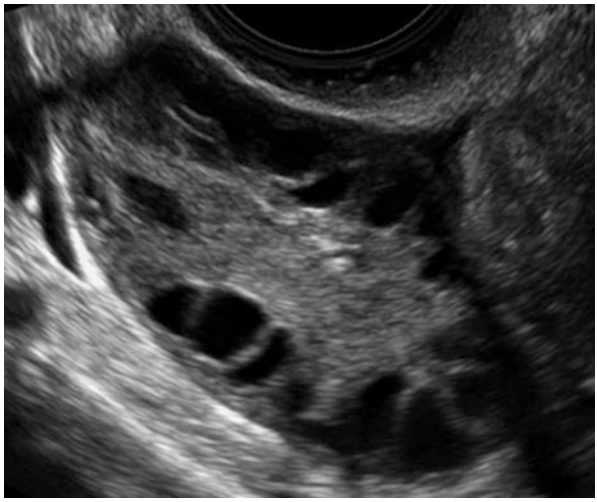


Fig. 5 Ultrasound image of human polycystic ovary: more than 12 follicles measuring 2–9 mm in diameter and surrounding an enlarged ovarian *stroma*

The aetiology of PCOS remains unclear, but evidence exists for a multifactorial origin with a genetic predisposition. In women with PCOS, we can find multiple abnormalities such as oligomenorrhea, hyperandrogenism, anovulatory infertility, and metabolic risk factors such as obesity, insulin resistance, dyslipidemia, impaired glucose tolerance, fatty liver, and obstructive sleep apnea.

Prevalence

PCOS is the commonest endocrine disorder in women of reproductive age and the leading cause of anovulatory infertility. It is estimated that around 20% of all IVF/ICSI cycles are performed in patients with PCOS (ESHRE Capri Workshop Group 2012).

Management

When lifestyle modifications are not successful in restoring ovulation, patients can be treated with ovarian stimulation. Typically, ovulation induction begins with the use of CC or letrozole. Subsequent steps are represented by administration of exogenous gonadotropins associated with timed intercourse or IUI and IVF/ICSI (ESHRE Capri Workshop Group 2012).

There is ongoing debate regarding the use of ovulation induction versus ART for the treatment of infertile women with PCOS. An RCT was conducted in order to compare the efficacy of IUI vs timed intercourse with CC as a first-line treatment for anovulatory infertility associated by Abu et al. One hundred eighty-eight women with PCOS received three consecutive cycles of ovulation induction with CC and IUI ($n = 93$, 259 cycles) or three consecutive cycles of CC with timed intercourse ($n = 95$, 266 cycles). The study showed comparable outcomes regarding the clinical pregnancy rate per cycle or per woman (8.49 vs 7.89% and 23.6 vs 22.1%; $p = 0.26$ and $p = 0.33$, respectively). Therefore, the authors concluded that ovulation induction with CC and timed intercourse is as effective as IUI in patients with PCOS and could represent the first treatment, being less invasive and less expensive than IUI (Abu et al. 2011).

A PCOS Consensus sponsored by ESHRE/American Society for Reproductive Medicine (ASRM) concluded that combining IUI with ovulation induction may be considered in anovulatory women with PCOS after failure to conceive despite successful induction of ovulation or in cases associated with male factor infertility. Such treatment has shown from 11% to 20% clinical pregnancy rate per cycle with a multiple pregnancy rate ranging from 11% to 36% (Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2008).

After failed ovulation induction or IUI, or in the presence of other infertility factors such as tubal damage, severe endometriosis, or male factor infertility, IVF/ICSI treatment in women with PCOS is recommended. At the same time, gynecologists should take into considerations other variables such as age. In fact, fertility

potential declines rapidly after 40 years of age; thus in these categories of women, clinicians need to avoid a delay in initiation of ovulation induction.

Women with PCOS are difficult to stimulate: sometimes they demonstrate resistance to stimulation, and in other cases they have exaggerated response. In PCOS women, the risk of moderate-to-severe OHSS has been evaluated of approximately 10% versus 0.5–4.0% in the general IVF population. A meta-analysis reported an OR of 6.8 (95% CI: 4.9–9.6) for the development of OHSS in ultrasound-determined PCOS patients compared with those with normal-appearing ovaries on baseline ultrasound. Moreover, although oocyte recruitment during ART is higher in these patients, quality and maturity are poor and maybe compromised (Baumgarten et al. 2013).

The goal of the ART is to find the optimal strategy to prevent OHSS and cycle cancellation. In 2016 the ASRM recommended, in women with PCOS who undergo ART, the use of "step-up" or "step-down" protocols, or a sequential scheme, where a low dose of exogenous FSH or combined gonadotropins are employed to tie up ovarian hyperstimulation (American Society for Reproductive Medicine 2016).

"Step-up" protocols consist in an initial low gonadotropin daily dose (37.5–50 IU/day), followed by a small incremental dose (augmented by 50–100%) until folliculogenesis is reached up as evidenced by a lead follicle on ultrasound and the rising of estradiol levels (Berger and Bates 2014).

"Step-down" protocol starts with a higher dose of FSH that is gradually decreased; dose reduction is progressive and based on the visualization of at least one follicle of 10 mm. Although mimicking a physiological cycle, the step-down protocol is associated with a higher risk of OHSS and cycle cancellation (Christin-Maitre et al. 2003).

Moreover, these two protocols could be combined in a "sequential protocol": FSH dose is gradually increased until a leading follicle reaches 14 mm diameter; then gonadotropin dose is decreased by 50%.

Strategies to Reduce OHSS Risk

Several interventions have been suggested in order to reduce the occurrence of OHSS while not influencing or even improving pregnancy outcomes:

- The use of GnRH antagonist protocol against conventional long GnRH agonist for pituitary suppression
- Coasting before oocyte triggering
- The use of GnRH agonist to trigger oocyte maturation
- "Freeze-all" embryo strategy
- Dopamine agonists, metformin, inositol, and aspirin

Coasting is the strategy of withholding exogenous gonadotropins at the end of ovarian stimulation: mature follicles will survive for a few days, while smaller follicles will reduce through atresia of granulosa cells. Low-quality evidence suggests that coasting reduces moderate-to-severe OHSS. Moreover, the optimal length of coasting has not been determined, with a limited number of studies suggesting 4 days (Nardo et al. 2006).

ASRM recommends the use of GnRH agonist (GnRHa) for the final oocyte maturation trigger in oocyte donation (evidence grade A). Nowadays, the use of GnRHa trigger is a well-established first-line treatment in oocyte donation and segmentation IVF/ICSI cycles (when egg collection and transfer are done through embryo cryopreservation and cryopreserved embryo transfer in a separate cycle). On the other hand, this procedure showed lower reproductive outcomes for fresh embryo transfer cycles. Essentially, this poor outcome is due to the low-circulating endogenous LH levels after the GnRHa trigger, leading to corpus luteum demise and consequently suboptimal progesterone levels at peri-implantation. This finding led to the development of modified luteal phase support protocols. Unfortunately, none of these has gained universal acceptance (American Society for Reproductive Medicine 2016).

Randomized study on infertile women with PCOS found that frozen-embryo transfer resulted in a higher rate of live births than fresh-embryo transfer, a difference that was attributed to a lower rate of pregnancy loss. Also, in the frozen-embryo group was found a lower frequency of the OHSS but a higher frequency of preeclampsia (Chen et al. 2016).

Dopamine agonists, cabergoline and quinagolide, reduce incidence of moderate or severe OHSS, based on moderate-quality evidence; administration of these treatments should start at the time of hCG trigger and continued for several days (6–8). There is no evidence that cabergoline or quinagolide influences pregnancy outcomes such as live birth rate, clinical pregnancy rate, multiple pregnancy rate, and miscarriage rate.

Another strategy being validated is the administration of aspirin during ovarian stimulation cycles; evidence about aspirin is based on a single randomized trial comparing aspirin alone to no treatment and another study comparing combined acetylsalicylic acid and steroid treatment with no treatment (American Society for Reproductive Medicine 2016).

Conclusions

Nowadays ART is available throughout most of the civilized world, and it is likely that continued enhancements will widen its appeal and applicability. The introduction of PGS/PGD allowed the screening of an IVFI-/CSI-created embryo for aneuploidy and for the testing of a genetic disorder, respectively. Moreover egg and sperm donation has allowed women with premature ovarian failure and men with intractable azoospermia to access the techniques. Although the advances in ART have opened new doors for infertile couples, treatment of patients with poor ovarian response and PCOs remains challenging and still presents many uncertainties with debated approaches. Moreover, the widespread of these techniques and the inclusion of a population of older women with comorbidities has posed the problem of managing obstetric complications following ART. Therefore more research is needed in assisted reproduction in order to optimize the chances of a safe pregnancy in certain groups of patients.

Cross-References

► The Polycystic Ovary Syndrome (PCOS)

References

Abu HH, Osama O, Ibrahim AE. Intrauterine insemination versus timed intercourse with clomifene citrate in polycystic ovary syndrome: a randomized controlled trial. Acta Obstet Gynecol. 2011;90:344–50. Scand.

Adams DH, Clark RA, Davies MJ, de Lacey S. A meta-analysis of sperm donation offspring health outcomes. J DevOrig Health Dis. 2017;8(01):44–55.

American Society for Reproductive Medicine. Fertil Steril. 2016;106:1634–47.

ASRM. https://www.asrm.org/news-and-publications/practice-committee-documents/

Baart EB, Martini E, Eijkemans MJ, Van Opstal D, Beckers NG, Verhoeff A, et al. Milder ovarian stimulation for in-vitro fertilization reduces aneuploidy in the human preimplantation embryo: a randomized controlled trial. Hum Reprod. 2007;22:980–8.

Balakier H, Sojecki A, Motamedi G, et al. Time-dependent capability of human oocytes for activation and pronuclear formation during metaphase II arrest. Hum Reprod. 2004;19:982–7.

Bartal MF, Sibai BM, Bart Y, Shina A, Mazaki-Tovi S, Eisen IS, et al. The impact of sperm and egg donation on the risk of pregnancy complications. Am J Perinatol. 2018;47:41.

Baumgarten M, Polanski L, Campbell B, Raine-Fenning N. Do dopamine agonists prevent or reduce the severity of ovarian hyperstimulation syndrome in women undergoing assisted reproduction? A systematic review and meta-analysis. Hum Fertil. 2013;16:168–74.

Berger JJ, Bates GW. Optimal management of subfertility in polycystic ovary syndrome. Int J Women's Health. 2014;6:613–21.

Boomsma CM, Heineman MJ, Cohlen BJ, Farquhar C. Semen preparation techniques for intrauterine insemination. Cochrane Database Syst Rev. 2007;(4). Art. No.: CD004507.Strandell et al. 2003.

Chen ZJ, Shi Y, Sun Y, Zhang B, Liang X, Cao Y, Yang J, Liu J, Wei D, Weng N, Tian L, Hao C, Yang D, Zhou F, Shi J, Xu Y, Li J, Yan J, Qin Y, Zhao H, Zhang H, Legro RS. Fresh versus Frozen Embryos for Infertility in the Polycystic Ovary Syndrome N Engl J Med. 2016, 11;375 (6):523–33.

Christin-Maitre S, Hugues JN, Recombinant FSH Study Group. A comparative randomized multicentric study comparing the step-up versus step-down protocol in polycystic ovary syndrome. Hum Reprod. 2003;18:1626–31.

Davar R, Neghab N, Naghshineh E. Pregnancy outcome in delayed start antagonist versus microdose flare GnRH agonist protocol in poor responders undergoing IVF/ICSI: an RCT. Int J Reprod Biomed (Yazd). 2018;16(4):255–60.

De Geyter C, Calhaz-Jorge C, Kupka MS, Wyns C, Mocanu E, Motrenko T et al. European IVF-monitoring Consortium (EIM) for the European Society of Human Reproduction and Embryology (ESHRE). ART in Europe, 2014: results generated from European registries by ESHRE: the European IVF-monitoring Consortium (EIM) for the European Society of Human Reproduction and Embryology (ESHRE). Hum Reprod. 2018.

Desmyttere S, De Rycke M, Staessen C, et al. Neonatal follow-up of 995 consecutively born children after embryo biopsy for PGD. Hum Reprod. 2012;27(1):288–93.

ESHRE fact sheets 3 January 2017.

ESHRE fact sheet, Updated 18 February 2018.

ESHRE Capri Workshop Group. Intrauterine insemination. Hum Reprod Update. 2009;15(3): 265–77.

ESHRE Capri Workshop Group. Health and fertility in World Health Organization group 2 anovulatory women. Hum Reprod Update. 2012;18:586–99.

Faddy M, Gosden R, Ahuja K, Elder K. Egg sharing for assisted conception: a window on oocyte quality. Reprod Biomed Online. 2011;22(1):88–93.
Fatemi HM, Kyrou D, Al-Azemi M, Stoop D, De Sutter P, Bourgain C. Ex-vivo oocyte retrieval for fertility preservation. Fertil Steril. 2011;95(5):1787.e15–7.
Fauser BC, Devroey P. Reproductive biology and IVF: ovarian stimulation and luteal phase consequences. Trends Endocrinol Metab. 2003;14:236–42.
Ferraretti AP, La Marca A, Fauser BC, Tarlatzis B, Nargund G, Gianaroli L, on behalf of the ESHRE working group on Poor Ovarian Response Definition. ESHRE consensus on the definition of "poor response" to ovarian stimulation for in vitro fertilization: the Bologna criteria. Hum Reprod. 2011;26:1616–24.
Forman EJ, Tao X, Ferry KM, et al. Single embryo transfer with comprehensive chromosome screening results in improved ongoing pregnancy rates and decreased miscarriage rates. Hum Reprod. 2012;27(4):1217–22.
Fragouli E, Alfarawati S, Goodall NN, Sanchez-Garcia JF, Colls P, Wells D. The cytogenetics of polar bodies: insights into female meiosis and the diagnosis of aneuploidy. Mol Hum Reprod. 2011;17:286–95.
Gleicher N, Weghofer A, Barad DH. Improvement in diminished ovarian reserve after dehydroepiandrosterone supplementation. Reprod Biomed Online. 2010;21:360–5.
Gonda KJ, Domar AD, Gleicher N, Marrs RP. Insights from clinical experience in treating IVF poor responders. Repr BioMedicine Online. 2018;36(1):12–9, ISSN 1472-6483.
Good Clinical Treatment in Assisted Reproduction – An ESHRE position paper. June 2008.
Hou H, Wang X, Yu Q, Li HY, Li SJ, Tang RY, Guo ZX, Chen YQ, Hu CX, Yang ZJ, Zhang WK, Qin Y. Evidence that growth hormone can improve mitochondrial function in oocytes from aged mice. Reproduction. 2019. REP-18-0529.R1.
Humaidan P, Alviggi C, Fischer R, Esteves SC. The novel POSEIDON stratification of "Low prognosis patients in Assisted Reproductive Technology" and its proposed marker of successful outcome. F1000Res. 2016;5:2911. Published 2016 Dec 23.
Keay SD, Liversedge NH, Mathur RS, Jenkins JM. Assisted conception following poor ovarian response to gonadotrophin stimulation. Br J Obstet Gynaecol. 1997;104(5):5217.. Review
Li J, Yuan H, Chen Y, Wu H, Wu H, Li L. A meta-analysis of dehydroepiandrosterone supplementation among women with diminished ovarian reserve undergoing in vitro fertilization or intracytoplasmic sperm injection. Int J Gynaecol Obstet. 2015;131(3):240–5.
Maheshwari A, McLernon D, Bhattacharya S. Cumulative live birth rate: time for a consensus? Hum Reprod. 2015;30:2703–7.
McGee EA, Hsueh AJW. Initial and cyclic recruitment of ovarian follicles. Endocr Rev. 2000;21(2):200–14.
Nardo LG, Cheema P, Gelbaya TA, Horne G, Fitzgerald CT, Pease EH, Brison DR, Lieberman BA. The optimal length of 'coasting protocol' in women at risk of ovarian hyperstimulation syndrome undergoing in vitro fertilization. Hum Fertil (Camb). 2006;9:175–80.
NICE. 2013. https://www.nice.org.uk/guidance/cg156/evidence/full-guideline-pdf-188539453
NICE Last updated: September 2017 – National Institute for Health and Care Excellence. Fertility problems: assessment and treatment.
Ombelet W, Cooke I, Dyer S, Serour G, Devroey P. Infertility and the provision of infertility medical services in developing countries. Hum Reprod Update. 2008;14(6):605–21.
Owen EJ, West C, Mason BA, Jacobs HS. Co-treatment with growth hormone of sub-optimal responders in IVF-ET. Hum Reprod. 1991;6:524–8.
Paulson RJ, Boostanfar R, Saadat P, Mor E, Tourgeman DE, Slater CC, et al. Pregnancy in the sixth decade of life: obstetric outcomes in women of advanced reproductive age. JAMA. 2002;288:2320–3.
Plachot M, Belaisch-Allarch J, Mayenga JM, Chouraqu A. Outcome of conventional IVF and ICSI on sibling oocytes in mild male factor infertility. Hum Reprod. 2002;17:362–9.
Polyzos NP, Devroey P. A systematic review of randomized trials for the treatment of poor ovarian responders: is there any light at the end of the tunnel? Fertil Steril. 2011;96:1058.e7–61.e7.

Reichman DE, Gunnala V, Meyer L, Spandorfer S, Schattman G, Davis OK, et al. In vitro fertilization versus conversion to intrauterine insemination in the setting of three or fewer follicles: how should patients proceed when follicular response falls short of expectation? Fertil Steril. 2013;100(1):94–9.

Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod. 2004;19:41.

Rubio C, Bellver J, Rodrigo L, et al. In vitro fertilization with preimplantation genetic diagnosis for aneuploidies in advanced maternal age: a randomized, controlled study. Fertil Steril. 2017;107(5):1122–9.

Siristatidis C, Salamalekis G, Dafopoulos K, Basios G, Vogiatzi P, Papantoniou N. Mild versus conventional ovarian stimulation for poor responders undergoing IVF/ICSI. In Vivo. 2017;31(2):231–7.

Song D, Shi Y, Zhong Y, Meng Q, Hou S, Li H. Efficiency of mild ovarian stimulation with clomiphene on poor ovarian responders during IVF\ICSI procedures: a meta-analysis. Eur J Obstet Gynecol Reprod Biol. 2016;204:36–43.

Sullivan-Pyke C, Dokras A. Preimplantation genetic screening and preimplantation genetic diagnosis. Obstet Gynecol Clin N Am. 2018;45(1):113–25.

Sunkara SK, Rittenberg V, Raine-Fenning N, Bhattacharya S, Zamora J, Coomarasamy A. Association between the number of eggs and live birth in IVF treatment: an analysis of 400 135 treatment cycles. Hum Reprod. 2011;26(7):1768–74.

The Practice Committee of the American Society for Reproductive Medicine. Diagnostic evaluation of the infertile female: a committee opinion. 2006. Fertil Steril. 2015;103(6):e44–50.

Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Consensus on infertility treatment related to polycystic ovary syndrome. Hum Reprod. 2008;23(3):462–77.

Tournaye H. Male factor infertility and ART. Asian J Androl. 2012;14(1):103–8.

Vaiarelli A, et al. What is new in the management of poor ovarian response in IVF? CurrOpinObstet Gynecol. 2018;30(3):155–62.

van der Gaast MH, Eijkemans MJ, van der Net JB, de Boer EJ, Burger CW, van Leeuwen FE, Fauser BC, Macklon NS. Optimum number of oocytes for a successful first IVF treatment cycle. Reprod Biomed Online. 2006;13:476–80.

van Tilborg TC, Torrance HL, Oudshoorn SC, Eijkemans MJC, Koks CAM, Verhoeve HR, et al. OPTIMIST study group. Individualized versus standard FSH dosing in women starting IVF/ICSI: an RCT. Part 1: the predicted poor responder. Hum Reprod. 2017;32(12):2496–505.

van Weert JM, Repping S, van Voorhis BJ, van der Veen F, Bossuyt PM, et al. Performance of the postwash total motile sperm count as a predictor of pregnancy at the time of intrauterine insemination: a meta-analysis. Fertil Steril. 2004;82:612–20.

Verberg MF, Eijkemans MJ, Macklon NS, Heijnen EM, Fauser BC, Broekmans FJ. Predictors of low response to mild ovarian stimulation initiated on cycle day 5 for IVF. Hum Reprod. 2007;22:1919–24.

Verberg MF, Eijkemans MJ, Macklon NS, Heijnen EM, Baart EB, Hohmann FP, et al. The clinical significance of the retrieval of a low number of oocytes following mild ovarian stimulation for IVF: a meta-analysis. Hum Reprod Update. 2009;15:5–12.

von Wolff M, Kollmann Z, Fluck CE, Stute P, Marti U, Weiss B, et al. Gonadotrophin stimulation for in vitro fertilization significantly alters the hormone milieu in follicular fluid: a comparative study between natural cycle IVF and conventional IVF. Hum Reprod. 2014;29:1049–57.

Part IV

Menopause

Premature Ovarian Insufficiency

15

M. N. Gunning, L. Troìa, F. J. Janse, S. Luisi, and Bart C. Fauser

Contents

M. N. Gunning (✉) · F. J. Janse · B. C. Fauser
Department of Reproductive Medicine and Gynecology, University Medical Center Utrecht, University of Utrecht, Utrecht, The Netherlands
e-mail: m.n.gunning@umcutrecht.nl; F.Janse-2@umcutrecht.nl; B.C.Fauser@umcutrecht.nl

L. Troìa · S. Luisi
Department of Molecular and Developmental Medicine, University of Siena, Siena, Italy
e-mail: libera.17@hotmail.it; stefano.luisi@unisi.it

F. Petraglia, B. C. Fauser (eds.), *Female Reproductive Dysfunction*, Endocrinology,
https://doi.org/10.1007/978-3-030-14782-2_15

Abstract

In women diagnosed with premature ovarian insufficiency (POI), the resting pool of primordial follicles is prematurely exhausted. These women present with absent menses and increased follicle-stimulating hormone (FSH) concentrations along with low estrogens before 40 years of age. The underlying etiology in most cases remains unknown. In a small proportion of cases, POI results from genetic defects, autoimmune disease, or iatrogenic origin (such as surgery, radiotherapy, or chemotherapy). In the absence of contraindications, hormone replacement therapy (HRT) is recommended to mitigate short- and long-term physical and mental symptoms. Little prospectively generated information is available regarding long-term health risks associated with POI. Since fertility is severely compromised in women diagnosed with POI, IVF along with oocyte donation or adoption may be offered. In women at risk for POI, fertility preservation options can be considered.

Keywords

Menopause · Genetics · Bone density · Vitamin D · Cardiovascular health · Hormonal replacement therapy · Egg cell donation · Fertility preservation

Introduction

Premature (or primary) ovarian insufficiency (POI) is also known as premature ovarian failure (POF) or premature menopause. The preferred term, however, is POI (Shifren et al. 2014). The prevalence of POI varies between approximately 0.2% and 2% (Cramer and Xu 1996; Luborsky et al. 2003; Lagergren et al. 2018). The accelerated follicle loss and premature cessation of ovarian function has implications for the entire female body. Besides physical and mental complications due to low estrogen concentrations, the psychological impact of this diagnosis is severe. Due to lack of awareness in the general population and among physicians, both diagnosis and treatment of women with POI are often delayed. This chapter will focus on the definition, etiology, diagnosis, treatment, and health implications (Webber et al. 2016).

Diagnostic Criteria

According to the ESHRE guideline 2016, the diagnosis of POI is based on the presence of oligomenorrhea or amenorrhea – often secondary – for at least 4 months, in combination with an elevated FSH level > 25 IU/l on two occasions, more than 4 weeks apart. It must be confirmed that elevated FSH concentrations are not measured during ovulation. Transvaginal or abdominal ultrasound and anti-Mullerian hormone (AMH) levels have no part in this definition (yet) (Webber et al. 2016). However, ultrasound in women with POI will show few or no follicles, and AMH concentrations will be low and therefore often undetectable. In a Dutch

cohort of women with POI ($n = 112$), all women showed AMH levels below the fifth percentile of normoovulatory controls ($n = 83$). AMH appeared to be more consistent than inhibin B or antral follicle count in assessing the extent of the follicle pool in young hypergonadotropic patients (Knauff et al. 2009).

Etiology of Premature Ovarian Insufficiency

Premature ovarian insufficiency is a heterogeneous, multicausal condition. POI arises from either the premature depletion of the ovarian follicles or ovarian follicle dysfunction. Premature depletion of the ovarian follicle pool may be due to destruction of primordial follicles by toxic agents, autoimmune response, activation of proapoptotic pathways, or accelerated follicular recruitment. In rare cases of follicular dysfunction, sufficient ovarian follicles are present upon ultrasonography or ovarian biopsy, but they do not function properly (Hubayter et al. 2010). Unfortunately, in a substantial proportion of women with POI, the cause remains unknown.

Genetics

Heritability of POI has long been under investigation. Population-based, retrospective studies suggest heritability for age at natural menopause (van Asselt et al. 2004). Moreover, POI tends to run in families in up to 30% of cases (Bachelot et al. 2009; Janse et al. 2010). Also, from sisters of women with POI in oocyte donation settings, it is known that there is an increase in cycle cancellation rates and lower ovarian responses, hereby also suggesting a hereditary component. Indeed, it is estimated that genetic causes account for approximately 20–25% of women with POI (Jiao et al. 2018). An overview of genes associated with POI is given in Table 1.

Chromosomal defects account for approximately 10–12% of women diagnosed with POI. The chance of finding an abnormal karyotype in women with POI is greater in those with primary amenorrhea compared to those with secondary amenorrhea (Jiao et al. 2012; Kalantari et al. 2013). However, there is no clear correlation between age of secondary amenorrhea and the probability of identifying a chromosomal abnormality.

In the great majority of women with POI caused by a chromosomal abnormality, this condition is related to an X-chromosome defect. X-chromosome monosomy (45, X) causes Turner syndrome. The incidence of Turner syndrome is 1 in 2,500 females. While Turner syndrome leads to miscarriages in the majority of these pregnancies, females surviving may carry any degree of mosaicism (Hook and Warburton 2014). Turner syndrome is typically characterized by linear growth restriction, cardiovascular abnormalities, renal system abnormalities, webbing of the neck, and a rapid decline of the primordial follicle pool. This either leads to primary amenorrhea or secondary amenorrhea at an early age (Zinn et al. 1993). In contrast, trisomy X (47,XXX) does not influence fertility so much, unless there is mosaicism with 45,X involved (such as 45,X/47,XXX). Moreover, structural

Table 1 Human genetics associated with POI

Human gene	Full gene name	Cytogenetic location	Syndrome (OMIM)	Reported incidence in POI
GPR3	G protein-coupled receptor 3	1p36.1-p35	*None*	0%
LHX8	LIM homeobox 8	1p31.1	*None*	Rare
HSD3B2	Hydroxy-δ-5-steroid dehydrogenase, 3β- and steroid δ-isomerase 2	1p13.1	*None*	Unknown
LMNA	Lamin A/C	1q22	Malouf syndrome (212112)	Rare
LHR	LH receptor	2p21	*None*	<1%
FSHR	FSH receptor	2p21-p16	*None*	<1%
FIGLA	Folliculogenesis specific basic helix-loop-helix	2p13.3	*None*	2%
EIF5B	Eukaryotic translation initiation factor 5B	2q11.2	*None*	Rare
INHA	Inhibin alpha	2q33-q36	*None*	0–7%
FOXL2	Forkhead box L2	3q23	Blepharophimosis ptosis epicanthus inversus syndrome (110100)	2.8%
TP73L	Tumor protein p63	3q27	Rapp-Hodgkin syndrome (129400)	Rare
BMPR1B	Bone morphogenetic protein receptor 1B	4q23–24	Demirhan syndrome (609441)	Rare
ADAMTS19	ADAM metallopeptidase with thrombospondin type 1 motif, 19	5q31	*None*	Polymorphism (not mutation)
SIL1	*S. cerevisiae* homolog 1	5q31	Marinesco-Sjogren syndrome (248800)	Rare
GDF9	Growth-differentiation factor 9	5q31.1	*None*	1.6%
MSH5	MutS homolog 5	6p21.3	*None*	4.9%
FOXO3A	Forkhead box O3A	6q21	*None*	2.2%
ESR1	Estrogen receptor α	6q25.1	*None*	Polymorphism (not mutation)
NOBOX	Newborn ovary homeobox	7q35	*None*	0–1%
WRN	RecQ protein-like 2	8p12-p11.2	Werner syndrome (277700)	Unknown
STAR	Steroidogenic acute regulatory protein	8p11.2	Lipoid congenital adrenal hyperplasia (600617)	Unknown

(continued)

Table 1 (continued)

Human gene	Full gene name	Cytogenetic location	Syndrome (OMIM)	Reported incidence in POI
GALT	Galactose-1-phosphate uridyltransferase	9p13	Galactosemia (230400)	Unknown
NR5A1	Nuclear receptor subfamily 5, group A, member 1	9q33	*None*	1.4–8%
CYP17A1	Cytochrome P450, family 17, subfamily A, polypeptide 1	10q24.3	Congenital adrenal hyperplasia due to 17α-OH deficiency (202110)	Unknown
FSHB	FSH β subunit	11p13	*None*	Rare
ATM	Ataxia telangiectasia mutated	11q22.3	Ataxia telangiectasia (208900)	Unknown
FOXO1A	Forkhead box O1A	13q14.1	*None*	1.1%
EIF2B2	Eukaryotic translation initiation factor 2B, subunit 2	14q24	Ovarian leukodystrophy (603896)	Unknown
CYP19A1	Cytochrome P450, family 19, subfamily A, polypeptide 1	15q21.1	Aromatase deficiency (107910)	Rare
POLG	DNA polymerase gamma	15q25	Progressive external ophthalmoplegia with mitochondrial DNA deletions (157640)	Unknown
BLM	DNA helicase RecQ protein-like-3	15q26.1	Bloom syndrome (210900)	Unknown
PMM2	Phosphomannomutase 2	16p13.3–13.2	Congenital disorder of glycosylation, type 1A (212065)	Unknown
FA	Fanconi anemia complementation groups	16q24.3	Fanconi anemia (227650)	Rare
Ybx2	Y box-binding protein 2	17p11.2–13.1	*None*	Unknown
NOG	Noggin	17q22	*None*	1%
NANOS3	Nanos homolog 3	19p13.12	*None*	0%
LHB	LH beta polypeptide	19q13.33	*None*	Rare
AIRE	Autoimmune regulator	21q22.3	Autoimmune polyendocrine syndrome 1 (240300)	Rare

(continued)

Table 1 (continued)

Human gene	Full gene name	Cytogenetic location	Syndrome (OMIM)	Reported incidence in POI
DMC1	DMC1 dosage suppressor of mck1 homolog, meiosis-specific homologous recombination	22q13.1	*None*	2.4%
BMP15	Bone morphogenic protein 15	Xp11.2	*None*	1.5–12%
AR	Androgen receptor	Xq11–12	*None*	4.5%
XIST	X-inactivation gene	Xq13.2	Familial skewed X-inactivation (300087)	Unknown
POF1B	Premature ovarian failure, 1B gene	Xq21.2	*None*	Unknown
DACH2	Homolog of *Drosophila* dachshund gene	Xq21.2	*None*	2.7%
DIAPH2	Diaphanous homolog 2	Xq21.33	*None*	Unknown
PGRMC1	Progesterone receptor membrane component 1	Xq22-q24	*None*	1.5%
BHLHB9	Basic helix-loop-helix domain containing, class B, 9	Xq25	*None*	Unknown
FMR1	Fragile X mental retardation gene 1	Xq27.3	Fragile X syndrome (309550)	0.8–13%
FRAXE (FMR2)	Fragile X mental retardation gene 2	Xq28	X-linked mental retardation (309548)	1.5%

When multiple gene names exist, ENTREZ gene name was chosen (available at http://www.ncbi.nlm.nih.gov/gene)
OMIM Online Mendelian Inheritance in Man
Adapted from Persani et al. (2010), de Vos et al. (2010), and Simpson (2008)

abnormalities of the X-chromosome also may cause POI. In case the abnormalities comprise terminal deletions or translocations within the proximal Xp or proximal Xq regions especially, this may result in POI (Jiao et al. 2012).

Besides chromosomal aberrations, monogenetic causes of POI have been studied by candidate gene approaches. As such, gene function and gene expression in folliculogenesis and ovarian development have been studied, some by using murine knockout models and others by applying phenotype characteristics. Many genes emerged as POI candidate genes, but unfortunately only a minority of these have been proven to play a causative role when validation of the findings was undertaken (Jiao et al. 2018; Tucker et al. 2016) (Table 1).

The most common mutation among women with POI is the fragile X premutation. The FMR1 gene is located on the X-chromosome (Xq27.3). Full mutations contain over 200 CGG repeats, and these lead to the fragile X syndrome, which is characterized by mental retardation primarily in males. However, women carrying the premutation, i.e., 55–200 repeats, are at increased risk (13–26%) for developing POI. The prevalence of FMR1 premutation in women with POI is estimated to be 0.7 up to 13% (Conway et al. 1996; Wittenberger et al. 2007; Murray et al. 2014).

Other relatively common X-linked monogenetic causes include mutations in the BMP15 gene (Xp11.2) associated with typical facial characteristics of the blepharophimosis, ptosis, and epicanthus inversus syndrome (Rossetti et al. 2009). Also, autosomal mutations such as those affecting the FOXL2 (3q23) and NOBOX (7q35) genes may play a causative role in POI. These genes are implicated in approximately 1–5% of women with POI (Crisponi et al. 2001; Jiao et al. 2018).

POI may also arise in relation to other conditions such as galactosemia, caused by mutations in the GALT gene (9p13.3), in which 80% of affected women will develop POI (Fridovich-Keil et al. 2011). Perrault syndrome, in which hearing impairs, is associated with POI as well and caused by mutations in several different genes (Tucker et al. 2016).

The large amount of candidate genes identified to cause POI underlines the heterogeneity of the condition. Despite numerous reports on the different candidate genes, these only explain a minority of women with POI. As such, candidate gene studies are driven by hypothesis, focusing on identifying genes affecting oogenesis and folliculogenesis. Contrastingly, genome-wide studies aim to identify genetic variants without such a priori hypothesis, thereby finding underlying pathways responsible for POI. Previously, SNP-based genome-wide association studies and copy number variants have been assessed, without clear findings due to limited sample sizes. For early menopause four SNPs reached genome-wide levels of significance (CRHR1, SLC25A13, MCM6, and MB21D1/C6ORF150) (Perry et al. 2013). Newer research focuses on whole exome sequencing, which may well lead to individual diagnostic testing in the future (Tucker et al. 2016).

Autoimmune

It is estimated that approximately 20% of women with POI have a history of autoimmune diseases, most frequently thyroid disease (Hoek et al. 1997). The most common autoimmune diseases associated with POI are hypothyroidism, type I diabetes mellitus, and Addison's disease (Luisi et al. 2015). However, a true ovarian autoimmune reaction may only be demonstrated in 4–5% of women with sporadic POI (Bakalov et al. 2005). Autoimmune oophoritis will lead to POI on the basis of ovarian follicle dysfunction or follicle depletion.

Autoimmune oophoritis may occur as part of the autoimmune polyendocrine syndrome (APS). APS type I, also known as autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy (APECED), has its onset in

childhood. First, mucocutaneous candidiasis (75%) and/or hypoparathyroidism (89%) and later adrenal insufficiency (60–79%) and POI (60%) may also occur. APS type II usually develops in adulthood and is manifested by autoimmune Addison's disease and thyroid autoimmunity or type I diabetes. In APS type II, 10% of women develop POI. Moreover, POI may also arise from an inflammatory autoimmune response against ovarian-specific antigens or regulatory factors, and circulating steroidogenic cell antibodies have been recorded (La Marca et al. 2010).

Infectious

Scarce data is available on the link between infectious disease and POI. However epidemic parotitis virus, which on its turn leads to mumps oophoritis, is linked to POI. Other infectious diseases which have been linked to POI are human immunodeficiency virus (HIV) and antiviral therapy, tuberculosis, malaria, varicella, and shigella (Morrison et al. 1975; Ohl et al. 2010). A causal relation between infectious diseases and POI remains controversial.

Iatrogenic

Iatrogenic POI is a result of pelvic surgery, radiotherapy, or chemotherapy. Surgical menopause is caused by bilateral oophorectomy. There is also evidence suggesting that extirpation of the uterus, without removal of the ovaries, results in an earlier menopause in comparison with women who did not undergo uterus extirpation. This is likely to be caused by perioperative damage to the ovarian blood supply (Farquhar et al. 2005). Radiotherapy and chemotherapy are commonly used to treat oncological disease in children and adults. The aforementioned therapeutic interventions are also known to cause ovarian function loss. Chemotherapeutic agents disturb essential intracellular processes and stop proliferation of cells (Maltaris et al. 2007). The risk for ovarian function loss increases with age post puberty. High risk is the combined chemotherapy and alkylating agents (non-cell cycle-specific) in oncology patients. Radiation therapy also has a destructive effect on ovarian function, even with the use of pelvic shielding. Severity of function loss is dependent on a woman's age, radiation dose, and field (Meirow and Nugent 2001). Radiotherapy below the diaphragm or irradiation directly applied to the ovaries is a high-risk treatment strategy for causing iatrogenic menopause (Larsen et al. 2003; Fleischer et al. 2011).

Idiopathic

Unexplained POI is the most common form of POI. Up to 60–90% of all POI is thought to be idiopathic (Fenton 2015).

Clinical Symptoms

Women with POI by definition experience symptoms of oligomenorrhea or amenorrhea. This complaint is possibly accompanied with symptoms related to the hypoestrogenic state. Common are hot flushes, night sweats, and physical stiffness. Sexual functioning is impaired by vaginal dryness, dyspareunia, and loss of libido. Dyspareunia is here defined as recurrent or persistent genital pain directly before, during, or shortly after sexual intercourse. A change in urinary frequency might also be noticed. Mental health is often compromised due to mood swings, lack of concentration, and sleeping disorders.

Nonreproductive Features Associated with POI

Metabolic Profile

POI is a significant risk factor for health and disease susceptibility, including increased mortality. It has been postulated that the incremental decline of ovarian function which is accompanied by endogenous estrogen deficiency in women with POI may contribute to an increased risk for cardiovascular diseases (CVD) and mortality. Multiple risk factors for CVD development are often present in women with POI: autonomic and endothelial dysfunction, abnormal lipid profile, insulin dysfunction, and metabolic syndrome development (Podfigurna-Stopa et al. 2016). Several studies have been performed to examine the metabolic profile in women affected by POI; in fact these patients present with unfavorable lipid profiles. Evidence is inconsistent on which lipoproteins specifically show abnormalities. In a Dutch population of women with POI ($n = 90$), higher triglyceride (TG) levels and lower high-density lipoprotein (HDL) cholesterol levels were reported in comparison with controls ($n = 198$) adjusted for age, body mass index, and smoking habits. Dutch women with POI showed higher total cholesterol (TC) and low-density lipoprotein (LDL) levels and a significant negative correlation between estrogen and TC levels ($r = -0.291$, $p = 0.047$) (Knauff et al. 2008).

In a Turkish population study, similar levels of glucose, insulin, homeostasis model of assessment-insulin resistance (HOMA-IR), low-density lipoprotein cholesterol (LDL-C), and triglycerides were observed in women with POI compared to controls. However, the presence of metabolic syndrome was significantly increased in women with POI (Ates et al. 2014). In contrast, other research groups reported increased serum glucose, insulin, and HOMA-IR in POI women ($n = 43$) versus controls ($n = 33$) (Kulaksizoglu et al. 2013). Regardless of the varying study results regarding lipid profiles and insulin resistance, the overall cardiovascular risk in POI women seems to be significantly increased (Kulaksizoglu et al. 2013). In a recent study, the lifetime estrogen exposure in women with POI was linked to the cardiovascular disease risk. It appeared that prolonged estrogen deprivation was associated with an increased estimated risk of CVD. This study result could support the policy of early use of hormonal replacement therapy (Christ et al. 2018). The risk for

ischemic heart disease mortality is roughly increased with 80% in women with POI compared to women with natural menopause age, at 49–55 years (Kalantaridou et al. 2004; Podfigurna-Stopa et al. 2016).

Bone Density

The association between estrogen deficiency in the postmenopausal period and osteoporosis has been clearly established by the North American Menopause Society (Schnatz 2011). The pioneering work of Fuller Albright presented a relationship between estrogen deficiency, menopause, and an increased incidence of fractures in women (Albright 1940). Young women exposed to hypoestrogenism and hypoandrogenemia also experience such consequences; these hormonal deficiencies have a detrimental effect on peak bone mass (PBM) formation and bone mineral density (BMD) status (Meczekalski et al. 2010). It appears that up to 90% of PBM is achieved before the age of 18. Therefore, hypoestrogenic state in women with POI has a tremendous negative impact on BMD, particularly because estrogen deficiency may appear in the phase of intensive gain in bone mass (Leite-Silva et al. 2009). BMD status is assessed by dual-energy X-ray absorptiometry (DEXA). In women with POI, a decrease of BMD is observed in the lumbar spine and femoral neck. Bone loss associated with hypoestrogenic state is greater in trabecular bone and less in cortical bone (Szeliga et al. 2018). In women with spontaneous POI, serum FSH concentrations, but not estradiol, are positively associated with bone mass loss in skeletal regions (both the spinal column and femoral neck) (Podfigurna-Stopa et al. 2016). A large study including 442 cases revealed that POI patients have a lower BMD compared to regularly menstruating women: 15% of POI patients had a Z-score below -2 compared to 3% of the controls ($p = 0.005$) (Popat et al. 2009). In a study of 50 Brazilian women with POI, a decrease in the lumbar spine and femoral BMD was observed. The lumbar part of the spine was the most affected by the BMD decrease. They reported that age generally, age of POI, and reproductive age were factors associated with the BMD of the lumbar spine (Leite-Silva et al. 2009). Total body BMD clearly corresponds to the duration of ovarian function in POI patients (Podfigurna-Stopa et al. 2016). Women with menopause between ages 40 and 44 years have lower vertebral BMD compared to those with natural menopause. While BMD decreased in both groups over time, the difference in vertebral BMD did not differ between those with early or natural menopause after age 55 years.

Chemotherapy in young age can lead to the temporary or permanent cessation of ovarian function. POI patients after chemotherapy due to gynecological malignancies had significantly decreased BMD when compared to controls (39% vs. 15%, respectively; $p = 0.009$) (Stavraka et al. 2013). Loss of BMD was not measured directly after chemotherapy but after more than 18 months of chemotherapy. This observation indicates that not chemotherapy on itself but decreased concentration of estrogens due to POI is the reason for the BMD decrease. Unfortunately, there is limited evidence available on fracture risk in POI patients. A Dutch study with over

4,700 postmenopausal women, 50–80 years of age, revealed that women experiencing early menopause had a significantly increased overall fracture rate (OR, 1.5; 95% CI, 1.2–1.8), in comparison with women who experience menopause at a normal age (van der Voort et al. 2003). Although studies have demonstrated greater bone loss in the initial years following estrogen deficiency in women with early menopause compared to women with menopause at average age, the results of studies assessing fracture risk in women with early versus average age menopause have been inconsistent (Hadjidakis et al. 2003; Faubion et al. 2015). The most contributing risk factors to BMD loss in POI are degree and duration of estrogen deficiency and nonadherence to estrogen replacement therapy (Webber et al. 2016). Additional risk factors for decreased BMD in POI are early diagnosis of POI, more than 1 year delay in diagnosis of POI, low concentration of serum vitamin D, low calcium intake, and lack of physical exercise (Szeliga et al. 2018).

Cardiovascular Disease Risk

In women with POI, the decreased life expectancy is mainly caused by cardiovascular disease. Endothelial dysfunction, measured as the flow-mediated dilation of the brachial artery, is one of the markers of early signs of cardiovascular disease. Endothelial function appears to be significantly reduced in POI women (Kalantaridou et al. 2004). Evidence on carotid intima media thickness and women with POI remains inconsistent (Yorgun et al. 2013, Daan et al. 2016,). The left ventricular diastolic function does seem deteriorated in women with POI, in comparison with healthy controls (Yorgun et al. 2013). Interestingly, hormonal therapy used for 6 months improves the flow-mediated dilation by 2.4-fold, similar to levels of the control population (Kalantaridou et al. 2004). The cardiovascular risk was worse in women who experienced early menopause compared to women with menopause at age 50 or older, with the most unfavorable risk in the subgroup of women with early bilateral salpingo-oophorectomy (BSO) (Atsma et al. 2006).

Iatrogenesis and spontaneous POI patients have a two times higher risk for angina and higher severity of angina 1 year post-myocardial infarction compared with women experiencing menopause at age 50 or later (Parashar et al. 2010). An association has been suggested between early menopause and heart failure risk. In the Multi-Ethnic Study of Atherosclerosis, the risk of heart failure was increased by 66% in those undergoing menopause before the age of 45 compared with women experiencing menopause after 45 years of age. A decrease of 4% in risk was observed for each year increase in menopausal age (Ebong et al. 2014). An increase in the incidence (36%) of heart failure was detected in women with natural menopause at age 40–45 compared with women with a menopausal age between 50 and 54 years of age. A decrease of 2% in heart failure risk was observed with each year increase of menopausal age. Whether a causal association exists between estrogen deficiency and the development of heart failure remains unclear. It also remains unclear whether other cardiovascular risk factors contribute to earlier age at menopause. Inconsistency in evidence must be noted here as well.

Not all studies report increased risk of stroke in women with premature menopause, but multiple observational studies have demonstrated that early-onset menopause is linked with an increased risk of ischemic stroke (Rocca et al. 2012). Although hormonal therapy poses an increased risk of stroke for older women, it is associated with reduced stroke in women before the age of 50 (Rocca et al. 2012). All together we may conclude that early menopause is related to increased risk of cardiovascular disease events and mortality. The greatest risk is observed in women undergoing early BSO.

Psychological Impact and Emotional Health

Psychosocial and emotional impact of POI is often overshadowed by its clinical impact. Their mood is often influenced negatively by concerns on their personal health and concerns on their fertility. Women with POI may show various psychological disturbances, depression and low self-esteem, which can effect sexuality negatively (Schmidt et al. 2011). The diagnosis of POI is often a devastating life event, from which women experience lots of anger, depression, anxiety, loss and sadness, hostility, and psychological distress. Directly after the diagnosis, women can experience shock and confusion; therefore all healthcare providers should offer support regarding the patient's altered self-image, sexual dysfunction, and neurocognitive decline (Podfigurna-Stopa et al. 2016). Low androgens – next to estrogens, which are also observed in women with POI – may also be related to impaired psychosexual dysfunction (Janse et al. 2012). An American research group investigated the presence of depression in women with spontaneous POI. They studied 174 women with spontaneous 46,XX POI and 100 women with Turner syndrome and compared prevalence of depressive symptoms in these patients with community-based controls. POI proved to be associated with an increased lifetime risk for major depression, higher than in Turner syndrome and controls ($p < 0.001$). The researchers advocated that more attention should be paid to symptoms of depression in POI. Moreover, the onset of depression frequently occurred after signs of altered ovarian function but before the diagnosis of POI (Schmidt et al. 2011).

Evidence on sexual well-being reveals that women with POI were less satisfied with their sexual life. They experienced fewer sexual fantasies and masturbated less frequently. Their sexual activities were associated with less sexual arousal, reduced lubrication, and increased genital pain. These observations were made despite of the fact that women with POI and controls had similar frequency of desire to have sex and similar frequency of actual sexual contact with a partner. Women with POI did have lower levels of estradiol, total testosterone, and androstenedione. But androgen levels had only a weak influence on sexual functioning; higher total testosterone levels were associated with increased frequency of desire for sexual contact, and higher androstenedione levels were associated with elevated frequency of sexual contact (Van Der Stege et al. 2008). A cross-sectional study comparing sexual function of 58 women with POI with regularly menstruating women demonstrated

that POI patients suffer from decreased sexual functioning: less satisfaction, lubrication, and orgasms and more pain. Sexual arousal however was similar in both groups (de Almeida et al. 2011). Women experiencing a surgical menopause are at an increased risk for hypoactive sexual desire disorder (HSDD), with less satisfaction regarding sexual contact, relationships, and negative emotional states, compared to premenopausal or naturally menopausal women (Dennerstein et al. 2006).

Early menopause is also associated with neurological dysfunction and higher risk for dementia. This increase is most apparent in the global cognitive domain and in verbal memory tests. Findings related to the loss of cognitive function after chemotherapy or GnRH analogue treatments are inconsistent (Nappi et al. 1999; Podfigurna-Stopa et al. 2016). Attentive and verbal memory performances were observed in physiological and surgical menopause. Surgical menopause appeared to affect short-term verbal memory more than physiological menopause. Furthermore, early age at surgical menopause was associated with cognitive deterioration and presence of Alzheimer's disease. The Mayo Clinic Cohort Study of Oophorectomy and Ageing reported on women who had undergone a bilateral oophorectomy before they went into natural menopause. The research group reported that women experienced a long-term increased risk of parkinsonism, cognitive impairment or dementia, and depressive and anxiety symptoms (Rocca et al. 2009). A dose effect of estrogen deficiency on dementia exists, and the age-dependent effect suggests that the younger brain is more vulnerable to estrogen deficiency (Phung et al. 2010; Podfigurna-Stopa et al. 2016).

Treatment

HRT

POI results in all the complications associated with the menopause but at a much younger age. The need for HRT in young women with POI extends beyond the need for symptom relief. The purpose of HRT is to maintain sufficient estradiol concentrations, so it will protect POI women against serious morbidity and earlier mortality related to prolonged estrogen deficiency. It should be taken until the age of ca. 50 years which is the median age of physiological menopause (Webber et al. 2016).

Little evidence is available on effects of all subtypes of estrogen replacement for women with POI. However, it is suggested that physiological sex steroid replacement therapy is preferred over combined oral contraceptive pill (COCP) use. COCP contains more steroid hormone than needed for replacement purposes and therefore increases the risk for negative side effects, such as dyslipidemia and risk for thromboembolic events. If better compliance is reached with COCP use, this would be a fair alternative. A wish for contraception may also encourage prescribing COCPs. On women with POI and a uterus in situ, there is no evidence available on progestogen replacement therapy. In postmenopausal women no difference was found between oral cyclical micronized natural progesterone and cyclical or

continuous medroxyprogesterone acetate for endometrial protection. It is suggested that natural progesterone has a positive influence on the cardiovascular profile and potentially a lower risk of mamma carcinoma (Luisi et al. 2015; Webber et al. 2016).

The majority of women with POI prefers a cyclical combined HRT regimen. Patients can decide individually how long they continue with the HRT each "cycle"; recommended is no longer than 12 weeks. The addition of cyclic progesterone for 10–12 days each month is protective against endometrial hyperplasia and endometrial cancer (Webber et al. 2016).

The evidence is not definitive; however, a tendency toward prescribing transdermal estrogens is supported, due to less side effects. Transdermal HRT may be preferred in women with coagulation disturbances and those more prone to thrombosis, such as patients with thrombophilias. The patient's preference on the administration route is most important (Webber et al. 2016).

Transdermal, oral, or transvaginal estradiol in doses of 100 micrograms daily is the therapy of choice to mimic a physiologic dose range and to achieve symptom relief. The dosage of progestogen depends on the regimen of estrogen replacement. Continuous regimens require a minimum dose of 1 mg of oral norethisterone daily or 2.5 mg medroxyprogesterone acetate (MPA) at the moderate to high doses of estrogen that should be provided for women with POI. Sequential regimens require 10 mg MPA for a minimum of 10–12 days per month or 200 mg micronized oral progesterone (Webber et al. 2016).

Data on androgen therapy is very scarce and long-term health effects are still unclear. Androgen replacement therapy may be used for the following indications: diminished sexual function, neurological complaints (working memory), and decreased bone density (Webber et al. 2016).

Vitamin D

Findings of the Nurses' Health Study cohort suggest that vitamin D intake from nutrition sources, dairy in specific, is associated with a lower risk of early menopause. Because vitamin D is obtained through dietary intake as well as sunlight exposure, concentrations of 25-hydroxyvitamin D [25(OH)D], the primary circulating metabolite of vitamin D, is a more reliable indicator of vitamin D status than dietary intake alone. Studies evaluating 25(OH)D concentrations and AMH are conflicting (Drakopoulos et al. 2017; Purdue-Smithe et al. 2018). Evidence suggest that levels of AMH vary with each season, which correlates with 25(OH)D concentrations and that vitamin D supplementation prevented a seasonal decline in AMH concentrations (Dennis et al. 2012). A study in immunocompromised American women found 25(OH)D to be positively associated with AMH concentrations (Merhi et al. 2012); however, two other studies did not report a significant association (Pearce et al. 2015; Drakopoulos et al. 2017). In a more recent study, authors reported no association between vitamin D metabolite and the risk of early menopause or with concentrations of plasma AMH. Contrariwise, vitamin D-binding protein concentrations were positively associated with an increased risk

of early menopause (Purdue-Smithe et al. 2018). Although further evaluation in large, ethnically diverse populations is needed to clarify the association between early menopause and vitamin D, adequate calcium and vitamin D intake is important for bone health of these patients at risk of fractures. The recommendations for calcium and vitamin D intake for women experiencing menopause are similar, regardless of age at menopause. The Institute of Medicine recommends a daily intake of 1,200 mg of calcium and 600 IU of vitamin D for women aged 51–70. The National Osteoporosis Foundation advocates counseling women to reduce their risk for osteoporosis and fracture. They recommend the following: stop smoking, avoiding excess alcohol intake, assessing risk of falling, healthy body weight, physical exercise, and a daily intake of 1,200 mg of calcium for women 51 years of age and older and 800–1000 IU of vitamin D, including supplements if needed (Cosman et al. 2014; Faubion et al. 2015). There is no clear evidence-based recommendation for timing of BMD testing in POI. Measurement of BMD must be considered at initial diagnosis by all women with POI. When there are any additional risk factors, a BMD is recommended. If BMD is normal and adequate HRT is prescribed, additional BMD testing has no added value (Webber et al. 2016). In women with mamma carcinoma, BMD testing is suggested within 3 months of therapy-induced menopause. In women who underwent risk-reducing BSO, who do not use estrogen therapy, a 2-year window for measurement of BMD has been recommended (Faubion et al. 2015).

Women diagnosed with POI should visit their physician to assess general health status and the effectiveness of the adopted treatment (Faubion et al. 2015).

IVF and Oocyte Donation

For many women with POI, infertility is the most devastating aspect of the diagnosis. Women with POI do not respond to traditional fertility treatments, such as ovarian stimulation with hormonal. Their options to start a family include adoption, a small potential for spontaneous pregnancy, donor embryo, or egg donation using in vitro fertilization. Spontaneous pregnancy will occur in about 2.5–10% of women with 46,XX POI (Webber et al. 2016; Sullivan et al. 2016).

Researchers suggested that estrogen administration may help reduce endogenous gonadotropin concentrations, which may lead to an upregulation of FSH receptors in remaining follicles and potentially ovulation (Bidet et al. 2008). Also, the use of 100 micrograms transdermal estradiol led to the normalization of serum LH levels in half of the study group with POI patients. It is hypothesized that the normalized LH levels might avoid untimely luteinization of follicles, thereby improving follicle function and chances of ovulation (Popat et al. 2008). Dehydroepiandrosterone supplement use has been suggested to increase the number of retrieved oocytes in assisted conception cycles, improve pregnancy rates, and decrease miscarriage rates. COCP has been used prior to ovarian stimulation to lower FSH levels, but unfortunately data are limited (Langrish et al. 2009; Maclaran and Panay 2015). Overall, studies investigating other options to improve fertility in women with POI have an

inadequate sample size and are non-randomized. Embryo transfer using donor oocytes has demonstrated high success rates and is considered a preferred treatment. The pregnancy rate after an oocyte donation cycle is around 40%, and cumulative pregnancy rates after four cycles reach 70–80% (Męczekalski et al. 2018). Patients with Turner syndrome are also eligible for oocyte donation. Due to their increased risk for cardiovascular anomalies, women with Turner Syndrome should undergo a medical screening to reduce maternal cardiovascular mortality before receiving an embryo transfer. Pregnancies resulting from oocyte donation are associated with an increased risk for gestational hypertension and preeclampsia (Savasi et al. 2016; Męczekalski et al. 2018).

Future Possibilities

Experimental studies have put a traditional paradigm to the test. That is, women possess a finite ovarian pool of primordial follicles which irreversibly decline during life. Ovarian tissue cryopreservation followed by transplantation is rapidly gaining ground as a fertility preservation and restoration strategy. Techniques to improve the life span and quality of grafted ovarian tissue as well as to avoid transmission of malignant cells are being developed. Lots of new techniques involve stem cells due to their self-renewal and regeneration potential. Several types of stem cells are used in studies on treatment for POI patients, for example, mesenchymal stem cells, stem cells from extraembryonic tissues, induced pluripotent stem cells, and ovarian stem cells (Stimpfel et al. 2013; Leng et al. 2015; Męczekalski et al. 2018). Transplantation of stem cells into ovaries of adult mice resulted in mature oocytes, ovulation, and fertilization with the production of viable embryos. Oocyte renewal has a major potential for further development and consequently future clinical perspectives (Sheikhansari et al. 2018; Song et al. 2018).

In young girls and women of reproductive age, fertility preservation and restoration play a large role before and after oncological treatment, due to the reproductive risk of chemotherapeutics and improved long-term survival of cancer patients. The Prevention of Early Menopause Study was developed to study the effect of goserelin (gonadotropin-releasing hormone (GnRH) agonists) injections prior to and during chemotherapy for estrogen receptor-negative mamma carcinoma. In the treatment group, 8% of the women suffered from ovarian insufficiency against 22% in the placebo study group. Pregnancies occurred more often in the treatment group compared to the placebo group: 22% versus 12%, respectively (Gerber and Ortmann 2014). The presumed working mechanism of GnRH agonists is that they cause a diminished ovarian blood circulation while activating GnRH receptors on oocytes or granulosa cells. This biochemical cascade might result into upregulation of anti-apoptotic pathways and inhibition of accelerated follicular atresia through interruption of FSH secretion.

Curcumin (CRC) and capsaicin (CPS) are naturally occurring phytochemicals that have been found to have significant health benefits as painkillers, anticancer agents, and anti-inflammatory agents. The mechanisms underlying these health

effects have been attributed especially to their anti-inflammatory effects, which involve modification of macrophage function by decreasing production of pro-inflammatory mediators, reactive oxygen species, metabolites of arachidonic acid, proteases, and lysosomal enzymes (Meczekalski et al. 2010). CRC and CPS treatment of rats with cyclophosphamide-induced POF had a beneficial effect on reducing ovarian damage by improving tissue oxidative stress marker levels, ovarian reserve marker levels, and histopathological parameters. The significant improvements in ovarian tissue histopathological damage and hormonal levels detected indicate that treatment with CRC or CPS might be a conservative treatment approach for cyclophosphamide-induced POF (Melekoglu et al. 2018).

Resveratrol, a natural non-flavonoid polyphenol compound, has been shown to enhance rat ovarian function by activating the PI3K/Akt/mTOR signaling pathway. It diminishes oxidative stress and impedes granulocyte apoptosis. It is hypothesized that early application of resveratrol combined with other medication may provide a new treatment strategy for POI (Li et al. 2018).

Another novel treatment is in vitro activation (IVA) of primordial and preantral follicles (Zhai et al. 2016). These follicles of POI patients develop rarely; therefore, patients are unlikely to conceive with their own oocytes. IVA implements ovarian fragmentation disruption of Hippo signaling pathway and treatment with PI3K stimulator to activate primordial and preantral follicles. One cycle of IVA comprises laparoscopic removal of the ovary, cutting the ovary into cortical strips and vitrification. After thawing of cryopreserved ovarian tissues, the ovarian strips are further fragmented and incubated for 2 days with PI3K stimulators. After that, the ovarian strips are laparoscopically placed back into the patient. Then the patient undergoes full protocol of ovarian stimulation and IVF procedure (Zhai et al. 2016; Męczekalski et al. 2018). Great interest has been awakened in recent years for the possibility of creating an "artificial ovary," with the objective of reducing the possibility of disseminating malignant cells and maximizing the chances of survival and growth of isolated follicles and ovarian cells, recreating their optimal microenvironment (Luyckx et al. 2014). The concept of the "artificial ovary" consists in the packaging of oocytes and follicular cells in a biodegradable scaffold to maintain the three-dimensional structure. The scaffold should be constituted of a matrix to maintain the interactions between the oocyte and granulosa cells while revascularization occurs. Further research focused on the study of optimal matter is needed to increase graft survival by reducing the damage resulting from ischemia and oxidative stress.

Conclusion

POI has a detrimental effect on mental and physical health. Causes vary from genetic, iatrogenic, or autoimmune. Fertility preservation is of great importance for women at risk for POI. Long- and short-term consequences of POI are diverse, varying from mental health, bone density, sexual functioning, and cardiovascular health. Timing of treatment is vital for the quality of life of women with POI.

Cross-References

- ▶ Climacteric Syndrome
- ▶ Infertility
- ▶ Menopause and Bone Metabolism

References

Albright F. Post-menopausal osteoporosis. Trans Assoc Am Phys. 1940;55:298–305.
Ates S, Yesil G, Sevket O, et al. Comparison of metabolic profile and abdominal fat distribution between karyotypically normal women with premature ovarian insufficiency and age matched controls. Maturitas. 2014;79(3):306–10.
Atsma F, Bartelink ML, Grobbee DE, van der Schouw YT. Postmenopausal status and early menopause as independent risk factors for cardiovascular disease: a meta-analysis. Menopause. 2006;13(2):265–79.
Bachelot A, Rouxel A, Massin N, et al. Phenotyping and genetic studies of 357 consecutive patients presenting with premature ovarian failure. Eur J Endocrinol. 2009;161(1):179–87.
Bakalov VK, Anasti JN, Calis KA, et al. Autoimmune oophoritis as a mechanism of follicular dysfunction in women with 46,XX spontaneous premature ovarian failure. Fertil Steril. 2005;84(4):958–65.
Bidet M, Bachelot A, Touraine P. Premature ovarian failure: predictability of intermittent ovarian function and response to ovulation induction agents. Curr Opin Obstet Gynecol. 2008;20(4):416–20.
Christ JP, Gunning MN, Palla G, et al. Estrogen deprivation and cardiovascular disease risk in primary ovarian insufficiency. Fertil Steril. 2018;109(4):594–600.e1.
Conway GS, Kaltsas G, Patel A, et al. Characterization of idiopathic premature ovarian failure. Fertil Steril. 1996;65(2):337–41.
Cosman F, de Beur SJ, LeBoff MS, et al. Clinician's guide to prevention and treatment of osteoporosis. Osteoporos Int. 2014;25:2359–81.
Cramer DW, Xu H. Predicting age at menopause. Maturitas. 1996;23(3):319–26.
Crisponi L, Deiana M, Loi A, et al. The putative forkhead transcription factor FOXL2 is mutated in blepharophimosis/ptosis/ epicanthus inversus syndrome. Nat Genet. 2001;27:159–66.
Daan NM, Muka T, Koster MP, van Lennep JER, et al. Cardiovascular risk in women with premature ovarian insufficiency compared to premenopausal women at middle age. J Clin Endocrinol Metab. 2016;101(9):3306–15.
de Vos M, Devroey P, Fauser BC. Primary ovarian insufficiency. Lancet. 2010;376(9744):911–21.
Dennerstein L, Koochaki P, Barton I, Graziottin A. Hypoactive sexual desire disorder in menopausal women: a survey of Western European women. J Sex Med. 2006;3(2):212–22.
Dennis NA, Houghton LA, Jones GT, et al. The level of serum anti-Müllerian hormone correlates with vitamin D status in men and women but not in boys. J Clin Endocrinol Metab. 2012;97(7):2450–5.
Drakopoulos P, Van de Vijver A, Schutyser V, et al. The effect of serum vitamin D levels on ovarian reserve markers: a prospective cross-sectional study. Hum Reprod. 2017;32(1):208–14.
Ebong IA, Watson KE, Goff DC Jr, et al. Age at menopause and incident heart failure: the multi-ethnic study of atherosclerosis. Menopause. 2014;21(6):585–91.
Farquhar CM, Sadler L, Harvery SA, Stewart AW. The association of hysterectomy and menopause: a prospective cohort study. BJOG. 2005;112(7):956–62.
Faubion SS, Kuhle CL, Shuster LT, Rocca WA. Long-term health consequences of premature or early menopause and considerations for management. Climateric. 2015;18(4):483–91.
Fenton AJ. Premature ovarian insufficiency: pathogenesis and management. J Midlife Health. 2015;6(4):147–53.

Fleischer RT, Vollenhoven BJ, Weston GC. The effects of chemotherapy and radiotherapy on fertility in premenopausal women. Obstet Gynecol Surv. 2011;66(4):248–54.
Fridovich-Keil JL, Gubbels CS, Spencer JB, et al. Ovarian function in girls and women with GALT-deficiency galactosemia. J Inherit Metab Dis. 2011;34(2):357–66.
Gerber B, Ortmann O. Prevention of Early Menopause Study (POEMS): is it possible to preserve ovarian function by gonadotropin releasing hormone analogs (GnRHa)? Arch Gynecol Obstet. 2014;290(6):1051–3.
Hadjidakis DJ, Kokkinakis EP, Sfakianakis ME, Raptis SA. Bone density patterns after normal and premature menopause. Maturitas. 2003;44(4):279–86.
Hoek A, Schoemaker J, Drexhage HA. Premature ovarian failure and ovarian autoimmunity. Endocr Rev. 1997;18(1):107–34.
Hook EB, Warburton D. Turner syndrome revisited: review of new data supports the hypothesis that all viable 45,X cases are cryptic mosaics with a rescue cell line, implying an origin by mitotic loss. Hum Genet. 2014;133(4):417–24.
Hubayter ZR, Popat V, Vanderhoof VH, et al. A prospective evaluation of antral follicle function in women with 46,XX spontaneous primary ovarian insufficiency. Fertil Steril. 2010;94(5):1769–74.
Janse F, Knauff EAH, Niermeijer MF, et al. Similar phenotype characteristics comparing familial and sporadic premature ovarian failure (POF). Menopause. 2010;17(4):758–65.
Janse F, Tanahatoe SJ, Eijkemans MJ, Fauser BC. Testosterone concentrations, using different assays, in different types of ovarian insufficiency: a systematic review and meta-analysis. Hum Reprod Update. 2012;18(4):405–19.
Jiao X, Qin C, Li J, et al. Cytogenetic analysis of 531 Chinese women with premature ovarian failure. Hum Reprod. 2012;27:2201–7.
Jiao X, KE H, Qin Y, Chen Z. Molecular genetics of premature ovarian insufficiency. Trends Endocrinol Metab. 2018;29(11):795–807.
Kalantari H, Madani T, Zari Moradi S, et al. Cytogenetic analysis of 179 Iranian women with premature ovarian failure. Gynecol Endocrinol. 2013;29(6):588–91.
Kalantaridou SN, Naka KK, Papanikolaou E, et al. Impaired endothelial function in young women with premature ovarian failure: normalization with hormone therapy. J Clin Endocrinol Metab. 2004;89:3907–13.
Knauff EA, Westerveld HE, Goverde AJ, et al. Lipid profile of women with premature ovarian failure. Menopause. 2008;15(5):919–23.
Knauff EA, Eijkemans MJ, Lambalk CB, et al. Anti-Mullerian hormone, inhibin B, and antral follicle count in young women with ovarian failure. J Clin Endocrinol Metab. 2009;94(3):786–92.
Kulaksizoglu M, Ipekci SH, Kebapcilar L, et al. Risk factors for diabetes mellitus in women with primary ovarian insufficiency. Biol Trace Elem Res. 2013;154(3):313–20.
La Marca A, Brozzetti A, Sighinolfi G, et al. Primary ovarian insufficiency: autoimmune causes. Curr Opin Obstet Gynecol. 2010;22(4):277–82.
Lagergren K, Hammar M, Nedstrand E, Bladh M, Sydsjö G. The prevalence of primary ovarian insufficiency in Sweden; a national register study. BMC Womens Health. 2018;18(1):175.
Langrish JP, Mills NL, Bath LE, et al. Cardiovascular effects of physiological and standard sex steroid replacement regimens in premature ovarian failure. Hypertension. 2009;53(5):805–11.
Larsen EC, Müller J, Schmiegelow K, Rechnitzer C, Andersen AN. Reduced ovarian function in long-term survivors of radiation- and chemotherapy-treated childhood cancer. J Clin Endocrinol Metab. 2003;88(11):5307–14.
Leite-Silva P, Bedone A, Pinto-Neto AM, Costa JV, Costa-Paiva L. Factors associated with bone density in young women with karyotypically normal spontaneous premature ovarian failure. Arch Gynecol Obstet. 2009;280(2):177–81.
Leng L, Tan Y, Gong F, et al. Differentiation of primordial germ cells from induced pluripotent stem cells of primary ovarian insufficiency. Hum Reprod. 2015;30(3):737–48.
Luborsky JL, Meyer P, Sowers MF, Gold EB, Santoro N. Premature menopause in a multi-ethnic population study of the menopause transition. Hum Reprod. 2003;18(1):199–206.
Luisi S, Orlandini C, Regini C, Pizzo A, Vellucci F, Petraglia F. Premature ovarian insufficiency: from pathogenesis to clinical management. J Endocrinol Investig. 2015;38(6):597–603.

Luyckx V, Dolmans MM, Vanacker J, et al. A new step toward the artificial ovary: survival and proliferation of isolated murine follicles after autologous transplantation in a fibrin scaffold. Fertil Steril. 2014;101(4):1149–56.
Maclaran K, Panay N. Current concepts in premature ovarian insufficiency. Womens Health (Lond). 2015;11(2):169–82.
Maltaris T, Seufert R, Fischl F, et al. The effect of cancer treatment of female fertility and strategies for preserving fertility. Eur J Obstet Gynecol Reprod Biol. 2007;130:148–55.
Meczekalski B, Podfigurna-stopa A, Genazzani AR. Hypoestrogenism in young women and its influence on bone mass density. Gynecol Endocrinol. 2010;26(9):652–7.
Męczekalski B, Maciejewska-jeske M, Podfigurna A. Reproduction in premature ovarian insufficiency patients – from latest studies to therapeutic approach. Gynecol Endocrinol. 2018;17(3):117–9.
Meirow D, Nugent D. The effects of radiotherapy and chemotherapy on female reproduction. Hum Reprod Update. 2001;7(6):535–43.
Melekoglu R, Ciftci O, Eraslan S, Cetin A, Basak N. Beneficial effects of curcumin and capsaicin on cyclophosphamide-induced premature ovarian failure in a rat model. J Ovarian Res. 2018;11(1):33.
Merhi ZO, Seifer DB, Weedon J, et al. Circulating vitamin D correlates with serum antimüllerian hormone levels in late-reproductive-aged women: Women's Interagency HIV Study. Fertil Steril. 2012;98(1):228–34.
Morrison JC, Givens JR, Wiser WL, Fish SA. Mumps oophoritis: a cause of premature menopause. Fertil Steril. 1975;26(7):655–9.
Murray A, Schoemaker MJ, Bennett CE, et al. Population-based estimates of the prevalence of FMR1 expansion mutations in women with early menopause and primary ovarian insufficiency. Genet Med. 2014;16:19–24.
Nappi RE, Sinforiani E, Mauri M, Bono G, Polatti FNG. Memory functioning at menopause : impact of age in ovariectomized women. Gynecol Obstet Investig. 1999;47(1):29–36.
Ohl J, Partisani M, Demangeat C, et al. Alterations of ovarian reserve tests in Human Immunodeficiency Virus (HIV)-infected women. Gynecol Obstet Fertil. 2010;38(5):313–7.
Parashar S, Reid KJ, Spertus JA, Shaw LJ, Vaccarino V. Early menopause predicts angina after myocardial infarction. Menopause. 2010;17(5):938–45.
Pearce K, Gleeson K, Tremellen K. Serum anti-Mullerian hormone production is not correlated with seasonal fluctuations of vitamin D status in ovulatory or PCOS women. Hum Reprod. 2015;30(9):2171–7.
Perry JR, Corre T, Esko T, et al. A genome-wide association study of early menopause and the combined impact of identified variants. Hum Mol Genet. 2013;22(7):1465–72.
Persani L, Rossetti R, Cacciatore C. Genes involved in human premature ovarian failure. J Mol Endocrinol. 2010;45(5):257–79.
Phung TK, Waltoft BL, Laursen TM, et al. Hysterectomy, oophorectomy and risk of dementia: a nationwide historical cohort study. Dement Geriatr Cogn Disord. 2010;30(1):43–50 61.
Podfigurna-Stopa A, Czyzyk A, Grymowicz M, et al. Premature ovarian insufficiency : the context of long term effects. J Endocrinol Investig. 2016;39(9):983–90.
Popat VB, Vanderhoof VH, Calis KA, Troendle JF, Nelson LM. Normalization of serum luteinizing hormone levels in women with 46,XX spontaneous primary ovarian insufficiency. Fertil Steril. 2008;89(2):429–33.
Popat VB, Calis KA, Vanderhoof VH, et al. Bone mineral density in estrogen-deficient young women. J Clin Endocrinol Metab. 2009;94(7):2277–83.
Purdue-Smithe AC, Whitcomb BW, Manson JE, et al. Vitamin D status is not associated with risk of early menopause. J Nutr. 2018;148(9):1445–52.
Rocca WA, Shuster LT, Grossardt BR, et al. Long-term effects of bilateral oophorectomy on brain aging: unanswered questions from the Mayo Clinic Cohort Study of Oophorectomy and Aging. Womens Health (Lond). 2009;5(1):39–48.
Rocca WA, Grossardt BR, Miller VM, Shuster LT, Brown RJ. Premature menopause or early menopause and risk of ischemic stroke. Menopause. 2012;19(3):272–7.

Rossetti R, Di Pasquale E, Marozzi A, et al. BMP15 mutations associated with primary ovarian insufficiency cause a defective production of bioactive protein. Hum Mutat. 2009;30(5):804–10.
Savasi VM, Mandia L, Laoreti A. Maternal and fetal outcomes in oocyte donation pregnancies. Hum Reprod Update. 2016;22(5):620–33.
Schmidt PJ, Luff JA, Haq NA, et al. Depression in women with spontaneous 46, XX primary ovarian insufficiency. J Clin Endocrinol Metab. 2011;96:278–87.
Schnatz PF. The 2010 North American Menopause Society position statement: updates on screening, prevention and management of postmenopausal osteoporosis. Conn Med. 2011;75(8):485–7.
Sheikhansari G, Aghebati-Maleki L, Nouri M, Jadidi-Niaragh F, Yousefi M. Current approaches for the treatment of premature ovarian failure with stem cell therapy. Biomed Pharmacother. 2018;102:254–62.
Shifren JL, Gass ML, NAMS recommendations for Clinical Care of Midlife Women Working Group. The North American Menopause Society recommendations for clinical care of midlife women. Menopause. 2014;21(10):1038–62.
Simpson JL. Genetic and phenotypic heterogeneity in ovarian failure: overview of selected candidate genes. Ann N Y Acad Sci. 2008;1135:146–54.
Song K, Cai H, Zhang D, et al. Effects of human adipose-derived mesenchymal stem cells combined with estrogen on regulatory T cells in patients with premature ovarian insufficiency. Int Immunopharmacol. 2018;55:257–62.
Stavraka C, Maclaran K, Gabra H, et al. A study to evaluate the cause of bone demineralization in gynecological cancer survivors. Oncologist. 2013;18(4):423–9.
Stimpfel M, Skutella T, Cvjeticanin B, et al. Characterization and differentiation of cells expressing pluripotent/multipotent markers from adult human ovaries. Cell Tissue Res. 2013; 354(2):593–607.
Sullivan SD, Sarrel PM, Nelson LM. Hormone replacement therapy in young women with primary ovarian insufficiency and early menopause. Fertil Steril. 2016;106(7):1588–99.
Szeliga A, Maciejewska-Jeske M, Męczekalski B. Bone health and evaluation of bone mineral density in patients with premature ovarian insufficiency. Prz Menopauzalny. 2018;17(3):112–6.
Tucker EJ, Gover SR, Bachelot A, et al. Premature ovarian insufficiency: new perspectives on genetic cause and phenotypic spectrum. Endocr Rev. 2016;37(6):609–35.
van Asselt KM, Kok HS, Pearson PL, et al. Heritability of menopausal age in mothers and daughters. Fertil Steril. 2004;82(5):1348–51.
Van Der Stege JG, Groen H, Van Zadelhoff SJN, Van Santbrink EJP, Apperloo MJA. Decreased androgen concentrations and diminished general and sexual well-being in women with premature ovarian failure. Menopause. 2008;15(1):23–31.
van der Voort DJ, van Der Weijer PH, Barentsen R. Early menopause: increased fracture risk at older age. Osteoporos Int. 2003;14(6):525–30.
Webber L, Davies M, Anderson R, et al. European Society for Human Reproduction and Embryology (ESHRE) guideline: management of women with premature ovarian insufficiency. Hum Reprod. 2016;31(5):926–37.
Wittenberger MD, Hagerman RJ, Sherman SL, et al. The FMR1 premutation and reproduction. Fertil Steril. 2007;87(3):738–44.
Yorgun H, Tokgözoğlu L, Canpolat U, et al. Cardiovascular effects of premature ovarian failure. Int J Cardiol. 2013;168(1):506–10.
Zhai J, Yao G, Dong F, et al. In vitro activation of follicles and fresh tissue auto-transplantation in primary ovarian insufficiency patients. J Clin Endocrinol Metab. 2016;10:4405–12.
Zinn AR, Page DC, Fisher EMC. Turner syndrome: the case of the missing sex chromosome. Trends Genet. 1993;9(3):90–3.

Climacteric Syndrome

16

Agnieszka Podfigurna, Anna Szeliga, and Błażej Męczekalski

Contents

Abstract

The climacteric is the phase in the aging of women characterized by transition from the reproductive phase to the nonreproductive state. A woman is considered postmenopausal when she is over the age of 45 and has gone at least 12 months without a spontaneous menstrual period. The climacteric is sometimes, but not indispensable, associated with symptomatology. Symptoms associated with climacteric, such as hot flushes, night sweats, fatigue, headache, dizziness,

A. Podfigurna · A. Szeliga · B. Męczekalski (✉)
Department of Gynecological Endocrinology, Poznan University of Medical Sciences, Poznan, Poland
e-mail: agnieszkapodfigurna@gmail.com; annamaria.szeliga@gmail.com; blazejmeczekalski@yahoo.com

F. Petraglia, B. C. Fauser (eds.), *Female Reproductive Dysfunction*, Endocrinology,
https://doi.org/10.1007/978-3-030-14782-2_16

numb, sore limbs, decreased attention, anxiousness and nervousness, insomnia, mood swings, and sorrow depression, are called "climacteric syndrome." Women during menopausal transition are particularly vulnerable to mood disturbances and depression. This period is regarded as the important risk of these disorders. Depressive symptoms present increasing tendency during menopausal transition and decreasing tendency after menopause. Mood changes during menopausal transition have detrimental effects on these women's quality of life. Decline of cognitive function during menopausal transition and menopause is referred to aging and to direct and indirect effects of hormonal changes on the brain. Direct effects concern direct influences of hormonal changes on the regions of neuronal system responsible for cognitive function control. Indirect effects are related to hormonal change influences on the brain which have impact on other symptoms like sleep, mood changes, and vasomotor symptoms. Vasomotor symptoms (VMS), which include hot flushes and night sweats, affect 70% of postmenopausal women <55 years of age. VMS often severely impact physical, sexual, and psychosocial life and overall well-being. Symptoms usually last from 6 to 10 years (median 7.4 years), and most women report that VMS are the most bothersome of all postmenopausal symptoms. Sleep disturbances are very meaningly affected by other menopausal symptoms such as hot flushes. Sleep disturbances may occur in the form of insomnia, sleep-disordered breathing, restless legs syndrome (RLS), mood and anxiety disorders, and other medical diseases associated with vasomotor symptoms and aging.

Keywords

Climacteric syndrome · Vasomotor symptoms · STRAW · Menopause · Hot flushes · Night sweats

Introduction

The climacteric is the phase in the aging of women characterized by transition from the reproductive phase to the nonreproductive state, while the menopause is defined as the final menstrual period. A woman is considered postmenopausal when she is over the age of 45 and has gone at least 12 months without a spontaneous menstrual period. A menopausal woman who has undergone at least 12 months of amenorrhea is unlikely to ever have another menstrual period; however, it can occur in about 10% of women.

Menopause occurs during the climacteric, currently around the age of 51. Many lifestyle factors that may affect timing of menopause include education, occupation, income, smoking, physical activity, and body mass index (BMI). Of these, smoking has been consistently recognized to have an association with earlier menopause (Schoenaker et al. 2014).

Menopause may be either spontaneous (natural menopause) or iatrogenic (secondary menopause). The latter includes ovarian failure caused by chemotherapy or radiotherapy as well as removal of both ovaries (surgical menopause). Menopause before age 40 years is considered to be pathological and is referred to as primary ovarian insufficiency (premature ovarian failure).

The climacteric is sometimes, but not indispensable, associated with symptomatology (Utian 2004). Symptoms associated with climacteric, such as hot flushes, night sweats, fatigue, headache, dizziness, numb, sore limbs, decreased attention, anxiousness and nervousness, insomnia, mood swings, and sorrow depression, are clinically called "menopause syndrome" or "climacteric syndrome" (Zhou et al. 2012).

STRAW

The 2001 Stages of Reproductive Aging Workshop (STRAW) proposed nomenclature and a staging system for ovarian aging including menstrual and hormonal criteria as a definition of each stage.

The STRAW classification separates a woman's life into seven segments, with segments −2, −1, and 0 including the early menopausal transition, the late menopausal transition, and the final menstrual period, respectively (Davis et al. 2015).

Taking into consideration the candidate biomarkers considered in 2001, FSH was only one parameter consistently measurable in a clinical setting; therefore, staging recommendations were based on menstrual cycle bleeding criteria and qualitative follicle-stimulating hormone (FSH) criteria. Stages of Reproductive Aging Workshop +10 (STRAW +10) developed in 2012 is more advanced and updated clinical tool for health providers. It characterizes critical changes in hypothalamic-pituitary and ovarian function that occur before and after the final menstrual period (Harlow et al. 2012). Based on FSH, antral follicle count (AFC), anti-Müllerian hormone (AMH), estradiol, and inhibin-B, STRAW +10 is a tool helping to determine the onset of late reproductive life and early menopausal transition and ensures criteria for staging postmenopause. The STRAW staging system can be also used as the evaluation tool for assessment of fertility or contraceptive needs.

Despite the fact that 2001 STRAW was a useful clinical tool, it was not applicable to certain groups of women, namely, women with a body mass index (BMI) greater than 30 kg/m^2, smokers, women after hysterectomy, women engaged in heavy aerobic exercise, and women with abnormal menstrual cycles, uterine or ovarian abnormalities, or significant illness such as cancer. Researches on ovarian function during menopausal transition conducted in 10 years after 2001 STRAW indicated that smoking and BMI, however, influence hormonal levels and the timing of transition, and they do not alter the trajectory of change in hormonal levels or bleeding patterns. This allowed to create STRAW +10 staging system as applicable to women regardless of age, demographic, BMI, or lifestyle characteristics.

STRAW +10

STRAW +10 divides women's lives into three main periods of time: reproduction, menopausal transition, and postmenopause (Fig. 1). Late reproductive stage is characterized by subtle changes in menstrual cycle characteristics, specifically shorter cycles. It is caused by increased and variable day 2–5 FSH concentration and becomes the first symptom of following menopausal transition. Early menopausal transition is associated with variability in menstrual cycle length exceeding 7 days in the length of consecutive cycles. Variability in this stage is usually persistent, which is defined as recurrence within ten cycles of the first variable length cycle. Increased variability is associated with low AMH levels and low AFC. Reduction in cycle length is followed by gradual prolongation of cycles, leading to amenorrhea periods lasting 60 days or longer, characteristic for late menopausal transition. More pronounced variations of FSH concentration are observed in this stage; nevertheless serum FSH higher than >25 IU/L is diagnostic for this stage. Late menopausal transition lasts usually 1–3 years, while the next stage, early menopause, is about 2-year long. It is characterized by stabilization of high FSH and low estradiol. Last period, remaining lifespan, is late menopause. Hormonal profile is usually stabile, although many years after menopause, there may be a further decline in levels of FSH in very old persons. Symptoms of vaginal dryness and urogenital atrophy become increasingly prevalent at this time.

Estradiol

Serum estradiol (E2) levels change significantly across the menopausal transition and into early postmenopause. Pronounced decline in E2 levels begins, on average, 2 years before the FMP and lasts until 2 years after FMP. In the time between 2 and 6 years after the FMP (early postmenopause +1b and +1c), the mean rate of change was insignificantly indicating stabile E2 concentration. Six to eight years after FMP, second decline in E2 concentration is observed; probably this change represents exhaustion of theca cells. Interestingly, women with a greater BMI (>30 kg/m^2) did not develop the second decline in E2 levels (Sowers et al. 2008a).

FSH

FSH stimulates folliculogenesis, a key in the dynamics of ovarian aging. Parallel with the age-related decline in the oocyte quality and quantity, there are progressively higher FSH levels observed. Second, the rise in FSH levels is a premise of significant declines in ovarian steroid secretion, including estradiol secretion. Changes in FSH concentration associated with menopausal status can be divided into 4 stages (Fig. 2). The major change in FSH concentration is present at 45 years, while the major deceleration in changes of FSH concentration occurs at the age 55 years (Sowers et al. 2008b).

	Reproductive		Menopausal transition			Postmenopause			
	Late reproductive		Early menopausal transition	Late menopausal transition		Early menopause			Late menopause
			Perimenopause						
	-3b	-3a	-2	-1		1a	1b	1c	+2
Lenght [years]	variable		variable	1-3 y		1 y	1y	3-6 y	remaining lifespan
Menstrual cycles	regular	shorter cycles	increased variability in menstrual cycle length (≥7 days) persisting > 10 cycles	amenorrhea of 60 days or longer; increased variability in cycle length		stabilization of amenorrhea Abnormal uterine bleeding- needs forther diagnosis			
FSH on day 2-5	N	increase/ variable	increased but variable	variable; greater than 25 IU/L in a random blood draw - characteristic of being in late transition		the period of rapid changes in mean FSH and estradiol levels.		stabilization of high FSH levels and low estradiol values	may be a further decline in levels of FSH in very old persons
AMH*	low	low	low	low		low		very low	
AFC	low	low	low	low		very low		very low	
Inhibin-B	low	low	low	low		low		very low	
Symptoms	N	N	N	symptoms, most notably vasomotor symptoms, are likely to occur during this stage		symptoms, most notably vasomotor symptoms, are likely to occur during this stage		vaginal dryness and urogenital atrophy become increasingly prevalent	

Final Menstrual Period (FMP)

Fig. 1 Stages of Reproductive Aging Workshop (STRAW) in women. Based on Harlow et al. (2012) and Davis et al. (2015)

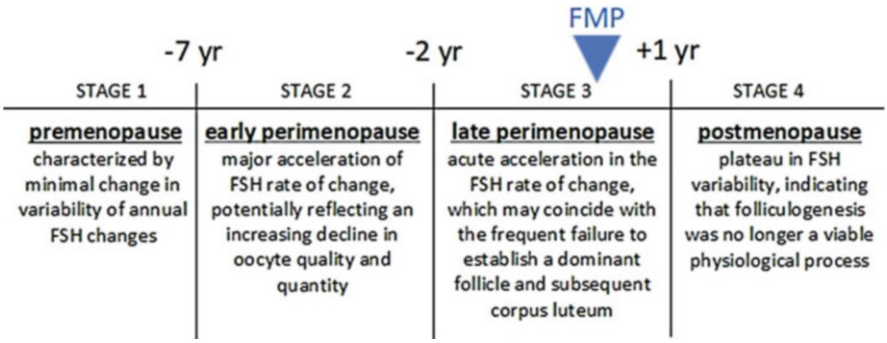


Fig. 2 FSH changes from perimenopausal state to postmenopause. Based on Sowers et al. (2008b)

AMH

In females, AMH, also known as Müllerian-inhibiting substance, is produced in the granulosa cells of ovarian follicles. It reflects the transition of small primordial follicles into growing follicles. It is produced at all follicle development stages until FSH dependency is achieved (6–7 mm in diameter of follicle). Taking this into consideration, AMH is a promising candidate to mark critical thresholds for menopausal transition.

AMH levels decrease markedly and progressively across the STRAW stages. AMH levels remain relatively stable until age 30. After this age linear decline in AMH concentration to values below detection at a time 5 years before the FMP is observed (La Marca et al. 2010; Sowers et al. 2008c). AMH level of 0.086 μg/L (detection limit of the assay: 0.026 μg/L) would seem to be a reliable cutoff for the menopausal transition (van Disseldorp et al. 2008).

Inhibin B

Inhibin B, produced by granulosa and theca cells of growing follicles, is a marker of the growth of small antral follicles. Concentration of inhibin B should be assessed in early follicular phase of cycle as its concentration may be influenced by cycle phase as well as body mass and perhaps ethnicity.

Physiologically, increased inhibin B concentration suppresses FSH secretion from the pituitary gland. Decrease in small follicle's number in menopausal transition causes decrease in inhibin B concentration, which in turn leads to increase in FSH concentration. The inverse relationship between declining inhibin B and rising FSH is consistent with loss of FSH suppression by inhibin B. Four to five years before the FMP inhibin B reaches a level in which the follicle number is too limited for successful recruitment or that follicles do not response to the elevated FSH levels about. Nevertheless, inhibin B concentration decelerates in a curvilinear manner; thus assessment of particular reproductive stage is more difficult when compared to

parameters declining in linear manner, like AMH. The absence of detectable inhibin B occurring 5 years before the FMP is a reflection of the diminishing FSH-sensitive antral follicle pool (Sowers et al. 2008c; Welt and Schneyer 2001).

Atrial Follicular Count (AFC)

Reproductive status is also represented by number of antral follicles assessed in ultrasonography (AFC). It is considered to reflect reproductive status because it is related to age at menopause and age at birth of the last child. However, in some studies no longitudinal changes in AFC were observed across examined fertile women over 4 years; thus, it is suspected that only low AFC provide clinically useful estimates of reproductive status. Moreover, AFC is particularly dependent on health provider experience and shows some cycle-to-cycle variation. AMH and AFC values have been shown to be highly correlated, and levels of AMH, which is produced by granulosa cells of small antral follicles in the size range of 2–7 mm, are considered to be a reflection of the size of the primordial follicle pool (Liu et al. 2011; Broekmans et al. 2009).

Climacteric Syndrome

Mood Changes

Mood is very important for proper functioning in the personal, professional, and social life. Regarding mood changes the most important problems concern depression. There is an important difference in the depression risk between sexes. Women present two times higher depression risk than men (Kessler et al. 2003). Major depression lifetime prevalence (in all age groups) in women is 21% compared to 11% in men. Considering women at age 45–54, major depression occurs in approximately 5,0% of women. Recurrent depression rate is indeed the highest in this group of women (45–54 years of age) (Soares 2017).

Background of such difference should be referred to differences in the hormonal and psychosocial environment. From this point of view, reproductive changes such as puberty, postpartum, and menopause can be of critical significance.

There is difference between low mood and depression. Low mood includes sadness, feeling anxious or panicky, worry, tiredness, low self-esteem, frustration, and anger. Generally low mood can be changed to normal mood or can be progressed to depression (Freeman 2015).

Symptoms of depression are more serious and are presented as:

- Low mood lasting 2 weeks or more
- Not getting any enjoyment out of life
- Feeling hopeless
- Feeling tired or lacking energy

- Not being able to concentrate on everyday things
- Comfort eating or losing appetite
- Sleeping more than usual or being unable to sleep
- Having suicidal thoughts or thoughts about harming yourself

Mood disturbances and depression can occur during menopausal transition. This period is regarded as the important risk of these disorders (about three times higher risk in comparison to menopause) (Carranza-Lira and Palacios-Ramírez 2019). Penn Ovarian Ageing Study revealed that depressive symptoms present increasing tendency during menopausal transition and decreasing tendency after menopause (Freeman and Sammel 2016). Mood changes during menopausal transition have detrimental effects on these women's quality of life. Women with previous history of depressive illness, those with severe hot flushes and sleep disorders, and those after surgical menopause are more prone to develop depression. Reciprocal influence of described problems is called "domino effect."

There are differences in depression presentation between women of reproductive age and women during menopause transition. Generally menopausal women present less tension and depressive symptoms but present more sleep disturbances, anger/hostility, or fatigue/inertia (Freeman 2015).

Etiopathogenesis

Hormonal changes during menopausal transition can contribute to mood changes' development. They can be referred to as decrease in sex steroid (mainly estradiol) levels. Estrogens have important impact on neurotransmitter secretion and action, their receptor expression, and membrane permeability.

Estrogens positively affect serotonin and norepinephrine action, which are neurotransmitters controlling mood status.

Specific effects of estrogen on mood can be listed as follows (Russell et al. 2019):

- Decrease of monoamine oxidase which impairs the breakdown of serotonin and norepinephrine
- Increase of serotonin synthesis
- Increase of norepinephrine activity
- Stimulation of the synthesis of brain-derived neurotrophic factor (BDNF)

Estrogen receptors are widely distributed in the brain regions (prefrontal cortex and hippocampus) responsible for mood regulations. Taking into consideration the biological role of estrogen on mood, the fluctuation of estradiol levels over time can have important impact on menopause-related depression.

Other symptoms typical for menopause as vasomotor symptoms (VMS) and sleep disorders may significantly impair mood in pre- and postmenopausal women (Raglan et al. 2019). Fragmented sleep can contribute to depression, but on the other hand, depression can worsen sleep quality in menopausal women.

Additionally, regarding etiopathogenesis of mood disorders in menopausal women, other associated factors should be considered. They include health problems

which occur at this time of life: cardiovascular disorders, urogenital problems, metabolic syndrome, and osteoporosis (Gracia and Freeman 2018).

Finally, psychosocial stressors which include poor social support and stressful life events occurring at this time of female life are important in relation to the background of menopausal mood changes.

Mood Change Evaluation

Specific tools to evaluate the depressive symptoms are very important from clinical point of view also in the field of menopause. These tools can be referred to rating scales, which are psychiatric instrument having descriptive words and phrases that indicate the severity of depression for a time period. Generally these scales can be divided into three categories: scales completed by researches, scales completed by patients, and scales completed by patients and researchers (Willi and Ehlert 2019).

Regarding the first category, the best example is Hamilton Depression Rating Scale. Other popular scales from this group are Montgomery-Asberg Depression Rating Scale and Raskin Depression Rating Scale.

The most popular scale completed by patients is the Beck Depression Inventory.

The Primary Care Evaluation of Mental Disorders (PRIME-MD) scale is a scale completed by patients and researchers.

Hamilton Depression Rating Scale was developed by Dr. Max Hamilton from the University of Leeds. It is composed of 21 items, but the scoring is based on first 17 items (Table 1) (Hamilton 1960).

Montgomery-Asberg Depression Rating Scale was developed to measure the severity of depressive episodes in patients with mood disorders. It was developed in 1978 as an improvement of some part of Hamilton Depression Rating Scale and is composed of ten items. Raskin Depression Rating Scale enables the evaluation of baseline depression level and changes in depression severity over time.

Beck Depression Inventory was developed by Aaron Beck in 1961. It is a multiple-choice self-reported inventory composed of 21 questions. Described rating scales for depression evaluation are very useful for assessment of mood changes in menopausal women (Table 2) (Georgakis et al. 2016; Beck 1961).

Primary Care Evaluation of Mental Disorders (PRIME-MD) is a questionnaire which is composed of 26 yes/no questions about the presence of symptoms and signs during the past month. This evaluation serves as an initial screen for five general groups of mental disorders commonly found in the general population.

Cognitive Functions

Cognitive functions can be understood as mental processes that allow a person to carry out any task using receiving, choosing, transforming, storing, processing, and retrieving of information. Decline of cognitive function and dementia become real problem for public health, because worldwide prevalence of dementia is supposed to increase three times by 2050. This decline of cognitive function can represent broad range from mild cognitive impairment (MCI) to dementia. Dementia can be

Table 1 Hamilton Rating Scale for Depression

Instructions: For each item select the "cue" which best characterizes the patient during the past week.
1. Depressed mood (sadness, hopeless, helpless, worthless)
0 Absent
1 These feeling states indicated only on questioning
2 These feeling states spontaneously reported verbally
3 Communicates feeling states nonverbally, i.e., through facial expression, posture, voice, and tendency to weep
4 Patient reports VIRTUALLY ONLY these feeling states in his spontaneous verbal and nonverbal communication
2. Feelings of guilt
0 Absent
1 Self-reproach, feels he has let people down
2 Ideas of guilt or rumination over past errors or sinful deeds
3 Present illness is a punishment. Delusions of guilt
4 Hears accusatory or denunciatory voices and/or experiences threatening visual hallucinations
3. Suicide
0 Absent
1 Feels life is not worth living
2 Wishes he were dead or any thoughts of possible death to self
3 Suicide ideas or gesture
4 Attempts at suicide (any serious attempt rates 4)
4. Insomnia – early
0 No difficulty falling asleep
1 Complains of occasional difficulty falling asleep, i.e., more than an hour
2 Complains of nightly difficulty falling asleep
5. Insomnia – middle
0 No difficulty
1 Patient complains of being restless and disturbed during the night
2 Waking during the night – any getting out of bed rates 2 (except for purposes of voiding)
6. Insomnia – late
0 No difficulty
1 Waking in early hours of the morning but goes back to sleep
2 Unable to fall asleep again if gets out of bed
7. Work and activities
0 No difficulty
1 Thoughts and feelings of incapacity, fatigue, or weakness related to activities, work, or hobbies
2 Loss of interest in activity, hobbies, or work – either directly reported by patient or indirect in listlessness, indecision, and vacillation (feels he has to push self to work or activities)
3 Decrease in actual time spent in activities or decrease in productivity. In hospital, rate 3 if patient does not spend at least 3 h a day in activities (hospital job or hobbies) exclusive of ward chores
4 Stopped working because of present illness. In hospital, rate 4 if patient engages in no activities except ward chores or if patient fails to perform ward chores unassisted

(continued)

Table 1 (continued)

8. Retardation (slowness of thought and speech; impaired ability to concentrate; decreased motor activity)
0 Normal speech and thought
1 Slight retardation at interview
2 Obvious retardation at interview
3 Interview difficult
4 Complete stupor
9. Agitation
0 None
1 "Playing with" hand, hair, etc.
2 Hand-wringing, nail-biting, biting of lips
10. Anxiety – psychic
0 No difficulty
1 Subjective tension and irritability
2 Worrying about minor matters
3 Apprehensive attitude apparent in face or speech
4 Fears expressed without questioning
11. Anxiety – somatic
0 Absent physiological concomitants of anxiety such as:
1 Mild gastrointestinal – dry mouth, wind, indigestion,
2 Moderate diarrhea, cramps, belching
3 Severe cardiovascular – palpitations, headaches
4 Incapacitating respiratory – hyperventilation, sighing, urinary frequency, sweating
12. Somatic symptoms – gastrointestinal
0 None
1 Loss of appetite but eating without staff encouragement. Heavy feelings in abdomen
2 Difficulty eating without staff urging. Requests or requires laxatives or medications for bowels or medication for GI symptoms
13. Somatic symptoms – general
0 None
1 Heaviness in limbs, back, or head, backaches, headache, muscle aches, loss of energy, and fatigability
2 Any clear-cut symptom rates 2
14. Genital symptoms
0 Absent 0 Not ascertained
1 Mild symptoms such as loss of libido
2 Severe menstrual disturbances
15. Hypochondriasis
0 Not present
1 Self-absorption (bodily)
2 Preoccupation with health
3 Frequent complaints, requests for help, etc.
4 Hypochondriacal delusions

(continued)

Table 1 (continued)

16. Loss of weight
A. When rating by history:
0 No weight loss
1 Probable weight loss associated with present illness
2 Definite (according to patient) weight loss
B. On weekly ratings by ward psychiatrist, when actual changes are measured:
0 Less than 1 lb. weight loss in a week
1 Greater than 1 lb. weight loss in a week
2 Greater than 2 lb. weight loss in a week
17. Insight
0 Acknowledges being depressed and ill
1 Acknowledges illness but attributes cause to bad food, climate, overwork, virus, need for rest, etc.
2 Denies being ill at all
Total score:

defined as decline in one or more such cognitive representations as language, learning and memory, complex attention, executive function, social cognition, and perceptual-motor function which impair daily function (Morgan et al. 2018). Alzheimer disease (AD) accounts for approximately 60–80% cases of dementia. MCI is an intermediate state between normal cognitive function and dementia.

Although presented problem is of great clinical significance, there are limited studies focusing on evaluation of cognitive functions during menopause transition. Data from epidemiological studies provide some indication of an association between increased lifetime endogenous exposure and decreased risk of poor cognition (Guarnieri 2019). However, these studies have some limitations due to numbers of participants and focusing on only small numbers of reproductive events such as age at menarche, age at menopause, and parity.

There is hypothesis that endogenous estrogen exposure (EEE) can protect against dementia. Dementia is understood as mental ability decline which impairs daily life. This hypothesis is supported by inverse correlation between indicators of lifetime EEE and late life cognitive functions. Additionally, observation comes from results of study which evaluated cognitive function in patients with spontaneous and surgically induced premature ovarian insufficiency. Large meta-analysis revealed that surgical menopause made by bilateral oophorectomy at <45 years of age may be associated with higher risk of dementia and cognitive functions decline (Georgakis et al. 2019). Results from numerous studies concerning menopausal women and dementia risk are contradictory. For instance, Paganinni-Hill (Paganini-Hill and Henderson 1994) reported that increased incidence of AD in older women may be related to estrogen deficiency. The largest study in this field from 2018 did not support hypothesis that EEE across the reproductive period affects the risk of dementia in late life (Prince et al. 2018).

Table 2 Beck's depression inventory

This depression inventory can be self-scored. The scoring scale is at the end of the questionnaire	
1.	0 I do not feel sad 1 I feel sad 2 I am sad all the time and I can't snap out of it 3 I am so sad and unhappy that I can't stand it
2.	0 I am not particularly discouraged about the future 1 I feel discouraged about the future 2 I feel I have nothing to look forward to 3 I feel the future is hopeless and that things cannot improve
3.	0 I do not feel like a failure 1 I feel I have failed more than the average person 2 As I look back on my life, all I can see is a lot of failures 3 I feel I am a complete failure as a person
4.	0 I get as much satisfaction out of things as I used to 1 I don't enjoy things the way I used to 2 I don't get real satisfaction out of anything anymore 3 I am dissatisfied or bored with everything
5.	0 I don't feel particularly guilty 1 I feel guilty a good part of the time 2 I feel quite guilty most of the time 3 I feel guilty all of the time
6.	0 I don't feel I am being punished 1 I feel I may be punished 2 I expect to be punished 3 I feel I am being punished
7.	0 I don't feel disappointed in myself 1 I am disappointed in myself 2 I am disgusted with myself 3 I hate myself
8.	0 I don't feel I am any worse than anybody else 1 I am critical of myself for my weaknesses or mistakes 2 I blame myself all the time for my faults 3 I blame myself for everything bad that happens
9.	0 I don't have any thoughts of killing myself 1 I have thoughts of killing myself, but I would not carry them out 2 I would like to kill myself 3 I would kill myself if I had the chance
10.	0 I don't cry any more than usual 1 I cry more now than I used to 2 I cry all the time now 3 I used to be able to cry, but now I can't cry even though I want to
11.	0 I am no more irritated by things than I ever was 1 I am slightly more irritated now than usual 2 I am quite annoyed or irritated a good deal of the time 3 I feel irritated all the time
12.	0 I have not lost interest in other people 1 I am less interested in other people than I used to be 2 I have lost most of my interest in other people 3 I have lost all of my interest in other people

(continued)

Table 2 (continued)

13.	0 I make decisions about as well as I ever could 1 I put off making decisions more than I used to 2 I have greater difficulty in making decisions more than I used to 3 I can't make decisions at all anymore
14.	0 I don't feel that I look any worse than I used to 1 I am worried that I am looking old or unattractive 2 I feel there are permanent changes in my appearance that make me look unattractive 3 I believe that I look ugly
15.	0 I can work about as well as before 1 It takes an extra effort to get started at doing something 2 I have to push myself very hard to do anything 3 I can't do any work at all
16.	0 I can sleep as well as usual 1 I don't sleep as well as I used to 2 I wake up 1–2 h earlier than usual and find it hard to get back to sleep 3 I wake up several hours earlier than I used to and cannot get back to sleep
17.	0 I don't get more tired than usual 1 I get tired more easily than I used to 2 I get tired from doing almost anything 3 I am too tired to do anything
18.	0 My appetite is no worse than usual 1 My appetite is not as good as it used to be 2 My appetite is much worse now 3 I have no appetite at all anymore
19.	0 I haven't lost much weight, if any, lately 1 I have lost more than 5 pounds 2 I have lost more than 10 pounds 3 I have lost more than 15 pounds
20.	0 I am no more worried about my health than usual 1 I am worried about physical problems like aches, pains, upset stomach, or constipation 2 I am very worried about physical problems and it's hard to think of much else 3 I am so worried about my physical problems that I cannot think of anything else
21.	0 I have not noticed any recent change in my interest in sex 1 I am less interested in sex than I used to be 2 I have almost no interest in sex 3 I have lost interest in sex completely

Interpreting the Beck Depression Inventory
Total score_____
Levels of depression
1–10_____These ups and downs are considered normal
11–16_____Mild mood disturbance
17–20_____Borderline clinical depression
21–30_____Moderate depression
31–40_____Severe depression
Over 40_____Extreme depression

Etiopathogenesis

Decline of cognitive function during menopausal transition and menopause is referred to aging and to direct and indirect effects of hormonal changes on the brain. Direct effects concern direct influences of hormonal changes on the regions of neuronal system responsible for cognitive function control.

Indirect effects are related to hormonal change influences on brain which have impact on other symptoms like sleep, mood changes, and vasomotor symptoms (Khadilkar and Patil 2019).

Specific regions of the brain like basal forebrain represent high numbers of estrogen receptors. Basal forebrain is a major source of cholinergic innervations to the hippocampus which is responsible for memory and learning. Estrogen augments acetylcholine synthesis through the stimulation activity of choline acetyltransferase. Similarly, estrogen stimulates glutamate system which is also responsible for processes related to memory and learning.

Other roles of estrogen in the aspect of cognitive functions include (Riedel et al. 2016):

- Neurotransmission modulation
- Neurotrophic regulation
- Neuroprotective action
- Maintenance of mitochondrial bioenergetics
- Attenuation of tau hyperphosphorylation and deposition of amyloid beta (main processes related to neurodegeneration in Alzheimer disease)

Cognitive Function Evaluation

There is now need to screen routinely menopausal and older women for cognition (who do not present symptoms) decline. When the screening is important at the beginning, it should include medical history (family history, drug history) (Wesnes 2006). Information which are presented by family members are very valuable. If needed neurological consultation should be realized. Cognitive testing also should be considered. It includes Mini-Mental State Examination (MMSE) or Montreal Cognitive Assessment (MOcA). MMSE is based on testing orientation, attention, memory, language, and visual-spatial skills. MOcA evaluates short-term memory, visuospatial ability, executive functions, attention and concentration, language ability, and orientation (Goldstein et al. 2014).

Additionally for this group of patients, screening for depression is important because in some cases depression is the cause of typical decline of cognitive functions. Patients with suspected dementia should undergo neuroimaging procedures (magnetic resonance of head or computerized tomography of head). Evaluation of vitamin B12 deficiency and hypothyroidism is also important.

Sleep Disturbances

The perimenopausal period and the numerous physiological symptoms associated with it most often begin about 4 years before the last menstrual period in life. Many

of these symptoms can have a huge impact on the quality of life for women during menopause.

One of these symptoms may be sleep disturbances, which significantly contribute to the deterioration of patients' quality of life. Sleep disturbances are very meaningly affected by other menopausal symptoms such as hot flushes.

Sleep disturbances may occur in the form of insomnia, sleep-disordered breathing, restless legs syndrome (RLS), mood and anxiety disorders, and other medical diseases associated with vasomotor symptoms and aging.

Sleep disorders are 1.3–1.8 times more common in women than men.

Due to the fact that hot flushes most often occur at night, they can indeed affect the sleep of patients, waking them from sleep during night. However, it should be noted that menopausal women also experience sleep disorders despite the absence of hot flushes.

The incidence of primary sleep disorders in the population of women during menopause is about 53%. Patients most commonly report sleep disturbances such as sleep apnea or restless legs syndrome.

In the early menopause, the incidence of sleep disorders is about 32–40%, reaching up to 46% of women over the years.

In addition, menopausal symptoms such as anxiety and depression may also contribute to the occurrence of sleep disorders and may even occur primarily to sleep disorders (Freedman and Roehrs 2007).

Insomnia is the most common type of sleep disorder in menopausal women. It can be both primary and secondary, caused by vasomotor symptoms (hot flushes), mood disorders (depression and anxiety), and other medical reasons such as diminished bone density or urogynecological disturbances.

Treatment of women for vasomotor symptoms can significantly improve sleep disorders. However, due to various causes of sleep disorders (such as primary sleep disorders, anxiety, and depression), this treatment is not always fully effective.

Sleep Disturbance Etiopathogenesis

The etiopathogenesis of sleep disorders in menopausal women most likely includes hormonal changes. An important hormone that has a very significant effect on sleep is progesterone. It is known that intravenous progesterone has direct sedative properties, stimulating benzodiazepine receptors, and has anxiolytic action. During the normal menstrual cycle, we observe a significant peak of progesterone secretion during the luteal phase which may be the cause of sleep disorders.

Additionally, estrogens clearly have a significant impact on sleep. Estrogens are involved in the metabolism of noradrenaline, serotonin, and acetylcholine, increasing REM cycles in humans. Hypoestrogenism during menopause may be responsible for increasing sleep latency, quantity of awakenings during sleep, and spontaneous arousals, thus reducing overall sleep time (Lukacs et al. 2004).

The effect of estrogen on sleep also occurs through its effect on cortisol. Menopausal women have an earlier morning cortisol peak that may be reflected in sleep disorders.

Estrogens are important in normalizing this morning cortisol concentration, thereby stabilizing sleep. In addition, menopausal women are significantly more sensitive to elevated cortisol levels at night.

Another hormone relevant to sleep disorders is melatonin, which is responsible for circadian rhythm. With age, there is a decrease in melatonin, which can lead to sleep disorders. Reduced melatonin levels have been found in postmenopausal women suffering from insomnia.

The effect of testosterone on sleep is associated with its negative effect on obstructive sleep apnea. Increased testosterone levels caused by exogenous administration of this hormone may increase apnea. However, the reason for this is not fully understood, so further research is needed in this area (Netzer et al. 2003).

Sleep Disorder Evaluation

Estimation of sleep disorders should be performed as objectively as possible. Routine sleep assessment is done using polysomnography or actigraphy, systems that enable the detection of movements or their absence by analyzing sleep. Polysomnography assesses the different stages of sleep using electroencephalography, electromyography, and electrooculography. During the test, by making measurements, electrical currents radiated from the scalp, muscles, and eyes are detected. Thanks to such a system as polysomnography, it is possible to obtain not only an objective measurement but also direct assessment of brain function and somatic activity during the sleep process.

Actigraphy refers to insomnia, periodic limb movements of sleep, and postmenopausal women (Kalleinen et al. 2008).

Sexual Function (Libido) Decrease

Sexual disorders are submitted by thereabout 40% of women worldwide.

It is known that for a significant group of women who are in long-term relationships, desire may be absent before any sexual activity, but it may occur during activity in response to sexual pleasure.

Sexual response should be considered and analyzed in an interpersonal context. For some women, it is desire that most often initiates sexual activity, while for a large proportion of women, sexual activity is motivated by other factors, such as maintaining emotional ties or strengthening relationships with their partner.

Pain in sexual dysfunction in women is a separate category of sexual dysfunction, often with well-defined causes and types of treatment. These disorders include genitourinary syndrome of menopause (including vulvar and vaginal atrophy), pelvic floor disorders (formerly known as vaginosis), and other forms of dyspareunia. Consequently, pain during sexual intercourse often leads to disorders of desire and excitement.

Sexual Function (Libido) Decrease Etiopathogenesis

Changes in sexual function in peri- and postmenopausal women occur due to the effect of hypoestrogenism on vulvovaginal tissues and pelvic floor. This is most commonly due to natural or surgical menopause but may also occur in other hypoestrogenism-related situations such as premature ovarian insufficiency, hypothalamic amenorrhea, postpartum or lactation period, or using antiestrogenic drugs.

Among premenopausal women, estradiol is the dominant form of circulating estrogen. Its serum levels in premenopausal women are relieved during the menstrual cycle.

Estrogen acts on its receptors that are found in the vagina, vulva, urethra, and bladder. Thanks to this action, optimal blood flow within the genitals is maintained, and adequate collagen content in the epithelium is ensured, affecting its thickness and elasticity. In addition, the action of estrogens in the vagina, vulva, urethra, and bladder is responsible for the maintenance of acid mucopolysaccharides and hyaluronic acid, which maintain the moisture of epithelial surfaces.

Adequate estrogen levels ensure suitable thickness of nonkeratinized stratified squamous epithelium of the vagina. That is why this vagina epithelium is rugated and rich in glycogen, which is the substrate for Döderlein's lactobacilli. Subsequently, glucose is being converted to lactic acid causing an acidic vaginal environment (pH: from 3.5 to 5.0). Adequately acidic vaginal pH maintains normal vaginal flora, protecting and preventing infections within the vagina and urinary tract.

Menopause inevitably and physiologically leads to a drastic reduction in estrogen secretion. Estrogen production decreases during menopause compared to the reproductive period by approximately 95%. In postmenopausal women, we observe that estrogen levels reach a certain type of plateau at an average level of 5 pg/ml in serum (Lee et al. 2006).

Of course, such a huge decrease in estrogen concentration results from the physiological aging process and is additionally responsible for a number of adverse changes, which include vaginal atrophy, among others.

However, changes in the genitourinary system usually develop gradually and last for years, and in many women, they remain until treatment begins.

Changes in the vagina resulting from hypoestrogenism include mainly thinning of the top layer of superficial epithelial cells, even leading to a situation in which this layer does not exist at all, as is the case with severe atrophy. The thinning of the vaginal epithelium reveals the underlying connective tissue which is more susceptible to inflammation or infection. Moreover, the epithelium thinning of vagina obviously increases the frequency of injuries, leading to bleeding and subsequently to ulceration under any pressure, which may be sexual intercourse or even performing of a Pap test.

Reduced glycogen content in the thinned vaginal epithelium may lead to an increase in vaginal pH by limiting lactic acid secretion by lactobacilli. Increased vaginal pH can be the reason of atrophic vaginitis because of inflammation modifications and flora changes.

The above changes in the vagina may cause overgrowth of nonacidophilic coliforms, reducing the content of lactobacillus species. As a consequence, there is an increased risk of infection with the rectal and skin flora (staphylococci, streptococci, coliforms, diphtheroids), as well as Trichomonas species (Miller et al. 2016).

Vaginal changes resulting from reduced estrogen levels may also apply reduced elasticity of the vaginal epithelium and decline of rugae. In addition, the vaginal canal may become shorter and narrower with loss of expansion.

Genitourinary syndrome of menopause (GSM) includes genitourinary symptoms resulting from decreases in estrogen levels. The term GSM includes symptoms from the labia, vagina, urethra, and bladder.

Symptoms of GSM include symptoms such as vaginal dryness, burning, and irritation, sexual symptoms of reduced hydration, reduced vascular congestion during sexual excitement, as well as pain. Numerous urinary tract symptoms such as pain when passing urine and recurrent urinary tract infections can also negatively affect female sexual activity (Portman and Gass 2014).

One of the components of GSM is vulvovaginal atrophy (VVA), which can often cause pain during sexual activity. It is caused by low serum estrogen levels, causing thinning of the vulva and vagina, and as a consequence dryness, pain, tissue fragility, and numerous minor abrasions are experienced by women during sexual intercourse.

In menopausal women, VVA symptoms have a very important negative impact on their sexual function.

In the sexual dysfunction of women during menopause, most likely, besides estrogens, androgens are also important. However, their role is not yet fully understood and proven. It is believed that supraphysiological doses of androgens in menopausal women may increase libido and the frequency of sexual intercourse (Shifren and Davis 2017).

In addition, vasomotor symptoms may also affect sexual function through negative effects on sleep.

Sexual Function (Libido) Decrease Evaluation

Assessment of sexual dysfunction can be performed on female patients by analyzing typical medical history and sexual history.

In addition, a detailed medical examination should always be carried out when assessing sexual dysfunction to discover the etiopathogenesis of the disorder.

Pelvic examination is only required in situations of pain assessment and diagnosis resulting from sexual activities.

The American Psychiatric Association (APA) has established diagnostic criteria for sexual disorders. According to these criteria, the disorder should last for a minimum of 6 months, be recurrent or persistent, cause personal distress, and not result from another diagnosis.

Sexual disorders most often involve many phases of the sexual response cycle.

The 2013 APA diagnostic criteria cover many categories, such as female sexual interest/arousal disorder, female orgasmic disorder, genito-pelvic pain/penetration disorder, substance-/medication-induced sexual dysfunction (American Psychiatric Association (APA) 2013).

Broadly understood sexual disorders can take various forms, such as lack of sexual desire, impairment or inability to reach orgasm, as well as pain during sexual activity. Sexual dysfunction can be a problem that affects young girls when they start sexual activity, or they can appear later in life after years of satisfactory sexual activity. Moreover, in the evaluation and treatment of sexual dysfunction, very helpful might be an understanding of sexual response.

The traditional definition of the sexual response cycle was divided into four phases: desire (libido), arousal (excitement), orgasm, and resolution. For each individual woman, the phases may differ in sequence, overlap, repeat, or be even missing during some or all sexual intercourses. Additionally, interestingly, the individual's subjective satisfaction with certain sexual experiences may also not apply to all stages of the reaction, including orgasm (American Psychiatric Association (APA) 2013).

Vasomotor Symptoms

Vasomotor symptoms (VMS), which include hot flushes and night sweats affect 70% of postmenopausal women <55 years of age, while 28.5% of women suffer from moderate to severe symptoms with at least 10% of women reporting them as intolerable(Prague et al. 2017). VMS often severely impact physical, sexual, and psychosocial life and overall well-being. Symptoms usually last from 6 to 10 years (median 7.4 years), and most women report that VMS are the most bothersome of all postmenopausal symptoms. The mean age of VMS onset is 47 years, and they usually achieve peak of severity in late transition period. In some cases, however, some women suffer from VMS even after 20 years or more since the menopause.

Hot flushes are sudden episodes of vasodilation in the face and neck, while night sweats are hot flushes that occur at night and often interfere with sleep. VMS last on average 1–5 min (ranges from a few seconds to 60 min) and are accompanied by profuse sweating. A hot flush is characterized by an intense feeling of heat, which often rises through the body and spreads upward or downward. Hot flushes are accompanied by peripheral cutaneous vasodilatation, palpitations, behavioral change, and reddening of the skin to reduce temperature (Prague et al. 2017). They may be spontaneous or triggered by a variety of common situations such as embarrassment, sudden ambient temperature change, stress, alcohol, caffeine, or a warm drink (Sturdee et al. 2017).

Etiopathogenesis

Postmenopausal decrease in estrogen concentration followed by significant FSH, and particularly LH, concentration seems to play the key role in etiopathogenesis of VMS. Function of hypothalamic-pituitary-ovarian axis in humans is regulated by the hypothalamic neurons containing colocalized kisspeptin, neurokinin B (NKB), and dynorphin receptors (KNDy neurons). Latest trials give evidence that dysregulation of KNDy neurons, and in particular NKB secretion, is implicated in the etiology of the menopausal hot flushes.

In young, ovulating women KNDy neurons play key role in proper functioning of reproductive axis. KNDy synthesize NKB which stimulates, and dynorphin, which diminishes kisspeptin secretion in autocrine and paracrine manner. Kisspeptin in turn acting on GnRH neurons in median eminence stimulates GnRH secretion and subsequent FSH and LH secretion (Szeliga et al. 2018).

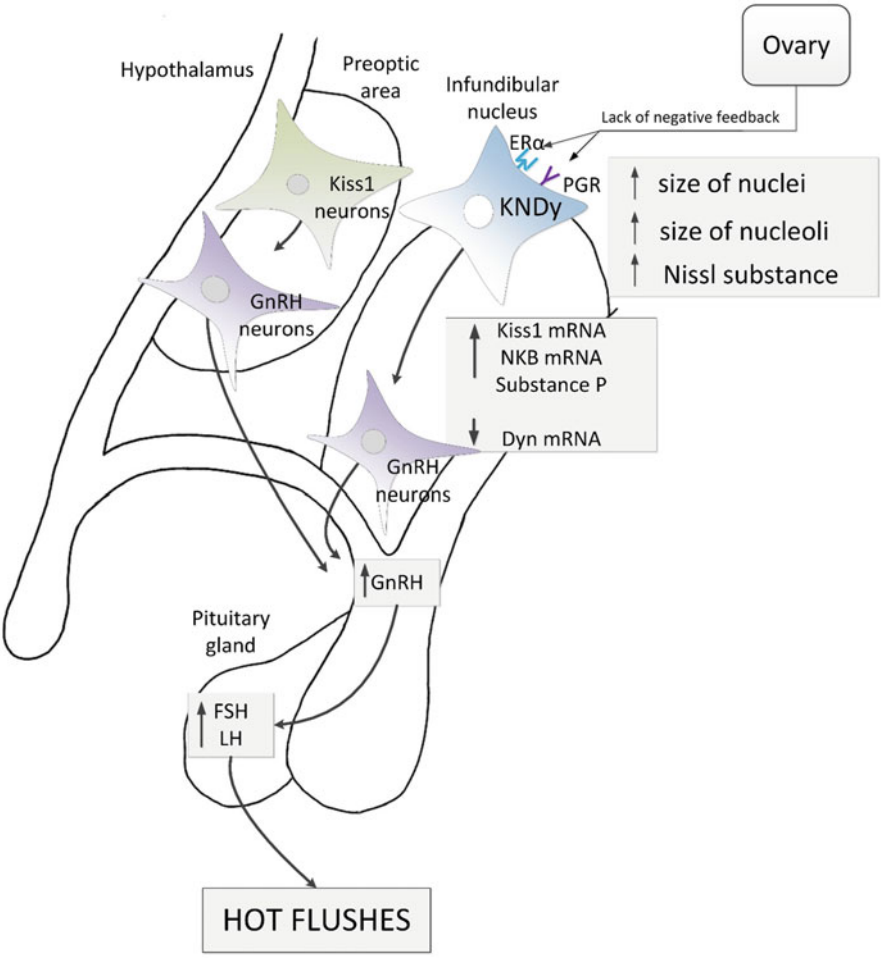


Fig. 3 Physiology of kisspeptin/neurokinin B/dynorphin neurons after menopause. (Reprinted from Szeliga et al. 2018)

It is observed that after menopause there is a hypertrophy of KNDy neurons in hypothalamic infundibular nucleus with subsequent hypersecretion of neurokinin B (NKB) and kisspeptin (Fig. 3). Increased amount of Nissl substance and enlargement of nuclei suggest increased neuronal activity and increased protein synthesis. Microscopic examination revealed a 30% increase in size of KNDy neurons. Similar processes are observed in ovariectomized monkeys, which may suggest that ovarian impairment and loss of estrogen negative feedback play the key role in this phenomenon.

Functional evidence has accumulated in parallel with synchronization of LH pulses and hot flushes. Taking into consideration that increased postmenopausal

LH secretion is associated with hyperstimulation by KNDy and GnRH neuron, it suggests that NKB signaling may play a key role in VMS pathogenesis. Further studies revealed that peripheral infusion of NK3R agonist caused a drop in core temperature and prevented acute tail vasoconstriction, which indirectly stimulated skin vasodilatation, heat dissipation, and creation of hot flushes in rats. Contrary, injection of selective toxin for NK3R-expressing neurons in median preoptic area (main site of thermoregulatory and reproductive axes integration) resulted in reduction of tail skin temperature in rodents. NKB intravenous administration to healthy premenopausal women stimulated hot flushes typical in location and duration to those described by postmenopausal women (Jayasena et al. 2015). Moreover, it was found that genetic variation in TACR3 gene, which encodes NK3R, may be responsible for variable intensity of hot flushes reported among menopausal women (Crandall et al. 2017). Taking into consideration those clinical premises, it was hypothesized that NKB antagonist may reduce generation of hot flushes. NK3R antagonist treatment decreases basal LH secretion (with no influence on FSH concentration) and reduces hot flush frequency, which may support a link between LH/GnRH pulsatility, NKB, and vasomotor symptoms (Skorupskaite et al. 2017). For the first time, NKB antagonist was used to treat menopausal hot flushes in postmenopausal women in 2017 by Prague et al. (2017). Administration of oral neurokinin 3 receptor antagonist (MLE4901) significantly reduced the total weekly number of hot flushes and weekly hot flush severity compared with placebo. The effect of decreased flushes by MLE4901 was confirmed by objective measurement using the skin conductance monitor and detection algorithm software. Mean weekly MENQOL domain scores showed that vasomotor symptoms improved when taking MLE4901 compared with placebo, as did the physical domain score and psychosocial domain score (Prague et al. 2017). Another study conducted by Prague et al. confirmed previous observations. MLE4901 rapidly reduced frequency, bother, severity and interference of vasomotor symptoms. Furthermore, similar improvements were seen in daytime and nighttime symptoms. Moreover participants experienced significant improvement in sleep (Prague et al. 2018).

Evaluation of VMS

Hot flushes are the most severe and fourth most prevalent menopausal symptom reported by women; thus, evaluation of their severity is a key factor in the diagnosis and tailored treatment. There are a few scales, which provide a clinical tool for VMS assessment which can be used by health providers.

The Greene Scale provides a brief measure of menopausal symptoms. It can be used to assess changes in psychological (divided in anxiety and depression), physical, and vasomotor domain both before and after menopause treatment. It takes 5 min to take this questionnaire which makes it a useful tool in diagnostic process.

The Women's Health Questionnaire (WHQ)

The WHQ is a 36-item questionnaire assessing 9 domains of physical and emotional health rated on 4-point scales. The following domains are covered by the questionnaire:

- Depressed mood (6 items)
- Somatic symptoms (7 items)
- Anxiety/fears (4 items)
- Vasomotor symptoms (2 items)
- Sleep problems (3 items)
- Sexual behavior (3 items)
- Menstrual symptoms (4 items)
- Memory/concentration (3 items)
- Attractiveness (3 items) (Hunter and Questionnairequality 2003)

The Menopause-Specific Quality of Life Questionnaire (MENQOL) was presented in 1996 as a tool to assess health-related quality of life in the postmenopausal period. It is specific to the menopausal state and demonstrated reliability and validity. Moreover, it includes all important domains of the menopause experience, and it is developed on women's own qualitative and quantitative menopausal symptoms. The MENQOL is self-administered and consists of 29 items. Each item assesses the impact of one of four domains of menopausal symptoms experienced over the last month: vasomotor (items 1–3), psychosocial (items 4–10), physical (items 11–26), and sexual (items 27–29) (Radtke et al. 2011).

Previously mentioned standard quality of life measures were intended as global measures of functioning or quality of life and were not designed to be specific to the impact of hot flushes. Hot Flash-Related Daily Interference Scale (HFRDIS) is the tool that was designed to measure specifically the impact of hot flushes on overall quality of life as well as on nine specific activities: work, social activities, leisure activities, sleep, mood, concentration, relations with others, sexuality, and enjoyment of life (Carpenter 2001).

Conclusion

The climacteric is the phase in the aging of women characterized by transition from the reproductive phase to the nonreproductive state. Women during menopausal transition are particularly vulnerable to mood disturbances and depression. Symptoms particularly associated with climacteric such as hot flushes, night sweats, fatigue, headache, dizziness, numb, sore limbs, decreased attention, anxiousness and nervousness, insomnia, mood swings, and sorrow depression are called "climacteric syndrome." Profound knowledge on pathophysiology of climacteric syndrome is necessary for correct treatment of VMS.

Cross-References

▶ Hormone Replacement Therapy (HRT)
▶ Long-Term Consequences of Menopause
▶ Menopause and Bone Metabolism

References

American Psychiatric Association (APA). Sexual dysfunctions. In: Diagnostic and statistical manual of mental disorders. 5th ed. Arlington: American Psychiatric Association; 2013.

Beck AT. An inventory for measuring depression. Arch Gen Psychiatry. 1961;4(6):561.

Broekmans FJ, Soules MR, Fauser BC. Ovarian aging: mechanisms and clinical consequences. Endocr Rev. 2009;30(5):465–93.

Carpenter JS. The hot flash related daily interference scale: a tool for assessing the impact of hot flushes on quality of life following breast cancer. J Pain Symptom Manag. 2001;22(6):979–89.

Carranza-Lira S, Palacios-Ramírez M. Depression frequency in premenopausal and postmenopausal women. Rev Med Inst Mex Seguro Soc. 2019;56(6):533–6.

Crandall CJ, Manson JE, Hohensee C, et al. Association of genetic variation in the tachykinin receptor 3 locus with hot flushes and night sweats in the Women's Health Initiative Study. Menopause. 2017;24(3):252–61.

Davis SR, Lambrinoudaki I, Lumsden M, et al. Menopause. Nat Rev Dis Primers. 2015;1(1):15004.

Freedman RR, Roehrs TA. Sleep disturbance in menopause. Menopause. 2007;14(5):826–9.

Freeman EW. Depression in the menopause transition: risks in the changing hormone milieu as observed in the general population. Women's Midlife Heal. 2015;1(1):2.

Freeman EW, Sammel MD. Methods in a longitudinal cohort study of late reproductive age women: the Penn Ovarian Aging Study (POAS). Women's Midlife Heal. 2016;2(1):1.

Georgakis MK, Thomopoulos TP, Diamantaras A-A, et al. Association of age at menopause and duration of reproductive period with depression after menopause. JAMA Psychiat. 2016;73(2):139.

Georgakis MK, Beskou-Kontou T, Theodoridis I, Skalkidou A, Petridou ET. Surgical menopause in association with cognitive function and risk of dementia: a systematic review and meta-analysis. Psychoneuroendocrinology. 2019;106:9–19.

Goldstein JM, Holsen L, Handa R, Tobet S. Fetal hormonal programming of sex differences in depression: linking women's mental health with sex differences in the brain across the lifespan. Front Neurosci. 2014;8:247.

Gracia CR, Freeman EW. Onset of the menopause transition. Obstet Gynecol Clin N Am. 2018;45(4):585–97.

Guarnieri B. Sleep disorders and cognitive alterations in women. Maturitas. 2019;126:25–7.

Hamilton M. A rating scale for depression. J Neurol Neurosurg Psychiatry. 1960;23(1):56–62.

Harlow SD, Gass M, Hall JE, et al. Executive summary of the stages of reproductive aging workshop +10: addressing the unfinished agenda of staging reproductive aging. Climacteric. 2012;15(2):105–14.

Hunter MS. The Women's Health Questionnaire (WHQ): Frequently Asked Questions (FAQ). Health Qual Life Outcomes. 2003;1(1):41.

Jayasena CN, Comninos AN, Stefanopoulou E, et al. Neurokinin B administration induces hot flushes in women. Sci Rep. 2015;5(1):8466.

Kalleinen N, Polo-Kantola P, Himanen S-L, et al. Sleep and the menopause – do postmenopausal women experience worse sleep than premenopausal women? Menopause Int. 2008;14(3):97–104.

Kessler RC, Berglund P, Demler O, et al. The epidemiology of major depressive disorder. JAMA. 2003;289(23):3095.

Khadilkar SV, Patil VA. Sex hormones and cognition: where do we stand? J Obstet Gynecol India. 2019;69(4):303–12.

La Marca A, Sighinolfi G, Radi D, et al. Anti-Mullerian hormone (AMH) as a predictive marker in assisted reproductive technology (ART). Hum Reprod Update. 2010;16(2):113–30.

Lee JS, Ettinger B, Stanczyk FZ, et al. Comparison of methods to measure low serum estradiol levels in postmenopausal women. J Clin Endocrinol Metab. 2006;91(10):3791–7.

Liu K, Case A, Cheung AP, et al. Advanced reproductive age and fertility. J Obstet Gynaecol Can. 2011;33(11):1165–75.

Lukacs JL, Chilimigras JL, Cannon JR, Dormire SL, Reame NE. Midlife women's responses to a hospital sleep challenge: aging and menopause effects on sleep architecture. J Women's Health. 2004;13(3):333–40.
Miller EA, Beasley DE, Dunn RR, Archie EA. Lactobacilli dominance and vaginal pH: why is the human vaginal microbiome unique? Front Microbiol. 2016;7:1936.
Morgan KN, Derby CA, Gleason CE. Cognitive changes with reproductive aging, perimenopause, and menopause. Obstet Gynecol Clin N Am. 2018;45(4):751–63.
Netzer NC, Eliasson AH, Strohl KP. Women with sleep apnea have lower levels of sex hormones. Sleep Breath. 2003;7(1):25–9.
Paganini-Hill A, Henderson VW. Estrogen deficiency and risk of Alzheimer's disease in women. Am J Epidemiol. 1994;140(3):256–61.
Portman DJ, Gass MLS. Vulvovaginal atrophy terminology consensus conference panel. Genitourinary syndrome of menopause. Menopause. 2014;21(10):1063–8.
Prague JK, Roberts RE, Comninos AN, et al. Neurokinin 3 receptor antagonism as a novel treatment for menopausal hot flushes: a phase 2, randomised, double-blind, placebo-controlled trial. Lancet. 2017;389(10081):1809–20.
Prague JK, Roberts RE, Comninos AN, et al. Neurokinin 3 receptor antagonism rapidly improves vasomotor symptoms with sustained duration of action. Menopause. 2018;25(8):862–9.
Prince MJ, Acosta D, Guerra M, et al. Reproductive period, endogenous estrogen exposure and dementia incidence among women in Latin America and China; A 10/66 population-based cohort study. PLoS One. 2018;13(2):e0192889. El Bcheraoui C, ed.
Radtke JV, Terhorst L, Cohen SM. The menopause-specific quality of life questionnaire: psychometric evaluation among breast cancer survivors. Menopause. 2011;18(3):289–95.
Raglan GB, Schulkin J, Micks E. Depression during perimenopause: the role of the obstetrician-gynecologist. Arch Womens Ment Health. 2019 (epub ahead of print)
Riedel BC, Thompson PM, Brinton RD. Age, APOE and sex: triad of risk of Alzheimer's disease. J Steroid Biochem Mol Biol. 2016;160:134–47.
Russell JK, Jones CK, Newhouse PA. The role of estrogen in brain and cognitive aging. Neurotherapeutics. 2019;16(3):649–65.
Schoenaker DAJM, Jackson CA, Rowlands JV, Mishra GD. Socioeconomic position, lifestyle factors and age at natural menopause: a systematic review and meta-analyses of studies across six continents. Int J Epidemiol. 2014;43(5):1542–62.
Shifren JL, Davis SR. Androgens in postmenopausal women. Menopause. 2017;24(8):970–9.
Skorupskaite K, George JT, Veldhuis JD, Millar RP, Anderson RA. Neurokinin 3 receptor antagonism reveals roles for neurokinin B in the regulation of gonadotropin secretion and hot flushes in postmenopausal women. Neuroendocrinology. 2017;106(2):148–57.
Soares CN. Depression and menopause. Psychiatr Clin North Am. 2017;40(2):239–54.
Sowers MR, Zheng H, McConnell D, et al. Estradiol rates of change in relation to the final menstrual period in a population-based cohort of women. J Clin Endocrinol Metab. 2008a;93(10):3847–52.
Sowers MR, Zheng H, McConnell D, et al. Follicle stimulating hormone and its rate of change in defining menopause transition stages. J Clin Endocrinol Metab. 2008b;93(10):3958–64.
Sowers MR, Eyvazzadeh AD, McConnell D, et al. Anti-Mullerian hormone and inhibin B in the definition of ovarian aging and the menopause transition. J Clin Endocrinol Metab. 2008c;93(9):3478–83.
Sturdee DW, Hunter MS, Maki PM, et al. The menopausal hot flush: a review. Climacteric. 2017;20(4):296–305.
Szeliga A, Czyzyk A, Podfigurna A, Genazzani ARAD, Genazzani ARAD, Meczekalski B. The role of kisspeptin/neurokinin B/dynorphin neurons in pathomechanism of vasomotor symptoms in postmenopausal women: from physiology to potential therapeutic applications. Gynecol Endocrinol. 2018;34(11):1–7.
Utian WH. Menopause-related definitions. Int Congr Ser. 2004;1266:133–8.

van Disseldorp J, Faddy MJ, Themmen APN, et al. Relationship of serum antimüllerian hormone concentration to age at menopause. J Clin Endocrinol Metab. 2008;93(6):2129–34.
Welt CK, Schneyer AL. Differential regulation of inhibin B and inhibin a by follicle-stimulating hormone and local growth factors in human granulosa cells from small antral follicles. J Clin Endocrinol Metab. 2001;86(1):330–6.
Wesnes KA. Cognitive function testing: the case for standardization and automation. Br Menopause Soc J. 2006;12(4):158–63.
Willi J, Ehlert U. Assessment of perimenopausal depression: a review. J Affect Disord. 2019;249:216–22.
Zhou B, Sun X, Zhang M, Deng Y, Hu J. The symptomatology of climacteric syndrome: whether associated with the physical factors or psychological disorder in perimenopausal/postmenopausal patients with anxiety–depression disorder. Arch Gynecol Obstet. 2012;285(5):1345.

Long-Term Consequences of Menopause

17

Rossella E. Nappi and Laura Cucinella

Contents

Abstract

The dramatic changes in sex hormone levels occurring during menopausal transition and beyond are responsible for long-term consequences, which are of paramount importance for healthy aging in women. Sex hormones have a vital physiological role to maintain health and normal functioning of several organs such as the bone, heart, and brain. Disease activity is strongly dependent on estrogen exposure, and cardiovascular and musculoskeletal disorders occur more frequently during post-reproductive life. Even cognitive decline is related to hormonal deprivation across menopausal transition. Several lines of evidence indicate that the presence, duration, and severity of menopausal symptoms, especially hot flashes, not only have an impact on quality of life, but they are biomarkers of increased risk for chronic conditions, which required preventive strategies, including menopause hormone therapy. Apart from nutrition, exercise, and other lifestyle measures, the use of appropriate hormonal treatments in

R. E. Nappi (✉) · L. Cucinella
Research Center for Reproductive Medicine, Gynecological Endocrinology and Menopause, Obstetrics and Gynecology Unit, IRCCS San Matteo Foundation, Department of Clinical, Surgical, Diagnostic and Pediatric Sciences, University of Pavia, Pavia, Italy
e-mail: renappi@tin.it

F. Petraglia, B. C. Fauser (eds.), *Female Reproductive Dysfunction*, Endocrinology,
https://doi.org/10.1007/978-3-030-14782-2_17

symptomatic women during the "window" of opportunity (under 60 years of age or within 10 years from menopause) may significantly counteract the aging process at many targets of the female body. In the meantime, an individualized menopause hormone therapy (MHT) may help postmenopausal women to overcome the burden of symptoms, including those related to the genitourinary syndrome of menopause.

Keywords

Aging · Osteoporosis · Fractures · Metabolic syndrome · Diabetes · Cardiovascular risk · Cognition · Dementia · Quality of life (QoL) · Genitourinary syndrome of menopause (GSM)

Introduction

The Stages of Reproductive Aging Workshop (STRAW) classification system and its update, STRAW+10, is a validated tool to identify when reproductive aging starts and to attempt separating the effect of menopause on women's health from the process of senescence (Soules et al. 2001; Harlow et al. 2012). Literally, menopause means the final menstrual period (FMP), which corresponds to the absence of menstrual bleeding for at least 12 months. However, the neuroendocrine phenomena associated with reproductive aging start well before the FMP with a transition period termed perimenopause, which is highly variable, lasting up to 10 years. Early postmenopause represents the period in which the endocrine hallmark of menopause (high follicle-stimulating hormone (FSH) and low estradiol [E2]) stabilizes, and it lasts approximately 5–8 years (Harlow et al. 2012). That being so, the journey of menopause is quite long with symptoms and signs showing peculiar trajectories depending on a vast array of biopsychosocial determinants, which are still under investigation in longitudinal studies (Santoro 2016; Davis et al. 2015). Menopause usually occurs between 48 and 52 of age with a little geographic variation possibly reflecting genetic and climatic factors (Palacios et al. 2010). Entering menopause at a certain point in the life span has been an advantage for human beings, and the long-term consequences of ovarian exhaustion have been less apparent until recent times (Lumsden and Sassarini 2019). Nowadays, in developed countries women expect to survive more than 30 years following spontaneous menopause and may manifest a series of pathological conditions associated with the decline in ovarian estrogen production (Monteleone et al. 2018). A complex interaction between genetic and epigenetic factors is responsible for the negative impact of menopause on women's health. Recent observations point to the evidence that menopause accelerates biological aging, especially when reproductive failure occurs prematurely (Faubion et al. 2015; Levine et al. 2016). Deprivation of androgens, produced both by the ovaries and by the adrenal glands, may contribute to the deterioration of several health domains, including bone, metabolism, cardiovascular system, and cognitive

performances, as well as sexual function and quality of life (QoL) (Davis and Wahlin-Jacobsen 2015).

In here, we will briefly summarize the impact of menopause on the most relevant chronic conditions occurring in women at midlife and beyond.

Menopausal Symptoms in the Context of Long-Term Consequences

Historically, there is a separation between the short-term consequences of menopause, including the symptoms most commonly observed around FMP, such as hot flashes, sleep changes, adverse mood, and vaginal dryness, and the long-term consequences arising from the definitive estrogen deprivation and, possibly, from the reduction in androgens. Even aging in itself contributes to long-term manifestations such as osteoporosis, increased cardiovascular disease, and cognitive decline, but menopause-related changes significantly inflect the individual pattern of several health domains throughout a vast array of mechanisms (Davis et al. 2015; Monteleone et al. 2018). However, recent reviews suggest that there is a link between short- and long-term consequences of the menopause, implying that presence, duration, and severity of symptoms are "red flags" to alert health-care providers (HCPs) to devote a special care to symptomatic women (Biglia et al. 2017; Genazzani and Simoncini 2013). Indeed, the aim of addressing symptoms, in particular hot flashes, is not only to improve QoL but to prevent cardiovascular risk, osteoporosis, and cognitive deterioration (Biglia et al. 2017). Whether these conditions co-occur as a common marker of a multi-organ response to sex hormone changes or, alternatively, some etiopathogenetic mechanisms involved in vasomotor symptoms (VMS) influence long-term sequelae is still under investigation. Other symptoms, such as sleep disturbances and mood changes, are part of the menopausal syndrome and can be risk factors for cardiovascular health and cognitive problems (Monteleone et al. 2018). Overall, these findings corroborate the hypothesis that there is a "window" of opportunity to set up preventive strategies for future healthy longevity by treating the appropriate sample of women (Genazzani and Simoncini 2013). Menopause hormone therapy (MHT) has shown a favorable risk to benefit ratio in those women suffering from moderate to severe symptoms, under 60 years of age or within 10 years from menopause (women at midlife in the age range from 50 to 64 years). In women with premature menopause, MHT is mandatory until at least the time FMP usually occurs (Baber et al. 2016).

Even vaginal dryness has clearly shown a strong relationship with low estrogens during and after the menopausal transition, as it is for hot flashes, and may occur very early around the time of FMP. Vaginal dryness is the cardinal symptom of vulvovaginal atrophy (VVA), recently renamed genitourinary syndrome of menopause (GSM) (Portman et al. 2014). VVA/GSM is a chronic condition, progressing with sex hormone deficiency (Lachowsky and Nappi 2009), chronological aging, and medical morbidity (Mitchell and Waetjen 2018), with a great impact on sexual

function and QoL in the post-reproductive life span (Nappi et al. 2016). Whether presence and severity of vaginal dryness at menopause may represent an early biomarker of long-term pathological conditions associated with VVA/GSM is conceivable, but it awaits for further evidence (Constantine et al. 2014).

Bone Health

The menopausal transition is a critical period of change in bone health because there is a significant acceleration of the age-dependent bone loss, which is crucial for development of osteoporosis and fracture susceptibility in older women. Changes in estradiol and follicle-stimulating hormone (FSH) levels during the menopausal transition relate to changes in bone mass with a rate of decline in white women of 2.5% per year in the lumbar spine and of 1.8% per year in the femoral neck (Greendale et al. 2012). Interestingly, by analyzing the medical records of more than 23,000 US women (aged 50–79 years), Crandall et al. showed that women with moderate/severe VMS had lower BMD (at the femoral neck and lumbar spine) and increased rates of hip fractures during more than 8 years of follow-up compared with women who did not have VMS (Crandall et al. 2015). Estrogen deficiency causes a decrease in bone mineral density (BMD) and alterations in the microarchitecture of the bone. Estrogens stimulate osteoblast proliferation and differentiation promoting deposition and mineralization of bone matrix by binding both ERα and ERβ isoforms. Moreover, they induce apoptosis in osteoclasts, and their absence results in a pro-inflammatory cytokine milieu with an increase in oxidative stress, which further induces bone resorption, and it may explain the link with hot flashes (Karlamangla et al. 2018). Circulating levels of bone resorption markers, such as *N*-telopeptide of type 1 collagen, C-terminal telopeptide of type 1 collagen, and pyridinoline crosslinks, increase by 90% after menopause, whereas markers of bone formation increase by only 45% (Eastell and Szulc 2017). In a 3-year period around FMP, a rapid phase of bone loss is the result of bone remodeling imbalance with greater increase in the level of osteoclast-mediated bone resorption than osteoblast-mediated bone formation. BMD begins to decline at approximately 1 year prior to the FMP and continues to decrease in early postmenopause, with a slight reduction in loss rate around 2 years after the FMP. Trabecular bone becomes thinner, and there is a loss of connectivity, which translate into the reduction in load-bearing capacity. There is less rapid cortical bone loss in the long bones and vertebrae after the menopause, and following 10 years slower age-related bone loss becomes prominent and continues for the rest of life. At the age of 50 years, the lifetime risk of hip fracture is 15% and of vertebral deformities is about 25%. However, many fractures occur in postmenopausal women with normal or only a slightly reduced BMD value indicating that many other additional risk factors play a role. Minority race/ethnicity status, low socioeconomic status, obesity, diabetes, certain types of drugs (psychoactive agents, angiotensin-converting enzyme inhibitors, thyroid replacement, etc.), smoking, and low vitamin D intake have been linked to increased fracture risk (Karlamangla et al. 2018). Osteoarthritis is affected by estrogen deprivation with a significant increase of deformity and pain because estrogens target cartilage,

periarticular bone, synovial lining, ligaments, and the joint capsule (Roman-Blas et al. 2009). A special subset of women are those with early, estrogen receptor-positive breast cancer commencing adjuvant endocrine therapy with aromatase inhibitors in postmenopause or selective estradiol receptor modulators such as tamoxifen in premenopause because of acceleration of bone loss and increased fracture risk (Ramchand et al. 2019).

At the beginning of menopause, evaluation of the subsequent risk of fracture is not so easy. Most screening tools are not accurate enough to identify those women who will fracture within the next 10 years. A history of a prior fracture and low bone mineral density are the only major consistently found predictors for the risk of fracture. Current guidelines (from the US Preventive Services Task Force) for osteoporosis screening during the "window of opportunity" recommend using the Fracture Risk Assessment Tool (FRAX) to estimate the 10-year risk for osteoporotic fracture as a first step and to proceed only if the estimated 10-year risk exceeds 9.3%. Unfortunately, in women between 50 and 64 years of age, only around one-third who would meet treatment criteria by BMD (T score ≤ -2.5) and only 25% who experience a fracture over the next 10 years would meet the FRAX-based threshold for screening (Trémollieres 2019). That being so, prevention of bone loss in postmenopausal women by using MHT is likely a strategy of true primary prevention to reduce fracture risk later in life. Indeed, benefit/risk balance is favorable in early postmenopause in women who require MHT to relieve VMS. The very recent guidelines of the Endocrine Society support the use of MHT and indicate that in postmenopausal women with low BMD and at high risk of fractures with osteoporosis, calcium and vitamin D should be used as an adjunct to osteoporosis therapies or as an alternative to prevent hip fractures in those women who cannot tolerate other specific treatments. Dietary intake with calcium is the safest and most appropriate approach for postmenopausal women undergoing treatment for osteoporosis. Recommended dose of calcium per day in diet and/or supplements is 1000–1200 mg. There is no consensus concerning a threshold level of vitamin D that should be reached when supplementing postmenopausal women. However, all postmenopausal women with a confirmed diagnosis of osteoporosis should be screened with a serum level of 25-hydroxyvitamin D. By ingesting 1000 IU of vitamin D per day, women can fulfill both European guidelines for women with osteoporosis (at least 20 ng/mL [50 nmol/L]) and Endocrine Society guideline (a threshold of 30 ng/mL [75 nmol/L]). In addition, resistance and balance exercises, smoking cessation, limited alcohol use, decreased use of drugs, and optimization of comorbid conditions that can harm bone for all postmenopausal women are recommended (Eastell et al. 2019).

Metabolic Syndrome

Women are at higher risk of metabolic syndrome (MetS) as compared to men, because of polycystic ovarian syndrome, gestation, and menopausal transition. A meta-analysis on the global prevalence of MetS in postmenopausal women indicated a pooled prevalence among postmenopausal women of 37.17%

(95% confidence interval [CI] 35.00%–39.31%), ranging from 13.60% (95% CI 13.55%–13.64%) to 46.00% (95% CI 1.90%–90.09%), depending upon the diagnostic criteria used. The overall pooled OR for MetS in postmenopausal women, compared with premenopausal women, was OR 3.54 (95% CI 2.92–4.30), but this ranged from OR 2.74 (95% CI 1.32–5.66) to OR 5.03 (95% CI 2.25–11.22), depending upon the criteria used. Furthermore, the odds of high fasting blood sugar (OR 3.51, 95% CI 2.11–5.83), low high-density lipoprotein cholesterol (OR 1.45, 95% CI 1.03–2.03), high blood pressure (OR 3.95, 95% CI 2.01–7.78), high triglycerides (OR 3.2, 95% CI 2.37–4.31), and high waist circumference (OR 2.75, 95% CI 1.80–4.21) were all found to be higher in postmenopausal women than in premenopausal women (Hallajzadeh et al. 2018). The MetS includes at least three of the following findings: obesity, hypertension, hyperglycemia, hypertriglyceridemia, and low plasma high-density lipoprotein cholesterol (HDL-C) levels. A number of events linked to menopausal status, including weight gain, changes in lifestyles, and endocrine adjustment, may be related to the prevalence of MetS, which seems more common in women suffering from hot flushes (Chedraui and Pérez-López 2019). Indeed, women with VMS had increased body mass index, larger waist circumference, a more adverse lipid profile, and a higher prevalence of MetS as compared to asymptomatic women, after adjusting for many confounders. Race/ethnicity are strongly linked to the hormonal pattern of women at midlife, which is in turn related to the trajectory of VMS over time and to the ability of accumulating visceral abdominal fat. VMS are also associated with insulin resistance, obesity, and type 2 diabetes mellitus (T2DM), which are significant components of the MetS. A lifetime history or current major depressive episode at baseline was associated with a higher risk of developing the MetS during the follow-up period. Even sleep disorders have been related to MetS because of circadian alterations and neuroendocrine rearrangements, involving orexigenic and anorexigenic mediators regulating caloric intake and energy expenditure. Absolute weight gain in women at midlife seems to be more related to aging rather than to menopause itself. However, across menopausal transition there was an absolute cumulative 6-year increase in fat mass of 3.4 kg and of about 5.7 cm in waist circumference with a rate of increase slowing 1 year after FMP. The prevalence of the MetS is higher among women with excessive weight or visceral adiposity, but it may occur also in lean individuals, and it is one of the main contributors to the pathogenesis of cardiovascular diseases (CVDs) (El Khoudary and Thurston 2018; Tuomikoski and Savolainen-Peltonen 2017). An imbalance of sympathetic nervous system, as well as an increase in both pro-inflammatory and thrombotic status and oxidative stress, may also play a role in the occurrence of the cardiovascular risk (Tuomikoski and Savolainen-Peltonen 2017). Data support the notion that MetS duplicates the risk of CVD while increasing by 1.5 times the mortality due to all causes. Finally, MetS may affect even cognition, especially when other risk factors are present, such as T2DM. Hyperglycemia and elevated blood pressure have been strongly associated with cognitive decline (Chedraui and Pérez-López 2019). Risk factor assessment in perimenopausal women is recommended, thereby permitting the timely introduction of lifestyle, hormonal, and therapeutic interventions to modify or reverse adverse

changes. Studies mostly indicate a reduction in overall fat mass with estrogen and estrogen-progestin therapy, improved insulin sensitivity, and a lower rate of development of T2DM. Such a positive effect influences positively health-related QoL and sexual function (Davis et al. 2012).

Cardiovascular Risk

There is a strong connection between MetS and CVD, which is the leading cause of death in women with an increased incidence following menopause. In comparison with men, there is a 10-year delay in the clinical manifestation of CVD because of the strong protection exerted by estrogens until FMP (Collins et al. 2016). CVD prevalence in women after age 75 exceeds that reported for men. In addition, short- to medium-term mortality after acute myocardial infarction (AMI) is higher in women than in men, and the impact of classical CV risk factors is also likely to differ in men and women. For example, T2DM is linked with increased morbidity and mortality from CVD in both sex but women with diabetes appear to be at a greater relative risk of CVD than men (Collins et al. 2016; Maffei et al. 2019). This occurs despite the fact that there is no clear evidence of an association between estradiol levels and glucose balance parameters during the menopausal transition (Park et al. 2017). CVD risk factors are affected by hormonal status and other common, female-specific comorbidities, including duration of reproductive life span, occurrence of reproductive disorders such as polycystic ovarian syndrome and miscarriage, and a positive history of hypertensive pregnancy disorders and gestational diabetes (Maffei et al. 2019). Menopause is an independent CVD risk factor, a finding further corroborated by evidences in women with premature menopause. A meta-analysis showed that bilateral oophorectomy is an independent risk factor for CV disease with a RR of 4.55, whereas early menopause was associated with a RR of 1.25 (Atsma et al. 2006). In the "Early Menopause Predicts Future Coronary Heart Disease and Stroke: The Multi-Ethnic Study of Atherosclerosis (MESA)," women with a history of early menopause had worse CHD and impaired stroke-free survival, after model adjustment for age and race/ethnicity (Wellons et al. 2012).

The vascular and cardioprotective role of estradiol displays a peculiar pattern over time, and its role is extremely fine-tuned, involving a wide range of mechanisms, from vasodilation to anti-inflammatory activity, antioxidant properties, and neuroendocrine modulation (Simoncini 2009). Increased deposition of visceral fat in postmenopausal women, especially heart fat, which is related to low estradiol levels, plays a crucial role in increasing women's risk of CVD later in life. Even FSH may have a role in controlling fat deposition, insulin resistance, diabetes development, lipid metabolism, and atherosclerosis. Blood lipid profiles tend to become atherogenic in women within the year following the FMP, with major increases in total cholesterol, LDL cholesterol, and apolipoprotein B (APOB), independent of ethnicity, age, or weight. On the other hand, the anti-atherogenic effect of HDL cholesterol is less effective after menopause even though it is still a good predictor of

cardiovascular mortality in women. Elevated triglycerides may be as well an independent risk factor for CHD mortality in women, particularly in women with low HDL cholesterol concentration (Matthews et al. 2009). Lack of exposure to estrogen during menopause can exert a negative effect on endothelial cell function resulting in the reduced release of cardioprotective nitric oxide and influencing the growth and proliferation of vascular smooth muscle cells. Moreover, estrogen deficiency leads to activation of the renin-angiotensin system, as well as upregulation of endothelin, a potent vasoconstrictor. Even the prevalence of salt sensitivity increases in postmenopausal women contributing to their increased risk of developing hypertension. That being so, the prevalence of hypertension in postmenopausal women is more than twice the prevalence in premenopausal women (Monteleone et al. 2018; El Khoudary and Thurston 2018). Interestingly, a higher frequency of hot flashes has been correlated with increased awake and sleep systolic blood pressure, independent of the menopausal status (Gerber et al. 2007). Moreover, late postmenopausal women with VMS have a higher prevalence of a previous diagnosis of hypertension supporting the notion that VMS is an early hallmark of endothelial dysfunction predicting CV risk in women (Biglia et al. 2017). Indeed, the severity of VMS is associated with decreased vascular response to endothelium-mediated dilatation, suggesting a decline in endothelial function and reduced vascular compliance. Another interesting finding is that surgical menopause causes an imbalance of the autonomic nervous control of the CV system with a decrease in cardiac vagal modulation and an increase in sympathetic activity. The same is evident in women at midlife during VMS. In addition, in women with frequent VMS, carotid intima-media thickness is higher, and in those during the menopausal transition who present with hot flashes, there is a higher incidence of arterial endothelial dysfunction, indicated by impaired flow-mediated dilation of the brachial artery. Finally, VMS are associated with both a procoagulant hemostatic and an unfavorable hemodynamic profile with a lower overall cardiac index and stroke volume index. These data support the evidence that women with current signs and symptoms of myocardial ischemia presenting with VMS during the transition have a higher mortality due to CVD and reduced endothelial function in comparison with women reporting VMS late in the menopause (Monteleone et al. 2018). Other aspects of menopausal well-being, such as sleep and mood disorders, may play a role as CV risk factors. Shorter duration and lower overall quality of sleep have been associated with a greater degree of aortic calcification and increased carotid atherosclerosis among women at midlife (El Khoudary and Thurston 2018). The National Health and Nutrition Examination Survey I study showed that women with depression had a higher relative risk of developing CVD than women without depression (Ferketich et al. 2000).

These data collectively indicate that the menopausal period and early menopause present an ideal opportunity to assess cardiovascular risk and to set up preventive strategies, from reduction of body weight to elimination of other traditional risk factors such as smoking and physical inactivity. The use of MHT may be beneficial in women reporting distressing VMS at midlife, and it should be initiated during the "window of opportunity in accordance with the so-called timing hypothesis" (Maffei et al. 2019).

Cognitive Decline

Cognitive decline and dementia are a growing public health problem, with the worldwide prevalence of dementia expected to triple by 2050. Alzheimer's disease (AD), the most common cause of dementia, occurs more frequently in women than in men (Hebert et al. 2013). This difference might be due to the longer life expectancy of women, but sex-specific differences in the incidence of AD might also exist. Indeed, increased risk of late-onset AD in women suggests pathophysiologic changes that may be mediated by endocrine transition states such as menopause, which is both female-specific and age-related. Perimenopause is a midlife neuroendocrine transition state; the symptoms of perimenopause are largely neurologic in nature, including disruption in estrogen-regulated systems such as thermoregulation, sleep, circadian rhythms, and sensory processing, as well as depression and impairment in multiple cognitive domains (Morgan et al. 2018). Estradiol may have a major role in cognitive performance because estrogens target areas of the central nervous system, such as the hippocampus and prefrontal cortex, which mediate episodic and working memory. Indeed, estrogens modulate the synthesis, release, and metabolism of several neurotransmitters (serotonin, dopamine, and acetylcholine) and a vast array of neuropeptides (β-endorphin and neurosteroids). Moreover, they can influence the electrical excitability, function, and morphological features of the synapses (Monteleone et al. 2018). Estrogens may be neuroprotective throughout several mechanisms, including promotion of cell growth and survival of neurons, elevation of dendritic spine density and synaptogenesis, and attenuation of neurodegenerative processes associated with AD. Even though brain aging and neurodegenerative diseases have a multifactorial nature, estrogen dysregulation during perimenopause significantly affects brain bioenergetic system, inflecting neurodegenerative processes (Mishra and Brinton 2018). Fluctuating levels of estradiol during perimenopause might cause the transient cognitive deficits clinically evident, especially when VMS are present (Morgan et al. 2018). Brinton (2016) have shown that perimenopause may induce a hypometabolic state associated with neurological dysfunction because of increased fatty acid catabolism, β-amyloid (a main constituent of senile plaques, a hallmark of AD pathology) deposition, and declines in synaptic plasticity in vulnerable women. All these changes adversely influence microglial function and antioxidant and clearance mechanisms, exacerbating neuroinflammation and eventually neurodegeneration. Even the high levels of FSH and LH in postmenopausal women have been linked to AD in these hormones might be responsible for increased production of β-amyloid (Morgan et al. 2018). However, both the compromised bioenergetic system of menopause and the chronic low-grade innate inflammation of aging contribute to neurodegenerative diseases, together with the increased metabolic and vascular risk, explaining the difficulties in linking MHT to cognitive benefits. Nevertheless, the perimenopausal transition represents a "window of opportunity" to prevent age-related neurological diseases because longitudinal studies documented decrements in cognitive function, such as reduced "learning effects" over repeated cognitive assessments rather than by a decline in cognitive performance. A lack of

improvement in verbal memory was reported in the early and late perimenopausal stages, and deficits in processing speed with repeated testing were seen in the late perimenopausal stage compared with the premenopausal and postmenopausal stages (Greendale et al. 2009). In spite of the evidence that cognitive deficits observed during perimenopause are independent of mood symptoms, the SWAN showed that women with more depressive symptoms displayed a worse processing speed and those with higher degrees of anxiety had a poorer verbal memory (Morgan et al. 2018), supporting the association between depression and AD (Ownby et al. 2006). VMS in addition to poor sleep quality seem to favor the onset of depressive symptoms which are very frequent during the menopausal transition (Maki et al. 2018). Even sleep disorders are associated with an increased risk of cognitive decline in menopausal women, particularly with respect to attention, episodic memory, and executive function. Moreover, early postmenopausal women at high risk of obstructive sleep apnea displayed a significant impairment of cognitive function compared with those at low risk of sleep apnea (Morgan et al. 2018). Studies conducted in surgical menopausal women, especially when ovariectomy is performed before the age of natural menopause, further corroborated the crucial role of endocrine deprivation on cognitive function (Rocca et al. 2007).

Collectively, these data should support the widespread use of MHT to prevent cognitive decline also in light of a 29% reduction in AD in meta-analyses of observational studies (Yaffe et al. 1998). However, the Women's Health Initiative Memory Study (WHIMS), the only randomized trial of MHT for prevention of AD, reported a doubling of the risk of all-cause dementia (Shumaker et al. 2004). In parallel to cardiovascular risk, a key consideration is the age at initiation of MHT, which in the general population is around 52 years but in WHIMS was 65 years and older. That being so, for women in early menopause with bothersome VMS, the use of MHT is cognitive safe and offers a "domino" benefit by relieving many symptoms which may contribute to cognitive decline at midlife and beyond.

Conclusion

Menopause is a time of considerable distress for the majority of women and brings about signs and symptoms with a great impact on QoL and general health. Addressing the multitude of changes that are directly or indirectly dependent from sex hormone deprivation is crucial to early recognition of risk factors for chronic conditions, which may be preventable by a comprehensive strategy including tailored treatments to the individual menopausal woman.

References

Atsma F, Bartelink ML, Grobbee DE, van der Schouw YT. Postmenopausal status and early menopause as independent risk factors for cardiovascular disease: a meta-analysis. Menopause. 2006;13(2):265–79.

Baber RJ, Panay N, Fenton A, IMS Writing Group. 2016 IMS Recommendations on women's midlife health and menopause hormone therapy. Climacteric. 2016;19(2):109–50. https://doi.org/10.3109/13697137.2015.1129166.

Biglia N, Cagnacci A, Gambacciani M, Lello S, Maffei S, Nappi RE. Vasomotor symptoms in menopause: a biomarker of cardiovascular disease risk and other chronic diseases? Climacteric. 2017;20(4):306–12. https://doi.org/10.1080/13697137.2017.1315089.

Brinton RD. Neuroendocrinology: oestrogen therapy affects brain structure but not function. Nat Rev Neurol. 2016;12(10):561–2. https://doi.org/10.1038/nrneurol.2016.147.

Chedraui P, Pérez-López FR. Metabolic syndrome during female midlife: what are the risks? Climacteric. 2019;22(2):127–32. https://doi.org/10.1080/13697137.2018.1561666.

Collins P, Webb CM, de Villiers TJ, Stevenson JC, Panay N, Baber RJ. Cardiovascular risk assessment in women – an update. Climacteric. 2016;19(4):329–36. https://doi.org/10.1080/13697137.2016.1198574.

Constantine GD, Bruyniks N, Princic N, Huse D, Palmer L, Lenhart G, et al. Incidence of genitourinary conditions in women with a diagnosis of vulvar/vaginal atrophy. Curr Med Res Opin. 2014;30(1):143–8. https://doi.org/10.1185/03007995.2013.850068.

Crandall CJ, Aragaki A, Cauley JA, Manson JE, LeBlanc E, Wallace R, et al. Associations of menopausal vasomotor symptoms with fracture incidence. J Clin Endocrinol Metab. 2015;100(2):524–34. https://doi.org/10.1210/jc.2014-3062.

Davis SR, Wahlin-Jacobsen S. Testosterone in women – the clinical significance. Lancet Diabetes Endocrinol. 2015;3(12):980–92. https://doi.org/10.1016/S2213-8587(15)00284-3.

Davis SR, Castelo-Branco C, Chedraui P, Lumsden MA, Nappi RE, Shah D, Writing Group of the International Menopause Society for World Menopause Day 2012, et al. Understanding weight gain at menopause. Climacteric. 2012;15(5):419–29. https://doi.org/10.3109/13697137.2012.707385.

Davis SR, Lambrinoudaki I, Lumsden M, Mishra GD, Pal L, Rees M, et al. Menopause. Nat Rev Dis Primers. 2015;1:15004. https://doi.org/10.1038/nrdp.2015.4.

Eastell R, Szulc P. Use of bone turnover markers in postmenopausal osteoporosis. Lancet Diabetes Endocrinol. 2017;5(11):908–23. https://doi.org/10.1016/S2213-8587(17)30184-5.

Eastell R, Rosen CJ, Black DM, Cheung AM, Murad MH, Shoback D. Pharmacological management of osteoporosis in postmenopausal women: an Endocrine Society∗ Clinical Practice Guideline. J Clin Endocrinol Metab. 2019;104(5):1595–622. https://doi.org/10.1210/jc.2019-00221.

El Khoudary SR, Thurston RC. Cardiovascular implications of the menopause transition: endogenous sex hormones and vasomotor symptoms. Obstet Gynecol Clin N Am. 2018;45(4):641–61. https://doi.org/10.1016/j.ogc.2018.07.006.

Faubion SS, Kuhle CL, Shuster LT, Rocca WA. Long-term health consequences of premature or early menopause and considerations for management. Climacteric. 2015;18(4):483–91. https://doi.org/10.3109/13697137.2015.1020484.

Ferketich AK, Schwartzbaum JA, Frid DJ, Moeschberger ML. Depression as an antecedent to heart disease among women and men in the NHANES I study. National Health and Nutrition Examination Survey. Arch Intern Med. 2000;160(9):1261–8.

Genazzani AR, Simoncini T. Pharmacotherapy: benefits of menopausal hormone therapy – timing is key. Nat Rev Endocrinol. 2013;9(1):5–6. https://doi.org/10.1038/nrendo.2012.228.

Gerber LM, Sievert LL, Warren K, Pickering TG, Schwartz JE. Hot flashes are associated with increased ambulatory systolic blood pressure. Menopause. 2007;14(2):308–15.

Greendale GA, Huang MH, Wight RG, Seeman T, Luetters C, Avis NE, et al. Effects of the menopause transition and hormone use on cognitive performance in midlife women. Neurology. 2009;72(21):1850–7. https://doi.org/10.1212/WNL.0b013e3181a71193.

Greendale GA, Sowers M, Han W, Huang MH, Finkelstein JS, Crandall CJ, et al. Bone mineral density loss in relation to the final menstrual period in a multiethnic cohort: results from the Study of Women's Health Across the Nation (SWAN). J Bone Miner Res. 2012;27(1):111–8. https://doi.org/10.1002/jbmr.534.

Hallajzadeh J, Khoramdad M, Izadi N, Karamzad N, Almasi-Hashiani A, Ayubi E, et al. Metabolic syndrome and its components in premenopausal and postmenopausal women: a comprehensive systematic review and meta-analysis on observational studies. Menopause. 2018;25(10):1155–64. https://doi.org/10.1097/GME.0000000000001136.

Harlow SD, Gass M, Hall JE, Lobo R, Maki P, Rebar RW, STRAW + 10 Collaborative Group, et al. Executive summary of the Stages of Reproductive Aging Workshop + 10: addressing the unfinished agenda of staging reproductive aging. J Clin Endocrinol Metab. 2012;97(4):1159–68. https://doi.org/10.1210/jc.2011-3362.

Hebert LE, Weuve J, Scherr PA, Evans DA. Alzheimer disease in the United States (2010–2050) estimated using the 2010 census. Neurology. 2013;80(19):1778–83. https://doi.org/10.1212/WNL.0b013e31828726f5.

Karlamangla AS, Burnett-Bowie SM, Crandall CJ. Bone health during the menopause transition and beyond. Obstet Gynecol Clin N Am. 2018;45(4):695–708. https://doi.org/10.1016/j.ogc.2018.07.012.

Lachowsky M, Nappi RE. The effects of oestrogen on urogenital health. Maturitas. 2009;63(2):149–51. https://doi.org/10.1016/j.maturitas.2009.03.012.

Levine ME, Lu AT, Chen BH, Hernandez DG, Singleton AB, Ferrucci L, et al. Menopause accelerates biological aging. Proc Natl Acad Sci U S A. 2016;113(33):9327–32. https://doi.org/10.1073/pnas.1604558113.

Lumsden MA, Sassarini J. The evolution of the human menopause. Climacteric. 2019;22(2):111–6. https://doi.org/10.1080/13697137.2018.1547701.

Maffei S, Guiducci L, Cugusi L, Cadeddu C, Deidda M, Gallina S, Working Group on "Gender difference in cardiovascular disease" of the Italian Society of Cardiology, et al. Women-specific predictors of cardiovascular disease risk – new paradigms. Int J Cardiol. 2019;286:190–7. https://doi.org/10.1016/j.ijcard.2019.02.005.

Maki PM, Kornstein SG, Joffe H, Bromberger JT, Freeman EW, Athappilly G, Board of Trustees for The North American Menopause Society (NAMS) and the Women and Mood Disorders Task Force of the National Network of Depression Centers, et al. Guidelines for the evaluation and treatment of perimenopausal depression: summary and recommendations. Menopause. 2018;25(10):1069–85. https://doi.org/10.1097/GME.0000000000001174.

Matthews KA, Crawford SL, Chae CU, Everson-Rose SA, Sowers MF, Sternfeld B, et al. Are changes in cardiovascular disease risk factors in midlife women due to chronological aging or to the menopausal transition? J Am Coll Cardiol. 2009;54(25):2366–73. https://doi.org/10.1016/j.jacc.2009.10.009.

Mishra A, Brinton RD. Inflammation: bridging age, menopause and APOEε4 genotype to Alzheimer's disease. Front Aging Neurosci. 2018;10:312. https://doi.org/10.3389/fnagi.2018.00312.

Mitchell CM, Waetjen LE. Genitourinary changes with aging. Obstet Gynecol Clin N Am. 2018;45(4):737–50. https://doi.org/10.1016/j.ogc.2018.07.010.

Monteleone P, Mascagni G, Giannini A, Genazzani AR, Simoncini T. Symptoms of menopause – global prevalence, physiology and implications. Nat Rev Endocrinol. 2018;14(4):199–215. https://doi.org/10.1038/nrendo.2017.180.

Morgan KN, Derby CA, Gleason CE. Cognitive changes with reproductive aging, perimenopause, and menopause. Obstet Gynecol Clin N Am. 2018;45(4):751–63. https://doi.org/10.1016/j.ogc.2018.07.011.

Nappi RE, Cucinella L, Martella S, Rossi M, Tiranini L, Martini E. Female sexual dysfunction (FSD): prevalence and impact on quality of life (QoL). Maturitas. 2016;94:87–91. https://doi.org/10.1016/j.maturitas.2016.09.013.

Ownby RL, Crocco E, Acevedo A, John V, Loewenstein D. Depression and risk for Alzheimer disease: systematic review, meta-analysis, and metaregression analysis. Arch Gen Psychiatry. 2006;63(5):530–8.

Palacios S, Henderson VW, Siseles N, Tan D, Villaseca P. Age of menopause and impact of climacteric symptoms by geographical region. Climacteric. 2010;13(5):419–28. https://doi.org/10.3109/13697137.2010.507886.

Park SK, Harlow SD, Zheng H, Karvonen-Gutierrez C, Thurston RC, Ruppert K, et al. Association between changes in oestradiol and follicle-stimulating hormone levels during the menopausal transition and risk of diabetes. Diabet Med. 2017;34(4):531–8. https://doi.org/10.1111/dme.13301.

Portman DJ, Gass ML, Vulvovaginal Atrophy Terminology Consensus Conference Panel. Genitourinary syndrome of menopause: new terminology for vulvovaginal atrophy from the International Society for the Study of Women's Sexual Health and the North American Menopause Society. Menopause. 2014;21(10):1063–8. https://doi.org/10.1097/GME.0000000000000329.

Ramchand SK, Cheung YM, Grossmann M. Bone health in women with breast cancer. Climacteric. 2019;21:1–7. https://doi.org/10.1080/13697137.2019.1580257.

Rocca WA, Bower JH, Maraganore DM, Ahlskog JE, Grossardt BR, de Andrade M, et al. Increased risk of cognitive impairment or dementia in women who underwent oophorectomy before menopause. Neurology. 2007;69(11):1074–83.

Roman-Blas JA, Castañeda S, Largo R, Herrero-Beaumont G. Osteoarthritis associated with estrogen deficiency. Arthritis Res Ther. 2009;11(5):241. https://doi.org/10.1186/ar2791.

Santoro N. Perimenopause: from research to practice. J Women's Health (Larchmt). 2016;25(4):332–9. https://doi.org/10.1089/jwh.2015.5556.

Shumaker SA, Legault C, Kuller L, Rapp SR, Thal L, Lane DS, Women's Health Initiative Memory Study, et al. Conjugated equine estrogens and incidence of probable dementia and mild cognitive impairment in postmenopausal women: Women's Health Initiative Memory Study. JAMA. 2004;291(24):2947–58.

Simoncini T. Mechanisms of action of estrogen receptors in vascular cells: relevance for menopause and aging. Climacteric. 2009;12(Suppl 1):6–11.

Soules MR, Sherman S, Parrott E, Rebar R, Santoro N, Utian W, et al. Executive summary: Stages of Reproductive Aging Workshop (STRAW). Climacteric. 2001;4(4):267–72.

Trémollieres F. Assessment and hormonal management of osteoporosis. Climacteric. 2019;22(2):122–6. https://doi.org/10.1080/13697137.2018.1555582.

Tuomikoski P, Savolainen-Peltonen H. Vasomotor symptoms and metabolic syndrome. Maturitas. 2017;97:61–5. https://doi.org/10.1016/j.maturitas.2016.12.010.

Wellons M, Ouyang P, Schreiner PJ, Herrington DM, Vaidya D. Early menopause predicts future coronary heart disease and stroke: the Multi-Ethnic Study of Atherosclerosis. Menopause. 2012;19(10):1081–7.

Yaffe K, Sawaya G, Lieberburg I, Grady D. Estrogen therapy in postmenopausal women: effects on cognitive function and dementia. JAMA. 1998;279(9):688–95.

Hormone Replacement Therapy (HRT)

18

Marta Caretto and Tommaso Simoncini

Contents

Abstract

Hormone therapy (HT) remains the most effective treatment for vasomotor symptoms (VMS) and the genitourinary syndrome of menopause (GSM) and has been shown to prevent bone loss and fracture. The risks of HT differ depending on type, dose, combination, duration of use, route of administration, and timing of initiation. Treatment should be individualized to identify the most

M. Caretto · T. Simoncini (✉)
Department of Clinical and Experimental Medicine, Division of Gynecology and Obstetrics, University of Pisa, Pisa, Italy
e-mail: tommaso.simoncini@med.unipi.it

F. Petraglia, B. C. Fauser (eds.), *Female Reproductive Dysfunction*, Endocrinology,
https://doi.org/10.1007/978-3-030-14782-2_18

appropriate HT type, dose, formulation, route of administration, and duration of use, using the best available evidence to maximize benefits and minimize risks, with periodic reevaluation of the benefits and risks of continuing or discontinuing HT.

For women aged younger than 60 years or who are within 10 years of menopause onset and have no contraindications, the benefit-risk ratio is most favorable for treatment of bothersome VMS and for those at elevated risk for bone loss or fracture.

For women who initiate HT more than 10 years from menopause onset or are aged 60 years or older, the benefit-risk ratio appears less favorable because of the greater absolute risks of coronary heart disease, stroke, and venous thromboembolism. Longer durations of therapy should be for documented indications such as persistent VMS or bone loss, with shared decision making and periodic reevaluation. New and emerging menopausal therapies have the potential to relieve menopausal symptoms and to create a target treatment.

Keywords

Breast cancer · Cardiovascular disease · Estrogen · Hormone replacement therapy · Menopause · Osteoporosis · Vaginal atrophy · Vasomotor symptoms

Introduction

The use of hormone replacement therapy (HRT) was first introduced in the 1940s, but the term "HRT" was replaced in the USA with "hormone therapy" or "menopausal hormone therapy" after the publication of Women's Health Initiative (WHI) study in the early 2000s (Lobo 2016). The term hormone therapy is used to refer to estrogen therapy (ET) and estrogen-progestogen therapy (EPT) and estrogen-receptor (ER) agonists or antagonists. Understanding the benefits and the risks of age at initiation or time since menopause, specific formulations and duration of HT, the need for monitoring during therapy, potential risks of continuation is essential to initiating or continuing HT. The use of HT could be suggested to different women, including those with surgical menopause, early menopause, or primary ovarian insufficiency (POI) and for women aged older than 65 years (The NAMS 2017 Hormone Therapy Position Statement Advisory Panel 2017).

HRT should be continued throughout the menopausal years to improve of vasomotor symptoms (VMS) with reductions in both frequency and severity in the order of 75% (MacLennan et al. 2004a), to confer optimal effects on bone, lipids, and the urogenital tract, and to offer a protection from cardiovascular disease, cerebrovascular disease, colon cancer, and neurodegenerative disorders such as Alzheimer's disease. HT may improve quality of life in symptomatic women (Welton et al. 2008).

History of HRT

Conjugated equine estrogens (CEE) were approved in the USA in 1942. The use of estrogen after menopause became popular in the late 1960s with the wrong idea that it would render women "feminine forever"(Lobo 2016) and then increased further after 1988 when its use was approved by the US Food and Drug Administration (FDA) for the prevention of osteoporosis (Osteoporosis 1984). During the 1990s, several observational data suggested a decrease not only in osteoporosis but also in coronary artery disease (Stampfer and Colditz 1991) and mortality (Grodstein et al. 1997), as well as cognitive function and dementia. In the mid-1970s, unopposed ET in women with a uterus was recognized to increase the risk of uterine cancer; however, the addition of progestogen to the hormone regimen was used to minimize or eliminate this risk (Woodruff and Pickar 1994).

The WHI hormone therapy trial was designed to assess the benefits and risks of MHT taken for chronic disease prevention in healthy postmenopausal women. The double-blinded, placebo-controlled, randomized clinical trial enrolled US postmenopausal women aged 50–79 years. The average age of patients in the trial was 63 years: nowadays, clinicians know that the average patient using HT is younger and usually the beginning of HT is not recommended for women who are aged >60 years. The first publications of the WHI hormone therapy trial in 2002 exposed HRT to a dramatic impact, troubling women's attitudes and physicians' prescribing practice throughout the world. This caused a general decrease of HT use. Publications that raised a word of caution about discontinuing use of HT were disregarded (Huber et al. 2002).

The WHI study tested the most common formulations of HT: CEE plus medroxyprogesterone acetate (MPA) for women with an intact uterus and CEE alone for women with hysterectomy. The CEE plus MPA trial was stopped after 5.6 years due to an increased risk of breast cancer; the CEE-alone trial was stopped after 7.2 years due to an increased risk of stroke. However, the trials confirmed some of the benefits of HRT such as a reduction in osteoporotic fractures and colon cancer, and with CEE alone, a reduction in breast cancer. In 2006, the first study from the WHI that included age stratification for coronary disease outcomes with the use of CEE was published. The findings showed that CEE had a protective effect in younger women (aged 50–59 years), but a significant trend of worsening outcomes was observed in older women (aged ≥60 years).

A number of studies have reported the effect of stopping HRT: in 2015, Mikkola et al. (2015) suggested that abrupt cessation of HRT leads to a significant increase in myocardial infarction and stroke, particularly in younger (aged <60 years) women. This study provided a confirmation of the benefits of HRT on the cardiovascular system, in particular for women younger than 60 years.

Nowadays, over 15 years after the first publication of WHI, it seems to be a revival of HT, which involve publications in leading medical journals (Huber et al. 2012) and outstanding worldwide organizations such as the International

Menopause Society, The North American Menopause Society, The British Menopause Society, and the Endocrine Society.

HRT for Early and Late-Menopause

Over the last decade, data show that timing of HRT initiation relative to age and time since menopause is critical, with beneficial effects demonstrated in perimenopausal and early postmenopausal women, and null or even negative effect in elderly patients.

The women in the WHI aged 50–59 years had a beneficial (protective) effect for CHD compared with the older groups of women, which included women up to age 79 years. CEE alone had a beneficial composite coronary score in younger (aged <60 years) women and, when both groups (CEE alone and CEE plus MPA) were combined, women aged 50–59 years in the WHI had a statistically significant reduction in mortality of 30%. The coronary benefit reported in the WHI was predominantly with CEE alone rather than CEE plus MPA. In a prospective trial, Early versus Late Intervention Trial with Estradiol (ELITE), that tested the timing hypothesis, carotid intima–media thickness as the primary end point, was shown to be significantly less over 5 years with oral estradiol (1 mg daily) than with placebo, which is consistent with estrogen causing an inhibition of atherosclerosis progression. However, this effect only occurred in women within 6 years of the onset of menopause and was not present in another group of women who were > 10 years from onset of menopause. Early initiation, but not late initiation, of HRT had a protective effect (Hodis et al. 2016).

HRT in Older Women: Is It Ever Too Late?

The primary objective of the care of symptomatic menopausal women is enhancement of quality of life (QoL). Women who have not been exposed to estrogen for many years may be particularly susceptible to estrogen-related side effects such as bloating, breast tenderness, and vaginal discharge. If a risk-benefit analysis favors HRT, it may be appropriate and acceptable to initiate systemic or local HRT in some older women to improve the QoL.

Vasomotor symptoms (VMS) are usually most troublesome in the perimenopause and early menopausal years, but generally improve spontaneously over a period of 2–5 years. In some women, VMS persist on average 7.4 years and sometimes for more than 10 years. In a study of Swedish women aged older than 85 years, 16% reported hot flashes at least several times per week (Vikstrom et al. 2013). HT can be considered for prevention of osteoporosis in women aged 65 years and older at elevated risk for fracture when bothersome VMS persist or when HT remains the best choice for QoL reasons or because of lack of efficacy or intolerance of other osteoporosis-prevention therapies. IMS Recommendations suggested that initiation of MHT in the age group 60–70 years requires individually calculated benefit/risk,

consideration of other available drugs, and the lowest effective dose. MHT should not be initiated after age 70 years. There is no mandatory time limit for duration of MHT provided that it is consistent with treatment goals. This is important as the protective effect of MHT on bone mineral density declines after cessation of therapy at an unpredictable rate, although some degree of fracture protection may remain after cessation of MHT (Baber et al. 2016). The decision to stop HT should be made on the basis of "extraskeletal" benefits and risks.

Lower doses of transdermal estrogen may represent a preferable route of ET administration for older or menopausal women who are obese or for those with elevated triglycerides or liver concerns. Ongoing monitoring for new health concerns, periodic trials of lower doses, transdermal formulations, or attempts at discontinuation may help healthcare providers and individual women aged older than 65 years clarify their decisions about continuing HT (The NAMS 2017 Hormone Therapy Position Statement Advisory Panel 2017).

The Beers criteria for Potentially Inappropriate Medication Use in Older Adults, by the American Geriatrics Society, include oral and transdermal estrogen, with or without a progestin (American Geriatrics Society Beers Criteria® Update Expert Panel 2019). However, the American Geriatrics Society's recommendation to a routinely discontinue systemic HT in women aged 65 years and older is not supported by data. In this regard, one of the most the ACOG's Practice Bulletin reported: "The decision to continue HT should be individualized and be based on a woman's symptoms and the risk-benefit ratio, regardless of age. Because some women aged 65 years and older may continue to need systemic HT for the management of vasomotor symptoms, the American College of Obstetricians and Gynecologists recommends against routine discontinuation of systemic estrogen at age 65 years. As with younger women, use of HT and estrogen therapy should be individualized, based on each woman's risk-benefit ratio and clinical presentation." (Practice bulletin no. 141 2014)

HRT After Prophylactic Risk-Reducing Salpingo-oophorectomy in BRCA1 and BRCA2 Mutation: Is It Possible?

Nowadays, prophylactic risk-reducing salpingo-oophorectomy (RRSO) is recommended in BRCA1 and BRCA2 patients, especially when childbearing is complete, because of its proven efficacy in reducing risk and mortality in BRCA mutation carriers. The surgical procedure is simple and feasible, but RRSO has several short and long term clinical consequences. Among these consequences, the occurrence of early menopause is the most feared by both patient and health care providers. In fact, early surgical menopause has an important impact on premenopausal woman's health, including her quality of life, sexual life, bone mineral density, cognitive impairment, cardiovascular disease. To mitigate these adverse effects, exogenous hormone prescription has been proposed with a favorable clinical feedback also in healthy BRCA mutation carriers who underwent RRSO before natural menopause. Marchetti and colleagues demonstrated that HRT seems to be a

safe therapeutic option in BRCA 1 and 2 mutation carriers undergoing RRSO. It seems that women who receive Estrogen-alone have a lower, not significant trend for breast cancer risk compared with those who receive estrogen plus progesterone. The perplexity on HRT administration is partially based on data derived from HRT trials in the overall postmenopausal population. In the WHI randomized trial, a statistically significant increase in breast cancer risk was observed among postmenopausal women submitted to estrogen plus progesterone therapy (Marchetti et al. 2018). But it should be underlined that women enrolled in these studies were from the overall population and were also postmenopausal women who extended their lifelong hormone exposure after menopause. This clinical scenario is completely different from premenopausal BRCA mutated and oophorectomized women. In fact, these women are usually and reasonably younger than those involved in WHI trials and experienced menopausal symptoms very earlier than natural. In BRCA mutation carries, Kostopoulos et al. found a possible protective effect in those women who used ET (Kotsopoulos et al. 2018).

HRT: Type and Combination

Estrogen deficiency is the principal pathophysiological mechanism that underlies menopausal symptoms and various estrogen formulations are prescribed as MHT, which remains the most effective therapeutic option available. The addition of progesterone aims to protect against the consequences of systemic therapy with estrogen only in women with intact uteri (Woods et al. 2013): namely, endometrial pathologies, including hyperplasia and cancer (Fig. 1). The risk-benefit ratios of all treatment options must be considered, taking into account the nature and severity of symptoms and individual treatment-related risks (Table 1).

Estrogen Alone or Combined with Progestin

The results of multiple studies support the effectiveness of HT for the management of menopausal vasomotor symptoms. A Cochrane meta-analysis of 24 randomized controlled trials (RCTs), which included 3,329 participants, found a 75% reduction in weekly hot flush frequency in users of oral estrogen or oral estrogen plus progestin compared with placebo and a reduction in symptom severity (MacLennan et al. 2004b). Greendale et al. proposed one of the largest studies investigating the effect of oral HT on vasomotor symptoms, the Postmenopausal Estrogen/Progestin Interventions trial, an RCT of 875 women treated with oral CEE alone or in combination with continuous or cyclic progestin versus placebo, which found a significant reduction in self-reported vasomotor symptoms in women in both the estrogen-alone arm and the estrogen-plus-progestin arm compared with placebo (Greendale et al. 1998).

There are a variety of preparations available for systemic estrogen therapy. Estrogen with or without progestin can be administered orally or transdermally in the forms of patches, gels, or sprays.

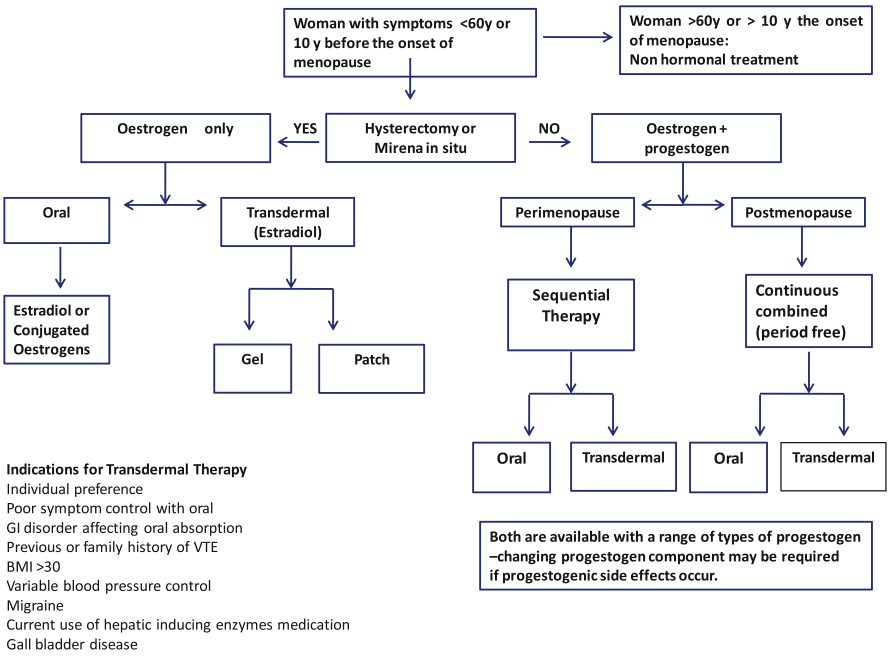


Fig. 1 HRT Treatment. (Modified by HRT Guide of British Menopause Society)

Studies of the efficacy of HT for the treatment of vasomotor symptoms have principally investigated standard doses of HT. Although HT is well tolerated by most women, standard doses may cause adverse effects, such as breast tenderness, vaginal bleeding, bloating, and headaches. Low-dose and ultra-low systemic doses of estrogen may be associated with a better adverse effect profile than standard doses and may reduce vasomotor symptoms in some women. Examples of low-dose systemic estrogen therapy formulations include 0.3–0.45 mg/d of oral CEE, 0.5 mg/d of oral micronized estradiol, 5 micrograms/d of ethinyl estradiol, and 0.025–0.0375 mg/week of transdermal estradiol (patch). There also are low-dose formulations of estradiol available in topical gels, creams, and sprays that are approved by the U.S. FDA.

There is good evidence that oral and transdermal low-dose estrogen regimens effectively relieve VMS in women. The Heart Outcomes Prevention Evaluation, a large trial that assessed the efficacy of different doses of HT on VMS in more than 2,500 postmenopausal women, found a similar reduction in VMS with a CEE dosage of 0.625 mg/d and all lower combination doses.

The results of studies that assessed the efficacy of ultra-low doses of estrogen (0.25 mg of oral micronized estradiol and 0.014 mg of transdermal estradiol) to treat VMS have been mixed, and currently these doses are not FDA approved for this indication. The degree of improvement of VMS from low-dose and ultra-low dose preparations has not been as extensively studied as with standard-dose preparations.

Table 1 Prescription therapy options for management of menopausal symptoms (Davis et al. 2015)

Treatment option Hormone therapy	Benefits	Risks	Patient-specific considerations
Systemic estrogen alone	• Symptom relief • Fracture risk reduction • Osteoporosis prevention • Improved QoL	• VTE • Stroke • CVD • Breast cancer (not seen in large clinical trials) • Gallstones	• Estrogen-related risk of stroke, VTE and CVD is exaggerated with advancing age and increasing time since onset of menopause • Comorbidities such as obesity, hypertension, and diabetes all increase the risk of VTE, CVD, and stroke • Risk reduction against VTE and gallstones achieved by using a transdermal route and reduced dose • For women with natural menopause and bothersome vasomotor symptoms who are aged <60 years or within 10 years of their menopause, the benefits of hormone therapy outweigh the potential for treatment-related harm • For those with early or premature ovarian insufficiency, benefits outweigh harm even in the absence of symptoms
Systemic estrogen and progestogen	• Symptom relief • Fracture risk reduction • Osteoporosis prevention • Improved QoL • Colon cancer risk reduction	• VTE • Stroke • CVD • Breastcancer • Gallstones	• Estrogen-related risk of stroke, VTE, and CVD is exaggerated with advancing age and increasing time since onset of menopause • Comorbidities such as obesity, hypertension, and diabetes all increase the risk of VTE, CVD, and stroke • Risk reduction against VTE may be achieved by using a transdermal route and reduced estrogen dose • Observational data suggest that progesterone may have less impact on breast cancer risk than MPA in menopausal women, although ideally this should be tested in an randomized trial • Endometrial cancer risk is associated with estrogen dose, as well as the type, dose, and duration of progestin • Progesterone is less effective than synthetic progestins at negating the proliferative effects of systemic estrogen • For women with natural menopause and bothersome vasomotor symptoms who are aged <60 years or within 10 years of their menopause, the benefits of hormone therapy outweigh the potential for treatment-related harm • For those with early or premature ovarian insufficiency, benefits outweigh harm even in the absence of symptoms

Low-dose vaginal estrogen	Effective against genitourinary syndrome of menopause	Negligible	Significant systemic absorption can transiently occur with excessive use of vaginal estrogen creams, particularly in the setting of an atrophic vaginal epithelium
Ospemifene (SERM)	Effective against moderate to severe dyspareunia due to vulvovaginal atrophy	VTE risks are deemed comparable to those of low-dose estrogen	Approved for management of moderate to severe dyspareunia due to vulvovaginal atrophy in the United States and Europe
TSEC (combination of conjugated equine estrogen and BZA (SERM))	Symptom relief	Similar risk profile to estrogen in terms of risk for VTE, stroke, CVD, and gallstones	• Approved in the United States and Europe • Indicated for management of menopausal symptoms in women with intact uteri • Neutral effects on the uterus and on breast tissue (SERM effect) • Potential for skeletal benefit as conjugated equine estrogens and BZA are effective for fracture risk reduction
Tibolone (synthetic steroid with estrogenic, progestogenic and androgenic activity)	• Symptom relief • Fracture risk reduction • Osteoporosis prevention • Improved QoL • Colon cancer risk reduction	• Risk of stroke in women aged >60 years • Risk of breast cancer may be lower than with other therapies	• Neutral effects on the endometrium; no need for concomitant progestogen therapy
Dehydroepiandrosterone [DHEA]	• Symptoms of menopause including bone loss, muscle loss • Type 2 diabetes • Fat accumulation • Osteoporosis • Hot flushes • Memory loss, cognition loss • Alzheimer's disease		• Neutral effects on the endometrium; no need for concomitant progestogen therapy

Although women will experience improvement in symptoms with low and ultra-low doses of HT, lower doses do not appear to be as effective as traditional doses of HT in alleviating VMS. Nonetheless, given the variable response to HT and the associated risks, it is recommended that health care providers individualize care and treat women with the lowest effective dose for the shortest duration that is needed to relieve vasomotor symptoms (Practice bulletin no. 141 2014).

The risks of systemic combined HT include thromboembolic disease and breast cancer. HT should be avoided in those with unexplained vaginal bleeding, active liver disease, previous breast cancer, coronary heart disease, stroke, personal history of thromboembolic disease, or known high inherited risk. CVD risk factors do not automatically preclude HT but should be taken into account. Upregulation of the hepatic synthesis of procoagulants is another known effect of oral estrogens. Transdermal estradiol does not seem to increase the risk of venous thromboembolic events. Evidence shows that trans-dermal estrogen ($\leq$50 μg) is associated with a lower risk of deep vein thrombosis, stroke, and myocardial infarction compared to oral therapy (The North American Menopause Society 2012) and may be the preferred mode of treatment in women with an increased thrombosis risk, such as obese women and smokers In addition, unlike oral estrogens, transdermal estradiol does not increase the risk of gallbladder disease (Olie et al. 2011; Høibraaten et al. 2000).

Genitourinary syndrome is a relatively new terminology describing vulvovaginal changes at menopause, as well as urinary symptoms of frequency, urgency, nicturia, dysuria, and recurrent urinary tract infections. Vaginal dryness is common after menopause and unlike VMS usually persists and may worsen with time. Urogenital symptoms are effectively treated with either local (vaginal) or systemic estrogen therapy (Santen 2014). Estrogen therapy effectively alleviates atrophic vaginal symptoms related to menopause, restores normal vaginal flora, lowers the pH, and thickens and revascularizes the vaginal lining. The number of superficial epithelial cells increases and symptoms of atrophy are alleviated. Given the documented efficacy and proven safety, vaginal estrogen is the first-line approach to treat symptoms of vaginal atrophy in the majority of women: vaginal estrogen is effective, while systemic absorption does occur, it does not induce endometrial hyperplasia. All low-dose systemic estrogen formulations are FDA-approved for the treatment of atrophic vaginitis. Oral dosages of CEE as low as 0.3 mg/d and transdermal estradiol dosages of 12.5 micrograms/d have demonstrated improvements in vaginal atrophy. Local vaginal estradiol and local CEE, which can be administered in cream, ring, and tablet formulations, are effective in treating atrophic vaginitis in menopausal women. Low-dose (10 micrograms) vaginal estradiol tablets improve vaginal symptoms. Typically, vaginal estrogen formulations are administered daily for 1–2 weeks as induction therapy and then may be used indefinitely at low doses for maintenance therapy. Studies indicate that the 3-month estradiol-releasing vaginal ring is preferred to cream because of greater comfort, ease of use, and satisfaction. Systemic absorption of vaginal estrogen has been documented in postmenopausal women with atrophy using a daily low-dose vaginal estrogen preparation with 25 micrograms of estradiol. Local estrogen therapy was not associated with an increased risk of

endometrial hyperplasia and the addition of progestins for endometrial protection is not needed. Use of progestin or local estrogen therapy does not require endometrial surveillance, unless women experience postmenopausal bleeding, which would require diagnostic evaluation. Because of variable rates of estrogen absorption associated with local estrogen therapy, there has been concern about the use of this treatment in women with a history of breast cancer (Practice bulletin no. 141 2014).

The relationship between HT and urinary incontinence depends on the delivery route. Systemic HT worsens urinary incontinence, but vaginal treatment may improve urge incontinence and prevent recurrent urinary tract infections. Using very low doses for the first few weeks is helpful if irritation occurs and indeed lower doses of vaginal ET, with less frequent administration, often yield satisfactory results (Roberts et al. 2014).

For how long should women take HT? Current international guidelines advise consideration of HT in healthy women within 10 years of their final menstrual period with moderate to severe VMS but there is less clarity on when to stop. This decision can be made on an individual basis and in the absence of contraindications some guidelines advise that it can be used for as long as the woman feels the benefits outweigh the risks for her. Balancing the increasing risk of breast cancer with duration of use (combined HT) and additional concerns about increased cardiovascular and cerebrovascular risk with age has meant that short-term use (under 5 years) may provide the best risk benefit ratio for most women. While overall use of HT has declined in most countries, recommendations about shorter periods of use mean that more women are navigating how and when to stop. Stopping HT commonly leads to recurrent VMS and may also trigger new onset VMS.

Initiation of HT is usually contraindicated in women with a personal history of breast cancer or venous thromboembolism, or those with a high risk for breast cancer, thrombosis, or stroke. Trans-dermal estrogen therapy may be considered and preferred when highly symptomatic women with type 2 diabetes mellitus or obesity, or those at high risk of cardiovascular disease, do not respond to non-hormonal therapies (Davis et al. 2015).

Progestin

In order to avoid undue chronic stimulatory effects on the endometrium, control menstrual bleeding, avoid abnormal bleeding, and avoid cancer development, the combination of the estrogen with a progestogen is needed. In women, progesterone is naturally produced in the ovaries (particularly the corpus luteum), in the placenta, and to a certain extend in the adrenals; there are a variety of synthetic progestogens. One of these progestogens, dydrogesterone, is a retro-progesterone, and another, drospirenone (DRSP), is spironolactone derivative. The "newer" progestogens belong to different classes based on their structure. For each of them, the progestogenic, as well as the antiestrogenic action, is common. The antiandrogenic effect is relevant for dienogest (DNG) and DRSP and minor for nomegestrol acetate (NOMAC). None of them have a glucocorticoid effect. DRSP is different due to

its strong antimineralocortocoid action and has a favorable effect on blood pressure. In addition, these progestogens do not interfere with the positive effect of estrogens on lipid and carbohydrate metabolism, do not augment hemostasis processes as monotherapy, and avoid induction of abnormal proliferation of the endometrium in doses clinically tested. Therefore, all three progestogens appear to be suitable for treatment of menopausal women (Schindler 2014). Although progestin is primarily used as an add-on agent to estrogen therapy to prevent endometrial hyperplasia and endometrial cancer in women with a uterus, there is some evidence that progestin may also improve vasomotor symptoms. There are limited data on the safety of progestin alone compared with combined estrogen and progestin preparations for the treatment of vasomotor symptoms. Because the risk of breast cancer was increased in the CEE and MPA arm of WHI and not in the CEE-alone arm, there is concern that the risk of breast cancer may be related to progestin use. Therefore, progestin alone is not considered a first-line therapy for the management of vasomotor symptoms, but its combination with estrogens is still indicated to prevent endometrial hyperplasia and cancer risk (Roberts et al. 2014).

Testosterone

Testosterone in combination with HT has been investigated for the treatment of menopausal symptoms. Testosterone for the treatment of vasomotor symptoms has shown no benefit and potential adverse effects including detrimental effects on lipid parameters, clitoromegaly, hirsutism, and acne. However, in the Cochrane meta-analysis, the pooled estimate suggested that the addition of testosterone to HT regimens improved sexual function scores and number of satisfying sexual episodes for postmenopausal women (Somboonporn et al. 2005). The role of testosterone for the treatment of postmenopausal desire or arousal disorders and the long-term implications of such a therapy in postmenopausal women remains unclear. This strategy may benefit a certain subset of women, such as those with surgically induced menopause, who have persistent sexual symptoms. Testosterone alone is currently not FDA-approved for use in women (Practice bulletin no. 141 2014).

Tibolone

Tibolone is a synthetic steroid that is rapidly converted to two metabolites with estrogenic activity and to a third metabolite characterized by a mixed progestogenic/androgenic activity. Tibolone controls hot flushes, sweating, mood symptoms and is effective in improving libido, due to its androgenic component.

Randomized, controlled studies show that tibolone increases bone mineral density and reduces fracture risk. These beneficial effects are seen over long-term treatments (Rymer et al. 2002) (over 10 years) and both in early and late postmenopausal women as well as in women with established osteoporosis.

The combined analysis of randomized clinical studies on tibolone indicates no increase in risk of breast cancer development compared with placebo. Tibolone treatment is associated with a reduction of proliferation and a stimulation of apoptosis in normal breast cells that is possibly attributable to the impact of this compound on the activity of estrogen-metabolizing breast enzymes (Valdivia et al. 2004). The metabolization of tibolone is tissue selective, and the conversion to the progestogenic metabolite is particularly active in the endometrium. Investigation of endometrial histology in women treated with tibolone shows no hyperplasia and a high level of atrophic endometrium, indicating no proliferative effect of this molecule. However, given its limited safety and efficacy data compared with HT, tibolone is not considered a first-line therapy for menopausal symptoms (Practice bulletin no. 141 2014).

The Emerging Therapy

In the past 2 years, new pharmaceutical preparations were approved in the United States and in Europe for the treatment of menopausal symptoms. An oral selective estrogen receptor modulator (SERM), ospemifene, has been approved for the treatment of moderate to severe pain during intercourse associated with vulvovaginal atrophy (Constantine et al. 2014), and a tissue-specific SERM–estrogen complex (a combination of oral conjugated equine estrogen and bazedoxifene (an SERM)) has been approved for the management of moderate to severe vasomotor symptoms in women with an intact uterus (Pinkerton et al. 2014).

Estrogen Agonists and Estrogen Antagonists: Ospemifene

Estrogen agonists and estrogen antagonists are synthetic compounds that selectively stimulate or inhibit the estrogen receptors of different target tissues. Such selectivity is possible because estrogen receptors in different target tissues vary in chemical structure. Because estrogen agonists and estrogen antagonists selectively stimulate or inhibit the estrogen receptors of different target tissues, their effects in some tissues can have a deleterious physiologic effect, including an increased risk of thromboembolic events, endometrial and vulvovaginal abnormalities, and vasomotor problems. Studies demonstrate that two currently available FDA-approved agents, raloxifene and tamoxifen, are not effective for the treatment of menopausal vaginal symptoms. However, the recent introduction of ospemifene, an orally available selective estrogen receptor modulator with the indication of prevention and treatment of VVA, without stimulating the endometrium, may represent a new option for those patients who are intolerant to long-term use of vaginal estrogens (Palacios et al. 2015). Common adverse effects of ospemifene reported during clinical trials included hot flushes, vaginal discharge, muscle spasms, genital discharge, and excessive sweating. The FDA approved ospemifene for treating moderate-to-severe dyspareunia in postmenopausal women.

TSEC (Combination of Conjugated Equine Estrogen and BZA (SERM))

The rationale for combining estrogens with a SERM is to retain beneficial effects of estrogens on VMS, VVA, and bone while incorporating the antiestrogenic effects of the SERM on the breast and endometrium to improve the overall safety profile. It was recently demonstrated that CEE and BZA can form ER heteroligand dimer complexes resulting in cooperative gene regulation. Furthermore, BZA has been found to degrade the ER in the endometrium and breast, suggesting it acts more like the pure antiestrogen Fulvestrant than like other SERMs in these tissues. BZA's antiestrogenic effects in endometrial tissue eliminate the need to include a progestin when combined with estrogens in women with a uterus (Pickar et al. 2009).

Five Phase III, randomized, double-blind Selective estrogens, Menopause, And Response to Therapy (SMART) trials established the efficacy and safety of CEE/BZA use for up to 2 years. CEE/BZA is generally well tolerated. Among women treated with CEE/BZA in the SMART studies, rates of ischemic stroke, cardiovascular events, and VTE were low and similar to placebo. The tissue selective estrogen complex (combination of 0.45 mg of oral CEE and 20 mg bazedoxifene) has been approved for the management of moderate to severe VMS in the USA and Europe (Genazzani et al. 2015).

Dehydroepiandrosterone (DHEA)

Over the past 15 years, hormone preparations of DHEA have been available over-the-counter and have been sold as the "fountain of youth"(Pluchino et al. 2013). DHEA serves as a precursor for estrogens and androgens from fetal life to postmenopause, and many people believe that DHEA is merely an inactive precursor pool for the formation of bioactive steroid hormones. On the contrary, DHEA also acts in its own right through dedicated receptors. In the brain, DHEA is a neurosteroid that acts as a modulator of neurotransmitter receptors. In addition, DHEA or DHEA sulfate (DHEAS) may also have effects through its more immediate metabolites, such as 7 α-hydroxy-DHEA. Higher concentrations of DHEA are found in brain in comparison with plasma values, with a brain-to-plasma ratio of 6.5. In human vessels, DHEA binds with high affinity to the endothelial cell membrane, and it is not displaced by structurally related steroids.

So, DHEAS represent the most abundant sex steroid in plasma in humans (more than 1000 times higher than estradiol and testosterone levels), but its serum concentration goes down to 10–20% of its maximum level by around the age of 70 years. The large difference between low and high serum DHEA levels has a major clinical impact. Among postmenopausal women with coronary risk factors, lower DHEAS levels were linked with higher mortality from cardiovascular disease and all-cause mortality. Similarly, women with lower DHEAS levels show increased risk of ischemic stroke and reduced flow-mediated dilation of the brachial artery compared

to women with normal DHEAS plasma values (Shufelt et al. 2010). Higher endogenous DHEAS levels are independently and favorably associated with executive function, concentration, and working memory (Davis et al. 2008).

Several studies had previously demonstrated that 1-year treatment (Genazzani et al. 2011; Pluchino et al. 2008), using administration of 10 mg DHEA daily in symptomatic postmenopausal women with lower (5th percentile) baseline DHEAS levels, improved climacteric and sexual symptoms and directly reversed some age-related changes in adrenal enzymatic pathways, including adrenal DHEA and progesterone synthesis.

In addition, before drawing definitive conclusions on DHEA replacement therapy, further aspects need to be better investigated, such as the genetics of DHEA intracrinology and adrenal aging as well their relation with climacteric symptoms (Pluchino and Genazzani 2015).

Another emerging hormonal therapeutic option for menopausal symptoms is a combination of oral prasterone (DHEA) and Acolbifene. Acolbifene is a SERM reported to have ER antagonist activity in the breast and uterus but estrogen agonist effects on bone. The rationale for combining DHEA with acolbifene is to potentially derive a product that combines the benefits of both components. For example, benefits with regard to prevention of osteoporosis may be additive given DHEA's anabolic effects (i.e., stimulation of bone formation) and acolbifene's ability to reduce bone loss. Use of DHEA to treat sexual function in postmenopausal women remains controversial due to conflicting results from randomized trials, many of which were small or had other methodologic limitations. Data from initial studies of acolbifene/DHEA are still awaited (Genazzani et al. 2015).

Conclusion

The role of HRT in the health care of women has gone through many changes. HRT remains the most efficacious of the available strategies for managing menopausal symptoms. In the majority of young and otherwise healthy early menopausal women who are within 10 years of onset of menopause, the benefits of systemic HT will probably outweigh any potential for harm. By contrast, in older women or women with comorbidities that increase the risk for cardiovascular disease and stroke (such as hypertension, obesity, longstanding smoking history, and/or a strong family history of stroke and premature atherosclerosis), the potential harm of systemic HT outweighs the benefits. New and emerging menopausal therapies have the potential to fill an unmet need in the post-WHI era for effective relief of menopausal symptoms with improved safety profiles. Treatment should always be individualized: the ultimate goal is to get closer to the profile of the ideal menopausal therapy that has to relieve bothersome menopausal symptoms and reduce the risk of osteoporosis and cardiovascular disease, improving QoL, without increasing the risk of endometrial or breast cancer.

Cross-References

- ▶ Hormonal Contraception
- ▶ Long-Term Consequences of Menopause
- ▶ Menopause and Bone Metabolism

References

American Geriatrics Society Beers Criteria® Update Expert Panel. American Geriatrics Society 2019 updated AGS Beers Criteria® for potentially inappropriate medication use in older adults. 2019.

Baber RJ, Panay N, Fenton A, The IMS Writing Group. Recommendations on women's midlife health and menopause hormone therapy. Climacteric. 2016;19(2):109–50.

Constantine G, Graham S, Portman DJ, Rosen RC, Kingsberg SA. Female sexual function improved with ospemifene in postmenopausal women with vulvar and vaginal atrophy: results of a randomized, placebocontrolled trial. Climacteric. 2014;18(2):226–32.

Davis SR, Shah SM, McKenzie DP, Kulkarni J, Davison SL, Bell RJ. Dehydroepiandrosterone sulfate levels are associated with more favorable cognitive function in women. J Clin Endocrinol Metab. 2008;93(3):801–8.

Davis SR, Lambrinoudaki I, Lumsden M, Mishra GD, Pal L, Rees M, Santoro N, Simoncini T. Menopause. PRIMER. 2015;I:4.

Genazzani AR, Stomati M, Valentino V, Pluchino N, Potì E, Casarosa E, Merlini S, Giannini A, Luisi M. Effect of 1-year, low-dose DHEA therapy on climacteric symptoms and female sexuality. Climacteric. 2011;14:661–8.

Genazzani AR, Komm BS, Pickar JH. Emerging hormonal treatments for menopausal symptoms. Expert Opin Emerg Drugs. 2015;20(1):31–46.

Greendale GA, Reboussin BA, Hogan P, Barnabei VM, Shumaker S, Johnson S, et al. Symptom relief and side effects of postmenopausal hormones: results from the Postmenopausal Estrogen/Progestin Interventions Trial. Obstet Gynecol. 1998;92:982–8.

Grodstein F, Stampfer MJ, Colditz GA. Postmenopausal hormone therapy and mortality. N Engl J Med. 1997;336:1769–75.

Hodis HN, Mack WJ, Henderson VW. Effects of early versus late postmenopausal treatment with estradiol. N Engl J Med. 2016;374:1221–31.

Høibraaten E, Qvigstad E, Arnesen H, Larsen S, Wickstrøm E, Sandset PM. Increased risk of recurrent venous thromboembolism during hormone replacement therapy—results of the randomized, double-blind, placebo-controlled estrogen in venous thromboembolism trial (EVTET). Thromb Haemost. 2000;84:961–7.

Huber J, Birkhäuser M, Metka M, Druckmann R, Rohr U, Winkler T. Europäische Gynäkologen analysieren die WHIStudie – Hormonstudie amerikanischer Kardiologen. J Menopause. 2002;9:7–13.

Huber J, Birkhäuser M, Metka M, Druckmann R, Rohr U, Winkler T, Schindler AE, Nelson HD, Walker M, Zakher B, Mitchell J. Menopausal hormone therapy for the primary prevention of chronic conditions: a systematic review to update the U.S. Services Task Force Recommendations. Ann Int Med. 2012;157:104–10.

Kotsopoulos J, Gronwald J, Karlan BY, Huzarski T, Tung N, Moller P, Armel S, Lynch HT, Senter L, Eisen A, Singer CF, Foulkes WD, Jacobson MR, Sun P, Lubinski J, Narod SA, Hereditary breast cancer clinical study group. Hormone replacement therapy after oophorectomy andand breast cancer risk among BRCA1 mutation carriers. JAMA Oncol. 2018;4(8):1059–65.

Lobo RA. Hormone-replacement therapy: current thinking. Nat Rev Endocrinol. 2016;13(4):220–31.

MacLennan AH, Broadbent JL, Lester S, Moore V. Oestrogen and combined oestrogen/progestogen therapy versus placebo for hot flushes. Cochrane Database Syst Rev. 2004a;4:CD002978.

MacLennan AH, Broadbent JL, Lester S, Moore V. Oral oestrogen and combined oestrogen/progestogen therapy versus placebo for hot flushes. Cochrane Database Syst Rev. 2004b;4:CD002978.

Marchetti C, De Felice F, Boccia S, Sassu C, Di Donato V, Pergnola G, … Benedetti Panici P. Hormone replacement therapy after prophylactic risk-reducing salpingo-oophorectomy and breast cancer risk in BRCA1 and BRCA2 mutation carriers: a meta-analysis. Crit Rev Oncol Hematol. 2018;132:111–115.

Mikkola TS, Tuomikoski P, Lyytinen H, Korhonen P, Hoti F, Vattulainen P, … Ylikorkala O. Increased cardiovascular mortality risk in women discontinuing postmenopausal hormone therapy. J Clin Endocrinol Metab. 2015;100(12):4588–4594.

Olie V, Plu-Bureau G, Conard J, Horellou MH, Canonico M, Scarabin PY. Hormone therapy and recurrence of venous thromboembolism among postmenopausal women. Menopause. 2011;18:488493.

Osteoporosis. In: National institutes of health consensus development conference statement, April 2–4, 1984. Department of Health & Human Services

Palacios S, Castelo-Branco C, Currie H. Update on management of genitourinary syndrome of menopause: a practical guide. Maturitas. 2015;82:308–13.

Pickar JH, Yeh IT, Bachmann G, Speroff L. Endometrial effects of a tissue selective estrogen complex containing bazedoxifene/conjugated estrogens as a menopausal therapy. Fertil Steril. 2009;92:1018–24.

Pinkerton JV, Abraham L, Bushmakin AG, Cappelleri JC, Racketa J, Shi H, Chines AA, Mirkin S. Evaluation of the efficacy and safety of bazedoxifene/conjugated estrogens for secondary outcomes including vasomotor symptoms in postmenopausal women by years since menopause in the Selective Estrogens, Menopause and Response to Therapy (SMART) trials. J Womens Health (Larchmt). 2014;23(1):18–28.

Pluchino N, Genazzani AR. DHEA replacement for postmenopausal women: have we been looking in the right direction? Climacteric. 2015;18:1–3.

Pluchino N, Ninni F, Stomati M, Freschi L, Casarosa E, Valentino V, Luisi S, Genazzani AD, Potì E, Genazzani AR. One-year therapy with 10mg/day DHEA alone or in combination with HRT in postmenopausal women: effects on hormonal milieu. Maturitas. 2008;59:293–303.

Pluchino N, Carmignani A, Cubeddu A, Santoro A, Cela V, Errasti T. Androgen therapy in women: for whom and when. Arch Gynecol Obstet. 2013;288:731–7.

Practice bulletin no. 141 management of menopausal symptoms. Obstet Gynecol 2014; 123 (1):202–216.

Roberts H, Hickey M, Lethaby A. Hormone therapy in postmenopausal women and risk of endometrial hyperplasia Cochrane review summary. Maturitas. 2014;77(1):4–6.

Rymer J, Robinson J, Fogelman I. Ten years of treatment with tibolone 2.5 mg daily: 283 effects on bone loss in postmenopausal women. Climacteric. 2002;5:390–8.

Santen RJ. Vaginal administration of estradiol: effects of dose, preparation and timing on plasma estradiol levels. Climacteric. 2014;17:1–14.

Schindler AE. The “newer” progestogens and postmenopausal hormone therapy (HRT). J Steroid Biochem Mol Biol. 2014;142:48–51.

Shufelt C, Bretsky P, Almeida CM, et al. DHEA-S levels and cardiovascular disease mortality in postmenopausal women: results from the National Institutes of Health–National Heart, Lung, and Blood Institute (NHLBI)-sponsored Women’s Ischemia Syndrome Evaluation (WISE). J Clin Endocrinol Metab. 2010;95:4985–92.

Somboonporn W, Bell RJ, Davis SR. Testosterone for peri and postmenopausal women. Cochrane Database Syst Rev. 2005;19;(4):CD004509.

Stampfer MJ, Colditz GA. Estrogen replacement therapy and coronary heart disease: a quantitative assessment of the epidemiologic evidence. Prev Med. 1991;20:47–63.

The NAMS 2017 Hormone Therapy Position Statement Advisory Panel. The 2017 hormone therapy position statement of The North American Menopause Society. Menopause. 2017;24 (7):728–53.

The North American Menopause Society. The 2012 hormone therapy position statement of the North American Menopause Society. Menopause. 2012;19:257–71.

Valdivia I, Campodonico I, Tapia A, Capetillo M, Espinoza A, Lavin P. Effects of 287 tibolone and continuous combined hormone therapy on mammographic breast density and 288 breast histochemical markers in postmenopausal women. Fertil Steril. 2004;81:617–23.

Vikstrom J, Spetz Holm A, Sydsjo G, Marcusson J, Wressle E, Hammar M. Hot flashes still occur in a population of 85-year-old Swedish women. Climacteric. 2013;16:453–9.

Welton AJ, Vickers MR, Kim J, Ford D, Lawton B, MacLennan AH, WISDOM team. Health related quality of life after combined hormone replacement therapy: randomised controlled trial. BMJ. 2008;337:a1190.

Woodruff JD, Pickar JH. The menopause study group. Am J Obstet Gynecol. 1994;170:1213–23.

Woods DC, White YA, Tilly JL. Purification of oogonial stem cells from adult mouse and human ovaries: an assessment of the literature and a view toward the future. Reprod Sci. 2013;20:7–15.

Menopause and Bone Metabolism

19

M. L. Brandi and L. Di Medio

Contents

M. L. Brandi (✉)
FirmoLab Fondazione F.I.R.M.O, Florence, Italy

University of Florence, Metabolic Bone Diseases Unit, University Hospital of Florence, Florence, Italy
e-mail: marialuisa.brandi@unifi.it

L. Di Medio
University of Florence, Metabolic Bone Diseases Unit, University Hospital of Florence, Florence, Italy
e-mail: lisadimedio80@gmail.com

F. Petraglia, B. C. Fauser (eds.), *Female Reproductive Dysfunction*, Endocrinology,
https://doi.org/10.1007/978-3-030-14782-2_19

Abstract

The hormonal changes that take place at menopause are the most important reason why women are at greater risk of osteoporosis than men.

Fragility fractures osteoporosis related are a relevant cause of disability and excess mortality in postmenopausal women.

General measures such as calcium and vitamin D supplementation or physical exercise could be very useful for prevention of fragility fractures.

The use of registered drugs is recommended when the health benefits overcome the risks. The FRAX® algorithm is a scientifically validated risk assessment tool that improves identification of patients at high risk of fracture.

Antiresorptive agents are the predominant therapeutic category for preventing fractures, and bisphosphonates are still the most commonly used. However, new and more effective therapies are nowadays available for prevention and treatment of bone loss.

Keywords

Postmenopause · Osteoporosis · Fragility fractures · Antiosteoporotic therapy

Introduction

Osteoporosis is an osteometabolic disease characterized by substantial loss of bone mass and microarchitecture deterioration of bone tissue.

The operational definition of osteoporosis is based on the finding of a reduced bone mineral density, measured with DEXA, of at least 25% compared to the healthy young adult population.

Bone maintenance is a delicate business. To preserve bone strength in postmenopause women, the daily removal of small amounts of bone mineral, a process called resorption, must be balanced by an equal deposition of new mineral. When this balance tips toward excessive resorption, bones weaken and become prone to fracture.

Hormones are possibly the most crucial modulators of bone formation. Estrogen, parathyroid hormone, and testosterone are essential for optimal bone development and maintenance. Of these, estrogen has the most direct effect on bone cells, interacting with specific receptors on the surface of osteoblasts and osteoclasts.

The hormonal changes that take place at menopause are one reason why women are at greater risk of osteoporosis than men.

As bones become more porous and fragile, the risk of fracture is greatly increased. The loss of bone occurs silently and progressively. Often there are no symptoms until the first fracture occurs.

Fragility fractures are associated with serious disability and excess mortality. However, general measures of prevention and treatment such as calcium and vitamin D supplementation, the guidance for fall prevention, and the practice of specific

physical exercises can be instituted before the manifestation of the disease and may promote other health benefits.

The use of drugs registered for the treatment of osteoporosis is recommended when the benefits overcome the risk.

FRAX is recognized as a useful tool for easily estimate the long-term fracture risk.

Pharmacological strategies may increase bone mineral density (BMD) and reduce the risk of osteoporotic fractures.

Antiresorptive agents are the predominant therapeutic category for preventing fractures, and bisphosphonates are still the most commonly used. Nowadays, new and more effective therapies are available to prevention and treatment of bone loss.

Postmenopausal Osteoporosis

Osteoporosis is an osteometabolic disease characterized by decreased bone mass and microarchitecture deterioration of bone tissue.

It is estimated over 200 million women have osteoporosis. Worldwide, one in three women and one in five men over the age of 50 will experience an osteoporotic fracture.

Since 1940 Albright suggested a causal relationship between the postmenopausal state and osteoporosis.

The hormonal changes that take place at menopause are one reason why women are at greater risk of osteoporosis than men.

Bone tissue loss generally begins after the age of about 40 years, when we are no longer able to replace bone tissue as quickly as we lose it. In women, however, the rate of bone tissue loss increases after menopause, when estrogen production stops and bones no longer benefit from its protective effect.

Bones are composed of two types of tissue: a hard outer layer called cortical (compact) bone, which is strong, dense, and tough and a spongy inner layer called trabecular (cancellous) bone. This network of trabeculae is lighter and less dense than compact bone. Bone is also composed of bone forming cells (osteoblasts and osteocytes), bone resorbing cells (osteoclasts), nonmineral matrix of collagen and noncollagenous proteins (osteoid), and inorganic mineral salts deposited within the matrix.

Our bones are living tissue and constantly changing. From the moment of birth until young adulthood, bones are developing and strengthening. During childhood and adolescence, the cartilage grows and is slowly replaced by hard bone. Our bones are at their most dense in our early 20s – called peak bone mass.

Osteogenesis (bone tissue formation) occurs by two processes: intramembranous and endochondral ossification. Intramembranous ossification involves the replacement of connective tissue membrane sheets with bone tissue and results in the formation of flat bones (e.g., skull, clavicle, mandible). Endochondral ossification involves the replacement of a hyaline cartilage model with bone tissue (e.g., femur, tibia, humerus, radius).

Bone modeling is when bone resorption and bone formation occur on separate surfaces (i.e., formation and resorption are not coupled). An example of this process is during long bone increases in length and diameter. Bone modeling occurs during birth to adulthood and is responsible for gain in skeletal mass and changes in skeletal form. Increase in length is due to continued endochondral bone formation at each end of the long bones. Increase in circumference of the bone shaft is achieved by formation of new bone on the outer surface of the cortical bone.

Bone remodeling is the replacement of old tissue by new bone tissue. This mainly occurs in the adult skeleton to maintain bone mass. This process involves the coupling of bone formation and bone resorption.

The balance between bone resorption and bone deposition is determined by the activities of osteoclasts and osteoblasts. At various stages throughout this process, the precursors, osteoclasts, and osteoblasts communicate with each other through the release of various "signaling" molecules. How these signaling molecules and various other endogenous (such as hormones) or external (such as diet and exercise) factors influence the cells involved in bone physiology is a topic of intense research activity.

Bone maintenance is a delicate business. In adults, the daily removal of small amounts of bone mineral, a process called resorption, must be balanced by an equal deposition of new mineral if bone strength is to be preserved. When this balance tips toward excessive resorption, bones weaken and over time can become brittle and prone to fracture.

This continual resorption and redeposition of bone mineral, or bone remodeling, is intimately tied to the pathophysiology of osteoporosis. Understanding how bone remodeling is regulated is the key to the effective prevention and treatment of osteoporosis.

As we age, daily remodeling leads to a gradual restructuring of the bone. Resorption of the minerals on the inside of the cortical layer and in the bone cavity itself leads to an inexorable loss of trabecular bone and a widening of the bone cavity. This is partly compensated for by the gradual addition of extra layers of mineral to the outside of the cortical layer.

The upshot is that overall the bones get slightly thicker. But the danger is that they are not getting any denser. In fact, peak bone mass, reached in early adulthood, gradually declines as people get older.

Bone architecture and continual remodeling combine to have a huge impact on the pathophysiology of osteoporosis. For example, young adults with wider femurs might be at higher risk for hip fractures late in life because, on average, wider bones tend to have thinner cortical layers. The thinner this layer is, the more susceptible it will be to resorption later in life (Seeman et al. 2006).

According to recent knowledge, postmenopausal osteoporosis is the result of the negative change in the balance between bone formation by osteoblasts and bone resorption by osteoclasts. Osteoblast and osteoclast proliferation and activity is regulated by systemic hormones (calciotropic hormones, estrogens, steroids, etc.) and by local factors (e.g., cytokines, prostaglandins, IGF, etc.).

Hormones are possibly the most crucial modulators of bone formation. It is well established that estrogen, parathyroid hormone, and to a lesser extent testosterone are essential for optimal bone development and maintenance.

Of these, estrogen is now believed to have the most direct effect on bone cells, interacting with specific proteins, or receptors, on the surface of osteoblasts and osteoclasts (Lips et al. 2006; Zallone et al. 2006).

Estrogen plays an important role in the growth and maturation of bone as well as in the regulation of bone turnover in adult bone. During bone growth, estrogen is needed for proper closure of epiphyseal growth plates both in females and in males. Also in young skeleton, estrogen deficiency leads to increased osteoclast formation and enhanced bone resorption.

Males have larger skeletal size and greater bone mass than females.

Osteoporosis is therefore more prevalent in women than in men, the fracture incidence increases after artificial or natural menopause, and estrogen replacement has a protective effect on bone mass loss.

Estrogen effects are mediated through one specific type of cell surface receptor called the estrogen receptor alpha (ERα), which binds and transports the hormone into the nucleus of the cell where the receptor-hormone complex acts as a switch to turn on specific genes. ERα receptors are found on the surface of osteoblasts, as is estrogen receptor-related receptor alpha (ERRα), which may play an auxiliary role in regulating bone cells.

Recent studies also suggest that sex hormone binding globulin (SHBG), which facilitates entry of estrogen into cells, may also play a supportive role.

During the accelerated bone loss in women during the first 4–8 years after menopause, the calcium fluxes resulting from skeletal and extraskeletal effects of estrogen deficiency are balanced, or nearly so, because serum PTH levels remain relatively constant. During this accelerated phase, the loss of cancellous bone is threefold to fivefold greater than the loss of cortical bone. The rapid cancellous bone loss is mediated by a large increase in the number and activity of osteoclasts, resulting in perforative resorption of trabecular plates with loss of structural elements.

In contrast, the continuous phase of bone loss in men is associated with a gradual but protracted decrease of serum bioavailable estrogen and testosterone of only about 15% per decade and, in postmenopausal women after the rapid phase of bone loss subsides, the already low levels of estrogen decrease little more.

This more gradual decline in serum bioavailable estrogen and testosterone results in slow bone loss that is mediated mainly by the extraskeletal effects of sex steroid deficiency.

In cortical bone the first response of estrogen withdrawal is enhanced endocortical resorption. Later, also intracortical porosity increases. These lead to decreased bone mass, disturbed architecture, and reduced bone strength. Moreover, osteoblasts and osteoclasts have estrogen receptors, suggesting a direct involvement of these hormones on bone formation and resorption.

At cellular level in bone, estrogen inhibits differentiation of osteoclasts thus decreasing their number and reducing the amount of active remodeling units.

This effect is probably mediated through some cytokines. These local factors, which also comprise some growth factors, act mainly as autocrines or paracrines on the proliferation or differentiation of bone cells. In particular interleukin I (ILl), IL 6 and tumor necrosis factor alpha (TNF-alpha) induce osteoblasts to stimulate mature osteoclasts and bone resorption via a promotion of osteoclast recruitment and differentiation from bone marrow precursors.

Furthermore, estrogens have been found to decrease the release of interleukin 6 (IL6) from human bone cells and that of ILl, TNF alpha, and granulocyte-macrophage colony-stimulating factor (GM-CSF) from human blood mononuclear cells.

This suggests that estrogens could also have indirect effects on the bone, perhaps by modulating the production of one or more of the local bone-regulating factors released by blood or bone cells. ILl and IL6 are cytokines produced predominantly by cells of the monocyte-macrophage lineage but also by other mesenchymal cells including those of bone lineage.

These cytokines play an important role in the pathogenesis of bone and cartilage destruction in several inflammatory diseases such as rheumatoid arthritis and in malignant osteolytic lesions.

Lots of investigations suggest that ILl and IL6 may be involved in the pathogenesis of osteoporosis and that estrogens could play a significant role in regulating their release.

The ILl release by circulating mononuclear cells increases in postmenopausal women and estrogens inhibit this phenomenon.

The normal values of cytokines observed in subjects treated with estrogens or estrogens and progesterone confirm that ovarian steroids play an important role in modulating the production of these factors which although not specific of bone, could regulate bone resorption.

Other systemic factors like parathormone (PTH) could stimulate the osteotropic factors, for example, 1,25(OH)2D3, Ill, and IL6.

An increase in serum PTH was found confirming the important role of this hormone in promoting bone resorption after the estrogen decrease. The increase in serum PTH could be due to the negative calcium balance for a deficit of 1,25(OH) 2D3 synthesis and a consecutive deficit of intestinal calcium absorption.

How any of these hormones impact bone remodeling depends on how they alter osteoclasts and/or osteoblasts activity. Recently, scientists have started to uncover specific cell surface receptors that help transmit signals from outside bone cells into the cell nucleus, where different genes that regulate cell activity can be switched on or off. These include receptors for bone morphogenetic proteins (BMPs), a family of proteins which are potent inducers of bone formation.

BMP receptors have been found on the surface of osteoblasts precursor cells (Mbalaviele et al. 2005).

Scientists have had more success piecing together various components that stimulate osteoclast activity. It was discovered that a cell surface receptor called RANK (for receptor activator of NFkB) prods osteoclasts precursor cells to develop into fully differentiated osteoclasts when RANK is activated by its cognate partner RANK ligand (RANKL).

RANKL, in fact, is produced by osteoblasts and is one of perhaps many signaling molecules that facilitate cross-talk between the osteoblasts and osteoclasts and help coordinate bone remodeling.

Osteoprotegerin, another protein released by osteoblasts, can also bind to RANKL, acting as a decoy to prevent RANK and RANKL from coming in contact. The balance of RANKL/osteoprotegerin may be crucial in osteoporosis.

Subtle differences in the genetic code might explain why one person's osteoblasts or osteoclasts are more active or responsive to their environment, and it might also lead to the discovery of unknown regulatory mechanisms. Environmental factors can also have an enormous impact on bone physiology.

Estrogen is made and secreted into the bloodstream some distance from bone and it also has profound effects on other tissues, such as the uterus and breast. But there are other, locally produced signaling molecules that have profound effects on bone physiology, for example, prostaglandins, particularly prostaglandin E2 (PGE2), that stimulate both resorption and formation of bone (Pilbeam et al. 2002). PGE2 may be required for exercise-induced bone formation.

Epidemiology

Osteoporosis is one of the most common and debilitating chronic diseases and a global healthcare problem.

Currently it is estimated that over 200 million people worldwide suffer from this disease. Around the world, one in three women and one in five men over the age of 50 will suffer an osteoporotic fracture (Fig. 1).

Although more common in older people, osteoporosis can also affect younger people. Approximately 30% of all postmenopausal women have osteoporosis in the United States and in Europe. At least 40% of these women and 15–30% of men will sustain one or more fragility fractures in their remaining lifetime.

Ageing of populations worldwide will be responsible for a major increase in the incidence of osteoporosis in postmenopausal women.

In women over 45 years of age, osteoporosis accounts for more days in hospital than may other diseases, including diabetes, myocardial infarction, and breast cancer.

In Sweden, osteoporotic fractures in men account for more hospital bed days than those due to prostate cancer.

It has been shown that an initial fracture is a major risk factor for a new fracture. An increased risk of 86% for any fracture has been demonstrated in people that have already sustained a fracture (Kanis et al. 2004).

Likewise, patients with a history of vertebral fracture have a 2.3-fold increased risk of future hip fracture and a 1.4-fold increase in risk of distal forearm fracture.

Vertebral fractures are rarely reported by physicians and remain most of the time remain undiagnosed. Fewer than 10% of vertebral fractures result in hospitalization, even if they cause pain and substantial loss of quality of life.

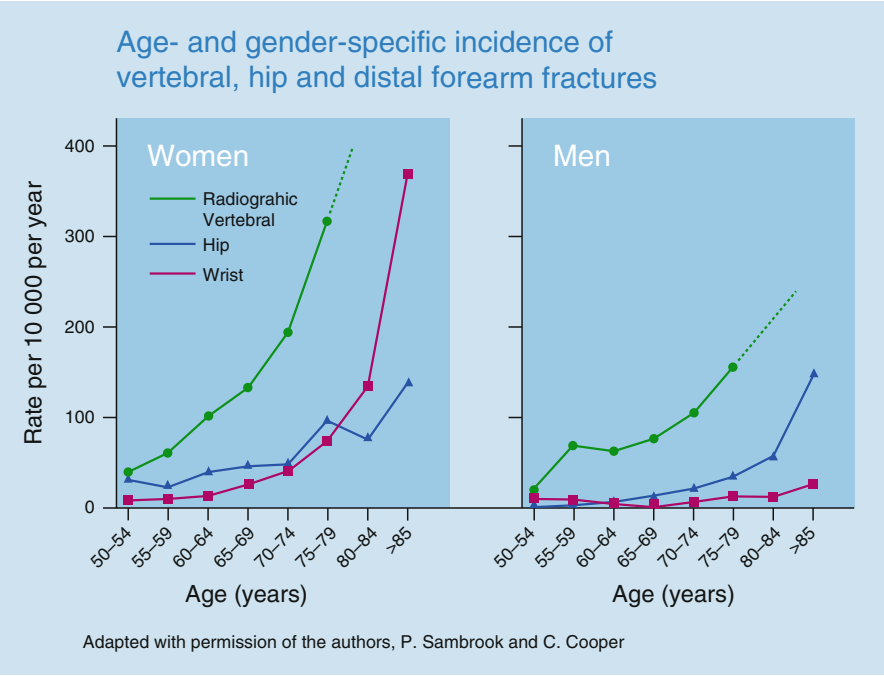


Fig. 1 Age-specific and sex-specific incidence of radiographic vertebral, hip, and distal forearm fractures (Sambrook et al. 2006)

Vertebral fractures are rarely reported by physicians and most of the time remain undiagnosed. Fewer than 10% of vertebral fractures result in hospitalization, even if they cause pain and substantial loss of quality of life.

In Europe, the prevalence defined by radiological criteria increases with age in both sexes and is almost as high in men as in women: 12% in females (range 6–21%) and 12% in males (range 8–20%). This fact could be explained by occupation-associated trauma in men.

New fractures are most likely in nearby vertebrae, and they occur more frequently in the mid-thoracic or thoracolumbar regions of the spine.

Hip fracture is associated with serious disability and excess mortality. Women who have sustained a hip fracture have a 10–20% higher mortality than would be expected for their age. After a hip fracture, about one-quarter of people die or never walk again.

The worldwide annual incidence of hip fracture is approximately 1.7 million.

Hip fracture rates vary markedly between populations. After age adjustment, hip fracture rates are more common in Scandinavian and North America than these observed in southern European, Asian, and Latin American countries. There are wide discrepancies between the incidence rate in women and men: the sex ratio F/M is 4/5 and 90% of the hip fractures occur in people over 50 years old (Melton III et al. 2006).

By 2050, the worldwide incidence of hip fracture is projected to increase by 240% in women and 310% in men.

The estimated number of hip fractures worldwide will rise from 1.66 million in 1990 to 6.26 million in 2050, even if age-adjusted incidence rates remain stable.

Wrist fractures are most likely to occur in women over 65 years old. An increase in age-adjusted incidence in white women between 45 and 60 years of age has been observed. Then the trend stabilizes or slightly increases. Only 15% of wrist fractures occur in men and this rate does not increase much with age.

In Europe, the annual incidence of distal forearm fractures in male and female was estimated 1.7 and 7.3 per 1000 person-years, respectively (EPOS Group 2002).

It is important to notice that distal forearm fractures are an early and sensitive marker of male skeletal fragility. Aging men carry a higher absolute risk for hip fractures than spinal fractures in comparison to women (Haentjens et al. 2004).

Instrumental Diagnosis

Dual-Energy X-ray Absorptiometry

Dual-energy X-ray absorptiometry (DXA) is a low radiation X-ray capable of detecting quite small percentages of bone loss. It is used to measure spine and hip bone density and can also measure bone density of the whole skeleton.

The World Health Organization has defined a number of threshold values for osteoporosis. The reference measurement is derived from bone density measurements in a population of healthy young adults (called a T-score). Osteoporosis is diagnosed when a person's BMD is equal to or more than 2.5 standard deviations below this reference measurement. Osteopenia is diagnosed when the measurement is between 1 and 2.5 standard deviations below the young adult reference measurement.

X-rays

Traditional X-rays cannot measure bone density, but they can identify spine fractures.

Back pain and loss of height can be the first symptoms of vertebral fractures induced by osteoporosis. In order to assess the severity of vertebral fractures, a semiquantitative method based on visual inspection has been developed (Fig. 2).

It has been extensively used in clinical trials and epidemiological studies. The severity of the fracture is assessed by measuring the extent of vertebral height reduction, by its morphological changes, and by differentiating the fracture from nonfracture deformities. Grades are assigned to each vertebra based on the degree of height reduction.

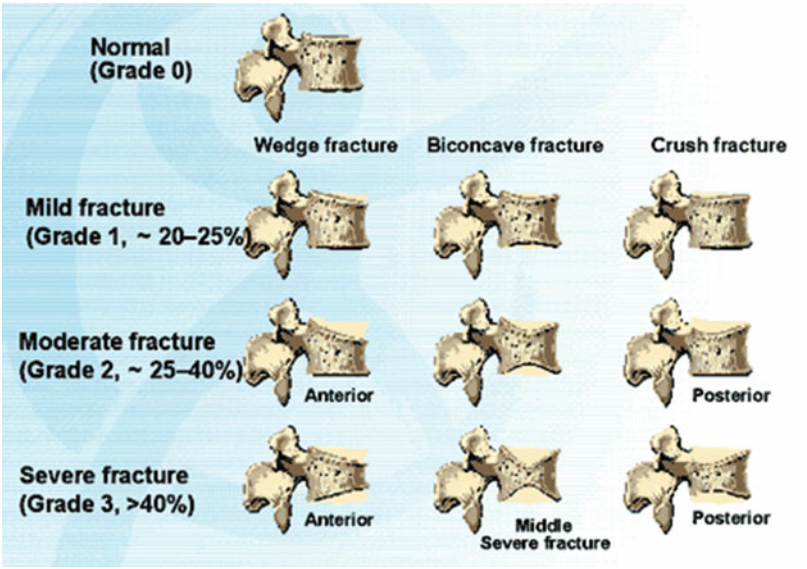


Fig. 2 Vertebral fractures semi-quantitative grading (Genant et al. 1996)

This method does not link the type of deformity with the grading of the fracture. One advantage of this method is by assessing the severity of the deformation, and new deformities occurring on a prevalent vertebral fracture can be assessed within the range of grading.

Quantitative methods such as morphometry or magnetic resonance imaging (MRI) have been developed over the past years and can be used to assess more precisely the features of vertebral fractures.

Peripheral Quantitative Computed Tomography

Until recently, treatment-induced bone microarchitectural changes could only be assessed by histomorphometric analyses of iliac crest bone biopsies.

The development of high-resolution peripheral quantitative computed tomography (HRpQCT), however, enables noninvasive assessments of cortical and trabecular bone volumetric density, geometry, and microarchitecture at peripheral anatomic sites in vivo. Only a few studies have investigated the effects of osteoporosis treatment on bone microarchitecture assessed by HRpQCT (Burghardt et al. 2010), and no published studies have assessed the microarchitectural and compartmental structural changes in response to the combination of antiresorptive and anabolic therapy in humans. Furthermore, recent technical advances now allow for the HRpQCT measurement of intracortical porosity, a parameter that is independently associated with prevalent hip fracture and is known to independently contribute to the age-related decrease in bone strength.

Bone Turnover Markers

Bone Turnover Markers (BTM) have been extensively used in clinical research to monitor the efficacy and mechanisms of action of new drugs. There are three categories of BTM depending on their origination from the bone mineral unit: bone resorption markers, bone formation markers, and markers of osteoclast regulatory proteins (Leeming et al. 2006).

These markers, measured in serum or urine, are not disease-specific. They assess alterations in skeletal metabolism, regardless of the underlying cause.

Combining BMD with BTM could improve fracture prediction in postmenopausal women. One advantage of biochemical markers compared to BMD is early estimation of treatment effect.

Significant changes in BTM can be seen during antiresorptive therapy after a few weeks of treatment, whereas individual monitoring with DXA usually requires 1–2 years to identify significant changes. As adherence is an important issue of long-term therapy in chronic disease, it has been suggested that BTM could be used in clinical practice to assess the patient's adherence to treatment and also provide feedback on the effectiveness of the medication.

Fragility Fractures

Osteoporosis can lead to fragility fractures, which result in clinical burden and increased mortality.

Even after a fracture, fewer than 25% of patients receive pharmacologic treatment for osteoporosis (Wilk et al. 2014).

After the discovery that sclerostin deficiency causes rare genetic conditions that are characterized by high bone mass and resistance to fracture, sclerostin became a therapeutic target for the treatment of osteoporosis. Sclerostin, a negative regulator of bone formation that is secreted by osteocytes, inhibits Wnt signaling, down-regulating this stimulus for osteoblast development and function (Poole et al. 2005).

Besides the instrumental approach, the osteoporotic patient workup requires the assessment of clinical risk factors for fragility fracture, facilitated by the use of specific algorithms (i.e., FRAX), and the integration with laboratory studies aiming to exclude secondary osteoporosis.

The antiosteoporotic treatment involves the use of pivotal interventions, such as educational, addressing lifestyle, and nutritional ones, including the appropriate calcium and vitamin D intake with the diet and, in case of insufficiency, with its supplementation.

Therapeutic management should include the integration of an antiosteoporotic drug in subjects at high risk for fragility fracture, which also takes into account patients comorbidity and compliance.

Because bone loss is gradual and painless, there are usually no symptoms to indicate a person is developing osteoporosis. This is why osteoporosis is often referred to as the silent disease. Often the first symptom of osteoporosis is a fracture.

Most commonly, osteoporotic fractures occur at the spine, the wrist, or the hip, although osteoporotic fractures can occur in other bones as well.

While most limb fractures (such as at the wrist or hip) are obvious, spinal fractures can be more difficult to diagnose. This is because they might be painless, or if there is pain, a person may not know it is caused by a fracture due to the many different causes of back pain. More obvious signs of spinal fractures are loss of height and development of a curved upper back.

Fragility fractures affect the muscle and the skeletal systems and cause chronic pain, loss of functional capacity, and compromise quality of life (Cosman et al. 2014).

Fragility fractures represent a significant challenge for Health Systems because of their increasing number, a factor directly related to the steady growth of the elderly population. Around 40% of the population experience a femur, vertebrae, or wrist fracture once in their lives, and in most cases this occurs after the age of 65 years, thus causing significant social and economic costs for both the health expenditure generated by the hospitalization, and the resulting disability and loss of independence; this is particularly noteworthy for femur fractures.

Aging is associated with dramatic increases in fracture risk. In men, the exponential increase in risk occurs approximately one decade later than in women (Gennari et al. 2007).

For the same age, morbidity and mortality rate after hip fracture was higher in men than in women.

The risk for a man over the age of 50 years of sustaining any type of osteoporotic fracture during the rest of his life ranges from 13.1% (in the USA) to 22.4% (in Sweden). Women have a significantly higher risk of suffering a similar fracture (over 53.2% in the UK).

Compared to women, men had fewer hip fractures (30% of total hip fractures), vertebral fractures (42% of the total number in this area), and forearm and humeral fractures (20% and 25% of the total number, respectively). However, in the rest of the skeleton, men sustained a greater number of fractures than women (54% vs. 46%) (Johnell and Kanis 2006).

Fractures in a context of osteoporosis in women over 45 years of age cause more hospitalization days than other important health conditions such as myocardial infarction, diabetes, or breast cancer. As a consequence, a reduction in the social and health impact of bone fragility would preserve the individual's motion independency, which is directly related to the quality of life of all elderly citizens, and would be associated with valuable cost savings at the health system level.

The social and health relevance of this condition has been realized only recently and this partly explains the delay in the implementation of organic health intervention plans, compared to those implemented for other diseases with a large impact on the population.

In the last 20 years, there have been enormous advances in our understanding of the many factors that contribute to fracture risk, and in elucidating the genetic, molecular, and cellular mechanisms that regulate bone metabolism, growth, and involution during the course of life. Based on this knowledge, we now have the

ability to identify in a more efficient and timely manner those individuals that have a high fracture risk and thus be in a position to start them on therapies that have, in recent years, proven effective in reducing the number of fractures.

However, when aiming at reducing the impact of bone fragility, we should consider factors other than just pharmacological therapy. Many fragility fractures are observed in individuals with only a moderate fracture risk, who represent a large percentage of the population. It is therefore necessary to develop primary prevention plans that promote a healthy and correct lifestyle in the younger population, with a goal to obtain a fracture risk reduction in a large proportion of the population, even with a mid- to long-term timeframe.

It is estimated that an increase of 5% in bone mass at the end of bone development a realistic objective that can be achieved by modifying the food habits and the physical activity of adolescents could translate to a 30% reduction of all fracture events in old age.

Only a small number of the patients that are hospitalized for a fragility fracture are then offered an appropriate diagnostic and therapeutic path after discharge, in spite of the high risk of recurrence that is typical in these patients. On the other hand, less than half of the patients that start on a pharmacological treatment adhere to their medication and therapy regimen after 1 year, thus generating an unacceptable system inefficiency and nullifying the chance of intervention efficacy.

These two issues may seem simple; however, they share the necessity of a common approach from a cultural and organizational point of view. Any patient that is hospitalized for acute treatment of a fragility fracture can be direct along an appropriate path whereby the correct clinical approach can only be guaranteed through the avoidance of intervention fragmentation.

The correct path would utilize all the synergies that the system provides, by applying a multidisciplinary approach along with a greater organizational efficiency following shared protocols and procedures (e.g., Fracture Unit) in order to maximize the existing expertise of our hospital staff and to grant higher standards of care to patients.

The effects of primary medicine need to integrate with an efficient health system that provides easy access to specialist intervention when appropriate, granting access to a network of second- and third-level centers that are necessary to grant high standards of care. This should be coupled with appropriate utilization of the more complex diagnostic resources and prescription of the more costly therapies.

Fracture Risk Assessment

Scientific research supports the use of proven therapies to prevent osteoporotic fractures based on the individual's probability of fracture as opposed to their bone density score alone. This relatively new concept has been validated to ensure its accuracy and reproducibility by a World Health Organization working group (established in 1998) in collaboration with International Osteoporosis Foundation and the US National Osteoporosis Foundation.

The result is an easy-to-use fracture risk assessment tool to use with patients of both sexes called FRAX® – Fracture Risk Assessment Tool. FRAX® is a scientifically validated risk assessment tool which has now been integrated into an increasing number of national osteoporosis guidelines around the world.

The FRAX® algorithms give the 10-year probability of hip and major osteoporotic fracture. It is based on specific individual risk factors, with or without bone mineral density values at the femoral neck.

It is a major milestone in helping health professionals to improve identification of patients at high risk of fracture.

Preventing Fracture

Osteoporosis can be diagnosed and treated and fractures often prevented through healthy lifestyle choices and appropriate medication for those in need.

Genetic factors play a significant role in determining whether an individual is at heightened risk of osteoporosis. However, lifestyle factors such as diet and physical activity also influence bone development in youth and the rate of bone loss later in life.

Bone mass acquired during youth is an important determinant of the risk of osteoporotic fracture during later life. The higher the peak bone mass, the lower the risk of osteoporosis.

During childhood and the beginning of adulthood, bone formation is more important than bone resorption. Later in life, however, the rate of bone resorption is greater than the rate of bone formation and results in net bone loss.

Bones are living tissue, and the skeleton grows continually from birth to the end of the teenage years, reaching a maximum strength and size (peak bone mass) in early adulthood, around the mid-20s. It is estimated a 10% increase of peak bone mass in children reduces the risk of an osteoporotic fracture during adult life by 50%.

Once peak bone mass has been reached, it is maintained by a process called remodeling. This is a continuous process in which old bone is removed (resorption) and new bone is created (formation). The renewal of bone is responsible for bone strength throughout life.

After mid-20s, bone thinning is a natural process and cannot be completely stopped. The thicker bones are, the less likely they are to become thin enough to break.

Any factor which causes a higher rate of bone remodeling will ultimately lead to a more rapid loss of bone mass and more fragile bones. Young women in particular need to be aware of their osteoporosis risk and take steps to slow its progress and prevent fractures.

There are a number of other risk factors associated with osteoporosis:

- A close family member diagnosed with osteoporosis
- A family history of fractures resulting from minor bumps and falls
- Frequent falls
- A previous fracture

- Long-term enforced bed rest
- Little physical activity
- Low body weight
- Loss in height
- Periods stop for more than 12 months
- A diet low in calcium and vitamin D
- High alcohol intake
- Smoking
- Certain medications in long-term use such as corticosteroids
- Age >60 years
- Chronic disorders such as anorexia nervosa, malabsorption syndromes including coeliac disease and Crohn's disease, chronic liver disease, primary hyperparathyroidism, post-transplantation, chronic renal failure, hyperthyroidism, Cushing's syndrome, arthritis.

Osteoporosis prevention should be based on the elimination of specific risk factors.

In particular, women in menopause should ensure a nutritious diet and adequate calcium intake (1200 mg/day), avoid under-nutrition, particularly the effects of severe weight-loss diets and eating disorders, maintain an adequate supply of vitamin D, participate in regular weight-bearing activity, avoid smoking and second-hand smoking, and avoid heavy drinking.

Physical exercise plays an important role in building and maintaining bone and muscle strength. It also helps to reduce falls by improving balance and aids rehabilitation from fractures. Muscles and bones respond and strengthen when they are "stressed." This can be achieved by weight bearing or impact exercises is an important stimulus for osteoporosis prevention and treatment (Kelley et al. 2013).

Bone tissue is continuously remodeled, and as a dynamic tissue, it adapts and responds to various stimuli, such as physical exercise and mechanical vibration.

During physical activity mechanical forces can be exerted on bones through ground reaction forces and by the contractile activity of muscles, resulting in maintenance or gain of bone mass. Studies have already pointed out many of the mechanical stimuli that are beneficial to bone tissue, including some physical activities as aquatic and ground exercises.

However, it is not clear yet which modality would be better to stimulate bone metabolism and enhance physical function of postmenopausal women.

Moderate to intense exercises, performed in a high speed during short intervals of time, in water or on the ground, can be part of a program to prevent and treat postmenopausal osteoporosis. Mechanical vibration has proven to be beneficial for bone microarchitecture, improving bone density and bone strength, as well as increasing physical function.

Walking as an exercise program can improve the femoral BMD in postmenopausal women, but not the spine BMD in this population. A combined exercise program (resistance + aerobic + impact) is recommended for an enhancement of spine BMD.

Mechanical vibration has also proven to stimulate bone metabolism and physical function in the postmenopausal period, being a very safe and feasible alternative to this population.

Although impact exercises are recognized as beneficial for bone tissue stimulation, it seems that other variables such as muscle strength, type of muscle contraction, duration, and intensity of exercises are also determinants to induce changes in bone metabolism of postmenopausal women. It is important to emphasize that not only osteogenic exercises should be recommended; activities aimed to develop muscle strength and body balance and improve the proprioception should be encouraged to prevent falls and fractures.

Postmenopausal women should engage in exercises with some precautions, to avoid the risk of injuries and fractures. For so, it is strictly recommended that after consulting with a physician, patient follows an exercise program prescribed by a specialist at the area: a physical education professional or a physiotherapist (Moreira et al. 2014).

Pharmacological Treatment

Osteoporosis is a chronic, progressive condition that generally requires long-term management.

In postmenopausal women with osteoporosis, the primary outcome investigated in pharmaceutical trials is the reduction of fracture. Risk reductions of between 30% and 70% have been demonstrated for vertebral fractures, around 15–20% for non-vertebral fractures and up to 40% for hip fracture.

A number of effective medications are approved for the prevention and treatment of postmenopausal osteoporosis. These medications must be tailored to a person's specific needs and used in conjunction with recommended lifestyle changes.

In addition to drug therapy, calcium and vitamin D supplements can be prescribed to ensure adequate intake and to ensure maximum effectiveness of the drug therapy.

Among the pharmacologic options to treat postmenopausal osteoporosis, two main classes are available: antiresorptive drugs, inhibiting bone resorption, and anabolic drugs, stimulating bone formation.

Bisphosphonates, selective estrogen receptor modulators (SERM), and the more recent denosumab and romosozumab belong to first category, while osteo-anabolic agents are teriparatide and strontium ranelate (which has also a slightly anti-resorptive action).

Antiresorptive agents are the predominant therapeutic category for the prevention and treatment of bone loss, and the nitrogen-containing bisphosphonates are the most commonly used. This results in an increase in BMD and reduction in risk for fracture.

Treatment options can only work if taken as recommended. It is common for people with osteoporosis to find taking medication challenging. As a result, up to half of all people stop their treatment after only 1 year (Reginster et al. 2006; Weycker et al. 2006).

Compared to people who adhere to their osteoporosis treatment, people with inadequate adherence will have smaller increases in bone mineral density weaker

suppression of bone resorption (the breakdown of bone by cells known as osteoclasts) and greater fracture risk.

Bisphosphonates

Bisphosphonates (BP) are potent inhibitors of bone resorption that inhibit the activity of osteoclasts. All approved bisphosphonates have been shown to reduce vertebral fracture risk and increase BMD, whereas some have demonstrated reductions in non-vertebral and hip fracture risk as well.

They are available in oral and intravenous formulations, with weekly, monthly, and annual dosing schedules, depending on the specific agent. Bisphosphonates significantly reduce bone turnover by binding to the mineralized surface of bone and inhibiting the bone-resorbing activity of mature osteoclasts.

Oral bisphosphonates are a commonly prescribed treatment for osteoporosis, but inconvenient dosing regimens and side effects can lead to low adherence (Silverman et al. 2011).

Suboptimal adherence to osteoporosis medication can reduce antifracture efficacy and increase health care use and costs. Although more extended dosing intervals can improve adherence, efficacy remains an influential determinant of patient preference for and adherence with osteoporosis medications (Silverman et al. 2011; Lee et al. 2011).

Approved bisphosphonates for postmenopausal osteoporosis are alendronate, risedronate, ibandronate, and zoledronic acid.

Alendronate

Alendronate decreases bone resorption and formation markers, increases BMD, and reduces the incidence of fractures by 30–50% in women with established osteoporosis. The antifracture efficacy has been shown both in women with prevalent vertebral fractures and in women with low BMD (T-score < -2) but without vertebral fractures.

Meta-analysis carried out on the basis of several studies in postmenopausal and elderly osteoporotic women showed that alendronate decreases the risk of hip fracture by about 45% (Papapoulos et al. 2005).

Bridging studies showed that once-weekly alendronate at the dose of 70 mg is therapeutically equivalent to the reference daily regimen (similar increase in BMD, similar decrease in bone turnover marker levels).

In early postmenopausal women, a smaller dose of alendronate (5 mg) prevents bone loss.

Risedronate

Risedronate decreases the incidence of new vertebral and peripheral fractures by the same extent as alendronate in women with low BMD and in women with prevalent vertebral fractures.

In osteoporotic women 70–79 years of age, risedronate decreased the incidence of hip fracture by 40%.

Bridging studies showed that alternative doses of risedronate (35 mg once a week, 75 mg on two consecutive days a month, 150 mg once a month) decrease the BTM levels and increase BMD to a similar extent as the daily regimen.

Ibandronate

Ibandronate is commercially available as an oral monthly regimen (150 mg once monthly) and intravenous form (3 mg intravenously every 3 months). In postmenopausal osteoporotic women, oral ibandronate induces a rapid increase in BMD and reduces the risk of vertebral fractures to a similar extent as weekly alendronate and risedronate.

In postmenopausal osteoporotic women, intravenous ibandronate (3 mg every 3 months) induces a greater increase in BMD and a similar reduction in the incidence of clinical fractures in comparison with oral ibandronate.

Meta-analyses of the results of all the existing studies showed that the annual cumulative exposure (ACE) to ibandronate higher than 10.8 mg was associated with a decrease in the incidence of the nonvertebral fractures by about 30–40% (Harris et al. 2008; Cranney et al. 2009).

Zoledronic Acid

Zoledronic acid administered intravenously to postmenopausal women with osteoporosis at a dose of 5 mg once-yearly induced a sustained decrease in bone turnover, a progressive increase in BMD and a significant decrease in the incidence of vertebral fractures by 70% and in the incidence of nonvertebral fractures by 25% (including a significant 40% decrease in the incidence of hip fractures) (Black et al. 2007).

In older men and women with recent low trauma hip fracture (2 weeks or later but less than 90 days after surgical repair), zoledronic acid increased BMD at the hip, decreased the incidence of clinical fractures (including a significant decrease in the incidence of hip fracture), and reduced the mortality rate by about 30% (Lyles et al. 2007; Eriksen et al. 2009).

In men and women treated with oral glucocorticoids, zoledronic acid induced a greater decrease in the rate of bone turnover and a greater increase in BMD compared with risedronate (Reid et al. 2009).

Side Effects and Limitations of Bisphosphonates

Orally administered BPs have a poor intestinal absorption and can induce mild intestinal disturbances.

Recently, several issues related to long-term use of BPs have been raised.

BPs are potent suppressors of bone resorption and may lead to a phenomenon called “severely suppressed bone turnover,” particularly in patients on glucocorticoid therapy and/or concomitant antiresorptive therapy, such as hormone replacement therapy (HRT). Such extreme inhibition of bone remodeling may theoretically lead to an accumulation of micro damage which might compromise bone strength and increase the risk of low trauma fracture or delay fracture healing.

However, iliac crest biopsies from women on long-term bisphosphonate do not show increased microdamage, and clinical trials of bisphosphonates did not show evidence of altered healing (Chapurlat et al. 2007).

To avoid potential side effects, many clinicians consider it appropriate to re-evaluate the patients fracture risk after 5 years of treatment and then consider whether to stop or continue the treatment. There is limited evidence to support this key clinical decision. In the FLEX trial, withdrawal of alendronate after 5 years of treatment was followed by a mild decrease in BMD (at some, but not all sites) and a mild increase in BTM levels (Black et al. 2006).

In another study, fracture incidence after BP discontinuation increased in women who took BPs for 2 years with a suboptimal adherence (Curtis et al. 2008).

By contrast, after discontinuation of the long term treatment with alendronate in the FLEX study, fracture incidence remained reduced for 5 years, except for a slightly higher risk of clinical vertebral fractures in comparison with women who took alendronate continuously (Black et al. 2006). However, there was no placebo group in this study, so it is difficult to draw firm conclusions. Thus, there are no evidence-based guidelines how long osteoporotic patients should take BPs. However, on the basis of the available clinical and preclinical data, it can be inferred that, in the vast majority of patients, stopping therapy is more likely to do harm than continuing therapy.

Osteonecrosis of the jaw (ONJ) is observed in patients with various malignancies who are treated for a long period of time with high doses of BPs (Hoff et al. 2008; Magopoulos et al. 2007).

By contrast, cases of ONJ in the osteoporotic patients are extremely rare – no case was found in more than 3000 patients participating in the clinical trials with zoledronate and alendronate(Black et al. 2007; Lyles et al. 2007) and no causal link between ONJ and BP therapy in these patients has been convincingly demonstrated. Precipitating factors for ONJ, which occurs in people who have never received BP therapy, include dental surgery, ill-fitted dental prosthesis, and aggravating factors (heavy smoking, infection) (Silverman et al. 2009; Yarom et al. 2007).

Clinical data do not support the use of BTM (e.g., serum CTX-I concentration) as predictors of the risk of ONJ in the bisphosphonate-treated patients (Baim et al. 2009).

Recent case reports have suggested a higher occurrence of atypical femoral shaft fractures (subtrochanteric or proximal diaphyseal fracture) in a select group of women and men treated long-term with alendronate, particularly in those receiving glucocorticoids and/or another antiresorptive medication such as estrogen (Abrahamsen et al. 2009a).

It is not clear if these fractures are related to long-term alendronate treatment or rather are a form of fragility fracture in osteoporotic patients. These fractures are generally thought to be low trauma fractures occurring in patients who have taken alendronate for several years (usually >5).

Prior to their fracture, these patients often, but not always, had experienced persistent pain in the thigh that was aggravated during standing and resistant to analgesics. Individuals with these types of fractures appear to have modest cortical

thickening of the femur diaphysis, and bone scintigraphy shows increased uptake of the radioisotope in the subtrochanteric area at the site of the cortical thickening which can be bilateral and which is consistent with a stress fracture.

From the practical point of view, two points are important. Firstly, a pain in the thigh not related to trauma and aggravating during standing needs further investigation when it is reported by a patient treated with alendronate. Secondly, it is advisable to discontinue alendronate in patients with normal BMD values on long-term glucocorticoid treatment. Obviously, these suggestions are always true, but particularly relevant in the context of the femoral shaft fractures. Additional studies are needed to determine the mechanism underlying these fractures and the characteristics of the few patients that may be at increased risk for this injury.

Treatment with BPs was associated with higher risk of atrial fibrillation in some, (Black et al. 2007; Abrahamsen et al. 2009b) but not all studies (Bunch et al. 2009; Sorensen et al. 2008).

The association between use of BPs and risk of atrial arrhythmia and its clinical significance remains to be elucidated. Women treated for osteoporosis may have a higher cardiovascular risk before the beginning of the BP treatment than non-osteoporotic women. During treatment with zoledronate, electrolyte imbalance does not seem to precipitate the atrial arrhythmia, because episodes of atrial fibrillation did not cluster in time after infusions, when serum electrolytes are most affected (Black et al. 2007).

Selective Estrogen Receptor Modulators

Selective estrogen receptor modulators (SERM) are synthetic molecules. They have the ability to bind to estrogen receptors throughout the body and act as estrogen agonists or antagonists depending upon the target organ. The concept of SERM is based on the observation that tamoxifen, used as an antiestrogen in the treatment of breast cancer, acts as an estrogen agonist on bone in postmenopausal women.

Approved SERM in postmenopausal osteoporosis treatment are raloxifene and bazedoxifene.

Raloxifene (60–120 mg daily) slows down bone turnover (decrease marker levels by 35%) and increases BMD by 2–3% at the lumbar spine and femoral neck.

It reduces the incidence of vertebral fractures by 40–50% (Ensrud et al. 2008; Barrett-Connor et al. 2006).

No effect was observed on nonvertebral fractures, except a 22% decrease in the incidence of major osteoporotic fractures in women with prevalent vertebral fractures, mainly severe vertebral fractures.

Raloxifene markedly reduces the risk of invasive estrogen-receptor positive breast cancer (Barrett-Connor et al. 2006). In most studies, raloxifene did not influence the risk of cardiovascular (coronary) events and, in some groups, may even decrease the risk of myocardial infarction or unstable angina (Barrett-Connor et al. 2006; Collins et al. 2009).

It increases the risk of venous thromboembolism to the same extent as HRT and increases the risk of fatal stroke mainly in women with high risk of stroke at baseline (Barrett-Connor et al. 2006; Collins et al. 2009; Mosca et al. 2009).

Another SERM, bazedoxifene (20–40 mg daily), decreases bone turnover marker (BTM) levels to a similar extent as 60 mg raloxifene daily, increases BMD of the lumbar spine by 2%, and prevents bone loss at the total hip (Silverman et al. 2008).

It decreases the risk of vertebral fracture by 40%, similarly to raloxifene, and decreases by 40% the risk of nonvertebral fracture in women at higher risk of fracture (low femoral neck T-score and presence of vertebral fractures at baseline).

In postmenopausal osteoporotic women at high risk of fracture assessed by FRAX®, bazedoxifene decreased the risk of morphometric vertebral fracture by 50% and the risk of all clinical fractures by 30% (Kanis et al. 2009).

In these studies, risk of cardiovascular events, cerebrovascular events, thromboembolism, and cancer was similar in the women treated with bazedoxifene, raloxifene, and placebo.

Denosumab

Denosumab is an antiresorptive agent that inhibits osteoclast-mediated bone resorption but works through a different pathway than bisphosphonates. Denosumab is a fully human monoclonal antibody that prevents the binding of receptor activator of nuclear factor-kB ligand (RANKL) to receptor activator of nuclear factor-kB (RANK) on the cells of the osteoclastic lineage. RANKL binds to RANK and stimulates osteoclast differentiation, activation, and survival.

The different mechanisms by which denosumab and alendronate inhibit bone resorption serve to raise the question as to how these agents compare with respect to efficacy measurements and safety profiles. Here, we describe results from a phase 3 randomized, double-blind study designed to evaluate efficacy and safety of the proposed therapeutic dose of denosumab (60 mg subcutaneously every 6 months) with alendronate (70 mg orally every week) through 12 months of treatment in postmenopausal women with low bone mass.

In published phase 2 and 3 studies that evaluated the effect of denosumab in postmenopausal women with low bone mass, denosumab treatment inhibited bone resorption and remodeling as measured by decreases in biochemical markers of bone turnover and increases in BMD at all measured skeletal sites (Bone et al. 2008; Miller et al. 2008).

In postmenopausal women with low BMD, denosumab administered subcutaneously 60 mg every 6 months increased BMD by 1–7% according to the skeletal site.

It inhibits bone resorption strongly and rapidly, for example, serum CTX-I decreases by more than 80% 1 week after denosumab injection. In postmenopausal osteoporotic women, denosumab decreased the risk of vertebral fracture by 70% (including a 60% decrease in the incidence of multiple vertebral fractures) and the risk of nonspine fractures by 20% (including a 40% decrease in the incidence of hip fracture) (Cummings et al. 2009).

In postmenopausal women with osteoporosis previously treated with oral bisphosphonates, denosumab is associated with greater BMD increases at all measured skeletal sites and greater inhibition of bone remodeling compared with zoledronate (Miller et al. 2016).

In postmenopausal women with hormone receptor-positive, early-stage breast cancer, treatment with adjuvant aromatase inhibitors is the standard of care, but it increases risk for osteoporosis and fractures. Denosumab constitutes an effective and safe adjuvant treatment for patients with postmenopausal hormone receptor-positive early breast cancer receiving aromatase inhibitor therapy and it significantly reduces clinical fractures.

Romosozumab

Romosozumab is a monoclonal antibody that binds and inhibits sclerostin, with a dual effect of increasing bone formation and decreasing bone resorption (McClung et al. 2014).

Sclerostin blocks canonical Wnt signaling, which results in decreased osteoblast-mediated bone formation and increased bone resorption, both of which are counteracted by romosozumab (van Bezooijen et al. 2007; Wijenayaka et al. 2011; Padhi et al. 2011; Ominsky et al. 2014).

Romosozumab increased levels of the bone-formation marker P1NP and decreased levels of the bone-resorption marker β-CTX within 12 months (Saag et al. 2017).

Romosozumab was associated with a risk of new vertebral fracture that was 73% lower than the risk with placebo at 12 months in the romosozumab group vs. 1.8% in the placebo group.

Romosozumab was also associated with a risk of clinical fracture that was 36% lower than the risk with placebo at 12 months.

Because vertebral and clinical fractures are associated with increased morbidity and considerable health care costs, a treatment that would reduce this risk rapidly could offer appropriate patients an important benefit.

Bone mass and structure are main determinants of bone strength.

Thus, a treatment approach employing a bone-forming agent prior to anti-resorptive therapy may provide benefits for patients at high risk for fracture. Indeed, it is increasingly appreciated that some patients at imminent risk of fracture (i.e., within 1–2 years, including those with recent prior fracture)(Johansson et al. 2017) may benefit from this approach. One year of romosozumab treatment in postmenopausal women with osteoporosis resulted in a lower risk of vertebral and clinical fractures than the risk with placebo.

It has been documented that the sequence of a bone-forming agent followed by antiresorptive therapy has the potential to provide substantially larger BMD improvements than treatment with an antiresorptive agent first (Cosman et al. 2017).

Hip fractures are less frequent with romosozumab followed by alendronate than with alendronate alone, suggesting an important benefit and challenging the common treatment practice of first-line use of alendronate in women who have had a previous fracture (Kenneth et al. 2017).

The sequence of romosozumab followed by denosumab results in large improvements in BMD, providing a stronger skeletal foundation and leading to fewer fractures upon transition to antiresorptive treatment with denosumab.

The effects of romosozumab and denosumab are reversible if discontinued without follow-on therapy, as has been observed for all osteoporosis treatments over variable offset timeframes (Cosman et al. 2018).

Indication to treatment with romosozumab is limited by serious cardiovascular adverse events, although these events are uncommon (Cosman et al. 2016).

Teriparatide

Recombinant 1–34 fragment of human parathyroid hormone (rhPTH(1–34) teriparatide) and recombinant human intact parathyroid hormone (PTH(1–84)) are effective stimulators of bone formation. They stimulate bone remodeling at the bone remodeling unit and bone modeling on quiescent bone surfaces. They induce a prompt increase in bone formation followed by a slower increase in bone resorption. As they strongly increase BMD in the trabecular compartment, the greatest increase in BMD is observed at the lumbar spine.In the cortical sites, they slightly decrease areal BMD measured by DXA (one-third distal radius) and volumetric BMD measured by QCT (femoral neck, total hip). By contrast, they increase cortical bone volume at the radius and femoral neck (Zanchetta et al. 2003; Black et al. 2005).

In osteoporotic women with prevalent vertebral fractures, rhPTH(1–34) decreases the incidence of new vertebral fractures by 65% and of nonvertebral fractures by 53%.

The fracture incidence remained significantly decreased for at least 30 months after discontinuation of teriparatide treatment (Prince et al. 2005).

However, these data should be interpreted cautiously, because during the follow-up after the discontinuation of teriparatide, patients and investigators were unblinded to the treatment and additional treatment for osteoporosis was allowed. The best candidates for the anabolic treatment are patients with preexisting osteoporotic fractures, patients with very low BMD, and those with unsatisfactory response to antiresorptive therapy (Hodsman et al. 2005).

In osteoporotic women taking alendronate for at least 1 year, continued alendronate plus rhPTH(1–34) subcutaneously daily for 3-month cycles alternating with 3 month periods without rhPTH(1–34) induced similar increase in BMD and in bone turnover marker (BTM) levels as continuous rhPTH(1–34) (Cosman et al. 2005).

Combined Therapy Teriparatide/Denosumab

Studies investigating the efficacy of combined bisphosphonates and parathyroid hormone or teriparatide generally demonstrated minimal or no greater increase in bone mass compared with monotherapy (Cosman et al. 2011; Finkelstein et al. 2010).

Recent studies demonstrate that combined denosumab and teriparatide therapy increases bone mineral density at the spine and hip more than either treatment and more than has been reported with any currently approved treatment for postmenopausal osteoporosis (Tsai et al. 2013). These studies demonstrate that 12 months of combined denosumab and teriparatide therapy improves peripheral cortical and total bone density, cortical microarchitecture, and bone strength more than either drug alone. Although superior efficacy is noted at both the tibia and the radius in the combination group, the advantage of the combined treatment is numerically greater at the tibia than at the radius (Tsai et al. 2015).

The mechanism underlying the differential effects at these two peripheral anatomic sites is uncertain but might reflect an ability of weight-bearing to amplify the skeletal benefits of teriparatide in humans, as previously documented in experimental animals.

Strontium Ranelate

Strontium ranelate (2 g daily) slightly inhibits bone resorption, slightly stimulates bone formation, and progressively dose-dependently increases BMD.

It decreases the incidence of vertebral fractures by about 40%. During long-term treatment (4 years), strontium ranelate decreased the vertebral fracture incidence by 33% (Meunier et al. 2009). Strontium also decreases the incidence of vertebral fractures by 35% in younger postmenopausal women (aged 65 or less) and by 32% in the elderly women aged 80 and over.

Strontium ranelate decreases the incidence of non-vertebral fractures by about 15% and even more (31%) in the oldest women.

Post hoc analyses demonstrated that strontium ranelate decreases the incidence of hip fracture by approximately 40% in high risk elderly women with severe osteoporosis.

Conclusion

Fragility fractures related to postmenopausal osteoporosis are nowadays a major cause of morbidity and mortality. This problem is becoming increasingly important due to the progressive aging of the population. We have effective systems for preventing and treating postmenopausal osteoporosis. However, additional data is required.

Cross-References

► Hormone Replacement Therapy (HRT)
► Long-Term Consequences of Menopause
► The Hypothalamus-Pituitary-Ovary Axis

References

Abrahamsen B, et al. Subtrochanteric and diaphyseal femur fractures in patients treated with alendronate: a register-based national cohort study. J Bone Miner Res. 2009a;24:1095–102.

Abrahamsen B, et al. Atrial fibrillation in fracture patients treated with oral bisphosphonates. J Intern Med. 2009b;265:581–92.

Baim S, et al. Assessing the clinical utility of serum CTX in postmenopausal osteoporosis and its use in predicting risk of osteonecrosis of the jaw. J Bone Miner Res. 2009;24:561–74.

Barrett-Connor E, et al. Effects of raloxifene on cardiovascular events and breast cancer in postmenopausal women. N Engl J Med. 2006;355:125–37.

Black DM, et al. One year of alendronate after one year of parathyroid hormone (1–84) for osteoporosis. N Engl J Med. 2005;353:555–65.

Black DM, et al. Effects of continuing or stopping alendronate after 5 years of treatment: the Fracture Intervention Trial Long-term Extension (FLEX): a randomized trial. JAMA. 2006;296:2927–38.

Black DM, et al. Once-yearly zoledronic acid for treatment of postmenopausal osteoporosis. N Engl J Med. 2007;356:1809–22.

Bone HG, et al. Effects of denosumab on bone mineral density and bone turnover in postmenopausal women. J Clin Endocrinol Metab. 2008;93:2149–57.

Bunch TJ, et al. Relation of bisphosphonate therapies and risk of developing atrial fibrillation. Am J Cardiol. 2009;103:824–8.

Burghardt AJ, et al. A longitudinal HR-pQCT study of alendronate treatment in postmenopausal women witeh low bone density: relations among density, cortical and trabecular microarchitecture, biomechanics, and bone turnover. J Bone Miner Res. 2010;25:2558–71.

Chapurlat RD, et al. Microcrack frequency and bone remodeling in postmenopausal osteoporotic women on long-term bisphosphonates: a bone biopsy study. J Bone Miner Res. 2007;22:1502–9.

Collins P, et al. Effects of the selective estrogen receptor modulator raloxifene on coronary outcomes in the Raloxifene Use for The Heart trial: results of subgroup analyses by age and other factors. Circulation. 2009;119:922–30.

Cosman F, et al. Daily and cyclic parathyroid hormone in women receiving alendronate. N Engl J Med. 2005;353:566–75.

Cosman F, et al. Effects of intravenous zoledronic acid plus subcutaneous teriparatide (rhPTH (1–34)) in postmenopausal osteoporosis. J Bone Miner Res. 2011;26:503–11.

Cosman F, et al. Clinician's guide to prevention and treatment of osteoporosis. Osteoporos Int. 2014;25(10):2359–2381.

Cosman F, et al. Romosozumab treatment in postmenopausal women with osteoporosis. N Engl J Med. 2016;375:1532–43.

Cosman F, et al. Treatment sequence matters: anabolic and antiresorptive therapy for osteoporosis. J Bone Miner Res. 2017;32:198–202.

Cosman F, et al. FRAME study: the foundation effect of building Bone with 1 year of Romosozumab leads to continued lower fracture risk after transition to Denosumab. J Bone Miner Res. 2018;33:1219–26.

Cranney A, et al. Ibandronate for the prevention of nonvertebral fractures: a pooled analysis of individual patient data. Osteoporos Int. 2009;20:291–7.

Cummings SR, et al. Denosumab for prevention of fractures in postmenopausal women with osteoporosis. N Engl J Med. 2009;361:756–65.

Curtis JR, et al. Risk of hip fracture after bisphosphonate discontinuation: implications for a drug holiday. Osteoporos Int. 2008;19:1613–20.

Ensrud KE, et al. Effects of raloxifene on fracture risk in postmenopausal women: the Raloxifene Use for The Heart trial. J Bone Miner Res. 2008;23:112–20.

EPOS Group, et al. Incidence of limb fracture across Europe: results from the European Prospective Osteoporosis Study (EPOS). Osteoporosis Int. 2002;13:565–71.

Eriksen EF, et al. Antifracture efficacy and reduction of mortality in relation to timing of the first dose of zoledronic acid after hip fracture. J Bone Miner Res. 2009;24:1308–13.

Finkelstein JS, et al. Effects of teriparatide, alendronate, or both in women with postmenopausal osteoporosis. J Clin Endocrinol Metab. 2010;95:1838–45.
Genant H, et al. Noninvasive assessment of bone mineral and structure: state of the art. J Bone Miner Res. 1996;11(6):707–30.
Gennari L, et al. Osteoporosis in men. Endocrinol Metab Clin North Am. 2007;36:399–419.
Haentjens P, On behalf of the Network on Male Osteoporosis in Europe (NEMO), et al. Evidence from data searches and life-table analyses for gender-related differences in absolute risk of hip fracture after Colles' or spine fracture: Colles' fracture as an early and sensitive marker of skeletal fragility in white men. J Bone Miner Res. 2004;19:1933–44.
Harris ST, et al. Ibandronate and the risk of non-vertebral and clinical fractures in women with postmenopausal osteoporosis: results of a meta-analysis of phase III studies. Curr Med Res Opin. 2008;24:237–45.
Hodsman AB, et al. Parathyroid hormone and teriparatide for the treatment of osteoporosis: a review of the evidence and suggested guidelines for its use. Endocr Rev. 2005;26:688–703.
Hoff AO, et al. Frequency and risk factors associated with osteonecrosis of the jaw in cancer patients treated with intravenous bisphosphonates. J Bone Miner Res. 2008;23:826–36.
Johansson H, et al. Imminent risk of fracture after fracture. Osteoporos Int. 2017;28:775–80.
Johnell O, et al. An estimate of the worldwide prevalence and disability associated with osteoporotic fractures. Osteoporosis Int. 2006;17(12):1726–33. Epub 2006 Sep 16.
Kanis JA, et al. A meta-analysis of previous fracture and subsequent fracture risk. Bone. 2004;35(2):375–82.
Kanis JA, et al. Bazedoxifene reduces vertebral and clinical fractures in postmenopausal women at high risk assessed with FRAX. Bone. 2009;44:1049–54.
Kelley GA, et al. Exercise and bone mineral density in premenopausal women: a meta-analysis of randomized controlled trials. Int J Endocrinol. 2013;2013:741639.
Kenneth G, et al. Romosozumab or alendronate for fracture prevention in women with osteoporosis. N Engl J Med. 2017;377:1417–27.
Lee S, et al. Efficacy, side effects and route of administration are more important than frequency of dosing of anti-osteoporosis treatments in determining patient adherence: a critical review of published articles from 1970 to 2009. Osteoporos Int. 2011;22:741–53.
Leeming DJ, et al. An update on biomarkers of bone turnover and their utility in biochemical research and clinical practice. Eur J Clin Pharmacol. 2006;62:781–92.
Lips P, et al. Vitamin D physiology. Prog Biophys Mol Biol. 2006;92(1):4–8.
Lyles KW, et al. Zoledronic acid and clinical fractures and mortality after hip fracture. N Engl J Med. 2007;357:1799–809.
Magopoulos C, et al. Osteonecrosis of the jaws due to bisphosphonate use. A review of 60 cases and treatment proposals. Am J Otolaryngol. 2007;28:158–63.
Mbalaviele G, et al. Beta-catenin and BMP-2 synergize to promote osteoblast differentiation and new bone formation. J Cell Biochem. 2005;94:403–18.
McClung MR, et al. Romosozumab in postmenopausal women with low bone mineral density. N Engl J Med. 2014;370:412–20.
Melton LJ III, et al. Epidemiology of vertebral fractures: implications for vertebral augmentation. Acad Ther. 2006;13:538–45.
Meunier PJ, et al. Effects of long-term strontium ranelate treatment on vertebral fracture risk in postmenopausal women with osteoporosis. Osteoporos Int. 2009;20:1663–73.
Miller PD, et al. Effect of denosumab on bone density and turnover in postmenopausal women with low bone mass after long-term continued, discontinued, and re- starting of therapy: a randomized blinded phase2 clinical trial. Bone. 2008;43:222–9.
Miller PD, et al. Denosumab or zoledronic acid in postmenopausal women with osteoporosis previously treated with oral bisphosphonates. J Clin Endocrinol Metab. 2016;101:3163.
Moreira LDF, et al. Physical exercise and osteoporosis: effects of different types of exercises on bone and physical function of postmenopausal women. Arq Bras Endocrinol Metabol. 2014;58(5):514–22.

Mosca L, et al. Effect of raloxifene on stroke and venous thromboembolism according to subgroups in postmenopausal women at increased risk of coronary heart disease. Stroke. 2009;40:147–55.
Ominsky MS, et al. Tissue-level mechanisms responsible for the increase in bone formation and bone volume by sclerostin antibody. J Bone Miner Res. 2014;29:1424–30.
Padhi D, et al. Single-dose, placebo-controlled, randomized study of AMG 785, a sclerostin monoclonal antibody. J Bone Miner Res. 2011;26:19–26.
Papapoulos SE, et al. Meta-analysis of the efficacy of alendronate for the prevention of hip fractures in postmenopausal women. Osteoporos Int. 2005;16:468–74.
Pilbeam C, et al. Prostaglandins and bone metabolism. In: Bilezikian JP, Raisz LG, Rodan GA, editors. Principles of bone biology. San Diego: Academic; 2002. p. 979–94.
Poole KE, et al. Sclerostin is a delayed secreted product of osteocytes that inhibits bone formation. FASEB J. 2005;19:1842–4.
Prince R, et al. Sustained nonvertebral fragility fracture risk reduction after discontinuation of teriparatide treatment. J Bone Miner Res. 2005;20:1507–13.
Reginster JY, Rabenda V, et al. Adherence to anti-osteoporotic treatment: does it really matter? Future Rheumatol. 2006;1(1):37–40.
Reid DM, et al. Zoledronic acid and risedronate in the prevention and treatment of glucocorticoid-induced osteoporosis (HORIZON): a multicentre, double-blind, double-dummy, randomised controlled trial. Lancet. 2009;373:1253–63.
Saag KG, et al. Romosozumab or alendronate for fracture prevention in women with osteoporosis. N Engl J Med. 2017;377:1417–27.
Sambrook P, et al. Lancet. 2006;367:2010–8.
Seeman E, et al. Bone quality – the material and structural basis of bone strength and fragility. N Engl J Med. 2006;354(21):2250–61.
Silverman SL, et al. Efficacy of bazedoxifene in reducing new vertebral fracture risk in postmenopausal women with osteoporosis: results from a 3-year, randomized, placebo-, and active-controlled clinical trial. J Bone Miner Res. 2008;23:1923–34.
Silverman SL, et al. Osteonecrosis of the jaw and the role of bisphosphonates: a critical review. Am J Med. 2009;122(2 Suppl):S33–45.
Silverman SL, et al. Oral bisphosphonate compliance and persistence: a matter of choice? Osteoporos Int. 2011;22:21–6.
Sorensen HT, et al. Use of bisphosphonates among women and risk of atrial fibrillation and flutter: population based case-control study. BMJ. 2008;336:813–6.
Tsai JN, et al. Teriparatide and denosumab, alone or combined, in women with postmenopausal osteoporosis: the DATA study randomized trial. Lancet. 2013;382:50–6.
Tsai JN, et al. Comparative effects of teriparatide, denosumab, and combination therapy on peripheral compartmental bone density, microarchitecture, and estimated strength, the DATA-HRpQCT Study. J Bone Miner Res. 2015;30(1):39–45.
van Bezooijen RL, et al. Wnt but not BMP signaling is involved in the inhibitory action of sclerostin on BMP-stimulated bone formation. J Bone Miner Res. 2007;22:19–28.
Weycker D, et al. Compliance with drug therapy for postmenopausal osteoporosis. Osteoporosis Int. 2006;17:1645.
Wijenayaka AR, et al. Sclerostin stimulates osteocyte support of osteoclast activity by a RANKL-dependent pathway. PLoS One. 2011;6:25900.
Wilk A, et al. Post-fracture pharmacotherapy for women with osteoporotic fracture: analysis of a managed care population in the USA. Osteoporos Int. 2014;25:2777–86.
Yarom N, et al. Osteonecrosis of the jaw induced by orally administered bisphosphonates: incidence, clinical features, predisposing factors and treatment outcome. Osteoporos Int. 2007;18:1363–70.
Zallone A, et al. Direct and indirect estrogen actions on osteoblasts and osteoclasts. Ann N Y Acad Sci. 2006;1068:173–9.
Zanchetta JR, et al. Effects of teriparatide (recombinant human parathyroid hormone (1–34)) on cortical bone in postmenopausal women with osteoporosis. J Bone Miner Res. 2003;18:539–43.

Part V

Pregnancy

Endocrinology of Maternal-Placental Axis

20

Viola Seravalli, Mariarosaria Di Tommaso, John Challis, and Felice Petraglia

Contents

Abstract

Pregnancy is a dynamic and complex state to which the mother's physiology makes substantial adaptations that enable her to provide all the needs of the growing fetus. The placenta is a highly active, transient endocrine organ and a central regulator of maternal-placental-fetal physiology. It produces steroid and protein hormones, growth factors, and cytokines from precursor provided by the mother as well as by the fetus. Cytotrophoblasts secrete hypothalamic

V. Seravalli (✉) · M. Di Tommaso
Department of Health Sciences, Division of Obstetrics and Gynecology, University of Florence, Florence, Italy
e-mail: viola.seravalli@unifi.it

J. Challis
Simon Fraser University, Vancouver, BC, Canada

Murdoch University, Murdoch, WA, Australia

F. Petraglia
Department of Biomedical, Experimental and Clinical Sciences, Division of Obstetrics and Gynecology, University of Florence, Florence, Italy

F. Petraglia, B. C. Fauser (eds.), *Female Reproductive Dysfunction*, Endocrinology,
https://doi.org/10.1007/978-3-030-14782-2_20

peptides and function in juxtaposition to syncytiotrophoblasts, which secrete the corresponding pituitary-like peptides in an anatomic arrangement analogous to the hypothalamic-pituitary axis. Some placental hormones play a predominant role at the beginning of pregnancy, favoring implantation and embryo development, while others exert their functions mainly in the second or third trimester, to maintain pregnancy and ensure appropriate fetal growth. Estrogens and progesterone are involved in pregnancy from before implantation to parturition. There are substances that are responsible of regulating maternal metabolic adaptation to pregnancy, in order to allow transfer of glucose and amino acids into the fetus. Many of these substances act in concert, in a complex interaction of hormones with one another, and in a fascinating cross talk between the placenta, the fetus, and the mother. The mechanisms involved in parturition are highly complex and involve endocrine factors, cytokines, oxytocin, and prostaglandins that lead the transition from a quiescent myometrium to a contractile state that allows delivery of the fetus. The endocrinology of the maternal-placental axis is still a progressive field of science, as some mechanisms are still incompletely understood.

Keywords

Placenta · Endocrinology of pregnancy · Estrogens · Progesterone · Maternal-placental axis

Introduction

The placenta is capable of synthesizing a large number of hormones, growth factors, and cytokines and is therefore considered as a transient endocrine organ, even though it does not have neural connections to the mother or the fetus (Liu 2014). Placental hormones are necessary for the establishment and maintenance of pregnancy, adaptation of the maternal organism to pregnancy, fetal growth, and well-being (Evain-Brion and Malassine 2003). They are also responsible for the development of the mechanisms that lead to parturition (Vannuccini et al. 2016). This chapter reviews the endocrinology of maternal-placental axis during pregnancy and parturition.

Endocrine Regulation in Early Pregnancy

The downregulation of progesterone and estrogen receptors induced by the corpus luteum production of progesterone marks the beginning of endometrial receptivity (Liu 2014). Various mediator, such as integrins, cytokines, metalloproteinases, and glycoproteins, localized to the embryo or the endometrium play a role in implantation (Liu 2014).

Trophoblastic invasion of the endometrium and myometrium, a fundamental process in the development of the placenta, is a complex phenomenon that is regulated by growth factors and interleukines (Bass et al. 1994; Librach et al. 1994). The trophoblasts form the chorionic villi, the functional units of the placenta, composed of connective tissue and capillaries connecting it with the fetal circulation. The syncytiotrophoblast, which forms the outer layer, and the cytotrophoblast, forming the inner layer, produce protein and peptide hormones. The syncytiotrophoblast also produces all of the steroid hormones (Liu 2014).

Embryo development and maintenance of early pregnancy require progesterone. This is produced almost entirely by the corpus luteum before 6 weeks of gestation; then its production shifts more to the placenta after the 7th week of pregnancy. Beyond 12 weeks, the placenta is the major source of progesterone. The maintenance of corpus luteum in early pregnancy depends on the presence of human chorionic gonadotropin (hCG). After implantation, hCG is produced principally by the syncytiotrophoblast and secreted into the intervillous space. hCG production begins 9–10 days after ovulation and increases progressively, with a peak around 10 weeks of gestation. Subsequently, the levels decrease to a plateau that is maintained throughout the duration of pregnancy. The rapid rising of hCG between 3 and 9 weeks of gestation coincides with the proliferation of immature trophoblastic villi and an extensive syncytial layer. Moreover, hCG is involved in autocrine and paracrine mechanisms regulating trophoblast differentiation. In particular, it has been observed that hCG enhances spontaneous differentiation of isolated cytotrophoblasts into syncytiotrophoblasts (Shi et al. 1993). hCG also seems to regulate maternal innate and adaptive immune responses allowing the formation of immune tolerance to the semi-allogenic fetus (Schumacher 2017).

Endocrine Functions of the Placenta

The placenta, which forms the functional interface between the maternal and fetal circulations, is a highly active endocrine organ during gestation, secreting a variety of hormones with physiological effects in the mother. It plays a key role in mediating adaptations in maternal physiology and at the same time influencing fetal growth and development (Napso et al. 2018). Placental hormones include members of the prolactin and growth hormone family, steroid hormones and neuroactive hormones, all of which drive physiological changes during pregnancy (Table 1).

Differently from the hypothalamus, the placenta does not receive direct neural inputs, and the exact mechanisms responsible for regulation of the secretion of hypothalamic-like placental peptides are still unknown. The placenta and the fetus produce and secrete peptides and steroids into the maternal circulation and stimulate maternal hormone production. Variations in maternal hormone concentrations determine the metabolic and immunologic changes required for successful outcome in pregnancy. The amounts of the fetal and placental hormones produced vary over the course of the gestational period.

Table 1 Placental hormones

Hormone	Abbreviation
Steroid hormones	
Progesterone	P
Estrogens	E1, E2, E3
Pituitary-like hormones	
Human chorionic gonadotropin	hCG
Human chorionic somatomammotropin	hCS
Placental growth hormone	GH
Placental adrenocorticotropic hormone	ACTH
Human chorionic thyrotropin	hCT
Hypothalamic-like hormones	
Placental gonadotropin-releasing hormone	GnRH
Placental corticotropin-releasing hormone	CRH
Placental thyrotropin-releasing hormone	TRH
Growth factors	
Inhibin and activin	
Insulin-like growth factors-1 and 2	IGF-1, IGF-2
Transforming growth factor	TGF
Epidermal growth factor	EGF

Steroid Hormones

Estrogens, androgens, and progesterone are involved in pregnancy from before implantation to parturition. Their synthesis and secretion involve both the placenta and the fetal adrenal cortex, two organs that present an interdependence and complementary steroidogenic function (Fig. 1): together, they exchange, metabolize, and secrete more steroids than any other human endocrine tissue (Hutchinson and DeCherney 1999).

Progesterone

Progesterone is synthetized in the syncytiotrophoblast from circulating maternal cholesterol carried in low-density lipoprotein (LDL) (Tulchinsky and Hobel 1973). Pregnenolone, originated from cholesterol, is converted to progesterone through the 3-beta-hydroxysteroid dehydrogenase, which is abundant in the placenta (Fig. 1) (Hutchinson and DeCherney 1999). Progesterone is released in the circulation and acts as a tocolytic in the maternal compartment, but it is also utilized in the fetal compartment for mineralocorticoid and glucocorticoid synthesis (aldosterone and cortisol). The fetus cannot make progesterone or androstenedione, the immediate precursor to the sex steroids, because the fetal adrenal cortex is functionally deficient in 3-hydroxysteroid dehydrogenase (Simpson et al. 1979).

In addition to its role in the maintenance of early pregnancy, progesterone is primarily responsible for maintaining uterine quiescence over the course of pregnancy, through its action on uterine smooth muscle cells (Blanks and Brosens 2012). The uterine relaxation properties of progesterone are the rationale for its use in

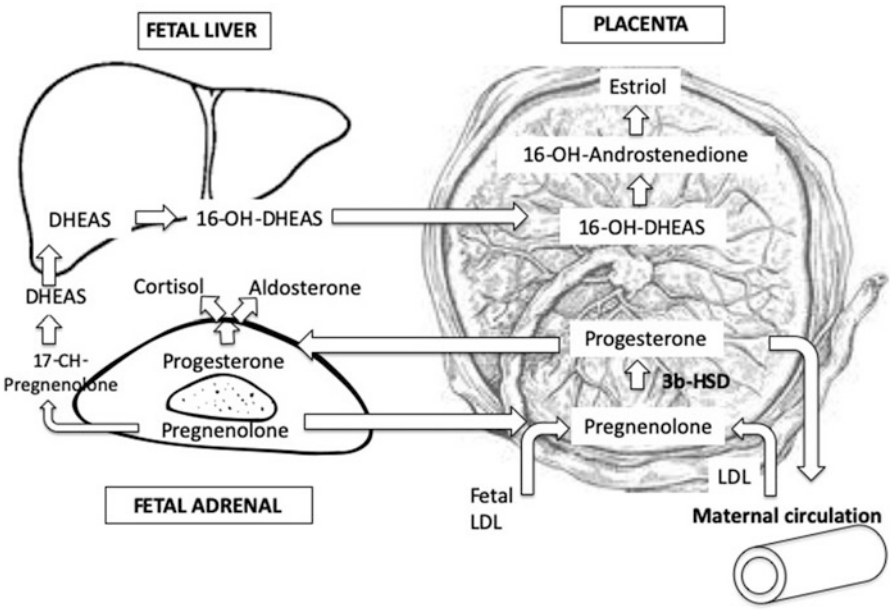


Fig. 1 Steroid hormones production and exchange between the placenta and the fetus. The enzyme 3-beta-hydroxysteroid dehydrogenase is present in the placenta, but not in the fetal cortex. Progesterone is produced by the placenta and then utilized in the fetal compartment for mineralocorticoid and glucocorticoid synthesis (aldosterone and cortisol). Estriol originates almost exclusively from the placenta and it derives principally from placental conversion of fetal 16-DHEA-S. DHEA-S, dehydroepiandrosterone sulfate; 3b-HSD, 3-beta-hydroxysteroid dehydrogenase; LDL, low-density lipoprotein

prevention of preterm birth in high-risk patients (Hassan et al. 2011; Meis et al. 2003). The effect of progesterone on the myometrium is known to be mediated by two nuclear progesterone receptor (PR) subtypes, PR-A and PR-B. Through the PR-B receptor, progesterone promotes uterine relaxation, while PR-A represses progesterone actions mediated by PR-B. Therefore, the extent of progesterone responsiveness is inversely related to the PR-A/PR-B expression ratio, and functional progesterone withdrawal in human parturition appears to be mediated by specific changes in myometrial PR expression, function, or both (Mesiano 2004). Moreover, progesterone appears to inhibit prostaglandin synthesis, thus favoring uterine quiescence and preventing premature cervical repening (Cane and Villee 1975; Carbonne et al. 2000).

Progesterone also inhibits T-lymphocyte-mediated tissue rejection, offering immunologic protection to the implanted conceptus and evolving placenta (Siiteri et al. 1977).

Estrogens

Human pregnancy is characterized by a typical hyperestrogenic state, where the placenta is the primary source of estrogens. Estrone (E1), estradiol (E2), and estriol

(E3) share the same basic 18-carbon estrogen nucleus, but they differ in the numbers and position of hydroxyl groups. Estriol is considered the primary estrogen responsible for augmented uterine blood flow during pregnancy (Resnik et al. 1974), and it is first detectable in maternal serum at 9 weeks. It originates almost exclusively from the placenta, and it derives principally from placental conversion of fetal 16-DHEA-S. Compared to concentrations of less than 0.01 ng/ml in nonpregnant women, E3 levels in pregnancy increase to approximately 10–30 ng/ml at term (Buster 1980). E3 production depends on the presence of a living fetus. In fact, DHEA-S, a critical fetal steroid precursor, is produced by the fetal adrenal cortex and first delivered to the fetal liver, where it is converted into 16-hydroxydehydroepiandrosterone sulfate (16-OH-DHEA-S), before being converted in the placenta first to 16-hydroxyandrostenedione and then further aromatized into estriol. E1 and E2 are synthesized in the placenta from conversion of both circulating maternal and fetal DHEA-S. Following conception, their levels increase gradually to a range of 6–30 ng/ml at term for E1 and 2–30 ng/ml at term for E2. Estrogens are then secreted into the maternal and fetal circulations. Estriol is conjugated in the maternal liver to form estriol sulfate, estriol glucosiduronate, and mixed conjugates, which are excreted in the maternal urine.

Estrogens during pregnancy have various functions, affecting uterine vasculature, placental steroidogenesis, and parturition. They stimulate placental angiogenesis, induce gap junction formation in the myometrium, and augment uterine blood flow. The anti-inflammatory TH2 responses in decidual stromal cells are promoted by estrogens, thus contributing to a successful pregnancy. Estrogens also increase expression of LDL receptors, and by regulating the availability of LDL cholesterol for conversion to pregnenolone, they control the biosynthesis of placental progesterone. However, placental estrogen synthesis, at least at high levels, does not seem to be essential for the maintenance of human pregnancy, as demonstrated by the fact that in some genetic diseases characterized by low estrogen levels, pregnancy nonetheless goes to term (Bedin et al. 1987).

Prior to labor, estradiol increases both oxytocin and a-adrenergic receptor concentration and stimulates the expression of prostaglandin E2 to induce labor (Noyola-Martinez et al. 2019).

Both estrogen and progesterone also play roles in regulating insulin and glucose homeostasis, lipid handling, and appetite regulation, and they may also facilitate some of the cardiovascular changes that accompany pregnancy (Napso et al. 2018).

Placental Protein and Peptide Hormones

Pituitary-Like and Hypothalamic-Like Hormones

Pituitary-like hormones, localized to the syncytiotrophoblast, include hCG, human chorionic somatomammotropin (hCS) [human placental lactogen (hPL)], placental GH, placental adrenocorticotropic hormone (ACTH), and human chorionic thyrotropin (hCT).

Hypothalamic-like hormones, produced by the cytotrophoblastic layer, include placental GnRH, placental corticotropin-releasing hormone (CRH), placental thyrotropin-releasing hormone (TRH).

Cytotrophoblasts, secreting hypothalamic peptides, function in juxtaposition to syncytiotrophoblast, which releases the corresponding pituitary-like peptide, in an arrangement analogous to the hypothalamic-pituitary axis.

Some of the hormones from these two classes are reviewed together in this section, because of their close relationships.

Human Chorionic Gonadotropin (hCG)

Placental hCG plays an important role in the maintenance of the ovarian corpus luteum in early pregnancy and may contribute to the regulation of fetal and placental steroidogenesis. In addition to its luteotropic function, hCG also promotes the biosynthesis and release of relaxin and inhibin by the corpus luteum, thus reducing spontaneous uterine activity and suppressing pituitary follicle-stimulating hormone (FSH) secretion (Hutchinson and DeCherney 1999). Moreover, hCG seems to be involved in the regulation of trophoblast differentiation (Shi et al. 1993), as well as maternal innate and adaptive immune responses, allowing the formation of immune tolerance to the fetus (Schumacher 2017).

HCG secretion throughout pregnancy is linked to many placental hormones: its production is primarily regulated by placental gonadotropin-releasing hormone (GnRH). HCG production is stimulated by glucocorticoids and suppressed by dehydroepiandrosterone sulfate (DHEA-S). Decidual inhibin and prolactin inhibit hCG production by term human trophoblasts, whereas decidual activin augments it (Buster and Carson 2002). Other growth factors (insulin-like growth factor 1 [IGF-1], IGF-2, transforming growth factor [TGF], and epidermal growth factor [EGF]) also influence gene regulation of the hCG system in the placenta (Buster and Carson 2002).

Gonadotropin-Releasing Hormone (GnRH)

Placental GnRH is similar in structure to the hypothalamic decapeptide GnRH. It has been localized both in the cytotrophoblast and syncytiotrophoblast, and it is the primary regulator of the release of hCG through a dose-dependent paracrine mechanism. Placental GnRH levels peak at around 8 weeks, to then decrease with advancing gestational age. The release of GnRH is stimulated by activin and inhibited by inhibin.

Human Chorionic Somatomammotropin (hCS)

Circulating levels of hCS, also known as human placenta lactogen, have been correlated with fetal and placental weight, and in fact its levels substantially increase from first to third trimester, making it the most abundant secretory product of the placenta. Its clearance from the circulation is very rapid, and it quickly becomes undetectable in serum after delivery of the placenta (Hutchinson and DeCherney 1999). It is produced in the syncytiotrophoblast and presents structural and biologic similarities to pituitary human GH and prolactin.

The major metabolic role of hCS is to ensure the nutritional needs of the fetus, particularly in the second and third trimester of pregnancy, because of the increased substrate requirements by the fetus. In the fetus, hCS acts via lactogenic receptors and possibly a unique placental lactogen receptor. hCS production is stimulated by hypoglycemia. It antagonizes insulin action, inducing glucose intolerance, lipolysis, and proteolysis in the maternal system and favoring the transfer of glucose and amino acids to the fetus. It has a critical role in shifting the pattern of energy metabolism from carbohydrates to one that is dependent on fats. HCS seems to exert its metabolic effects through stimulation of IGF-1. The regulation of hCS, however, remains poorly understood, and, interestingly, normal pregnancy outcomes have been reported in women without the hCS gene, suggesting that it might not be essential for pregnancy.

Placental Growth Hormone (GH)

During pregnancy, pituitary GH (hGH-N) expression in the mother is suppressed; and hGH-V, a GH variant expressed by the placenta and released in a non-pulsatile fashion, becomes the predominant GH in the mother. Maternal serum levels of this hormone increase throughout pregnancy, and it is also detectable in cord blood and in the amniotic fluid. Human placental GH allows maternal metabolic adaptation to pregnancy in order to allow fetal nutrition and to prevent maternal blood glucose variation. It exerts this function by stimulating IGF-1 production, acting in concert with hCS in a complex interaction of the hormones with one another and with other growth factors, leading to an increase in the availability of glucose and amino acids to the fetus (Handwerger and Freemark 2000). Through its action on IGF-1 levels, it therefore indirectly stimulates glyconeogenesis, lipolysis, and anabolism in maternal organs and influences fetal growth, placental development, and maternal adaptation to pregnancy (Velegrakis et al. 2017). Expression in invasive extravillous trophoblasts suggests that the physiological role of placental GH might also include a direct influence of this hormone on placental development via a potential combination of autocrine and paracrine mechanisms (Evain-Brion and Malassine 2003; Velegrakis et al. 2017). Lower circulating levels of placental GH are observed in pregnancies complicated by fetal growth retardation. The hGH-N is expressed by the fetal pituitary, but it has small or no physiological until late in pregnancy, due to the lack of functional GH receptors.

Placental Corticotrophin-Releasing Hormone (CRH) and Placental Adrenocorticotropic Hormone (ACTH)

During pregnancy, the placenta synthetizes CRH and releases it into maternal circulation at increasing levels to reach concentrations 1,000 to 10,000 times higher than those found in nonpregnant women (Thomson 2013). Placental CRH, which appears to be structurally similar to the hypothalamic CRH peptide, is released by the cytotrophoblasts and stimulates placental ACTH release in a dose-dependent relationship. Placental ACTH, also structurally similar to pituitary ACTH, is produced by the syncytiotrophoblasts and secreted into maternal circulation. ACTH travels to the cortex of the adrenals where it stimulates the synthesis and releases

steroid hormones including cortisol. CRH has been linked to numerous functions. It has been found to modulate glucose transporter (GLUT) proteins in placental tissue, therefore potentially influencing fetal growth. CRH is released into the fetal circulation in response to fetal stress and in conditions leading to growth restriction, including fetal hypoxemia. An increase in CRH levels has also been described in preeclampsia, fetal asphyxia, and premature labor. Finally, CRH is involved in the signaling systems that control uterine contractions and the timing of birth, as it stimulates prostaglandin synthesis in fetal membranes and placenta.

A paradoxical relationship exists between placental ACTH and CRH and their end-organ product, cortisol: this consists in a positive feedback mechanism that allows an increase in glucocorticoid secretions in condition of stress, such as hypoxia and infection. In fact, cortisol augments placental CRH synthesis via activation of nuclear factor kappa B (Thomson 2013). Placental CRH, once released into circulation, stimulates maternal and fetal pituitary glands as well as the placenta to secrete ACTH, which in turn stimulate more glucocorticoid secretion (Hutchinson and DeCherney 1999).

The enzyme 11beta-hydroxy steroid dehydrogenase type 2 (11BHSD-2), expressed in placental syncytiotrophoblast, critically regulates the transplacental transfer of bioactive cortisol from mother to fetus and maintains higher circulating cortisol levels in the maternal than in the fetal circulation. The activity of this placental enzyme may be reduced in certain conditions, including infection, hypoxemia and maternal undernutrition. In those circumstances the fetus (and placenta) may be exposed to inappropriately high levels of cortisol derived by transplacental transfer from the mother. This may contribute to fetal growth restriction, potentially by inhibition of growth-promoting factors in fetal tissues and programming predisposition to disease during later life development.

Placental Thyrotropin-Releasing Hormone (TRH) and Human Chorionic Thyrotropin (hCT)

Placental TRH is released by the cytotrophoblast and stimulates hCT production in the syncytiotrophoblast. HCT is produced by the placenta in small amounts, and its physiologic role remains unclear. Its structure is similar to pituitary TSH, but it does not possess the common *a* subunit. Furthermore, hCG also has thyrotropic activity, and it seems to have a more significant effect on maternal thyroid function than does hCT.

Other Pregnancy-Related Peptides

In addition to the pituitary- and hypothalamic-like peptides, the placenta produces a group of glycoproteins that are unique of pregnancy, as they do not have analogues in the nonpregnant state.

Pregnancy-Associated Plasma Protein-A (PAPP-A)

PAPP-A is a large protein secreted by the syncytiotrophoblast, whose levels in maternal plasma initially rise rapidly and then continue to rise more slowly until term. This glycoprotein seems to have an immunosuppressive role in pregnancy,

and decreased levels have been associated with early pregnancy failure. PAPP-A increases the bioavailability of insulin-like growth factor, which in turn mediates trophoblast invasion and modulates glucose and amino acids transport in the placenta. PAPP-A is also used as a first-trimester screening marker for aneuploidies. Decreased levels of PAPP-A before the 14th week of gestation are in fact associated with an increased risk for trisomy 21, trisomy 18, and trisomy 13. Low levels of PAPP-A have also been shown to be associated with increased risk of developing pre-eclampsia.

Placental Protein-5 (PP5)

PP5 is produced in the syncytiotrophoblasts. It is first detected about 6 weeks after ovulation, and then it continues to rise until term. PP5 has antithrombin and antiplasmic activities, and therefore it acts as a natural blood coagulation inhibitor at the implantation site.

Placental Pregnancy-Specific Beta 1-Glycoprotein (SP1)

This glycoprotein secreted from trophoblastic cells is first detected 4–5 weeks after ovulation, and it reaches peak concentrations of 100–200 ng/ml at term. SP-1 plays an immunomodulatory role in pregnancy: it is an immunosuppressor of lymphocyte proliferation and may help to prevent rejection of the conceptus. It is also a possible inducer of proangiogenic growth factors known to play an important role in the establishment of the vasculature at the maternal-fetal interface (Ha et al. 2010).

Growth Factors

Numerous growth factors have been identified in the placenta and the proliferative activities that they induce appear to overlap.

Inhibin and Activin

Inhibin and activin are heterodimeric glycoproteins synthetized by the placenta. Their biologic roles in pregnancy have been derived from in vitro studies. Activin appears to increase the release of GnRH, hCG, and progesterone, whereas inhibin produces the opposite effects. Inhibin release starts about 12 days after conception and peaks at 8–10 weeks, when it is released by the corpus luteum. The main source in the third trimester is the placenta.

Placental Insulin-Like Growth Factor-1 and Factor-2 (IGF-1 and IGF-2)

IGF-1 and IGF-2 regulate cell proliferation. The most important site of IGF-1 and IGF-2 production is the placenta. The IGF system is recognized to be crucial for fetal growth, as experiments in knockout mice have shown. Its release during pregnancy is regulated by placental growth hormone (GH), a variant of pituitary GH. In women with a fetoplacental unit disorder, low placental GH levels resulted in low IGF-1 and in a secondary pituitary GH increase (Caufriez et al. 1993).

IGF-2 is important in the process of first-trimester trophoblast invasion and fetal growth (Street et al. 2008), and it is also a metabolic regulator, with metabolic

actions in muscle and fat. Moreover, it stimulates prolactin synthesis in human decidual cells and has a role in steroidogenesis.

Transforming Growth Factor (TGF) and Epidermal Growth Factor (EGF)

These peptides regulate a variety of cellular functions, including cell proliferation, differentiation, apoptosis, migration/invasion, matrix synthesis, and immune response.

TGF has been purified from placenta and is thought to be a paracrine regulator of mesenchyme-epithelium interactions. Recent findings suggest that TGF-beta expression plays a key role in trophoblast invasion and that an altered expression of such molecule is responsible for inadequate trophoblast invasion observed in preeclampsia (Simpson et al. 2002). Epidermal growth factor (EGF) is produced by the syncytiotrophoblast. It exerts a gestational age-dependent dual action on the first-trimester placenta: it stimulates trophoblast proliferation at 4–5 weeks and then stimulates differentiated trophoblast function at 6–12 weeks (Maruo et al. 1992).

Endocrinology of Parturition

The complex interactions existing between the mother, the placenta, and the fetus during pregnancy culminate in the mechanisms leading to parturition. Such mechanisms involve some of the endocrine factors that have been discussed in this chapter, such as estrogens, progesterone, and CRH, as well as other mediators, in particular cytokines, growth factors, oxytocin, and prostaglandins, which lead the transition from a quiescent myometrium to a contractile state that allows delivery of the fetus. Inflammation and neuronal and immune pathways are also involved in this process.

The placenta is an active endocrine organ not only during the duration of pregnancy, when it allows normal growth and development of fetal and maternal tissues, but also during parturition. It releases molecules that can exert their biologic effects on the placenta itself but also, once they enter maternal and fetal circulation, can act as autocrine, paracrine, and endocrine factors (Vannuccini et al. 2016; Petraglia et al. 1996). Progesterone and estrogens are antagonistic in the parturition process. Progesterone is essential for the maintenance of uterine quiescence, together with other factors such as relaxin, prostacyclins, and nitric oxide. It produces uterine relaxation stabilizing lysosomal membranes and inhibiting prostaglandin synthesis. Estrogens, on the other hand, have the opposite effect on lysosome and stimulate prostaglandin release and expression of prostaglandin and oxytocin receptors in the myometrium. The ratios of estradiol and progesterone are closely related to the stimulation of myometrial gap junction formation, and an alteration in the estrogen/progesterone ratio is thought to take part in the initiation of labor, rather than a fall in progesterone plasma levels, which is not observed in human labor. The estrogens do not themselves cause uterine contractions in parturition; rather they enhance the capacity of the myometrium to generate contractions. Functional progesterone withdrawal in human parturition appears to be mediated by specific changes in

myometrial PR expression or function and in particular by an increase in PR-A/PR-B ratio (Mesiano 2004).

Another hormone involved in labor is oxytocin, even though its role in pregnancy and in the initiation of parturition remains unclear. Circulating levels of oxytocin are low throughout pregnancy and increase markedly only during the second stage of labor. However, an increase in the sensitivity of the myometrium to oxytocin, indicated by the increase in expression and number of oxytocin receptors present in the myometrium, is observed before the onset of labor. In addition to cause contraction, oxytocin also induces prostaglandin production by the decidua. Despite that, oxytocin cannot be considered an initiator of parturition, as suggested by experimental observation that mice deficient in oxytocin have a normal pregnancy and labor (Nishimori et al. 1996). The increased secretion of oxytocin through a neuroendocrine reflex occurs in stage 2 and 3 of labor and contributes to expulsion of the placenta and uterine involution.

Among the neuropeptides involved in the cascade of parturition, CRH is certainly one of the most important. The CRH/urocortin (Ucn) pathway influences uterine contractility, blood vessel tone, and immune function during pregnancy and undergo major changes at parturition (Petraglia et al. 2010). While the placenta represents the major source for CRH, the fetus abundantly secretes Ucn and adrenal dehydroepiandrosterone. Placental CRH synthesis is stimulated by fetal cortisol through a positive feedback mechanism. CRH effects on the onset of labor are mediated by the increase in estrogen release and altered progesterone/estrogen ratio induced by CRH and also by the effect that it has on myometrial contractility, through the increase of myosin light chain phosphorylation. CRH also modulates prostaglandin production by the placenta and fetal membranes, and it also has a pro-inflammatory effect, by inducing the release of chemokines and cytokines in the myometrium at term. The inflammation cascade can lead to activation of uterine contractility.

Ucn is synthesized and secreted by placenta and fetal membranes, and its levels increase after the onset of labor. It exerts similar actions as CRH, increasing matrix metalloproteinase, ACTH and prostaglandin secretion, and inflammation, thus contributing to myometrial contractility.

Prostaglandins, particularly PGE2 and PGF2, have a major role in initiation of human parturition, and they represent the endpoint of the CRH cascade. Their production in fetal membranes and uterine tissues increases with labor, and it is influenced by various factors, such as estrogens, CRH, and cytokines.

Among others, neurohormones, relaxin, parathyroid hormone-related protein, opioids, neurosteroids, and monoamines are also expressed and secreted from placental tissues and are involved in the complex phenomenon of parturition.

Conclusion

The placenta is a highly active endocrine organ and a central regulator of maternal–placental–fetal physiology during pregnancy and parturition. The complex interaction of hormones with one another allows a fascinating crosstalk between the

placenta, the fetus, and the mother. The endocrinology of the maternal–placental axis is still a progressive field of science, as some mechanisms are still incompletely understood.

Cross-References

- ▶ The "Great Obstetrical Syndromes"
- ▶ The Hypothalamus-Pituitary-Ovary Axis

References

Bass KE, Morrish D, Roth I, et al. Human cytotrophoblast invasion is up-regulated by epidermal growth factor: evidence that paracrine factors modify this process. Dev Biol. 1994;164(2):550–61.

Bedin M, Fournier T, Cabrol D, et al. Incidence of placental sulfatase deficiency on the mode of termination of pregnancy. Gynecol Obstet Invest. 1987;24(2):86–91.

Blanks AM, Brosens JJ. Progesterone action in the myometrium and decidua in preterm birth. Facts Views Vis Obgyn. 2012;4(3):33–43.

Buster JE. Fetal adrenal cortex. Clin Obstet Gynecol. 1980;23(3):803–24.

Buster JE, Carson SA. Endocrinology and diagnosis of pregnancy. In: Niebyl SGGJR, Simpson JL, editors. Obstetrics: normal and problem pregnancies. 4th ed. Philadelphia: Churchill Livingstone; 2002. p. 3–36.

Cane EM, Villee CA. The synthesis of prostaglandin F by human endometrium in organ culture. Prostaglandins. 1975;9(2):281–8.

Carbonne B, Dallot E, Haddad B, et al. Effects of progesterone on prostaglandin E(2)-induced changes in glycosaminoglycan synthesis by human cervical fibroblasts in culture. Mol Hum Reprod. 2000;6(7):661–4.

Caufriez A, Frankenne F, Hennen G, et al. Regulation of maternal IGF-I by placental GH in normal and abnormal human pregnancies. Am J Physiol. 1993;265(4 Pt 1):E572–7.

Evain-Brion D, Malassine A. Human placenta as an endocrine organ. Growth Horm IGF Res. 2003;13(Suppl A):S34–7.

Ha CT, Wu JA, Irmak S, et al. Human pregnancy specific beta-1-glycoprotein 1 (PSG1) has a potential role in placental vascular morphogenesis. Biol Reprod. 2010;83(1):27–35.

Handwerger S, Freemark M. The roles of placental growth hormone and placental lactogen in the regulation of human fetal growth and development. J Pediatr Endocrinol Metab. 2000;13(4):343–56.

Hassan SS, Romero R, Vidyadhari D, et al. Vaginal progesterone reduces the rate of preterm birth in women with a sonographic short cervix: a multicenter, randomized, double-blind, placebo-controlled trial. Ultrasound Obstet Gynecol. 2011;38(1):18–31.

Hutchinson K, DeCherney AH. Endocrinology of pregnancy. In: Reece EA, Hobbins JC, editors. Medicine of the fetus & mother. 2nd ed. Philadelphia: Lippincott-Raven Publishers; 1999. p. 85–103.

Librach CL, Feigenbaum SL, Bass KE, et al. Interleukin-1 beta regulates human cytotrophoblast metalloproteinase activity and invasion in vitro. J Biol Chem. 1994;269(25):17125–31.

Liu JH. Endocrinology of pregnancy. In: Creasy RK, Resnik R, Iams JD, Lockwood CJ, Moore TR, Greene MF, editors. Creasy & Resnik maternal-fetal medicine. Philadelphia: Elsevier Saunders; 2014. p. 100–11.

Maruo T, Matsuo H, Murata K, et al. Gestational age-dependent dual action of epidermal growth factor on human placenta early in gestation. J Clin Endocrinol Metab. 1992;75(5):1362–7.

Meis PJ, Klebanoff M, Thom E, et al. Prevention of recurrent preterm delivery by 17 alpha-hydroxyprogesterone caproate. N Engl J Med. 2003;348(24):2379–85.
Mesiano S. Myometrial progesterone responsiveness and the control of human parturition. J Soc Gynecol Investig. 2004;11(4):193–202.
Napso T, Yong HEJ, Lopez-Tello J, et al. The role of placental hormones in mediating maternal adaptations to support pregnancy and lactation. Front Physiol. 2018;9:1091.
Nishimori K, Young LJ, Guo Q, et al. Oxytocin is required for nursing but is not essential for parturition or reproductive behavior. Proc Natl Acad Sci USA. 1996;93(21):11699–704.
Noyola-Martinez N, Halhali A, Barrera D. Steroid hormones and pregnancy. Gynecol Endocrinol. 2019;35(5):376–84.
Petraglia F, Florio P, Nappi C, et al. Peptide signaling in human placenta and membranes: autocrine, paracrine, and endocrine mechanisms. Endocr Rev. 1996;17(2):156–86.
Petraglia F, Imperatore A, Challis JR. Neuroendocrine mechanisms in pregnancy and parturition. Endocr Rev. 2010;31(6):783–816.
Resnik R, Killam AP, Battaglia FC, et al. The stimulation of uterine blood flow by various estrogens. Endocrinology. 1974;94(4):1192–6.
Schumacher A. Human chorionic gonadotropin as a pivotal endocrine immune regulator initiating and preserving fetal tolerance. Int J Mol Sci. 2017;18(10):2166.
Shi QJ, Lei ZM, Rao CV, et al. Novel role of human chorionic gonadotropin in differentiation of human cytotrophoblasts. Endocrinology. 1993;132(3):1387–95.
Siiteri PK, Febres F, Clemens LE, et al. Progesterone and maintenance of pregnancy: is progesterone nature's immunosuppressant? Ann N Y Acad Sci. 1977;286:384–97.
Simpson ER, Carr BR, Parker CR Jr, et al. The role of serum lipoproteins in steroidogenesis by the human fetal adrenal cortex. J Clin Endocrinol Metab. 1979;49(1):146–8.
Simpson H, Robson SC, Bulmer JN, et al. Transforming growth factor beta expression in human placenta and placental bed during early pregnancy. Placenta. 2002;23(1):44–58.
Street ME, Grossi E, Volta C, et al. Placental determinants of fetal growth: identification of key factors in the insulin-like growth factor and cytokine systems using artificial neural networks. BMC Pediatr. 2008;8:24.
Thomson M. The physiological roles of placental corticotropin releasing hormone in pregnancy and childbirth. J Physiol Biochem. 2013;69(3):559–73.
Tulchinsky D, Hobel CJ. Plasma human chorionic gonadotropin, estrone, estradiol, estriol, progesterone, and 17 alpha-hydroxyprogesterone in human pregnancy. 3. Early normal pregnancy. Am J Obstet Gynecol. 1973;117(7):884–93.
Vannuccini S, Bocchi C, Severi FM, et al. Endocrinology of human parturition. Ann Endocrinol (Paris). 2016;77(2):105–13.
Velegrakis A, Sfakiotaki M, Sifakis S. Human placental growth hormone in normal and abnormal fetal growth. Biomed Rep. 2017;7(2):115–22.

The "Great Obstetrical Syndromes"

21

Salvatore Andrea Mastrolia and Irene Cetin

Contents

Abstract

The Great Obstetrical Syndromes, namely, preterm labor, preterm prelabor rupture of membranes (pPROM), fetal demise, preeclampsia, and intrauterine growth restriction (IUGR), affect more than 15% of pregnancies, posing the mother and the fetus at high risk for adverse pregnancy outcomes. These pregnancy complications may be responsible for both short- and long-term health outcomes.

Many pathways leading to their development have been suggested. Inflammation is the factor under higher evaluation from a clinical and academic point of view, offering a number of aspects to be assessed, due to the complexity

S. A. Mastrolia · I. Cetin (✉)
Department of Obstetrics and Gynecology, Ospedale dei Bambini "Vittore Buzzi", University of Milano, Milano, Italy
e-mail: mastroliasa@gmail.com; irene.cetin@unimi.it

F. Petraglia, B. C. Fauser (eds.), *Female Reproductive Dysfunction*, Endocrinology,
https://doi.org/10.1007/978-3-030-14782-2_21

of this process as well as of the immune system and its physiologic and pathologic changes during pregnancy. This process has been described in sterile conditions, in the presence of infection from pathogens, as well as in other conditions such as obesity, another extremely actual challenge for health systems.

Indeed, from implantation to parturition, inflammation in conjunction with the immune system function plays a significant role during normal pregnancy. Moreover, inflammation has to be tightly controlled since an exacerbation of this process can negatively affect tissue function, leading to the development of pregnancy complications, as suggested in obese women or those with gestational diabetes mellitus.

Due to the importance of the topic, the need for preventive strategies to reduce the incidence of the Great Obstetrical Syndromes is of extreme importance. Unfortunately, while we have available tools to perform an adequate secondary prevention of women at high risk for these syndromes, there is still a lack of effective screening tools for primary prevention.

Keywords

Gestational diabetes mellitus · Immune system · Inflammation · Metabolomics · Microbiome · Obesity · Pregnancy complications · Preterm labor · Toll-like receptor

Defining the Great Obstetrical Syndromes

The term "Great Obstetrical Syndromes" was coined by Roberto Romero (2009) to reframe the concept of obstetrical diseases, identifying conditions with the following characteristics: (1) multiple etiologies, (2) a long preclinical period, (3) adaptive in nature, (4) with fetal involvement, and (5) the result of complex interactions between the maternal and fetal genome and the environment (Parimi et al. 2008). This term comprises obstetrical complications such as preterm labor, preterm prelabor rupture of membranes (pPROM), fetal demise, preeclampsia, and intrauterine growth restriction (IUGR). The Great Obstetrical Syndromes affect more than 15% of pregnancies, posing the mother and the fetus at high risk for adverse pregnancy outcomes. These pregnancy complications can affect short- and long-term health outcomes for both the mother and fetus.

To understand the impact of these conditions, preterm birth, defined as birth before 37 weeks of gestation, affects 5–18% of pregnancies, representing the leading cause of neonatal death and the second cause of childhood death below the age of five (Liu et al. 2012). About 15 million preterm neonates are born every year, and the highest rates occur in Africa and North America (Blencowe et al. 2012). Neonates born preterm are at an increased risk of complications attributed to the immaturity of multiple organ systems as well as neurodevelopmental disorders, such as cerebral palsy, intellectual disabilities, and vision and hearing impairments (Mwaniki et al. 2012). Preterm birth is a leading cause of disability-adjusted life years (the number

of years lost because of ill health, disability, or early death), and the annual cost in the United States is at least 26.2 billion dollars per year and rising (Hutchinson-Colas and Segal 2015).

Although the Great Obstetrical Syndromes are academically described as distinct conditions, they may occur together increasing maternal and fetal/neonatal risk for adverse outcomes occurring during pregnancy and puerperium. These syndromes are the clinical endpoint of several underlying mechanisms, including intrauterine or systemic maternal infection/inflammation, uterine ischemia, placental vascular lesions, abnormal allograft reaction, and others (Romero 2009). In addition, many of the obstetrical syndromes are associated with an increased thrombin generation (Erez et al. 2009, 2010).

Gestational Diabetes Mellitus: Does It Deserve to Be Mentioned Among the Great Obstetrical Syndromes?

Of interest, the concept of a pathologic condition that adversely interacts with the maternal-fetal unit to initiate a subclinical pathology which progresses to clinical manifestations (Romero 2009) has been suggested also for gestational diabetes mellitus (GDM).

The placenta represents the interface between the maternal and fetal compartments and, in the first trimester, placenta-mediated maternal adaptations ensure preferential nutritional supply to the placenta and fetus. This is achieved through a metabolic state that is characterized by a relative increase in insulin resistance and anabolism, which is linked to the rise in human chorionic gonadotropin and human placental lactogen. This principal change in the metabolic handling of nutrients results in higher postprandial glucose levels and increased placental glucose utilization, contributing to a rapid fall of fasting glucose levels accompanied by a rise in free fatty acids (Metzger and Freinkel 1987). While there is a compensatory increase in pancreatic β-cell response and insulin secretion, the opposing effects of placental hormones, cortisol, and progesterone on metabolism remain progressive and prevalent so that the maintenance of placental/fetal nutrition prevails at the expense of maternal glycemic control. The recognition that pregnancy is a diabetogenic state is based on these metabolic changes that can exceed the threshold to precipitate the development of overt gestational diabetes (Gabbay-Benziv and Baschat 2015), a metabolic disease where maternal predisposition and placental factors result in progressively impaired glucose tolerance leading to secondary maternal and fetal effects that typically manifest in the third trimester. Gestational diabetes is associated with maternal and fetal short-term and long-term complications. Well-recognized complications are the increased risk of type 2 diabetes for the mother, accelerated fetal growth, and polyhydramnios. Less common but not less important are adverse fetal outcomes such as fetal demise, delivery complications attributable to macrosomia, and neonatal metabolic complications. Late-onset metabolic complications for the offspring are associated with endothelial/vascular dysfunction that may later cause obesity, hypertension, type 2 diabetes mellitus, and metabolic syndrome.

At least some of these complications may be related to aberrant placental structure or function caused by the metabolic milieu of the diabetic mother (Gabbay-Benziv and Baschat 2015).

Diagnostic and therapeutic recommendations for GDM are based on the evidence of the short-term risks associated with GDM for the offspring (i.e., a 3.5-fold higher risk of macrosomia, an elevated risk of shoulder dystocia, and hypoglycemia) and the increased maternal risks (i.e., cesarean section, polyhydramnios, gestational hypertension, and preeclampsia) (Cabero Roura and Hod 2015).

Identifying and providing good care for women with GDM might have a substantial impact on population health in both high- and low-income countries.

In addition to these clinical considerations, placental effects of impaired glucose metabolism in women with GDM should be analyzed, although the relationship between the degree of glycemic control and placental changes remains unclear. While some studies support the concept that the degree of damage is proportional to the degree of hyperglycemia, others have shown that, even in tight glycemic control, there are still histological differences in the diabetic placentas compared to nondiabetic ones (Madazli et al. 2008). Macroscopically, the diabetic placenta is characterized by increased size and weight resulting in an increased placental to fetal weight ratio (Taricco et al. 2003, 2009). Microscopically, a number of lesions have been found with increased frequency in placentas of diabetic women compared to controls. Villous fibrinoid necrosis, a condition where villous stroma is replaced by fibrinoid material, and villous immaturity with a decreased formation of terminal villi are observed. These changes may affect maternal-fetal nutrient delivery and gas exchange being potential precursors to otherwise unanticipated fetal complications such as fetal demise (Evers et al. 2003), while the interference with angiogenesis sets the stage for maternal hypertensive disorders.

As a result to the abovementioned mechanisms, the diabetic placenta is subjected to the opposing stimuli of glucose-mediated growth acceleration and impaired decreased vascular development, with an increased risk of local ischemic changes as well as of a mismatch which may predispose the fetus to abnormal gas and nutrient exchange (Gabbay-Benziv and Baschat 2015).

Due to all of the above, being associated with placental and clinical implications potentially affecting pregnancy, GDM can be included with merit among those conditions named as the Great Obstetrical Syndromes (Gabbay-Benziv and Baschat 2015).

Risk Factors for the Great Obstetrical Syndromes

The task of assessing the risk factors for the development of the Great Obstetrical Syndromes is of crucial importance. A number of factors have been identified such as low socioeconomic status, maternal age, maternal hypertension, GDM, alcohol/drug consumption, autoimmune diseases, thyroid malfunction, immunodeficiency states, infections during pregnancy, placental/cord abnormalities, genetic diseases or predisposition, multiple gestation, low maternal weight and abnormal weight gain

during pregnancy, as well as miscellaneous causes such as short inter-pregnancy intervals and the mother's physical activity level (Kim 2014).

Due to their high prevalence and strong impact on healthcare systems, mechanisms of disease on which risk factors can trigger the development of the Great Obstetrical Syndromes deserve exploration to identify possible preventive strategies and therapeutic interventions.

Pathogenesis of the Great Obstetrical Syndromes

Many pathways leading to the development of the Great Obstetrical Syndromes have been suggested in the past. Inflammation is the current topic under higher evaluation from a clinical and academic point of view, offering a number of aspects to be assessed, due to the complexity of this process as well as of the immune system and its physiologic and pathologic changes during pregnancy (Challis et al. 2000). This process has been described in sterile conditions, in the presence of infection from pathogens, as well as in other conditions such as obesity, another extremely actual challenge for the health systems.

Indeed, from implantation to parturition, inflammation in conjunction with the immune system function plays a significant role during normal pregnancy. Moreover, inflammation has to be tightly controlled since an exacerbation of this process can negatively affect tissue function, leading to the development of pregnancy complications (Denison et al. 2010).

The Immune System During Pregnancy: The Misbalance Toward Inflammation in the Great Obstetrical Syndromes

Pregnancy poses a challenge to the maternal immune system. The semi-allogeneic fetus, placenta, and chorioamniotic membranes continuously interact with maternal immune cells in the uterus and those in the maternal circulation determining an immune-privileged site. During implantation and placentation, there is a continuous immune recognition and modulation of the maternal immune system by trophoblasts at the maternal-fetal interface (Mor and Cardenas 2010). Moreover, there is a continuous transfer of fetal cells and trophoblastic debris into the maternal circulation, which leads to microchimerism and an increase in systemic maternal inflammation during pregnancy (Redman and Sargent 2008). Therefore, normal pregnancy is associated with a "physiological" mild inflammatory state (Hahn et al. 2012). This status is significantly pronounced in preeclampsia, where the activation state of neutrophils is higher than in sepsis (Gervasi et al. 2001). Overtly activated neutrophils are also implicated in recurrent fetal loss or bacterially induced abortions (Hahn et al. 2012). Inflammation results implicated also in GDM, and this syndrome needs to be evaluated according to the presence of obesity, due to the impact of this risk factor on the development of the Great Obstetrical Syndromes.

Cytokines and T Cell Function

Central to the immune adaptation are changes in cytokine production. Structurally, cytokines can be divided into four groups: the 4α helix family members (interleukin 2 [IL-2], interferon gamma [IFN-g], and IL-10), IL-1 family, IL-17 family, and chemokines.

However, a more helpful division that has been utilized in the past is the functional grouping into those involved in Th1 reactions (cell-mediated immunity) and those involved in Th2 reactions (humoral immunity). T lymphocytes (CD4+) play a key role in regulating the immunological response by producing cytokines: (1) Th1 cells produce mainly IL-1, IL-2, IL-6, IL-12, IL-15, IL-18, IFN-g, and tumor necrosis factor α (TNF-α) and promote strong cell-mediated responses, and (2) Th2-cells are the source for IL-4, IL-5, IL-10, IL-13, and granulocyte-macrophage colony-stimulating factor (GM-CSF) and are engaged mainly in the regulation of humoral response. Local T cells produce cytokines, but major sites of Th2 cytokine production appear to be nonlymphoid tissues including the placental/decidual tissues, particularly the trophoblast (Chaouat 1999). During normal pregnancy, Th1/Th2 activity balance is strongly shifted toward Th2 activity (so-called Th2 phenomenon) (Mor 2008; Challis et al. 2009), which plays a potential protective role in the feto-maternal relationship. Inflammatory/infection processes alter the balance of Th1 and Th2 cytokines causing a shift toward a Th1 predominance, which initiates and enhances the cascade of inflammatory cytokine production involved in preterm delivery, preeclampsia, and IUGR (Challis et al. 2009).

Moreover, a better insight in the inflammatory imbalance of the Great Obstetrical Syndromes has been provided assessing the T cell system as Th1/Th2/Th17/T regulator. In addition to the previous functional assessment of T cell function, this underlines the pro-inflammatory effect of IL-17, definable as an inflammatory inducer, produced by Th17 cells, and the action of T regulator cells.

In early pregnancy, IL-17 production is restricted to the CD4+ T cells in both the periphery and decidua. Th-17 cells are typically reduced in healthy pregnancies. T regulator cells play a central role for immune regulation and induction of tolerance by inhibiting proliferation and cytokine production in both CD4+ and CD8+ T cells, immunoglobulin production by B cells, cytotoxic activity of natural killer (NK) cells, and maturation of dendritic cells. Normal pregnancy is associated with increased levels of T regulator cells in the periphery and at the maternal-fetal interface, while reduction in T regulator cell numbers has been observed in pre-eclampsia (Saito et al. 2010).

Toll- Like Receptor Function During Normal Pregnancy and in Pregnancy Complications

The trophoblast expresses pattern recognition receptors (PRRs) that recognize the presence of bacteria, viruses, dying cells, and damaged tissue. Trophoblast cells identify and respond to pathogens through TLRs. All ten TLRs are expressed in the placenta (Mor 2008; Patni et al. 2009).

The variation in toll-like receptors (TLRs) expression has an important impact on when microorganisms pose the greatest threat to the developing fetus (Mor 2008).

A microorganism is a threat to the fetus if the syncytiotrophoblast layer lacking TLRs is breached and the microorganism makes its way into the decidual or the placental villous compartments. Upon recognition, the trophoblast secretes specific cytokines that act on decidual immune cells (Mor 2008).

Depending on gestational age and stage of development of the trophoblast, different TLRs are expressed, and their activation causes distinct cell responses. For example, TLR-6 is expressed during the third trimester instead of the first. TLR-2 and TLR-4 are expressed by the villous cytotrophoblast and extravillous trophoblast cells, but not by the syncytiotrophoblast in the first trimester. At term, the placenta expresses TLR-2 and TLR-4 on the cytoplasm of the syncytiotrophoblast (Patni et al. 2009).

Patni and colleagues suggested that the human term placenta can respond to TLR-3, TLR-5, TLR-7, and TLR-8 agonists (Patni et al. 2009). In placentas of women developing preeclampsia, TLR-2, TLR-4, TLR-5, and TLR-6 expression has been identified, with TLR-2, TLR-4, TLR-5, and TLR-6 significantly increased on the maternal side and TLR-2 on the fetal side (Dabagh-Gorjani et al. 2014). As a confirmation to this observation, in vitro studies have demonstrated that TLR-4 activation promotes cytokine production, whereas activation of TLR-2 leads to first-trimester trophoblast cell apoptosis, indicating that trophoblast cell death may be directly induced through TLR-2 pathogens, an observation recognized in several pregnancy complications, including spontaneous abortion, IUGR, and preeclampsia (Romero et al. 2007).

Inflammation in Obese Women

In the last decade, obesity has become a global problem. Maternal obesity is expanding exponentially worldwide to almost epidemic proportions, with an additional 5–10% of pregnant women with diabetes, representing a significant risk factor for adverse pregnancy outcomes (Myatt and Maloyan 2016) with both immediate and long-term consequences. Maternal obesity is associated with increased morbidity and mortality for both the mother and offspring. Antenatal risks include gestational diabetes, hypertensive disorders including preeclampsia, as well as thromboembolic complications. Maternal obesity increases the lifetime risk of obesity in offspring and a tendency to develop metabolic syndrome in childhood and adolescence (Denison et al. 2010).

A study by Denison et al. (2010) suggests that maternal obesity may affect placental transport and substrate availability. These authors suggest that obese mothers may have increased levels of placental macrophages that can promote placental inflammation.

Similarly, a study by Segovia et al. (Denison et al. 2010) showed that maternal obesity causes chronic low-grade inflammation characterized by accumulation of adipose tissue macrophage and systemic proinflammatory cytokine levels. White adipose cells produce over 50 adipokines, which contribute to low-grade inflammation in obesity. It also produces anti-inflammatory cytokines, such as adiponectin and IL-10 and IL-1R1. In addition, the effect of obesity on inflammation has been studied assessing oxidative stress in women with periodontal disease (Zambon et al. 2018).

Proinflammatory cytokines can cross the placenta, exposing the developing fetus to an inflammatory environment that can lead to developmental programming of metabolic disorders in the offspring (Segovia et al. 2014).

In a study by Challier et al., placental macrophages and maternal peripheral blood mononuclear cells were functionally and phenotypically characterized in obese versus normal weight women. The number of resident CD68+ and CD1+ cells was increased two- to threefold in the placenta of obese versus normal weight women. The macrophage population was characterized by a marked phenotypic heterogeneity with complex subsets of CD14+, CD68+, and CD11b+ (mac-1) cells associated with an increased expression of the proinflammatory cytokines IL-1, TNF-a, and IL-6. Placental inflammation was associated with increased peripheral blood mononuclear cell gene expression with an increase in the monocyte differentiation and maturation markers CD14+ and CD68+ in maternal peripheral blood mononuclear cells. The inflammatory changes were associated with higher plasma concentrations of C-reactive protein (CRP) and IL-6 in obese compared with lean women. Their work showed that chronic inflammation seen with prepregnancy obesity can extend to the fetus in utero with accumulation of a heterogeneous macrophage population and proinflammatory mediators in the placenta (Challier et al. 2008).

Inflammatory cytokines such as TNF-a and IL-6, which are secreted by the adipose tissue, are constitutively elevated in the serum of obese subjects and are known to cause insulin resistance and thus link obesity to the onset of GDM (Wolf et al. 2004). In addition, Ramsay et al. (2002) showed that obesity in pregnancy is associated with marked hyperinsulinemia, dyslipidemia, impaired endothelial function, high blood pressure, and inflammatory upregulation (by measuring CRP and IL-6).

Inflammation in Women with GDM

Women with GDM are at increased risk for maternal and perinatal complications. Perinatal complications include macrosomia, shoulder dystocia, birth injuries, nerve palsy, jaundice, and neonatal hypoglycemia. Offsprings born from these women are at an increased risk of developing long-term glucose intolerance, obesity, and having intellectual disability (Crowther et al. 2005).

Women with GDM are at an increased risk of developing type 2 diabetes and displaying features associated with metabolic syndrome, such as hypertension, dyslipidemia, microalbuminuria, and endothelial dysfunction (Wolf et al. 2004).

Prospective studies have shown that an increased leukocyte count as a marker of inflammation is associated with the development of type 2 diabetes, but it is not entirely clear how inflammation increases the risk of GDM. In a prospective study, Wolf et al. found that an increased leukocyte count early in pregnancy was independently and linearly associated with results of third-trimester GDM screening tests and the risk of GDM, indicating an association between inflammation and these obstetrical syndromes. This effect was independent from known risk factors for GDM, such as advanced maternal age, non-Caucasian race, obesity, hypertension, and multiple gestations (Wolf et al. 2004).

Altered carbohydrate metabolism in GDM leads to vascular damage and glomerular filtration dysfunction, which predisposes to preeclampsia.

Hyperglycemia impairs the invasive and proliferative abilities of first-trimester cytotrophoblast cells. These cells are essential for building an optimal blood flow to the placenta, and its disrupted invasion alters arterial remodeling (Cawyer et al. 2014). Serine proteases, cathepsin, and metalloproteinases are involved in this invasive process.

Cytotrophoblast invasion is facilitated from the conversion of plasminogen to plasmin by urokinase plasminogen activator (uPA), which is regulated by plasminogen activator inhibitor. Both are inhibited in preeclampsia. Cawyer et al. (2014) found that hyperglycemia disrupts the invasive properties of cytotrophoblast cells by decreasing uPA and PA1-I expression, downregulating VEGF and PIGF, and upregulating sEng and sFlt-1.

In addition, hyperglycemia impedes cytotrophoblast function by inducing stress pathway signaling (p38 MAPK and peroxisome proliferator activated receptor gamma [PPARγ]) followed by MMP-9 inhibition, which impairs migration and invasion of cytotrophoblast cells and causes oxidative stress, leading to placental hypoxia, and IL-6 elevation, followed by an angiogenic imbalance. All of these changes appear to converge in a common pathway that leads to abnormal placentation and preeclampsia (Cawyer et al. 2014).

Cytokine-induced insulin resistance is a primary mechanism associating inflammation and glucose intolerance with each other, and both adipose tissue and the placenta are important sources of these cytokines. It is believed that subclinical inflammation present in normal pregnancy is amplified in GDM. IL-6 and TNF-α can directly inhibit insulin-stimulated tyrosine phosphorylation at the insulin receptor. Inflammatory cytokines stimulate secretion of cortisol, growth hormone, and counter regulatory hormones that contribute to insulin resistance and hyperglycemia (Wolf et al. 2004).

Adipose cells play an important role in regulating insulin sensitivity by secreting adipokines involved in insulin resistance pathogenesis in pregnancy, especially in obese women. In fact, obesity plays a role in GDM through chronic subclinical inflammation, low-grade activation of the acute-phase response, and dysregulation of adipokines.

Examples of adipokines include adiponectin and leptin. Leptin has pro-inflammatory qualities, while adiponectin is anti-inflammatory and promotes insulin sensitization (Crowther et al. 2005).

Low adiponectin levels during the first trimester have been associated with GDM. Leptin increases the production of TNF-α and IL-6 by monocytes and stimulates the production of chemokine ligands. TNF-α, as part of a viscous circle, increases leptin production (Xu et al. 2014).

Endothelial dysfunction is common in GDM, although the effect on the endothelium of cytokines produced in GDM is unclear. Mrizak and colleagues determined forearm skin blood flow reduction in nonobese GDM women, with a negative correlation with TNF-α and IL-6 and a positive correlation with adiponectin, indicating some degree of endothelial dysfunction was present in nonobese pregnancies

complicated by GDM and endothelial dysfunction appeared to be a direct result of inflammatory mediators (Mrizak et al. 2013).

The Effect of Obesity and Impaired Glycemic Control on Placental Mitochondria

The placenta is a metabolically active organ with multiple functions. Mitochondrial oxidative phosphorylation and substrate oxidation represent the main energy source for placental function. Therefore, mitochondrial function plays an important role in metabolic health and cellular fate (Montgomery and Turner 2015). In human and rodent placenta, both nutritional and hypoxic stresses can alter mitochondrial biogenesis, function, and morphology leading to placental dysfunction (Mando et al. 2016a).

Placental alterations can affect fetal metabolism and development possibly leading to higher risk of developing disease in the future adult.

However, molecular mechanisms underlying programming effects have been only partially identified. Impaired placental transfer and metabolism of energy substrates in maternal obesity and/or diabetes mellitus have been reported (Cetin et al. 2012). A lipotoxic placental environment is indeed recognized in maternal obesity, with an altered metabolome profile, increased inflammation and oxidative stress, as well as decreased regulators of angiogenesis with a less efficient fetal/placental ratio (Zambon et al. 2018; Cetin et al. 2012; Fattuoni et al. 2018; Mando et al. 2016b).

This might alter mitochondrial function, resulting in excessive production of reactive oxygen species and oxidative stress, in a vicious cycle leading to placental dysfunction and impaired pregnancy outcomes. In a study by Mando et al. (2018) the effect of maternal pregestational body mass index (BMI) and glycemic levels on placental mitochondria has been assessed by measuring mitochondrial content and morphology in term placentas sampled at elective cesarean section. This study included Caucasian women carrying a singleton spontaneous pregnancy and delivering at term by elective cesarean section performed for breech presentation, repeated cesarean section, or maternal request. A significantly lower mtDNA content in placental tissue was measured in women with normal weight compared to obese women without GDM. In addition, an inverse correlation was observed between pregestational BMI and mtDNA content. This correlation was significant among women with normal weight versus obese women without GDM. The two analyses were not significant when comparing normal weight women to obese women with GDM. Of great interest, placental mitochondria from placentas of obese women with GDM showed morphological abnormalities at electron microscopic analysis (Mando et al. 2018).

The conclusions of this study support the hypothesis that placental mitochondria can be altered by elevated maternal BMI and/or by metabolic alterations occurring in gestational diabetes mellitus.

A compensatory increase in mitochondrial biogenesis can be explained by the endocrine stimuli due to high intracellular fatty acid levels and oxidative stress occurring in the lipotoxic environment of placentas in obese women (Hastie and Lappas 2014). Indeed, altered levels of mtDNA, as well as the impairment of nutrient transport systems, have been reported in previous studies in the placental tissue of different pregnancy pathologies characterized by elevated oxidative stress and inflammation levels, such as intrauterine growth restriction and preeclampsia (Mando et al. 2011, 2013).

Indeed, oxidative stress is one of the hallmark responses to intracellular lipid overload. High levels of free fatty acids impact the mitochondrial membrane structure, causing the release of reactive oxygen species (ROS) that can react with macromolecules and damage intracellular membranes and DNA (Agarwal et al. 2008). These alterations can, in turn, affect mitochondrial structure and function, in a vicious cycle of mitochondrial abnormalities and ROS formation, possibly representing a key mechanism of placental dysfunction in a disease condition.

In addition, several animal models of maternal obesity report mitochondrial dysfunctions in pancreatic islets, liver, or skeletal muscle of the offspring (Borengasser et al. 2011). However, maternal obesity and diabetes are not always associated with obvious fetal distress, and the possible placental adaptation may explain this finding.

Relatively to the effect of GDM on placental mitochondria, GDM has been associated with impaired placental development showing villous immaturity or alterations in villous branching, as well as impaired placental angiogenesis, villous vasculature, and uteroplacental perfusion (Gauster et al. 2012). Oxidative stress markers have also been reported in GDM placentas (Coughlan et al. 2004), possibly affecting the physiology of the placental vasculature and mitochondrial morphology.

Insulin resistance has been correlated in several tissues with a decrease in mitochondrial function and mitochondrial DNA copy number, with alterations of mitochondrial size and density. The possible role of epigenetic regulation is emerging for these alterations (Zheng et al. 2015). Indeed, some authors recently hypothesized that insulin resistance acts on the expression of proteins involved in the methylation of both nuclear and mitochondrial DNA, affecting the expression of genes involved in mtDNA replication, thus leading to decreased mitochondrial biogenesis (Zheng et al. 2015). However, other studies report either no impairment or a compensatory increase of mitochondrial function and oxidative capacity in conditions of insulin resistance (Turner et al. 2007). Hence, the relationship between mitochondria and insulin action is highly complex, and there is still much to learn in this area (Montgomery and Turner 2015).

Of interest, White et al. showed that, in addition to the dysregulation of glucose metabolism, GDM obese women compared with non-GDM obese women exhibit exaggerated dyslipidemic profiles prior to the GDM diagnosis, at week 17, when placentation still occurs. This possibly reflects enhanced insulin resistance in peripheral tissues of GDM women and a consequently reduced suppression of lipolysis, affecting lipid metabolism pathways.

Prevention of the Great Obstetrical Syndromes: Potential Strategies and Future Studies

In light of the evidence presented above, the need for preventive strategies to reduce the incidence of the Great Obstetrical Syndromes is of extreme importance. Unfortunately, while we have available tools to perform an adequate secondary prevention of women at high risk for these syndromes, there is still a lack of effective screening tools for primary prevention. Metabolomics, the global quantitative assessment of endogenous metabolites within a biological system, has proven to be a rapid approach in the identification of biomarkers predictive of the outcome of both a pathological condition and the individual's response to pharmacological treatment.

These syndromes, such as GDM, preeclampsia, and IUGR, are an example of syndromes occurring during pregnancy in which the application of metabolomics, with its potential of metabolic profiling of maternal biofluids for the identification of prenatal biomarkers, can be used for the prevention and monitoring of these diseases (Dessi et al. 2015).

In addition to metabolomics, the emergence of the concept of the microbiome, as the genomes of microorganisms and their hosts and the relationships between them, has strongly entered the field of research on human health and disease.

Metabolomics Applied to Pregnancy Complications

Possible sources of biological tissues that can be studied in pregnancy and puerperium are both from the mother (plasma, urine, vaginal fluids, milk), the fetus (amniotic fluid, umbilical cord blood), and the newborn (plasma, urine, placenta, saliva, other fluids). Different technologies might be generally adopted: nuclear magnetic resonance (NMR) spectroscopy, gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-mass spectrometry (LC-MS). The phase of data analysis and interpretation requires highly complex data mining software and an ample database of metabolites to describe the nodes and networking of metabolic pathways (Fanos et al. 2012).

Metabolomic analysis performed on plasma of pregnant women has been reported in several studies (Kenny et al. 2010) addressing different pregnancy complications. Plasma samples were analyzed with NMR or LC-MS. Lower plasma betaine and trimethylamine-*N*-oxide concentrations (Diaz et al. 2011) were observed in maternal plasma in case of fetal malformation. Additional different significant findings were reported for amino acids involved in gluconeogenesis, for *cis*-aconitate, acetone, 3-hydroxybutyric acid, and hypoxanthine. These data suggest that malformed fetuses demand enhanced gluconeogenesis and tricarboxylic acids cycle (Krebs Cycle), possibly due to hypoxic metabolism. Significant differences were observed also between normal pregnancies and cases which would later develop to gestational diabetes. Changes in 3-hydroxyisovalerate and

2-hydroxyisobutirrate suggested early changes in biotin status and altered amino acid and/or gut metabolism. Preeclampsia has been a major focus in metabolomics technologies applied to maternal-fetal medicine. Bahado-Singh et al. (2012, 2013) studied metabolomics of early- and late-onset preeclampsia. NMR-based metabolomics analysis was performed on first-trimester maternal serum at 11–13 weeks of gestation to assess the possible prediction of late preeclampsia. A parsimonious genetic computing model proved yielded 60% sensitivity at 96.6% specificity for late preeclampsia prediction. Two metabolites were notable for their differences between preeclamptic and normal pregnant women: glycerol and carnitine. Glycerol and carnitine are both important for lipid metabolism and mitochondrial energy productions based on lipids. More significantly carnitine inhibits oxidative stress. All these metabolic pathways are overexpressed in metabolic syndrome and its acute expression in late preeclampsia.

The changes observed in the amniotic fluid related to preterm delivery include increase of allantoin (a marker of oxidative stress) and a decrease of myoinositol that promotes fetal lung maturation. Two cross-sectional metabolomics studies reported the importance of the amniotic fluid metabolic signature for preterm labor with or without intra-amniotic infection or inflammation (Romero et al. 2010). Amniotic fluid biomarkers were able to discriminate three groups based on pregnancy outcome (Romero et al. 2010): (1) patients with preterm labor who delivered at term, (2) patients with preterm labor without intra-amniotic infection who delivered preterm, and (3) patients with preterm labor with intra-amniotic infection who delivered preterm (characterized by an increase in amino acid metabolites). A reduction of carbohydrates in the amniotic fluid was associated with preterm delivery (with or without intra-amniotic infection). Methyladenine and diaminopimelic acid, components of bacterial processes and bacterial wall, respectively, were the most significant biomarkers in this clinical condition. These studies show the potential of human amniotic fluid metabolome analysis as the basis for the development of rapid tests to differentiate between patients at risk of impending preterm birth (with or without infection/inflammation) and patients who will deliver at term, and this concept may be applied also to other obstetrical syndromes.

Human Microbiome and Great Obstetrical Syndromes

Currently, accumulating evidence links microorganisms to the etiology of various maternal-fetal conditions and to some of the Great Obstetrical Syndromes (i.e., preterm delivery, IUGR, GDM, and fetal demise). The implementation of molecular-based (culture-independent) techniques and methods enables characterization and reveals the great diversity of the microbiomes of the reproductive tract, challenging the concept of "normal microbiota," increasing the understanding of the role of the uterine microbiome in adverse obstetric outcomes. Furthermore, transmission of maternal microbiome to the neonate, according to vaginal delivery or cesarean section, is shown to affect health from birth to adulthood (Solt 2015).

Of the microorganisms comprising human microbiomes, 90% are estimated as uncultivable (Dethlefsen et al. 2007). The development of DNA-sequencing technology has led to the establishment of metagenomics, which enables identification of the host genome, together with that of microorganisms. The 16S ribosomal RNA (rRNA) gene is generally used for polymerase chain reaction (PCR) amplification, due to its presence in all bacteria and its inclusion of both highly conserved and heterogenic sequences.

In pregnant women, *Lactobacillus* species were more abundant and other phylotypes less so, and the bacterial community group that is not dominated by *Lactobacillus* was less common (Aagaard et al. 2012). Despite the relative stability of the vaginal microbiota of pregnant women, communities tended to shift from dominance by one *Lactobacillus* species to another. The authors suggested that the enhanced stability may increase resilience and thus protect against ascending infection and its sequela, though the mechanism of such protection remains to be elucidated (Aagaard et al. 2012).

The uterus was traditionally considered sterile. The documentation in 1927 of positive bacterial cultures in the amniotic fluid of women undergoing cesarean sections who had been in labor for >6 h was for long considered an indication of infection or disease. In one study that refuted the paradigm of the sterile uterus, conducted to identify the possible existence of upper reproductive tract (endometrial) bacteria in asymptomatic women with no history of previous pelvic infection and a normal pelvic examination, only six cultures isolated from 55 endometrial biopsies were sterile; 231 bacterial species were detected from the positive cultures (Solt 2015). Investigations of the uterine microbiome of healthy, term pregnancies remain scanty, even with the availability of culture-independent techniques. Han et al. (2009) identified nine bacterial species that presented in almost half of amniotic fluid samples that tested positive with PCR but were not cultivated. Compared to culture-dependent methods, culture-independent methods are estimated to have increased bacterial detection in the amniotic cavity by about 30–50% and increased the number of detected species by up to fivefold (Mendz et al. 2013). Among them, anaerobic bacteria belonging to the family *Fusobacteriaceae* are now recognized as a common inhabitant of amniotic fluid. The detection of bacteria in the intrauterine cavity by PCR, in the absence of signs of infection, confirms the proposition of a non-sterile uterus. Further, evidence accumulated from the animal models indicates the universality of microbial transmission from mothers to newborns, of commensal bacteria, and not only pathogens (Funkhouser and Bordenstein 2013). More investigation is needed to characterize the members of the healthy uterine microbiome, interactions among these members, and diversity among populations.

The sites of maternal infection that may come in direct contact with the fetus are amniotic fluid, the placenta, fetal membranes, and the umbilical cord. In addition, hematogenous transmission from the mother to the fetus may involve microbiomes that are not in direct contact with the fetus, such as those of the gastrointestinal, respiratory, and oronasal tracts.

Uterine Microbiome and Adverse Obstetric Outcomes

Bacterial invasion of the amniotic cavity has been indicated as the main cause of neonatal mortality worldwide (Lawn et al. 2005). Cultivated bacterial pathogens from the amniotic cavity have been associated with miscarriage, chorioamnionitis, PROM, and preterm delivery. Culture-independent techniques have contributed greatly to our understanding of the associations between infection, inflammation, and adverse obstetric outcomes. Culture-independent technologies have detected bacterial sequences in the fetal membranes of preterm deliveries and term deliveries, both with and without labor (Fortner et al. 2014; Steel et al. 2005), as well as in up to 70% of women undergoing elective cesarean sections at term, thus indicating that the presence of bacteria does not in itself cause preterm labor (Steel et al. 2005).

The detection of elevated levels of proinflammatory cytokines in the amniotic fluid of women who delivered before 34 weeks of gestation supported the etiology of intra-amniotic infection in preterm delivery (Hillier et al. 1993). However, proinflammatory cytokines were also detected in culture-negative amniotic fluid (Hitti et al. 1997). Though such a finding may result from treatment with antibiotics, culture-independent techniques have revealed bacterial presence and species that were not identified by cultivation and that may induce inflammatory responses. Gardella et al. (2004) detected 16S ribosomal DNA (rDNA) sequences in 36% of culture-negative amniotic fluid samples of women in preterm labor who had elevated levels of the pro-inflammatory cytokine interleukin-6 (IL-6). The culture-negative amniotic fluid specimens that contained 16s rDNA of *Leptotrichia* or *Fusobacterium* had particularly high IL-6 levels. Han et al. (2009) reported that about two-thirds of the bacterial species detected in amniotic fluid by culture-independent methods were not isolated by culture. Further, PCR-positive bacterial identification has itself been associated with elevated IL-6 levels in amniotic fluid, as well as histological chorioamnionitis and funisitis, and early-onset neonatal sepsis (Han et al. 2009). The observations of a shorter amniocentesis-to-delivery interval among women who were PCR positive and a dose response association between bacterial rDNA abundance and gestational age at delivery support the possibility of a causal relationship (DiGiulio et al. 2008). In a systematic review of women delivering before term, culture-independent techniques revealed intrauterine bacterial infections in 349/761 cases (46%) (Mendz et al. 2013).

The capability of detecting greater numbers and species of bacteria in amniotic fluid raises the expectations of deciphering associations between microorganisms in amniotic fluid and adverse perinatal outcomes. Microbial invasion of the amniotic cavity has been assessed as polymicrobial in 24–67% of the cases. The clinical significance of a positive PCR of amniotic fluid appears to be equivalent to that of a positive culture (DiGiulio 2012). Still, the role of bacteria in preterm delivery is not yet fully understood. A recent investigation of bacteria from fetal membranes of women who delivered preterm or term, with or without labor, reported an association of increased bacterial presence with a thinner chorion, regardless of infection, labor status, or gestational age. This suggests that bacterial presence may lead to chorion thinning and ultimately to pPROM.

Bacterial ascent from the vaginal tract to the uterus, possibly crossing the placental barrier, is recognized by both culture-dependent (Romero et al. 1988) and culture-independent (DiGiulio 2012) studies as the primary source of intrauterine infection. The finding that bacterial species common to the genital tract were among the most common bacteria detected in the uterine cavity supports this pathogenesis (Mendz et al. 2013).

Placental Microbiome and Adverse Obstetric Outcomes

The high rate of recurrence of preterm delivery (Goldenberg et al. 2006) suggests a sustained risk factor. This led to investigation of a placental microbiome and, specifically, the investigation of the microflora in the basal plate of the placenta, which is the tissue layer directly at the maternal-fetal interface (Stout et al. 2013). Bacteria of diverse morphologies were detected in basal plates of 27% of placentas examined, in 54% of women who delivered spontaneously preterm, before 28 weeks, and in 26% of those who delivered at term.

Conclusion

In conclusion, and of great importance, the presence of bacteria in normal-term deliveries, as well as the absence of bacteria in many cases of extreme prematurity, suggests that preterm delivery is determined not only by the presence or absence of bacteria but also by other factors, such as the relationships between bacterial types, characteristics of the host tissue and host responses, as well as general characteristics of individuals, such as ethnicity (Ravel et al. 2011). Other factors that warrant more investigation as to their effect on the vaginal, as well as other microbiomes in pregnant women, are age, genetic background, health and immune status, diet, and nutritional status. Using 16S rRNA, marked differences were detected in the composition of the different microbiomes of women developing the Great Obstetrical Syndromes (Acuna et al. 2011).

References

Aagaard K, Riehle K, Ma J, et al. A metagenomic approach to characterization of the vaginal microbiome signature in pregnancy. PLoS One. 2012;7:e36466.

Acuna JG, Cohavy O, Solt I, et al. Preliminary observations on the microbial phylogeny of the oral, vaginal, and rectal microbiome in gestational diabetes and healthy pregnancies. Am J Obstet Gynecol. 2011;204:S254.

Agarwal A, Gupta S, Sekhon L, Shah R. Redox considerations in female reproductive function and assisted reproduction: from molecular mechanisms to health implications. Antioxid Redox Signal. 2008;10:1375–403.

Bahado-Singh RO, Akolekar R, Mandal R, et al. Metabolomics and first-trimester prediction of early-onset preeclampsia. J Matern Fetal Neonatal Med. 2012;25:1840–7.

Bahado-Singh RO, Akolekar R, Mandal R, et al. First-trimester metabolomic detection of late-onset preeclampsia. Am J Obstet Gynecol. 2013;208:58.e1–7.
Blencowe H, Cousens S, Oestergaard MZ, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet. 2012;379:2162–72.
Borengasser SJ, Lau F, Kang P, et al. Maternal obesity during gestation impairs fatty acid oxidation and mitochondrial SIRT3 expression in rat offspring at weaning. PLoS One. 2011;6:e24068.
Cabero Roura L, Hod M. Identification, prevention, and monitoring of the "great obstetrical syndromes". Best Pract Res Clin Obstet Gynaecol. 2015;29:145–9.
Cawyer CR, Horvat D, Leonard D, et al. Hyperglycemia impairs cytotrophoblast function via stress signaling. Am J Obstet Gynecol. 2014;211:541.e1–8.
Cetin I, Parisi F, Berti C, Mando C, Desoye G. Placental fatty acid transport in maternal obesity. J Dev Orig Health Dis. 2012;3:409–14.
Challier JC, Basu S, Bintein T, et al. Obesity in pregnancy stimulates macrophage accumulation and inflammation in the placenta. Placenta. 2008;29:274–81.
Challis JRG, Matthews SG, Gibb W, Lye SJ. Endocrine and paracrine regulation of birth at term and preterm. Endocr Rev. 2000;21:514–50.
Challis JR, Lockwood CJ, Myatt L, Norman JE, Strauss JF, Petraglia F. Inflammation and pregnancy. Reprod Sci. 2009;16:206–15.
Chaouat G. Regulation of T-cell activities at the feto-placental interface – by placenta? Am J Reprod Immunol. 1999;42:199–204.
Coughlan MT, Vervaart PP, Permezel M, Georgiou HM, Rice GE. Altered placental oxidative stress status in gestational diabetes mellitus. Placenta. 2004;25:78–84.
Crowther CA, Hiller JE, Moss JR, et al. Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. N Engl J Med. 2005;352:2477–86.
Dabagh-Gorjani F, Anvari F, Zolghadri J, Kamali-Sarvestani E, Gharesi-Fard B. Differences in the expression of TLRs and inflammatory cytokines in pre-eclamptic compared with healthy pregnant women. Iran J Immunol. 2014;11:233–45.
Denison FC, Roberts KA, Barr SM, Norman JE. Obesity, pregnancy, inflammation, and vascular function. Reproduction. 2010;140:373–85.
Dessi A, Marincola FC, Fanos V. Metabolomics and the great obstetrical syndromes – GDM, PET, and IUGR. Best Pract Res Clin Obstet Gynaecol. 2015;29:156–64.
Dethlefsen L, McFall-Ngai M, Relman DA. An ecological and evolutionary perspective on human-microbe mutualism and disease. Nature. 2007;449:811–8.
Diaz SO, Pinto J, Graca G, et al. Metabolic biomarkers of prenatal disorders: an exploratory NMR metabonomics study of second trimester maternal urine and blood plasma. J Proteome Res. 2011;10:3732–42.
DiGiulio DB. Diversity of microbes in amniotic fluid. Semin Fetal Neonatal Med. 2012;17:2–11.
DiGiulio DB, Romero R, Amogan HP, et al. Microbial prevalence, diversity and abundance in amniotic fluid during preterm labor: a molecular and culture-based investigation. PLoS One. 2008;3:e3056.
Erez O, Gotsch F, Mazaki-Tovi S, et al. Evidence of maternal platelet activation, excessive thrombin generation, and high amniotic fluid tissue factor immunoreactivity and functional activity in patients with fetal death. J Matern Fetal Neonatal Med. 2009;22:672–87.
Erez O, Romero R, Vaisbuch E, et al. High tissue factor activity and low tissue factor pathway inhibitor concentrations in patients with preterm labor. J Matern Fetal Neonatal Med. 2010; 23:23–33.
Evers IM, Nikkels PG, Sikkema JM, Visser GH. Placental pathology in women with type 1 diabetes and in a control group with normal and large-for-gestational-age infants. Placenta. 2003; 24:819–25.
Fanos V, Barberini L, Antonucci R, Atzori L. Pharma-metabolomics in neonatology: is it a dream or a fact? Curr Pharm Des. 2012;18:2996–3006.
Fattuoni C, Mando C, Palmas F, et al. Preliminary metabolomics analysis of placenta in maternal obesity. Placenta. 2018;61:89–95.

Fortner KB, Grotegut CA, Ransom CE, et al. Bacteria localization and chorion thinning among preterm premature rupture of membranes. PLoS One. 2014;9:e83338.
Funkhouser LJ, Bordenstein SR. Mom knows best: the universality of maternal microbial transmission. PLoS Biol. 2013;11:e1001631.
Gabbay-Benziv R, Baschat AA. Gestational diabetes as one of the "great obstetrical syndromes" – the maternal, placental, and fetal dialog. Best Pract Res Clin Obstet Gynaecol. 2015;29:150–5.
Gardella C, Riley DE, Hitti J, Agnew K, Krieger JN, Eschenbach D. Identification and sequencing of bacterial rDNAs in culture-negative amniotic fluid from women in premature labor. Am J Perinatol. 2004;21:319–23.
Gauster M, Desoye G, Totsch M, Hiden U. The placenta and gestational diabetes mellitus. Curr Diab Rep. 2012;12:16–23.
Gervasi MT, Chaiworapongsa T, Pacora P, et al. Phenotypic and metabolic characteristics of monocytes and granulocytes in preeclampsia. Am J Obstet Gynecol. 2001;185:792–7.
Goldenberg RL, Andrews WW, Faye-Petersen O, Cliver S, Goepfert AR, Hauth JC. The Alabama Preterm Birth Project: placental histology in recurrent spontaneous and indicated preterm birth. Am J Obstet Gynecol. 2006;195:792–6.
Hahn S, Giaglis S, Hoesli I, Hasler P. Neutrophil NETs in reproduction: from infertility to preeclampsia and the possibility of fetal loss. Front Immunol. 2012;3:362.
Han YW, Shen T, Chung P, Buhimschi IA, Buhimschi CS. Uncultivated bacteria as etiologic agents of intra-amniotic inflammation leading to preterm birth. J Clin Microbiol. 2009;47:38–47.
Hastie R, Lappas M. The effect of pre-existing maternal obesity and diabetes on placental mitochondrial content and electron transport chain activity. Placenta. 2014;35:673–83.
Hillier SL, Witkin SS, Krohn MA, Watts DH, Kiviat NB, Eschenbach DA. The relationship of amniotic fluid cytokines and preterm delivery, amniotic fluid infection, histologic chorioamnionitis, and chorioamnion infection. Obstet Gynecol. 1993;81:941–8.
Hitti J, Riley DE, Krohn MA, et al. Broad-spectrum bacterial rDNA polymerase chain reaction assay for detecting amniotic fluid infection among women in premature labor. Clin Infect Dis. 1997;24:1228–32.
Hutchinson-Colas J, Segal S. Genitourinary syndrome of menopause and the use of laser therapy. Maturitas. 2015;82:342–5.
Kenny LC, Broadhurst DI, Dunn W, et al. Robust early pregnancy prediction of later preeclampsia using metabolomic biomarkers. Hypertension. 2010;56:741–9.
Kim C. Maternal outcomes and follow-up after gestational diabetes mellitus. Diabet Med. 2014;31:292–301.
Lawn JE, Cousens S, Zupan J, Lancet Neonatal Survival Steering Team. 4 million neonatal deaths: when? Where? Why? Lancet. 2005;365:891–900.
Liu L, Johnson HL, Cousens S, et al. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. Lancet. 2012;379:2151–61.
Madazli R, Tuten A, Calay Z, Uzun H, Uludag S, Ocak V. The incidence of placental abnormalities, maternal and cord plasma malondialdehyde and vascular endothelial growth factor levels in women with gestational diabetes mellitus and nondiabetic controls. Gynecol Obstet Invest. 2008;65:227–32.
Mando C, Tabano S, Colapietro P, et al. Transferrin receptor gene and protein expression and localization in human IUGR and normal term placentas. Placenta. 2011;32:44–50.
Mando C, Tabano S, Pileri P, et al. SNAT2 expression and regulation in human growth-restricted placentas. Pediatr Res. 2013;74:104–10.
Mando C, Razini P, Novielli C, et al. Impaired angiogenic potential of human placental mesenchymal stromal cells in intrauterine growth restriction. Stem Cells Transl Med. 2016a;5:451–63.
Mando C, Calabrese S, Mazzocco MI, et al. Sex specific adaptations in placental biometry of overweight and obese women. Placenta. 2016b;38:1–7.
Mando C, Anelli GM, Novielli C, et al. Impact of obesity and hyperglycemia on placental mitochondria. Oxid Med Cell Longev. 2018;2018:2378189.

Mendz GL, Kaakoush NO, Quinlivan JA. Bacterial aetiological agents of intra-amniotic infections and preterm birth in pregnant women. Front Cell Infect Microbiol. 2013;3:58.
Metzger BE, Freinkel N. Accelerated starvation in pregnancy: implications for dietary treatment of obesity and gestational diabetes mellitus. Biol Neonate. 1987;51:78–85.
Montgomery MK, Turner N. Mitochondrial dysfunction and insulin resistance: an update. Endocr Connect. 2015;4:R1–15.
Mor G. Inflammation and pregnancy: the role of toll-like receptors in trophoblast-immune interaction. Ann N Y Acad Sci. 2008;1127:121–8.
Mor G, Cardenas I. The immune system in pregnancy: a unique complexity. Am J Reprod Immunol. 2010;63:425–33.
Mrizak I, Arfa A, Fekih M, et al. Inflammation and impaired endothelium-dependant vasodilatation in non obese women with gestational diabetes mellitus: preliminary results. Lipids Health Dis. 2013;12:93.
Mwaniki MK, Atieno M, Lawn JE, Newton CR. Long-term neurodevelopmental outcomes after intrauterine and neonatal insults: a systematic review. Lancet. 2012;379:445–52.
Myatt L, Maloyan A. Obesity and placental function. Semin Reprod Med. 2016;34:42–9.
Parimi N, Tromp G, Kuivaniemi H, et al. Analytical approaches to detect maternal/fetal genotype incompatibilities that increase risk of pre-eclampsia. BMC Med Genet. 2008;9:60.
Patni S, Wynen LP, Seager AL, Morgan G, White JO, Thornton CA. Expression and activity of toll-like receptors 1–9 in the human term placenta and changes associated with labor at term. Biol Reprod. 2009;80:243–8.
Ramsay JE, Ferrell WR, Crawford L, Wallace AM, Greer IA, Sattar N. Maternal obesity is associated with dysregulation of metabolic, vascular, and inflammatory pathways. J Clin Endocrinol Metab. 2002;87:4231–7.
Ravel J, Gajer P, Abdo Z, et al. Vaginal microbiome of reproductive-age women. Proc Natl Acad Sci USA. 2011;108(Suppl 1):4680–7.
Redman CW, Sargent IL. Circulating microparticles in normal pregnancy and pre-eclampsia. Placenta. 2008;29(Suppl A):S73–7.
Romero R. Prenatal medicine: the child is the father of the man. 1996. J Matern Fetal Neonatal Med. 2009;22:636–9.
Romero R, Mazor M, Wu YK, et al. Infection in the pathogenesis of preterm labor. Semin Perinatol. 1988;12:262–79.
Romero R, Espinoza J, Goncalves LF, Kusanovic JP, Friel L, Hassan S. The role of inflammation and infection in preterm birth. Semin Reprod Med. 2007;25:21–39.
Romero R, Mazaki-Tovi S, Vaisbuch E, et al. Metabolomics in premature labor: a novel approach to identify patients at risk for preterm delivery. J Matern Fetal Neonatal Med. 2010;23:1344–59.
Saito S, Nakashima A, Shima T, Ito M. Th1/Th2/Th17 and regulatory T-cell paradigm in pregnancy. Am J Reprod Immunol. 2010;63:601–10.
Segovia SA, Vickers MH, Gray C, Reynolds CM. Maternal obesity, inflammation, and developmental programming. Biomed Res Int. 2014;2014:418975.
Solt I. The human microbiome and the great obstetrical syndromes: a new frontier in maternal-fetal medicine. Best Pract Res Clin Obstet Gynaecol. 2015;29:165–75.
Steel JH, Malatos S, Kennea N, et al. Bacteria and inflammatory cells in fetal membranes do not always cause preterm labor. Pediatr Res. 2005;57:404–11.
Stout MJ, Conlon B, Landeau M, et al. Identification of intracellular bacteria in the basal plate of the human placenta in term and preterm gestations. Am J Obstet Gynecol. 2013;208:226.e1–7.
Taricco E, Radaelli T, Nobile de Santis MS, Cetin I. Foetal and placental weights in relation to maternal characteristics in gestational diabetes. Placenta. 2003;24:343–7.
Taricco E, Radaelli T, Rossi G, et al. Effects of gestational diabetes on fetal oxygen and glucose levels in vivo. BJOG. 2009;116:1729–35.
Turner N, Bruce CR, Beale SM, et al. Excess lipid availability increases mitochondrial fatty acid oxidative capacity in muscle: evidence against a role for reduced fatty acid oxidation in lipid-induced insulin resistance in rodents. Diabetes. 2007;56:2085–92.

Wolf M, Sauk J, Shah A, et al. Inflammation and glucose intolerance: a prospective study of gestational diabetes mellitus. Diabetes Care. 2004;27:21–7.

Xu J, Zhao YH, Chen YP, et al. Maternal circulating concentrations of tumor necrosis factor-alpha, leptin, and adiponectin in gestational diabetes mellitus: a systematic review and meta-analysis. ScientificWorldJournal. 2014;2014:926932.

Zambon M, Mando C, Lissoni A, et al. Inflammatory and oxidative responses in pregnancies with obesity and periodontal disease. Reprod Sci. 2018;25:1474–84.

Zheng LD, Linarelli LE, Liu L, et al. Insulin resistance is associated with epigenetic and genetic regulation of mitochondrial DNA in obese humans. Clin Epigenetics. 2015;7:60.

Part VI

Breast

Hormones, Breast Disorders, and Lactation 22

Nicoletta Biglia, Valentina Elisabetta Bounous, Marta D'Alonzo, Michela Villa, and Andrea Villasco

Contents

N. Biglia (✉)
Gynaecology and Obstetrics, Umberto I – Mauriziano Hospital, Turin, Italy

Gynaecology and Obstetrics, University of Turin School of Medicine, Turin, Italy
e-mail: nicoletta.biglia@unito.it

V. E. Bounous · M. D'Alonzo · M. Villa · A. Villasco
Gynaecology and Obstetrics, Umberto I – Mauriziano Hospital, Turin, Italy
e-mail: valentinabounous@hotmail.com; martadalonzo@libero.it; michelavilla.23@gmail.com; andrea.villasco@unito.it

F. Petraglia, B. C. Fauser (eds.), *Female Reproductive Dysfunction*, Endocrinology,
https://doi.org/10.1007/978-3-030-14782-2_22

Abstract

The breast is an organ, equal and symmetrical, of the human body. It develops, grows, acquires functions, and involutes according to the stage of life: puberty, pregnancy, lactation, and menopause. These modifications are induced by the hormonal variations that a woman experiences during the course of her life. Besides the normal evolution of the breast, hormones are responsible for many breast disorders, be them benign or malignant.

The term benign breast disease is used to describe a wide variety of mammary clinical entities. Benign breast diseases are often referred to as para-physiological conditions in normal breast development and their prevalence peaks during the first four decades of the woman's life. Hormonal dysregulations are thought to play a key role in the development of such disorders. Similarly, endogenous and exogenous hormonal modifications are listed as major risk factors for the development of breast malignancies: the link between sex hormones and breast tumors has long been known and confirmed in experimental biological models and epidemiological observations. The incidence curve of breast cancer grows with advancing age but plateaus over the years after menopause, in parallel with the cessation of ovarian hormone production. At this stage of life, concerns have been raised over the possible deleterious effect of hormonal replacement therapy (HRT) on breast benign diseases and breast cancer risk since the results of the WHI were published in 2001. The same issues are reported in literature for the previous consumption of combined oral contraceptives (OCs). Beginning from normal breast development, physiology, and pathology, the effect of OCs and HRT on breast benign disease and on the risk of breast cancer will be the subject of this chapter.

Keywords

Hormone replacement therapy · Oral contraceptives · Benign breast diseases · Breast cancer · BRCA

Introduction

Breast Anatomy

Breasts lay anteriorly on the thoracic cage between the second and sixth rib in the vertical axis and between the sternal edge and the midaxillary line in the horizontal axis. Cranially, no precise limit can be identified, whereas caudally they are bounded by the inframammary groove. They lay deeply onto the pectoral fascia, which covers the pectoralis major muscle. Between the two breasts there is a wide groove corresponding to the body of the sternum.

The breast consists of three main elements: the skin, the nipple-areola complex, and the gland. The skin of the breast is thin and contains hair follicles, sebaceous glands, and exocrine sweat glands. The areola is a round cutaneous surface in which

the skin is pigmented, with a diameter of 3.5–6 cm. The surface is irregular due to the presence of 12–20 voluminous sebaceous glands, the Montgomery glands, which constitute the tubercles of Morgagni. The nipple, located in the center of the areola, has a variable shape and size, conical or cylindrical, with small depressions that represent the independent outlet of 15–20 milk ducts at its apex. The diameter is variable from 5 to 8 mm.

The mammary gland includes the proper glandular tissue and a stroma composed of connective and adipose tissue. The gland is comprehended between the superficial and deep sheets of the superficial fascia: connecting these two fascial layers are fibrous bands called the Cooper suspensory ligaments. These ligaments are accompanied by vessels, nerves, and lymphatics and represent the natural means of support for the breast. Histologically the mammary gland is a modified apocrine gland formed by 15–20 lobes wrapped in adipose and connective tissue. Each lobe is functionally independent and terminates at the level of the nipple with its own milk duct. In each lobe there are about 20–40 lobules, each composed of 10–100 alveoli, which constitute the true secretory units. The milk ducts are constituted of one innermost bilayer epithelium of cuboidal cells and an outermost layer of contractile myoepithelial cells resting on basement membrane. At the level of the nipple, they have a dilated portion called the lactiferous sinus.

The breast receives blood mainly from the perforating branches of the internal mammary artery and from the branches of the lateral thoracic artery which enters the axilla. Lymphatic drainage is important to better understand the possible spread of malignant diseases. In physiological conditions, the lymphatic flow in the breast goes from the surface towards the depth of the gland heading then to the axilla and the internal mammary lymphatic chain. The axillary lymph nodes, which are located at the level of the axillary vein, can be divided into three groups in relation to their position compared to the small pectoralis muscle:

- Level I: lateral lymph nodes up to the lateral border of the small pectoralis muscle
- Level II: lymph nodes posterior to the small pectoralis muscle
- Level III: medial lymph nodes up to the medial border of the small pectoralis muscle

Normal Breast Development

Human breast development initiates during embryonic life. Between the fifth and the sixth week of development, the ectodermal crest, called the “milk line,” develops bilaterally and goes from the axilla to the groin. The segments then merge into the nipples at the level of the fifth intercostal space. At birth, the breasts are rudimental and are made of 15 primitive ductal elements located beneath the nipple-areola complex.

Puberty marks the beginning of breast maturation and normally starts when hypothalamic gonadotropin-releasing hormone (GnRH) acquires a pulsatory secretion pattern. Variation in the pulses of GnRH induces the production and release of

follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which promote recruitment and maturation of the ovarian follicles and their secretion of estrogens.

During puberty the rudimental ducts experience progressive growth, spreading and branching into numerous ductules (alveolar buds) accompanied by an increase in the stromal component. When 10–12 alveolar buds cluster around a terminal duct, they form a lobule, called type 1 lobule. The number of alveolar buds keeps growing through puberty and adulthood leading to the formation of type 2 and type 3 lobules, but full differentiation of the mammary gland can only be attained with a pregnancy onset (type 4 lobules).

Physiology, Pregnancy, and Lactation

The breast tissue undergoes cyclical variations similar to those of the endometrium following the hormonal variations that characterize the ovarian cycle. During follicular phase, estrogen levels begin to rise leading to the proliferation of the epithelium of the ductules and of the ducts. After ovulation, during the luteal phase of the ovarian cycle, under the influence of progesterone, the proliferation of the structures of the terminal duct continues, with an increase in the mitotic index of the basal cells and the appearance of cytoplasmic vacuolation in the secreting epithelium. The stromal component of the lobule also proliferates and tends to swell: in fact, edema of the intralobular stroma can be observed during this phase. The combined stimulating effect of estrogens and progesterone on the lobular elements is responsible for the sensation of breast tenderness that the woman experiences in the premenstrual phase of the cycle. With the arrival of menstruation, the fall in hormonal blood levels is followed by desquamative phenomena affecting the epithelium, atrophy of the intralobular connective tissue, disappearance of the edema, and overall reduction in the volume of the lobules.

However, it is only with pregnancy that the breast reaches its complete morphological and functional maturation. There is a new wave of spreading and branching of the ductal tree and each alveolar bud sprouts giving rise to clusters of secretory glands, the acini; this leads to an inversion of the stroma/gland ratio so that, at the end of pregnancy, the breast is made up almost entirely of glands interposed with only a small amount of stromal tissue. During pregnancy the body prepares for breastfeeding whether or not the woman has planned to breastfeed her baby. Starting from the 6th–8th week of pregnancy, the breasts increase in volume because they increase the quota of mammary fat and the number of milk-producing glands. The breasts slowly increase in volume and weight and become firm and painful. Even the nipples and areolas become darker, the nipples may protrude more, and the areolas become larger. Around the fourth or fifth month of gestation, the nipple can sometimes lose a small amount of colostrum, a thick yellowish liquid. Colostrum is also the first milk secreted after the baby is born and it contains proteins and other substances that nourish the baby (sugars, vitamins, and fats) and antibodies to provide protection from infections. Within a few days of giving birth, the colostrum

will change into milk. When the baby starts sucking the nipple, let-down reflex is triggered: this causes oxytocin levels to peak leading to milk ejection from the nipple. This reflex occurs about 2–3 min after the baby starts sucking and can be slowed down by embarrassment, pain, anxiety, or stress. It can also be triggered by listening to the baby cry, looking at the baby or even thinking about him. At the end of breastfeeding, lactogenic hormone deprivation and local signals to undergo glandular involution induce the glands to regress: the caliber of the ducts is reduced and the volume of the entire mammary gland decreases, without however returning to that of the pregestational breast.

Final breast involution occurs after menopause resulting in progressive atrophy of the glandular elements and a marked decrease in the number of lobules which may as well disappear completely. Concurrently, the fibrous connective tissue component of the stroma decreases, and adipose tissue accumulates.

Hormonal Influences on Breast Development

Estrogens and progesterone are responsible of the proliferation of the breast epithelium. Estrogens act locally on the mammary gland promoting the branching and the alveolar buds' formation. Progesterone acts in conjunction with estrogens to regulate breast development through its specific receptor on breast epithelial cells. The proliferative activity of the mammary epithelium depends on the number of ER and PR receptors expressed by epithelial cells, number that depends on the degree of lobular differentiation. In fact, the highest level of proliferative activity is observed in the undifferentiated type 1 lobule, present in great percentage in the breast of nulliparous females. On the contrary, the predominant structure in the breast of parous women is the most differentiated type 3 lobule (70–90% of all lobules). This fact could explain the protective effects exerted on the risk of breast cancer development by a full-term pregnancy.

Benign Breast Diseases (Guray and Sahin 2006)

The term "benign breast disease" (BBD) comprehends a wide variety of mammary clinical entities, most of them considered para-physiological conditions in normal breast development. As mentioned above, during the first four decades of life, the development of lobules is still an active process and any new breast lesion has a very high probability of being a benign lesion. This probability progressively decreases with advancing age due to a lower mammary dynamism. (Visvanathan et al. 2009) Table 1.

Among all benign breast diseases, the most common are:

- Fibrocystic breast changes
- Benign breast neoplasms

Table 1 Breast cancer risk associated with proliferative breast disease and atypical hyperplasia

Risk factor	Frequency (%)	Relative risk of developing invasive breast cancer	95% CI
Non proliferative breast benign diseases	70	1.17	0.94–1.47
Proliferative breast benign diseases without atypia	26	1.76	1.58–1.95
Atypical hyperplasia	4	3.93	3.24–4.76

Fibrocystic Breast Changes

Fibrocystic disease is the most frequent acquired benign breast disease, affecting up to 50% of premenopausal women in the first five decades of life, whereas the onset of these lesions is rare before puberty and after menopause. It comprises a spectrum of alterations of the breast connective tissue and the specialized glandular epithelium of terminal ducts and lobules, usually seen multifocally and bilaterally. It is believed that hormonal imbalances, particularly hyperestrogenism, are the basis of these heterogeneous modifications. (Vorherr 1986) Relative hyperestrogenism is due to a deficiency of progesterone, as occurs after repeated anovulatory cycles. The most frequent presenting symptoms are pain, breast tenderness, and appearance, sometimes rapid, of breast lumps that usually tend to increase in the premenstrual phase and to involute after menstruation has ended. This disease presents two distinct evolutionary phases: the first phase, that normally occurs during puberty, is characterized by the increase of the stroma (fibroblastic proliferation due to the action of estrogens) and by intra- and perilobular fibrosis. During this phase, the breast is very painful ("mastalgia phase") because of the edema of the perilobular connective tissue. The second phase ("adenosis phase") is due to the stimulation that the estrogens exert on the epithelial component. Many attempts have been carried out to classify this heterogeneous group of disorders, and they have been eventually subgrouped in three histological categories: nonproliferative lesions, proliferative lesions without atypia, and atypical hyperplasia. (Dupont and Page 1985) The latter ones have been found to bear an increased risk of breast cancer development.

Nonproliferative Breast Benign Diseases

Nonproliferative lesions include cysts, ductal ectasia, and periductal fibrosis.

- *Breast cysts*
  Cysts are round or ovoidal formations filled with liquid derived from the terminal duct lobular unit. They are found in 30% of women between 35 and 50 years of age. Most of them are subclinical, but in one quarter of cases, they become palpable. Cysts cannot reliably be differentiated from other breast lumps by clinical examination or mammograms: they are normally diagnosed with breast ultrasound as anechoic formations with regular wall and posterior enhancement.

  Breast cysts have not been shown to be associated with an increased risk of breast cancer development, and hence the current consensus on the management

includes regular clinical and radiological follow-up without any further therapy. Only in case of pain due to cystic fluid pressure the cystic content may be drained with a syringe. Rarely, when there is echographic evidence of cystic septations, thickened or irregular cystic wall, absent posterior enhancement or an intracystic mass is present, cysts are described as atypical or complex (Degnim et al. 2007). They are individuated in approximately 5% of all breast ultrasound examinations. In these cases, fine needle aspiration or core biopsy should be performed to rule out a malignancy and subsequent treatment should be carried out accordingly.

- *Ductal ectasia*
  Mammary ductal ectasia is an inflammatory breast disease characterized by the dilation of the milk ducts caused by intraluminal plugs of histiocytes and periductal inflammation. The frequency of mammary ductal ectasia is highly variable according to the diagnostic method used, which might be clinical, histopathological, or autoptic. Mammary ductal ectasia typically occurs in females entering menopause, but can also occur in younger females, males, and infants.
- *Periductal fibrosis*
  Periductal fibrosis represents a part of normal involutional process of the breast following the resolution of an inflammatory process. It is normally diagnosed as an incidental finding on a breast biopsy or a surgical specimen.

Proliferative Breast Benign Diseases Without Atypia

They include radial scar, ductal hyperplasia of the usual type, intraductal papilloma and papillomatosis, sclerosing adenosis.

- *Ductal hyperplasia of the usual type*
  As mentioned above, milk ducts are normally constituted of one innermost bilayer epithelium of cuboidal cells and outermost layer of contractile myoepithelial cells resting on basement membrane. The term hyperplasia is used to describe an increase in cell number within the ducts. Histopathological classification is based on the architectural growth pattern and cytologic features of the proliferating cells. Usual ductal hyperplasia is composed of an increased number of cells without architectural distortion and it is not associated with an increased risk for breast cancer. It does not normally require surgery nor medical treatment.
- *Sclerosing adenosis*
  Sclerosing adenosis is a common benign breast lesion characterized by numerous distorted lobules with fastened acini, abundant myoepithelium, and stromal fibrosis. The biology of sclerosing adenosis has not yet been fully clarified. In a large cohort study, sclerosing adenosis was found in 28% of all benign biopsies and was associated with a twofold higher risk of breast cancer development.
- *Radial Scar*
  Radial scars are benign lesions of uncertain significance and unknown origin. They develop around a fibroelastotic core that entraps breast ducts giving them a radial aspect and lobules showing epithelial hyperplasia, adenosis, ductal ectasia, and papillomatosis. The incidence of radial scars has been increasing over time along with screening programs implementation. It has been hypothesized that

radial scars may harbor atypical epithelial proliferations leading to the development of lobular carcinoma in situ and ductal carcinoma in situ. The radiologic features of radial scars are nonspecific and may resemble those of invasive breast cancer. Since malignancy cannot be reliably excluded with a single core biopsy, a lesion suggestive of radial scar at mammography needs to be excised.

- *Intraductal papilloma and papillomatosis*
  Intraductal papillomas are benign tumors of the ductal epithelium that can grow at any point in the ductal system, even if they show a predilection for the lactiferous sinuses and the terminal ductules. Central papillomas tend to be solitary, whereas the peripheral ones are usually multiple. Their most common presenting symptom is serous or serosanguinous nipple discharge. Papillomas consist of normal ductal epithelium protruding into the ductal lumen supported by a fibrovascular pedunclе. The epithelial component can harbor many morphologic alterations ranging from metaplasia to atypical intraductal hyperplasia and in situ carcinoma.

  Papillomatosis is a condition that consists of a minimum of five separate papillomas in a single segment of breast tissue. Multiple papillomas are more likely to occur bilaterally. The data available in literature suggest that the finding of a single, benign, centrally located ductal papilloma is not associated to an increased risk of subsequent breast cancer, in contrast to papillomatosis which bears a slightly higher risk.

Proliferative BBDs with Atypia

Proliferative lesions with atypia include atypical ductal and lobular epithelial proliferations.

- *Atypical Ductal Hyperplasia*
  Atypical ductal hyperplasia (ADH) is characterized by an increased number of ductal epithelial cells whose uniform morphology mimics the one of low-grade ductal carcinoma in situ. Most lesions of ADH are small and localized, involving only few small ducts. The incidence of ADH has been increasing over time along with screening programs implementation: one third of core biopsies performed on mammographically detected microcalcifications show atypical ductal hyperplasia. (Kahlenborn et al. 2006) A patient diagnosed with atypical ductal hyperplasia has a fivefold risk of developing ipsilateral or contralateral invasive breast cancer in the next decade, reaching a nearly ten times higher risk if the patient has familiar history of breast cancer. (Gierisch et al. 2013) The risk for breast cancer in women with atypical ductal hyperplasia is also related to the patient's menopausal status: premenopausal women with atypical ductal hyperplasia have a substantially higher risk of developing invasive cancer. Routine imaging of both breasts is recommended. Treatment options, such as chemoprevention, should be evaluated and discussed with patients based on the global risk of developing a subsequent breast cancer.
- *Lobular epithelial proliferation*
  Atypical lobular hyperplasia and lobular carcinoma in situ are collected under the name "lobular neoplasia" because they show very similar histologic features, the

only difference between the two of them being the extent of epithelial proliferation. Both lesions are considered and managed as a risk factor for future development of invasive breast cancer, which they increase by about fourfold and tenfold, respectively, rather than precursor lesions. Lobular neoplasia tends to be multifocal e frequently bilateral. The prevalence of lobular neoplasia is highest in perimenopausal women and is usually identified incidentally in core biopsies for screening detected breast lesions. Although breast cancer can arise in either breast without a direct relationship to the first localization of the disease, the development of invasive carcinoma after the diagnosis of a lobular neoplasia is three times more likely to arise in the ipsilateral breast than in the opposite breast. Accurate routine clinical assessment and appropriate risk definition is recommended for patients with lobular neoplasia, in order to evaluate the need for chemoprevention.

Breast Benign Neoplasms

- *Fibroadenoma*
  Fibroadenoma is the most common neoplastic lesion of the young breast, affecting 25% of asymptomatic women between 15 and 35 years of age. The lesion is a hormone-dependent benign neoplasm that secretes during lactation and involutes along with the rest of the breast after menopause. Fibroadenoma presents as a firm, highly mobile, often palpable, and frequently unilateral breast lump. Fibroadenoma develops from the special stroma of the lobule proliferating around tubular glands. Macroscopically, the lesion appears as an ovoid, sometimes lobulated circumscribed mass, usually less than 3 cm in diameter. If the neoplasm assumes greater dimension (> 5–10 cm), it is called "giant fibroadenoma," but they represent only the 4% of all fibroadenomas.

  Patients with fibroadenoma have a modest increase in the risk of developing an invasive breast cancer compared to the general population (RR 1.5). In particular, the risk increases when there is a coexistence of fibroadenoma with other alterations, such as atypical hyperplasia in the context of the lesion itself ("complex fibroadenoma") or in the neighboring breast tissue, or in patients who have a positive family history of breast cancer.

  Surgical excision is indicated only in cases of giant fibroadenomas, fast-growing forms, and dubious lesions over the age of 35, even if they are negative to all cytological examination.
- *Lipoma*
  Lipomas are neoplasms consisting of adipose tissue which can be accompanied by epithelial (adenolipoma), vascular (angiolipoma) or cartilaginous (chondrolipoma) elements. Clinically, they appear as mobile breast lumps of soft consistency and rounded shape. The differential diagnosis is usually eased by the typical clinical features and the radiolucent appearance on mammography. Surgical excision of a lipoma is indicated if there are diagnostic doubts, or if the size of the lesion is such to recommend removal for aesthetic reasons.

- *Adenoma*
  An adenoma is an epithelial neoplasm of the breast that includes many histological variants: lactating, tubular, ductal, apocrine, and pleomorphic.

  Lactating adenoma is the most prevalent form of adenoma and the most common cause of breast mass during pregnancy and puerperium and will be discussed in the lactation disorders section. Tubular adenoma or pure adenoma of the breast presents as well-defined, solitary, firm mass. At X-ray mammography, it may mimic the appearance of noncalcified fibroadenoma. Histologically it is characterized of closely packed acinar structures very regularly disposed in a cellular stroma. Other types of breast adenomas are rare.

Clinical Implication of Breast Benign Diseases

Given the high prevalence of breast benign diseases, one practitioner will often have to confront to patients' age-related concerns on the breast condition. Young women, for example, could fear the effect of oral contraceptives consumption for the possible harm that hormones could exert on breast tissue, favoring the development or the worsening of breast benign diseases. Similarly, with advancing age, women with breast benign diseases may show anxiety towards the risk of developing breast cancer if a hormonal replacement therapy was to be prescribed to alleviate menopausal symptoms.

Breast Benign Diseases and Oral Contraceptives

Studies concerning oral contraceptives are justified by the wide use these drugs have all over the world: it is estimated, in fact, that about 64% of women of child-bearing age, married, or in a relationship, make use of it, with usage incidence peak that varies according to the different geographical areas. (Technical paper No 2017)

The correlation between oral contraceptives use and benign breast diseases incidence has been repeatedly investigated: the first studies date back to the Seventies of 1900; back then, however, the oral contraceptives formulation was different from the recent ones. This difference could undermine the applicability of the results to the current clinical practice.

Fasal et al. in 1975 published one of the first studies aimed at evaluating the risk of developing breast benign diseases associated with oral contraceptives use. They concluded that the relative risk of developing a benign breast disease among ever-users of oral contraceptives was 0.8 (significantly reduced), and it further decreased with longer duration of use down to 0.2 for women using oral contraceptives for 8 years or more. (Fasal and Paffenbarger Jr 1975)

In 1993 Charreau I. et al. reported the results of a French hospital-based case-control study designed to analyze the relationship between the use of oral contraceptives and the risk of benign breast disease. The cases were 286 women, between 1982 and 1985, less than 46 years old, with histologically proven benign breast

disease. Controls were 382 patients, matched to cases on year of birth and month of interview, who were hospitalized for a nonmalignant disease other than breast benign diseases. The risk of breast benign disease was found to decrease significantly with a longer use of oral contraceptives before the first full-term pregnancy (FFTP), but there was no association between the risk of BBD and the duration of OC use after FFTP. Oral contraceptives use before first full-term pregnancy reduced the risk of nonproliferative disease but did not significantly affect the risk of proliferative disease. These results did not depend on the amount of estrogens (0.05 mg or more vs < 0.05 mg) contained in OC. (Charreau et al. 1993)

A more recent cohort study by Rohan and Miller in 1999 observed an inverse association between use of oral contraceptives and risk of all types of benign breast disease combined, confirming the result previously obtained. Nevertheless, when the patients were examined by histological subcategory, the reduction in risk was confined largely to proliferative forms of breast benign diseases, and in particular, to those without histological atypia, in which there was a progressive reduction in risk with increasing duration of use. (Rohan and Miller 1999)

In 2007 Vessey M. and Yates D. published an update of the findings from the Oxford-Family Planning Association study including 17,032 women, first reported in 1981, on the relationship between oral contraceptives and benign breast disease. The update focused on the correlation between modern oral contraceptives containing <50 mcg estrogen and benign breast diseases in order to point out if they showed a protective effect similar to the earlier higher-dose preparations. The findings indicated that modern oral contraceptives use had an important negative association with hospital referral for breast benign diseases (fibroadenomas and fibrocystic breast changes) and that the association was stronger in women with a long duration of use and in recent users. No evidence was found on beneficial effect of progestin-only contraceptives instead. (Vessey and Yeates 2007)

The data on the effect of steroid hormones on benign breast disease come from observational studies with several potential bias and hence should be considered with caution. Anyways, based on the results presented in literature, even if some studies date back many decades, the use of oral contraceptive appears safe and helpful in reducing the incidence of breast benign diseases, especially fibroadenomas and fibrocystic breast changes, with the exception of proliferative breast disease with atypia, for which other medical treatment should be sought in order to reduce the risk breast cancer development. Based on the data presented, the US Medical Eligibility Criteria for Contraceptive Use and the US Selected Practice Recommendations for Contraceptive Use stated that there is no limitation for consuming OC in benign breast diseases. (Curtis et al. 2016a, b)

Breast Benign Diseases and Hormone Replacement Therapy

With the publication of the results of the WHI observational study in 2001, which concluded that there was an increase in the risk of developing breast cancer in women using hormone replacement therapy, there has been a drastic drop in HRT

prescriptions worldwide. In the general population, estrogen- and progestins-based hormone replacement therapy taken for more than 5 years slightly increases the risk of breast cancer development (RR: 1.26). On the other hand, estrogen-only therapy, which is commonly prescribed in menopausal women who had previously undergone hysterectomy, reduces both breast cancer incidence and mortality.

The studies on the use of hormone replacing therapy and the risk of developing benign breast diseases are few and outdated. Significant data derive from the WHI study which highlights how the reduction in breast cancer incidence associated with the use of estrogen-only replacement therapy, is not found in female users with a history of histologically established benign breast disease or with positive familiar history for breast cancer. Therefore, estrogen-only replacement therapy loses its protective value in women with BBD, but does not increase the risk of developing breast cancer.(Anderson et al. 2012a) In a study by Harvey in 1999 on a sample of 1133 postmenopausal women, 699 of whom used HRT, benign breast diseases were more frequent among users (27%) compared to nonusers (21%). For example, breast cysts were found in a statistically significant higher proportion among women using HRT (7%) compared to 1% of nonusers. (Harvey 1999)

In 2000, Friedenreich C. et al. published a study with aimed at establishing the risk factors for benign proliferative disease development. In this study, hormone replacement therapy was associated with a slight, but not statistically significant increase in the risk of BBD. (Friedenreich et al. 2000)

Two interesting papers published in 1997 and 2001 showed that HRT does not influence the clinical pattern of the preexisting benign breast diseases in postmenopausal women who decide to start replacement treatment. Both papers only showed a positive association with a dimensional increase in existing cystic formations or fibroadenomas if HRT was initiated. (Del Favero et al. 1997; Finkelde et al. 2001) Table 2.

Atypical hyperplasia deserves separate consideration. It has been observed that, unlike the other types of BBD, the incidence of atypical hyperplasia increases with age, particularly among postmenopausal women. The widespread availability of the screening programs for breast cancer has led to an increased incidental discovery of this disease. It has therefore been questioned if the use of hormone replacement therapy could play a role in its development. Guyet et al. were the first to study the phenomena, publishing the results in 2003. They observed that the incidence rate of atypical hyperplasia increased between 1994 and 1999, while other benign breast diseases globally decreased. Atypical hyperplasia proportion among other benign breast diseases also increased over time in women aged 40–69 years and this was

Table 2 Other studies evaluating the association between BBDs and HRT

Author	Year	BBD type	RR	95% CI
Trapido	1984	Fibroadenomas Fibrocystic breast changes	1.6 1.4	0.8–3.5 1.1–1.8
Pastides	1987	Any BBDs	2	1–3.9
Canny	1988	Fibroadenomas	2.83	1.21–6.60
Rohan	1999	Any BBDs	1.70	1.06–1.72

significantly higher among HRT users (OR = 2.05; 95% CI: 1.43–2.93), leading to the conclusion that HRT use might increase the development of atypical hyperplasia. (Gayet et al. 2003)

Menes TS. et al. obtained the same results observing retrospectively the incidence of atypical hyperplasia when hormone replacement therapy prescriptions began to drop after WHI results were presented. In fact, they observed that postmenopausal HT use decreased significantly from 35% to 11% between 1999 and 2005 and so did the incidence rate of ADH, from a peak incidence of 5.5/10,000 mammograms in 1999 to 2.4/10,000 in 2005. (Menes et al. 2009)

In summary, hormonal replacement therapy could play a role in inducing fibrocystic changes and fibroadenomas, but since these lesions are not associated with an increased breast cancer risk, they should not be regarded as an obstacle toward HRT use when necessary. Association of hormonal replacement therapy with atypical hyperplasia has not been proved and increases in malignancy rates have not been reported to date. Nevertheless, because these lesions are high-risk, and probably hormone-dependent, it seems wise not to prescribe HRT to patients with AH. (Eskandari and Alipour 2019)

Chemoprevention

Atypical breast lesions, including atypical ductal hyperplasia, atypical lobular hyperplasia (ALH), and lobular carcinoma in situ (LCIS), confer a high risk of cancer. In fact, after 25 years of follow-up, 30% of women in the Mayo Clinic cohort and 27.5% of Nashville Breast Cohort women with previous atypical breast hyperplasia developed breast carcinoma in situ or invasive breast cancer. (Degnim et al. 2007; Sanders et al. 2006)

The incidence of breast cancer can be significantly reduced by chemoprevention. Two selective estrogen receptor modulators (SERMs), Tamoxifen and Raloxifene, and 2 aromatase inhibitors, Exemestane and Anastrozole, have been proven to reduce breast cancer risk in several randomized clinical trials.(Coopey et al. 2012) It is to be noted that this beneficial effect was only seen in hormone receptor positive and therefore good prognosis tumors. A meta-analysis recently showed a 38% relative reduction of breast cancer risk (invasive and noninvasive) among all the study participants who were enrolled in the SERM randomized trials (HR, 0.62; 95% CI, 0.56 to 0.69) and a 31% reduction in the incidence of ductal carcinoma in situ. Analyses of data from the subgroup of women with atypical hyperplasia were performed in four of the placebo-controlled trials (NSABP P-1, MAP.3, IBIS-I, and IBIS-II). A total of 2009 women with atypical hyperplasia were randomly assigned to receive an active agent or placebo in those trials. Relative-risk reductions in the atypical hyperplasia subgroup ranged from 41 to 79%, which suggested an even greater benefit than in the total population treated with active agent in those trials. (Hartmann et al. 2015)

Even though the benefit/risk ratios for chemopreventive medications are favorable for many women, studies show that these agents are infrequently prescribed and

infrequently used. Waters et al., using data from the National Health Interview Survey (2010), found a 0.03% prevalence of Tamoxifen use for chemoprevention among US women 35–79 years of age and a 0.21% prevalence of Raloxifene use among women 50–79 years of age at high risk of developing breast cancer. (Hartmann et al. 2015; Waters et al. 2012) Research in patient decision making has shown that patients' perceived level of risk is a major determinant of their willingness to accept chemoprevention. In particular, it is likely that the low rate of the use of chemoprevention among women with atypical hyperplasia depends on the insufficient understanding of their cumulative risk of breast cancer. (Hartmann et al. 2015; Waters et al. 2012; Ozanne et al. 2010) It is therefore of great importance to address patients' preferences for interventions and risk perception during risk assessment and counseling consultations since informed decision making improves decision quality and is an important step towards improved patient outcomes.

Breast Disorders During Lactation

Most breast lesions diagnosed during pregnancy and lactation are benign; however, the differential diagnosis between the different disorders can be challenging due to the physiological modification that breast tissue undergoes during these phases.

- *Lactating adenoma*
  Lactating adenoma is the most prevalent form of adenoma and the most common cause of breast mass during pregnancy and puerperium. It presents as a solitary or multiple, mobile well circumscribed and lobulated palpable breast mass of usually less than 3 cm. Microscopically, it is composed of numerous hyperplastic secreting lobules. Lactating adenoma may also develop in heterotopic breast tissue. Although the tumor normally results in spontaneous involution, surgical removal may be needed to alleviate the mass effect. This neoplasm does not tend to recur and shows no proven malignant potential. It is difficult to distinguish between a lactating adenoma and a fibroadenoma by imaging. A radiolucent or hyperechoic area, which indicates fat content of breast milk, according to lactation hyperplasia, can be seen on mammography or ultrasonography and is useful in diagnosis. (Yu et al. 2013)
- *Galactocele*
  Galactocele is a benign lesion frequently diagnosed during the third trimester of pregnancy, lactation, and even after the end of lactation. It is a cystic milk-filled formation lined by epithelial and myoepithelial cells. Most of the galactoceles are found as painless, gross, palpable mass. At ultrasound examination, they appear as liquid-filled, hypoechoic, cystic formations with limited background enhancement and sometimes irregular margins. Needle aspiration biopsy is helpful for diagnosis. (Yu et al. 2013)
- *Puerperal Mastitis*
  Currently, in Western countries, puerperal mastitis and lactating abscesses are much less common than they were before, the reason for this being probably the

improvement in the hygienic conditions of both mothers and newborns, changes in lactation procedures, and early treatment of infections with antibiotic therapy. It is not certain whether the infection derives from saprophytic germs of the maternal skin or present in the oral cavity of the newborn. During the first weeks of breastfeeding, the breast is particularly vulnerable to bacterial infections due to fissures that form around the nipple. *Staphylococcus aureus* is primarily responsible, but Staphylococcus epidermidis and streptococci have also been occasionally isolated. Staphylococcus tends to produce a localized inflammatory lesion that can progress to form single or multiple abscesses. Streptococcus, on the other hand, tends to cause a widespread infection that extends rapidly to the entire mammary gland. Patients may present with pyrexia, tachycardia, and leukocytosis. If an abscess forms, the pus can be drained with a small incision and the residual cavity irrigated with saline solution. Once the infection is resolved, the woman can continue breastfeeding.

Effect of Lactation on Breast Benign Disease

Unlike the well-established protective effect of lactation on the risk of breast cancer risk, little is known on its relationship with breast benign diseases. In one study published in 1998 by Yuko Minam, the risk of BBD associated with lactation was examined based on the history of lactation for the last child: no association appeared to be present between history of lactation itself and the risk of BBD in either histopathological type. (Minami et al. 1998)

More recently, Bernardi S. et al. conducted a retrospective study on 105 women with BBD and 98 controls, focusing on their reproductive history and breast-feeding. They concluded that there seemed to be no difference in breast-feeding among BBDs types, but lactation might have influenced the number of fibroadenomas. (Bernardi et al. 2012) The little data available and presented seem to confer a neutral effect to lactation on breast benign diseases, but prospective studies are needed to better define the real influence of breast feeding on such disorders.

Breast Cancer and Hormones

Hormonal Risk Factors for Breast Cancer

Breast cancer is the most frequent cancer in the world and is the leading cause of cancer-related death in women. Incidence in Italy is slightly growing (+ 0.3% annually), whereas mortality is significantly decreasing (− 0.8% annually). The etiology of breast cancer is heterogenous and multifactorial. The risk of breast cancer increases with age with a probability of developing the disease by 2.3% up to 49 years, by 5.4% in the 50–69 years age group, and by 4.5% in the 70–84 years age group. The incidence curve exponentially grows until the menopause age and then slows down reaching a plateau after menopause; then it raises again after the age

of 60. This trend correlates both to the woman's endocrinological history and to the screening programs.

Reproductive factors are associated with breast cancer risk: a long fertile period, with an early menarche and a late menopause, exposes for a longer time the breast tissue to proliferative stimulation of ovarian estrogens. For each year younger at menarche and for each years older at menopause, breast cancer risk increases by a factor of 1.050 (95% CI 1.044–1.057, $p < 0.0001$) and 1.029 (CI 95% 1.025–1.032, $p < 0.0001$), respectively. Furthermore, early menarche is associated with many other known risk factors for breast cancer such as parity, age at first pregnancy and body mass index (BMI). Analyzing tumor characteristic, endogenous ovarian hormones are more relevant for estrogen receptor-positive disease than for estrogen-receptor negative and for lobular than for ductal tumors. (Primic-Žakelj and Košmelj 2012)

In literature several studies have focused on delineating the role of endogenous hormones in risk of breast cancer. A strong positive association between breast cancer risk and circulating levels of sex hormones has been confirmed in literature. In premenopausal women higher circulating testosterone levels are associated with an increase risk, but no significant risk association is observed for estradiol and progesterone. (Kaaks et al. 2014) In postmenopausal women for all sex steroids, androgens, as well as estrogens, elevated serum levels are associated with breast cancer risk (testosterone RR = 1.5, estradiol RR = 2.28). (Kaaks et al. 2005) Proposed mechanisms of androgens include a direct effect on epithelial cell proliferation and an indirect effect as a precursor for estrogen synthesis. Surgical menopause has a decreased impact on breast cancer risk maybe because of the reduction in androgen levels in oophorectomized women.

Factors associated with pregnancy could also play some etiological role in the development of breast cancer. First pregnancy leads to final breast maturation rendering the gland less susceptible to carcinogens. Age at the birth of first child is strongly associated with breast cancer incidence too: pregnancy at early age may reduce the probability of tumor initiation, whereas the same event at a later age may promote the growth of existing tumor cells. The increase in risk associated with delayed childbearing was more consistently observed for ER positive than ER negative tumors. Several case-control and cohort study demonstrate that higher parity is associated with a decrease in the risk of breast cancer. Risk estimates ranged from 0.5 to 0.8. In a meta-analysis the protective effect of parity was confined to women with estrogen and progestin receptor-positive breast cancer. Parous women who have had four or more full-term pregnancies have an approximately 50% reduction in the risk of breast cancer compared with women who have never had a full-term pregnancy.

One of the oldest and most enduring hypothesis concerning breast cancer is that lactation protects against its development. Lactation increases the proportion of differentiated cells in the breast and reduces risk of malignant transformation. Furthermore, during the lactation, levels of estrogen were lower and oxytocin, released as a response to suction, seems to inhibit cell proliferation and tumor growth. Having breastfeed each child on average of more than 3 months and up to 6 months decreases risk by 20%.

Overweight and obesity are prevalent risk factors for breast cancer. The excess of adipose tissue in post-menopause is the main source of circulating estrogen synthesis resulting in an excessive hormonal stimulation on the mammary gland. Furthermore, reduction of sex hormone binding globulin (SHBG) leads to increase of free testosterone circulating levels. The metabolic syndrome, which include conditions such as obesity, hypertension, and insulin resistance, contributes to increasing the risk of breast cancer. Insulin resistance causes an increase in the levels of insulin that acts on the insulin-like grow factor-1 (IGF-1) receptor activating intracellular signaling pathways promoting neoplastic transformation. Modifying lifestyle with regular physical activity and a balanced diet, it could be possible to reduce the risk of developing breast cancer by improving the metabolic and hormonal status of the woman. Excess body weight does not impact only on breast cancer risk but also on tumor characteristics and survival. Obese women have 1.7 and 1.8 fold increased risk of advanced stage disease and grade 3–4 tumors, respectively. Obesity negatively influences hormone-positive breast cancer-specific survival too: mortality increases from 1.78 to 2.16 fold in obese women compared to normal weight patient. (Blair et al. 2019)

Oral Contraceptives and Breast Cancer

Oral contraceptives are widely used in the world population. Despite previous research, the supposed correlation between pill use and breast cancer remains one of the more important aspects regarding hormonal contraceptives.

Several possible mechanisms have been hypothesized to explain the impact on breast cancer. In breast tissue the combination of estrogen and progestin has a proliferative effect on cancer cells, which could cause progression of breast cancer. Estrogens may also enhance angiogenesis and stromal cell recruitment promoting tumor growth. Studies in literature have found either no effect or a slight increase in risk. In 1996 the Collaborative Group on Hormonal Factors in Breast Cancer published a reanalysis of data from 54 epidemiological studies conducted in 25 countries for a total of 53,297 patients with breast cancer and 100,239 controls. The study revealed a small increased in the risk of having breast cancer among ever users compared to never users (RR 1.07, $p = 0.00005$). (Collaborative Group on Hormonal Factors in Breast Cancer 1996) In 2006 the Pennsylvania study reported an increased risk of premenopausal breast cancer in OCs users (OR 1.19, CI 95% 1.09–1.29). (Kahlenborn et al. 2006) A more recent systematic review exclusively focused on 44 publications since 2000 confirms a slightly increased risk in ever-use OCs users compared with never users (OR 1.08, CI 95% 1.00–1.17) with an estimated lifetime absolute risk of breast cancer increased of 0.89%. (Gierisch et al. 2013) Other studies show discordant results with a less impact on breast cancer risk. The Royal College of General Practioners' Oral Contraception Study including 46,022 women, after 44 years of follow-up, reports an incidence rate in current and recent users close to unit (IRR 1.04, 95% 0.91–1.17) and this effect is lost within 5 years stopping oral contraception. Results show a time-dependent relationship as a

function of time since last oral contraceptive use, with the highest risk seen in recent users (within the previous 5 years). (Hannaford et al. 2007)

This correlation with time interval since last pill administration has already been demonstrated in 1996: the relative risk (RR) of breast cancer was 1.24 (95% CI, 1.15–1.33; $p < 0.00001$) for current users, 1.16 (95%CI, 1.08–1.23; $p = 0.00001$) 1–4 years after stopping, 1.07 (95%CI, 1.02–1.13; $p = 0.009$) 5–9 years after stopping, and 1.01 (95%CI, 0.96–1.05; $p = 0.00001$) 10 or more years after stopping pill use. (Marchbanks et al. 2002) Another aspect that needs to be considered is the duration of use. In a Danish study among 1.8 million women, aged 15–49 years and using hormonal contraception, risk increased from 1.09 (95% CI 0.96–1.23) with less than 1 year of use to 1.38 (95% CI 1.26–1.51) with more than 10 years of use ($P = 0.002$). Fig. 1.

However, the overall absolute increase is only 1 extra breast cancer for 7690 users during 1 year. (Mørch et al. 2017) Also a meta-analysis of Zhu et al. of 13 studies evidences a linear significant increase of risk with long lasting OCs assumption: every 5 years of use the risk increase of 7% (RR 1,07 95% CI 1,03–1,11 $p < 0,01$) and every 10 years of 14% (RR 1,14 95% CI 1,05–1,23 $p < 0,01$). (Zhu et al. 2012) In some studies, the increased risk is relevant only when age at first assumption is under 20 years old (Collaborative Group on Hormonal Factors in Breast Cancer 1996) and when OCs is used before first pregnancy (OR 1.44, CI 95% 1.28–1.62). (Kahlenborn et al. 2006) In a large population-based, case-control study, current oral contraceptive use is associated with increased risk of lobular carcinoma (RR 2.6, 95% CI 1.0–7.1), whereas OCs use is not clearly associated with ductal carcinoma (RR 1.2, 95% CI 0.8–1.9). (Newcomer et al. 2003)

From the introduction of oral contraceptive in clinical practice, there have been progressive changes in terms of age of contraceptive formulations. Many of the previous study were performed on patient taking old contraceptive formulations in which the estrogenic component was represented by ethinylestradiol at a daily dose of 50 mcg and the progestin component was a derivate of testosterone, often levonorgestrel. In recent oral contraceptive estrogen dose has been reduced by

Duration of Use of Hormonal Contraceptive	Relative Risk of Breast Cancer (95% CI)		
	<1 Yr since Recent Use	1 to <5 Yr since Recent Use	5 to 10 Yr since Recent Use
<1 yr	0.96 (0.78–1.19)	0.96 (0.85–1.09)	1.01 (0.88–1.15)
1 to <5 yr	1.04 (0.88–1.23)	1.06 (0.96–1.18)	1.07 (0.94–1.20)
5 to 10 yr	1.33 (1.11–1.59)	1.16 (1.02–1.33)	1.30 (1.06–1.58)
>10 yr	1.52 (1.17–1.98)	1.16 (0.89–1.49)	NA†

Fig. 1 Correlation between duration of use of OCs and risk of breast cancer (Mørch et al. 2017)

about 50% (20–30 mcg) and new progestins have been introduced, such as gestodene and desogestrel. Data about the clinical relevance of estrogen and progestin types and doses are still conflicting and inconclusive. In the large Danish study including 1.8 million women, although the great number of preparations available, there are not major differences in risk between them. (Mørch et al. 2017) A discordant result emerges from the Nurses' Health Study II: current use of OCs leads to an increased risk (RR 1.33, 95% CI 1.03–1.73), especially triphasic formulation with levonorgestrel as progestin (RR 3.3, 95% CI 2.0–4.66). (Hunter et al. 2010) However, in the multicenter case-control study of Marchbancks et al., the same formulation does not increase the risk. No evidence of variation of risk with different OCs formulation was found in this study. Any formulation among the 10 analyzed is associated with a significative increase of risk of breast cancer. (Marchbanks et al. 2002) The increasing use of preparations with progestin has raised questions on the potential impact on the breast. The recent Danish study includes valuable data on levonorgestrel-releasing IUD, which provide risk of similar size to OCs. (Mørch et al. 2017) OCs may also have an impact on other type of cancer. Evidence of reasonably good level suggests that users of oral contraceptives are protected from colorectal (IRR 0.81), endometrial (IRR 0.66), and ovarian cancer (IRR 0.67) for many years after stopping, especially for colorectal and ovarian cancer. (Iversen et al. 2017)

Women should be informed that there seems to be a real risk, although small, of breast cancer with the use of the pill, but that the risk faints soon after last use, and that there are long-term benefits on ovarian cancer risk extending at least up to 30 years after the interruption of the OCs.

Hormone Replacement Therapy and Breast Cancer

Hormone replacement therapy containing estrogens remains the most effective treatment for vasomotor symptoms and genitourinary syndrome of menopause and has been shown to prevent bone loss and fracture. Progestogen is added to provide endometrial protection in women with uterus. After the publication of the initial WHI (Women's Health Initiative) results in 2002 reporting an overall increased risk of breast cancer, heart disease, stroke, and venous thromboembolism, many women discontinued HRT. HRT prescriptions in the United States rapidly decreased over 1 year from approximately 40–20%. (Chlebowski and Anderson 2012) The WHI remains the largest randomized controlled trial of HRT, but it only compared conjugated equine estrogens (CEEs) and medroxyprogesterone acetate (MPA) versus placebo in patient with an elevated average age. The design led to an overestimation of the risks for women aged less than 60 years and at low risk of cardiovascular disease and breast cancer. Despite the subsequent reanalysis of the results by age and years since menopause and the updates with extended follow-up, much controversy remains.

The WHI study results suggested a breast cancer risk increase in HRT users (HR 1.26, C.I. 1.00–1.59), but no data about mortality were reported because of the short

follow-up period (mean of 7.9 years). In HRT users for 5 or more years, nine additional breast cancer patients per 10,000 women have been seen. (Rossouw et al. 2002) A breast cancer increase in HRT users had already been observed 20 years before, in 1997, in the Collaborative Group Study; this review of 50 observational studies evidenced a relative risk of 1.14 (95%CI, 1,11–1.17) for ever users and 1.35 (C.I. 1.21–1.49) for women who had used HRT for 5 years or longer. No significant excess of breast cancer had been seen 5 or more years after cessation of HRT use or in relation to duration of use. The increase in breast cancer risk appeared to be limited to lean women, since obese postmenopausal women have already achieved the maximum hormone-related risk due to their endogenous estrogen production. (Beral et al. 1997) The North American Menopause Society (NAMS) position statement of 2017 asserts that different type of estrogen or progestogen, as well as different formulation, doses, duration, time of initiation, and patient characteristics, may have a significant impact on the risk of developing breast cancer. Garnet et al. randomized women aged between 50 and 79 years with prior hysterectomy to conjugated equine estrogen (CEE) therapy versus placebo. Patients receiving estrogen-alone had a significant reduction in breast cancer incidence after an extended follow-up of 11.8 years (HR 0.77, C.I. 0.62–0.95, $p = 0.02$). CEE use was associated with significant reduced risk of infiltrating ductal carcinoma (HR 0.67, C.I. 0.51–0.88). No differences merged for receptors positive and negative tumors. Results also showed a reduction in deaths for breast cancer in CEE group (HR 0.37, C.I. 0.13–0.91, $p = 0.03$). However, the risk reduction was restricted to patients without benign breast disease ($p = 0.01$) and family history of breast cancer ($p = 0.02$). (Anderson et al. 2012b) The update of WHI trial outcomes published in 2013 with extended postintervention follow-up of 13 years analyzed 27,347 patients treated with conjugated equine estrogen (CEE, 0.625 mg/day) with medroxyprogesterone acetate (MPA, 2.5 mg/day) or CEE alone. During intervention phase, hazard ratio (HR) for breast cancer was 1.24 (C.I. 1.01–1.53) with CEE-MPA use and 0.79 (C.I. 0.62–1.02) with CEE use. Estrogen-progestin use significantly increased risk of breast cancer during cumulative follow-up (HR 1.28, C.I. 1.11–1.48), even if a year-to-year reductions after stopping. By contrast the use of unopposed estrogen seemed to significantly reduce risk during cumulative follow-up (HR 0.79, C.I. 0.65–0.97). (Manson et al. 2013) Breast cancer risk is also influenced by duration of use and interval between menopause and starting hormone therapy. Beral et al. demonstrated that risk of breast cancer is greater in current users (RR 1.68, C.I. 164–1.72) than in past users (RR 1.08, C.I. 1.04–1.12) and it declines rapidly after use ceased. Several studies have reported that the time interval between onset of the menopause and start of HRT treatment may influence breast cancer risk with a higher risk in women who starting HRT within 1 year after onset of the menopause. (Beral et al. 2011)

Different routes of administration can have a different impact on breast cancer risk. Oral estrogens are associated with a significant reduction of circulating IGF-1 levels, as they inhibit the hepatic IGF-1 production, whereas IGF-1 modifications during transdermal estrogen administration tend to be biphasic, since IGF-1 levels decrease in women with high basal values and increased in those with low basal

values. Therefore, transdermal estrogens may have peculiar pharmacokinetic and metabolic properties compared to oral estrogens, and this could modify the hormone effects on breast tissue proliferation. (Sismondi et al. 2002) In The Million Women Study no statistically significant differences were found in relation to route of administration (RR 1.63, C.I. 1.04–2.56, and RR 1.34, C.I. 1.08–1.66, for oral and transdermal, respectively). (Beral 2003) However, trials comparing effects of oral and transdermal estrogens on breast cancer risk do not exist yet. In combined estro-progestin therapies a large variety of progestins can be used and this may impact on breast cancer risk. Some studies have shown a certain variability of results that may be attributed to different biological actions of these compounds, especially linked to the androgenic activity of some of them, like nor-testosterone derivatives. Large epidemiological studies, such as the French E3N Study and Cecile study, indicate that natural progesterone and dydrogesterone may be associated with a more favorable risk profile compared to the other progestins (RR 1.16 CI. 0.94–1.43 versus 1.69 CI. 1.50–1.91, respectively), especially those structurally related to testosterone show a higher risk.

Several studies investigated the relationship between tumor risk and type of combined hormonal treatment: continuous estrogen-progestogen therapy appears to confer a higher risk compared to sequential therapy. (Cordina-Duverger et al. 2013) New class of molecules have been developed to protect against effects of estrogens alone on breast tissue and endometrium. TSECs (Tissue-Selective Estrogen Complexes) are a combination of estrogens and SERMs (Selective Estrogen Receptor Modulators): CE 0.45 mg plus Bazedoxifene 20 mg is available in Italy since 2015. Bazedoxifene operates as an estrogen receptor antagonist and in the breast antagonizes estrogens-stimulated cell proliferation; in this way it guarantees a safety profile on endometrium. This combination demonstrated effects on bone mineral density and VMS with a significant improvement in vaginal dryness, dyspareunia, and sexual function compared to placebo. In addition, Bazedoxifene does not affect age related changes in breast density. It could be a safer alternative to estro-progestin therapy, even if data form large randomized trials are lacking. (Pickar et al. 2018)

Mammography density changes with age and in response to exposure to hormones. Increased breast density may obscure mammographic interpretation and is more strongly associated with the risk of aggressive breast tumor subtypes, especially among postmenopausal women using combined estrogen plus progesterone therapy. In the WHI trial the abnormal mammography rate that required further testing was higher among women treated with estrogens plus progestins than placebo: they had a 4% greater risk of having further investigation, such as biopsy, after 5 years ($p < 0.001$). Estrogens alone have a lower impact on mammography density. Mechanism for the association between breast cancer risk and breast density is unknown, but recently was postulated to result from an increase in tissue synthesis of estrogen from aromatase in dense tissue. (Yaghjyan et al. 2017) HRT can also influence the prognosis of breast cancer. Data on the effect of exogenous hormones on the prognosis of breast cancer are conflicting. Some studies found better outcome data with prediagnostic HRT use in breast cancer patients, while others saw no

difference in HRT users who subsequently developed breast cancer. The Collaborative Group on Hormonal Factors in Breast Cancer study reported that breast cancer detected in HRT users gave less frequently lymph node or distant metastases when compared to controls. (Beral et al. 1997) Although this positive effect might derive from an earlier diagnosis as a consequence of improved breast surveillance among hormone users, the large majority of the studies have shown that women treated with HRT develop breast cancers with better histologic differentiation, lower proliferation rate, and more favorable clinical course, thus suggesting a biological effect of HRT on the growth of less aggressive tumors. In a retrospective study, postmenopausal HRT users had significantly more early tumor stages ($p < 0.001$) and HRT was associated with longer time to progression (HR 0.81, 95%CI 0.55–1.19, $p = 0.28$) and overall survival (HR 0.68, 95%CI 0.45–1.02, $p = 0.059$). Large prospective study is lacking about this argument. (Baumgärtner et al. 2011) The cancer risks of HRT differ depending on a lot of factors, so treatment should be individualized to identify the most appropriate dose, regimen, duration, and route of administration, using the best available evidence, with periodic reevaluation of the woman's benefit-risk profile.

Hormone Therapies in High Risk Women for Breast Cancer

BRCA1 and BRCA2 are the two major susceptibility genes involved in hereditary breast and ovarian cancer. These genes appear to participate in a common pathway that is involved in the control of homologous recombination and in the maintenance of genomic integrity. BRCA1 and BRCA2 mutation carriers have a 54–85% and 45% lifetime risk of developing breast cancer, respectively, and a 18–60% and 11–27% lifetime risk of developing ovarian cancer, respectively. (King et al. 2003) Estrogens play a major role in the development and progression of breast cancer in general population. These hormones can enhance breast carcinogenesis by increasing the number of errors occurring during DNA replication, as well as by causing DNA damage via their genotoxic metabolites produced during oxidation reactions. In BRCA mutation carriers, these damages to DNA cannot be repaired. Furthermore, there are some biological evidences of interactions between estrogens and BRCA proteins. BRCA1 expression can be induced by estradiol in experimental models, and BRCA1 can modify the regulatory effects of the estrogen receptor α.

Oral Contraceptives

According to guidelines BRCA 1 and BRCA 2 mutation carriers should consider taking OCs as a chemoprevention for ovarian cancer. In general population, it is known that oral contraceptives can significantly reduce the risk of ovarian cancer. This reduction of risk increases with long-term use and persists for 30 years after discontinuation. Oral contraceptive use may reduce ovarian cancer risk even in BRCA mutation carriers. A meta-analysis including 18 studies updated to March

2010 investigated the associations between OCs use and risk of cancer among ascertained BRCA carriers. Results show a significant reduced risk for ovarian cancer (RR 0.50) and the risk decreases by 36% each additional 10 years of OCs use. (Iodice et al. 2010) Another meta-analysis including studies published since 2000 to 2012 confirms a reduction in ovarian cancer risk (RR 0.58, 95% CI 0.46–0.73). (Moorman et al. 2013) The potential increase risk of breast cancer has to be considered in prescribing OCs in this particular population. The impact of oral contraceptive use has been investigated not only in BRCA mutation carriers, but also in women with breast cancer family history. A collaborative reanalysis of individual data from 52 epidemiological studies including 58,209 women with breast cancer and 101,986 women without the disease reveals a risk ratio increase in women with positive family history. For women with 0, 1, or 2 affected first-degree relatives, the cumulative incidence of breast cancer up to age 50 is 1.7%, 3.7%, and 8.0%, respectively. However, there is no excess risk for breast cancer among OCs users with a history of breast cancer in a first-degree relative. (Collaborative Group on Hormonal Factors in Breast Cancer 2001) Similar result is showed by a review of all studies published from 1966 to 2008: OCs did not appear to substantially modify the risk of breast cancer in women with a family history of the disease. An elevated risk remained only for women who used OCs before 1975 (RR = 3.3, 95% CI: 1.5–7.2). (Gaffield et al. 2009) In a recent retrospective cohort study on 2527 women (4.5% BRCA mutation carriers, 72.2% high risk, and 23.3% intermediate risk), the use of OCs is not associated with an increased risk of breast cancer, regardless of the duration of use. If estrogen dose not influence the risk of breast cancer, the type of progestin might influence the risk: gestodene (P 0.028) and cyproterone acetate (P 0.031) are associated with an even greater reduced risk. (Grandi et al. 2018) In the World Health Organization (WHO) Medical Eligibility Criteria for contraceptive use, no restriction on the use of OCs for women who have a family history of breast cancer is reported.

In BRCA mutation carriers data are controversial. Some studies show a modestly increased risk. The risk appears to be greater for women who took OCs for at least 5 years and who took OCs before the age of 30 years. The meta-analysis of Iodice et al. does not show significant breast cancer increased risk for ever users neither correlation with duration of use. The summary relative risk is 1.13 (95% CI 0.88–1.45). (Iodice et al. 2010) Other studies analyzed if there are any difference between BRCA1 and BRCA2 mutation carriers. The meta-analysis of Moorman et al. identifies a risk for both groups nonsignificantly elevated (OR 1.21, 95% CI 0.93–1.58) with a modestly higher point estimate for BRCA2 (OR 1.36, 95% CI 0.89–2.10) as compared to BRCA1 mutation carriers (OR 1.19, 95% CI 0.92–1.55). (Moorman et al. 2013) The largest study concerning the effects of oral contraceptives on breast cancer risk in BRCA1 mutation carriers focused on timing of use: women who started the pill before 20 years old had an increased risk of breast cancer (OR 1.45; 95% CI 1.20–1.75; $P = 0.0001$) and the risk increased by 11% with each additional year of pill use when initiated prior to age 20. Caution should be taken when advising women with a BRCA1 mutation to take an oral contraceptive prior to age 25. (Kotsopoulos et al. 2014)

The inverse association between OCs use and ovarian cancer is considerably stronger than the positive association with breast cancer; hence, the chemo-preventive effect of OCs can be exploited in BRCA 1 and BRCA 2 mutation carriers.

Hormone Replacement Therapy

The absence of reliable methods of early diagnosis and the poor prognosis associated with advanced ovarian cancer have supported the performance of bilateral risk reduction salpingo-oophorectomy (RRSO) in BRCA1 and BRCA2 mutation carriers. An optimal age for RRSO is difficult to find. At present, the guidelines for ovarian cancer risk management recommend bilateral salpingo-oophorectomy at the completion of childbearing in women at high risk. In BRCA1 mutation carriers, RRSO is usually performed from the age of 35 years and definitely by 40 years, because below the age of 40 years, the risk of ovarian cancer is only 2%. In those with BRCA2 gene mutations, there is growing acceptance that women have until the age of 45 years to undergo surgery because their cumulative risk of ovarian cancer by age 50 years is only 0–1%. (Menon et al. 2018) RRSO leads these women to a premature menopause and it is demonstrated in literature that menopausal symptoms are more severe in young patient with premature forced menopause. Nonhormonal therapies may palliate some of these symptoms, but only hormone replacement therapy can relief all the menopausal symptoms. Many concerns and worries remain about the use of hormone replacement therapy in patients with BRCA mutations. In a large study including 462 women with BRCA1–2 mutations after bilateral prophylactic oophorectomy, short-term HRT (mean use of 3.2 years) does not negate the protective effect on subsequent breast cancer risk (HR = 0.37; 95% CI = 0.14–0.96). (Rebbeck et al. 2005) A matched case-control study of 472 postmenopausal women with a BRCA1 mutation seems to confirm this result: a higher proportion of control subjects who did not develop breast cancer than case patient had used HRT at some times (29% vs. 20% respectively); in this population HRT use was not associated with increased risk of breast cancer. (Eisen et al. 2008) In another case-control study, a research questionnaire was administered to 432 matched pairs of women with a BRCA1 mutation in order to obtain detailed information on HRT use after menopause and results show that short course of HRT should not be contra-indicated for BRCA1 mutation carriers who have undergone menopause and who have no personal history of cancer. The quality of life of patients using HRT after RRSO results improved with approximately the same levels of endocrine symptoms and sexual functioning as women who did not have surgery. (Kotsopoulos et al. 2016) Last NICE recommendations state to offer HRT to women with no personal history of breast cancer who have either BRCA1 or BRCA2 mutation or a family history of breast cancer and who have had a bilateral salpingo-oophorectomy up until the time they would have expected natural menopause. 2019 NCCN guidelines confirm that HRT does not affect the breast cancer risk reduction obtained with salpingo-oophorectomy; however, they place more caution on this argument, underlying that large randomized trial about this argument does not exist yet.

Conclusions

The correlation between breast diseases and hormones has been extensively analyzed and confirmed in many studies. Benign breast diseases have been subgrouped in three histological categories: nonproliferative lesions, proliferative lesions without atypia, and atypical hyperplasia. The latter ones have been found to bear an increased risk of breast cancer development. The use of oral contraceptive appears safe and helpful in reducing the incidence of breast benign diseases and HRT should not be discouraged in patients with BBDs, with the exception of atypical hyperplasia. Reproductive factors are associated with breast cancer risk: a long fertile period, with an early menarche and a late menopause, nulliparity, and a late pregnancy expose to an increased breast cancer risk. The correlation between OCs and breast cancer risk is unclear. Several studies evidence a small increase of the risk, but this risk is lost soon after last use. The cancer risks of HRT differ depending on multiple factors, such as formulation, doses, duration, and time of initiation, so the treatment should be individualized with periodic reevaluation of the woman's benefit-risk profile. In women who have a family history of breast cancer there is no restriction on the use of OCs. In BRCA1 and BRCA2 mutation carriers data are controversial, but the protective effect on ovarian cancer is considerably stronger, hence the chemo-preventive effect of OCs can be exploited. Prophylactic RRSO leads BRCA mutated women to a premature menopause. Guidelines state that HRT can be offered to symptomatic women with no personal history of breast cancer; HRT does not affect the breast cancer risk reduction obtained with salpingo-oophorectomy.

Cross-References

► Hormonal Contraception
► Hormone Replacement Therapy (HRT)

References

Anderson GL, Chlebowski RT, Aragaki AK, Kuller LH, Manson JE, Gass M, Bluhm E, Connelly S, Hubbell FA, Lane D, Martin L, Ockene J, Rohan T, Schenken R, Wactawski-Wende J. Conjugated equine oestrogen and breast cancer incidence and mortality in postmenopausal women with hysterectomy: extended follow-up of the Women's Health Initiative randomised placebo-controlled trial. Lancet Oncol. 2012a;13(5):476–86.

Anderson GL, Chlebowski RT, Aragaki AK, Kuller LH, Manson JE, Gass M, et al. Conjugated equine oestrogen and breast cancer incidence and mortality in postmenopausal women with hysterectomy: extended follow-up of the Women's Health Initiative randomised placebo-controlled trial. Lancet Oncol. 2012b;13(5):476–86.

Baumgärtner AK, Häusler A, Seifert-Klauss V, Schuster T, Schwarz-Boeger U, Kiechle M. Breast cancer after hormone replacement therapy–does prognosis differ in perimenopausal and postmenopausal women? Breast. 2011;20(5):448–54.

Beral V. Breast cancer and hormone-replacement therapy in the Million Women Study. Lancet. 2003;362:419.

Beral V, Calle EE, Heath CW, Coates RJ, Liff JM, Franceschi S, Talamini R, Košmelj K, Primic-Žakelj M, Ravnihar B, Stare J, Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer. Lancet. 1997;350(9084):1047–59.

Beral V, Reeves G, Bull D, Green J. Breast cancer risk in relation to the interval between menopause and starting hormone therapy. J Natl Cancer Inst. 2011;103(4):296–305.

Bernardi S, Londero AP, Bertozzi S, Driul L, Marchesoni D, Petri R. Breast-feeding and benign breast disease. J Obstet Gynaecol. 2012;32(1):58–61.

Blair CK, Wiggins CL, Nibbe AM, Storlie CB, Prossnitz ER, Royce M, et al. Obesity and survival among a cohort of breast cancer patients is partially mediated by tumor characteristics. NPJ Breast Cancer. 2019;5:33.

Charreau I, Plu-Bureau G, Bachelot A, Contesso G, Guinebretiére JM, Lê MG. Oral contraceptive use and risk of benign breast disease in a French case-control study of young women. Eur J Cancer Prev. 1993;2(2):147–54.

Chlebowski RT, Anderson GL. Changing concepts: menopausal hormone therapy and breast cancer. J Natl Cancer Inst. 2012;104(7):517–27.

Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and hormonal contraceptives: collaborative reanalysis of individual data on 53297 women with breast cancer and 100239 women without breast cancer from 54 epidemiological studies. Lancet. 1996;347:1713–27.

Collaborative Group on Hormonal Factors in Breast Cancer. Familial breast cancer: collaborative reanalysis of individual data from 52 epidemiological studies including 58,209 women with breast cancer and 101,986 women without the disease. Lancet. 2001;358(9291):1389–99.

Coopey SB, Mazzola E, Buckley JM, et al. The role of chemoprevention in modifying the risk of breast cancer in women with atypical breast lesions. Breast Cancer Res Treat. 2012;136:627–33.

Cordina-Duverger E, Truong T, Anger A, Sanchez M, Arveux P, Kerbrat P, et al. Risk of breast cancer by type of menopausal hormone therapy: a case-control study among post-menopausal women in France. PLoS One. 2013;8(11):e78016.

Curtis KM, Tepper NK, Jatlaoui TC, Berry-Bibee E, Horton LG, Zapata LB, et al. US medical eligibility criteria for contraceptive use, 2016. MMWR Recomm Rep. 2016a;65:1–103.

Curtis KM, Jatlaoui TC, Tepper NK, Zapata LB, Horton LG, Jamieson DJ, et al. US selected practice recommendations for contraceptive use, 2016. MMWR Recomm Rep. 2016b;65:1–66.

Degnim AC, Visscher DW, Berman HK, et al. Stratification of breast cancer risk in women with atypia: a Mayo Cohort Study. J Clin Oncol. 2007;25:2671–7.

Del Favero C, Rossini G, Tufarulo L, Ciancio ML, Sopransi M, Martegani A, Borghi C, Campi R, Fugazzola C. Mammography changes associated with hormone replacement therapy in post-menopausal patients. Radiol Med. 1997;93(3):210–3.

Dupont WD, Page DL. Risk factors for breast cancer in women with proliferative breast disease. N Engl J Med. 1985;312:146.

Eisen A, Lubinski J, Gronwald J, Moller P, Lynch HT, Klijn J, et al. Hormone therapy and the risk of breast cancer in BRCA1 mutation carriers. J Natl Cancer Inst. 2008;100(19):1361–7.

Eskandari A, Alipour S. Hormone replacement therapy and breast diseases: a matter of concern for the gynecologist. Arch Breast Cancer. 2019;6:113–9.

Fasal E, Paffenbarger RS Jr. Oral contraceptives as related to cancer and benign lesions of the breast. J Natl Cancer Inst. 1975;55(4):767–73.

Finkelde DT, Kitchen PR, Hayes PR, McKinlay MR, Henderson MA. Symptomatic benign breast disease and hormone replacement therapy. Breast. 2001;10(2):127–30.

Friedenreich C, Bryant H, Alexander F, Hugh J, Danyluk J, Page D. Risk factors for benign proliferative breast disease. Int J Epidemiol. 2000;29(4):637–44.

Gaffield ME, Culwell KR, Ravi A. Oral contraceptives and family history of breast cancer. Contraception. 2009;80:372–80.

Gayet A, Estève J, Séradour B, Piana L, Jacquemier J. Does hormone replacement therapy increase the frequency of breast atypical hyperplasia in postmenopausal women? Results from the Bouches du Rhone district screening campaign. Eur J Cancer. 2003;39(12):1738–45.

Gierisch JM, Coeytaux RR, Urrutia RP, Havrilesky LJ, Moorman PG, Lowery WJ, et al. Oral contraceptive use and risk of breast, cervical, colorectal, and endometrial cancers: a systematic review. Cancer Epidemiol Biomark Prev. 2013;22(11):1931–43.

Grandi G, Toss A, Cagnacci A, Marcheselli L, Pavesi S, Facchinetti F, et al. Combined hormonal contraceptive use and risk of breast cancer in a population of women with a family history. Clin Breast Cancer. 2018;18(1):e15–24.

Guray M, Sahin AA. Benign breast diseases: classification, diagnosis, and management. Oncologist. 2006;11:435.

Hannaford PC, Selvaraj S, Elliott AM, Angus V, Iversen L, Lee AJ. Cancer risk among users of oral contraceptives: cohort data from the Royal College of General Practitioner's oral contraception study. BMJ. 2007;335(7621):651.

Hartmann LC, Degnim AC, Santen RJ, Dupont WD, Ghosh K. Atypical hyperplasia of the breast–risk assessment and management options. N Engl J Med. 2015;372(1):78–89.

Harvey JA. Use and cost of breast imaging for postmenopausal women undergoing hormone replacement therapy. AJR Am J Roentgenol. 1999;172(6):1615–9.

Hunter DJ, Colditz GA, Hankinson SE, Malspeis S, Spiegelman D, Chen W, et al. Oral contraceptive use and breast cancer: a prospective study of young women. Cancer Epidemiol Biomark Prev. 2010;19(10):2496–502.

Iodice S, Barile M, Rotmensz N, et al. Oral contraceptive use and breast or ovarian cancer risk in BRCA1/2 carriers: a meta-analysis. Eur J Cancer. 2010;46:2275–84.

Iversen L, Sivasubramaniam S, Lee AJ, et al. Lifetime cancer risk and combined oral contraceptives: the Royal College of General Practitioners' Oral Contraception Study. Am J Obstet Gynecol. 2017;216(6):580.e1–9.

Kaaks R, Rinaldi S, Key TJ, Berrino F, Peeters PHM, Biessy C, et al. Postmenopausal serum androgens, oestrogens and breast cancer risk: the European prospective investigation into cancer and nutrition. Endocr Relat Cancer. 2005;12(4):1071–82.

Kaaks R, Tikk K, Sookthai D, Schock H, Johnson T, Tjønneland A, et al. Premenopausal serum sex hormone levels in relation to breast cancer risk, overall and by hormone receptor status – results from the EPIC cohort. Int J Cancer. 2014;134(8):1947–57.

Kahlenborn C, Modugno F, Potter DM, Severs WB. Oral contraceptive use as a risk factor for premenopausal breast cancer: a meta-analysis. Mayo Clin Proc. 2006;81(10):1290–302.

King MC, Marks JH, Mandell JB, New York Breast Cancer Study Group. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. Science. 2003;302:643–6.

Kotsopoulos J, Lubinski J, Moller P, et al. Timing of oral contraceptive use and the risk of breast cancer in BRCA1 mutation carriers. Breast Cancer Res Treat. 2014;143:579–86.

Kotsopoulos J, Huzarski T, Gronwald J, Moller P, Lynch HT, Neuhausen SL, et al. Hormone replacement therapy after menopause and risk of breast cancer in BRCA1 mutation carriers: a case-control study. Breast Cancer Res Treat. 2016;155(2):365–73.

Manson JE, Chlebowski RT, Stefanick ML, Aragaki AK, Rossouw JE, Prentice RL, et al. The Women's Health Initiative hormone therapy trials: update and overview of health outcomes during the intervention and post-stopping phases. JAMA. 2013;310(13):1353–68.

Marchbanks PA, McDonald JA, Wilson HG, Folger SG, Mandel MG, Daling JR, Bernstein L, Malone KE, Ursin G, Strom BL, Norman SA, Wingo PA, Burkman RT, Berlin JA, Simon MS, Spirtas R, Weiss LK. Oral contraceptives and the risk of breast cancer. N Engl J Med. 2002;346:2025–32.

Menes TS, Kerlikowske K, Jaffer S, Seger D, Miglioretti DL. Rates of atypical ductal hyperplasia have declined with less use of postmenopausal hormone treatment: findings from the Breast Cancer Surveillance Consortium. Cancer Epidemiol Biomark Prev. 2009;18(11):2822–8.

Menon U, Karpinskyj C, Gentry-Maharaj A. Ovarian cancer prevention and screening. Obstet Gynecol. 2018;131(5):909–27.

Minami Y, Ohuchi N, Taeda Y, Fukao A, Hisamichi S. Risk factors for benign breast disease according to histopathological type: comparisons with risk factors for breast cancer. Jpn J Cancer Res. 1998;89(2):116–23.

Moorman PG, Havrilesky LJ, Gierisch JM, et al. Oral contraceptives and risk of ovarian cancer and breast cancer among high-risk women: a systematic review and meta-analysis. J Clin Oncol. 2013;31:4188–98.

Mørch LS, Skovlund CW, Hannaford PC, Iversen L, Fielding S, Lidegaard Ø. Contemporary hormonal contraception and the risk of breast cancer. N Engl J Med. 2017;377(23):2228–39.

Newcomer LM, Newcomb PA, Trentham-Dietz A, Longnecker MP, Greenberg ER. Oral contraceptive use and risk of breast cancer by histologic type. Int J Cancer. 2003;106:961–4.

Ozanne EM, Wittenberg E, Garber JE, Weeks JC. Breast cancer prevention: patient decision making and risk communication in the high risk setting. Breast J. 2010;16(1):38–47.

Pickar JH, Boucher M, Morgenstern D. Tissue selective estrogen complex (TSEC): a review. Menopause. 2018;25(9):1033–45.

Primic-Žakelj M, Košmelj K. Menarche, menopause, and breast cancer risk: individual participant meta-analysis, including 118 964 women with breast cancer from 117 epidemiological studies. Lancet Oncol. 2012;13(11):1141–51.

Rebbeck TR, Friebel T, Wagner T, Lynch HT, Garber JE, Daly MB, et al. Effect of short-term hormone replacement therapy on breast cancer risk reduction after bilateral prophylactic oophorectomy in BRCA1 and BRCA2 mutation carriers: the PROSE Study Group. J Clin Oncol. 2005;23(31):7804–10.

Rohan TE, Miller AB. A cohort study of oral contraceptive use and risk of benign breast disease. Int J Cancer. 1999;82(2):191–6.

Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. JAMA. 2002;288(3):321–33.

Sanders ME, Page DL, Simpson JF, Schuyler PA, Dale Plummer W, Dupont WD. Interdependence of radial scar and proliferative disease with respect to invasive breast carcinoma risk in patients with benign breast biopsies. Cancer. 2006;106(7):1453–61.

Sismondi P, Biglia N, Ponzone R, Ambroggio S. Hormone replacement therapy, insulin-like growth factor I and breast cancer. In: Genazzani AR, editor. Hormone replacement therapy and cancer. The current status of research and practice. Boca Raton: The Parthenon Publishing Group; 2002. p. 78–85.

Technical paper No. 2017–12 – Who collects what on the current use of contraception? A review of survey data available for the estimation of contraceptive prevalence.

Vessey M, Yeates D. Oral contraceptives and benign breast disease: an update of findings in a large cohort study. Contraception. 2007;76(6):418–24.

Visvanathan K, Chlebowski RT, Hurley P, Col NF, Ropka M, Collyar D, Morrow M, Runowicz C, Pritchard KI, Hagerty K, Arun B, Garber J, Vogel VG, Wade JL, Brown P, Cuzick J, Kramer BS, Lippman SM, American Society of Clinical Oncology. American society of clinical oncology clinical practice guideline update on the use of pharmacologic interventions including tamoxifen, raloxifene, and aromatase inhibition for breast cancer risk reduction. J Clin Oncol. 2009;27(19):3235–58.

Vorherr H. Fibrocystic breast disease: pathophysiology, pathomorphology, clinical picture, and management. Am J Obstet Gynecol. 1986;154:161–79.

Waters EA, McNeel TS, Stevens WM, Freedman AN. Use of tamoxifen and raloxifene for breast cancer chemoprevention in 2010. Breast Cancer Res Treat. 2012;134(2):875–80.

Yaghjyan L, Tamimi RM, Bertrand KA, Scott CG, Jensen MR, Pankratz VS, et al. Interaction of mammographic breast density with menopausal status and postmenopausal hormone use in relation to the risk of aggressive breast cancer subtypes. Breast Cancer Res Treat. 2017;165(2):421–31.

Yu JH, Kim MJ, Cho H, Liu HJ, Han SJ, Ahn TG. Breast diseases during pregnancy and lactation. Obstet Gynecol Sci. 2013;56(3):143–59.

Zhu H, Lei X, Feng J, Wang Y. Oral contraceptive use and risk of breast cancer: a meta-analysis of prospective cohort studies. Eur J Contracept Reprod Health Care. 2012;17(6):402–14.

Index

F. Petraglia, B. C. Fauser (eds.), *Female Reproductive Dysfunction*, Endocrinology,
https://doi.org/10.1007/978-3-030-14782-2