Reproductive Medicine for Clinicians

Joseph G. Schenker · John J. Sciarra
Liselotte Mettler · Andrea R. Genazzani
Martin Birkhaeuser *Editors*

Reproductive Medicine for Clinical Practice

Medical and Surgical Aspects

The International Academy of Human Reproduction

Springer

Reproductive Medicine for Clinicians

This series will focuses on and presents developments in knowledge and practice within all aspects of reproductive medicine.
It will help to cover the important gap between the new possibilities offered by the most recent investigations and technical developments and the application in clinical practice.
The series will be a useful tool for professionals and practitioners in the fields of Gynecology, Obstetrics, and Human Reproduction. Trainees interested in the most complete information on the developments of reproductive medicine will benefit as well.

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Foreword

This first volume of the series of the International Academy of Human Reproduction focuses on new aspects of reproductive medicine, from the professional responsibility model of ethics to the areas of high clinical involvement in human reproduction, such as endometriosis, polycystic ovary, family planning, and postcoital contraception.

The book discusses fertility and assisted reproductive techniques in the context of genetics and epigenetics as well as psychosomatic and longevity aspects. In addition, it presents new technologies and therapeutic strategies to improve IVF results and prevent ovarian hyperstimulation syndrome (OHSS), as well as the new challenges and the future of imaging in reproduction. Menopause and the effects of estrogens on atherosclerosis prevention and mood and more generally the reproductive hormones' impact on dementia and healthy aging are also covered. Further, it includes a section devoted to innovative aspects of gynecological surgery, discussing the treatments of vaginal aplasia, reproductive microsurgery, and technological breakthroughs in pelvic organ prolapse surgery. Last but not least, it examines the syndromic aspects of preterm birth.

This volume is a useful and comprehensive tool for gynecologists, obstetricians, endocrinologists, and all specialists who deal with women's reproductive health.

Jerusalem, Israel — Joseph G. Schenker

Series Preface

The issue introduces a new series book *Reproductive Medicine for Clinicians* published on behalf of the International Academy of Human Reproduction.

The main objectives of the academy are to extend the knowledge in all aspects of human reproduction, to encourage clinical experience and promote scientific thoughts and investigation, and to consider the ethical and social implications of the current practice of human reproduction. The fellows of the academy are elected based on their significant contribution to the field and must be acknowledged as world leaders in the discipline.

The *Reproductive Medicine for Clinicians* book series will cover the clinical science and medical aspects of reproductive physiology, pathology, and endocrinology, including andrology, gonad function, gametogenesis, fertilization, embryo development, implantation, early pregnancy, genetics, genetic diagnosis, oncology, infectious disease, surgery, contraception, infertility treatment, psychology, ethics, and social issues. This series focuses on and presents developments in knowledge and practice within all aspects of reproductive medicine. The contents include original articles, reviews, and views.

It will help to cover the important gap between the new possibilities offered by the most recent investigations and technical developments and the application in clinical practice.

The series will be a useful tool for professionals and practitioners in the fields of gynecology, obstetrics, and human reproduction. Trainees interested in the most complete information on the developments of reproductive medicine will benefit as well.

The books will maintain the highest clinical and scientific standards in these matters under the guidance of active editorial board.

The *Reproductive Medicine for Clinicians* book series will be published twice per year.

On behalf of the International Academy of Human Reproduction (IAHR), I trust you will support to sustain a high-quality book series devoted to human reproduction.

Jerusalem, Israel — Joseph G. Schenker

Preface

Human reproductive medicine deals with the prevention, diagnosis, and management of reproductive problems.

Many advances in this field have come about mainly as a result of discoveries by scientists working in completely different fields, such as physiology, biochemistry, endocrinology, immunology, genetics, and the pathology of reproduction in both human and other animal species.

Reproductive medicine addresses medical conditions related to puberty, infertility, contraception, family planning, menopause, fertility preservation, and sexual dysfunction.

Advances in human reproductive medicine were not only scientifically important but also significant steps in female empowerment.

Sellman Aschheim and Bernard Zondek developed the "Pregnancy test," a major product of reproductive endocrinology. Invented in Berlin in 1927, it launched the modern era of obstetric knowledge, allowing women to know if they are pregnant in the early stages of gestation.

The introduction of "The Pill" allowed for the separation of sex and procreation and as such gave women more control over their bodies and improved the well-being of single women as well as those in marriages or relationships. The development of medical contraceptive technology created a shift in the balance of power between men and women by affecting fertility decision rights.

Global data in 2010 showed that 1.9% of women aged 20–44 suffered from primary infertility and 10.5% from secondary infertility. Infertility is a central issue in the lives of the individuals who suffer from it. It is a source of social and psychological suffering for both men and women.

Since the birth of Louise Brown, the first child born as a result of *in vitro* fertilization in 1978 (Edwards and Steptoe), IVF has become a routine and widely accepted treatment for infertility. Since her birth, around eight million children have been born worldwide as the result of assisted reproductive technologies (ART).

Assisted reproductive technology (ART) is defined as all treatments or procedures that include the *in vitro* handling of both human oocytes and sperm, or of embryos, for the purpose of establishing a pregnancy. This includes, but is not limited to, *in vitro* fertilization and embryo transfer, gamete intrafallopian transfer, zygote intrafallopian transfer, tubal embryo transfer, gamete and embryo cryopreservation, oocyte and embryo donation, and gestational surrogacy.

Assisted reproductive technology brings about a complete separation of reproduction from sex. It opens up new possibilities for reproduction without sex, some of which give rise to important ethical, legal, religious, and social questions.

The International Academy of Human Reproduction (IAHR) has decided to publish a series entitled *Reproductive Medicine for Clinical Practice*.

The objectives of the academy are (1) to extend the knowledge in all aspects of human reproduction, fertility and infertility, and family planning, as well as population affairs; (2) to encourage exchange of clinical experience and promotion of scientific thought and investigation; and (3) to consider the ethical and social implications of the current practice of human reproduction and reproductive biology.

The members of the academy are selected from among applicants from the fields of clinical medicine, medical education, medical and biological sciences, and other fields related to reproductive health and medicine. Members are elected based on their singular and significant contributions to the field and must be acknowledged as world leaders in their discipline.

Starting in 1974 in Rio de Janeiro, the IAHR has held successful congress every 3 years in Europe, Asia, Africa, the Americas, and Australia. Our congress promotes excellence in reproduction and aims to bridge the gap between the expansion of information and its implementation in clinical practice.

The series *Reproductive Medicine for Clinical Practice*, published by Springer, will provide background to many areas of human reproduction and highlight the issues of women's health.

To facilitate this, the chapters will be written by acknowledged pioneers and experts from each area of human reproduction.

The volumes of *Reproductive Medicine for Clinical Practice* will be of enormous value to clinicians, scientists, all students of the biomedical sciences, and other individuals interested in women's health issues.

Jerusalem Israel — Joseph G. Schenker
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The Professional Responsibility Model of Ethics in Obstetrics and Gynecology

1

Frank A. Chervenak and Laurence B. McCullough

1.1 Introduction

Obstetrician-gynecologists providing reproductive medical care to female and pregnant patients are well aware that they need to be prepared to identify and responsibly manage ethical challenges in clinical practice. Guidance for doing so is provided by the professional responsibility model of ethics in obstetrics and gynecology [1]. In this paper, we identify the key components of this model and its implications for managing threats to professionally responsible reproductive medical care.

1.2 The Role of Professional Virtues in the Professional Responsibility Model of Ethics in Obstetrics and Gynecology

Two remarkable eighteenth-century physicians were the first in the history of medical ethics to articulate the ethical concept of medicine as a profession and the professional virtues that should guide physicians. John Gregory (1724–1773) [2, 3] and Thomas Percival (1740–1804) [4] aimed to correct the entrepreneurial, self-interested, and guild-interest practice of British medicine in the eighteenth century

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and transform medicine into a true profession [2]. Physicians in reproductive medicine face similar challenges, especially in countries such as the United States in which there is comparatively little regulation of clinical practice by the state.

Gregory studied medicine in Edinburgh and then Leiden. Both medical schools were deeply influenced by Francis Bacon's (1561–1626) philosophy of medicine. Bacon called for physicians to practice medicine on the basis of "experience," the carefully observed results of natural and controlled experiments in patient care [2, 3].

Gregory became professor of medicine at the University of Edinburgh in 1766, where he gave lectures on medical ethics before to his students before they began their clinical instruction at the Royal Infirmary of Edinburgh [2, 3]. His goal was to end clinical practice based on relentless pursuit of self-interest in a fiercely competitive market. "Man midwives," physicians trained in obstetrics and exclusive users of forceps, faced intense competition from female midwives. Gregory's concern was that pregnant women were exploited by the pursuit of self-interest, which needed to be replaced by professionalism in clinical practice [2, 3].

Gregory defines the professional physician by the commitment to intellectual excellence, which requires the physician to become and remain scientifically and clinically competent. Physicians should submit clinical judgment and practice to the discipline of Bacon's "experience"-based reasoning, thus anticipating what is now known as evidence-based medicine. The professional physician is also defined by the commitment to moral excellence, which requires the physician to make the protection and promotion of the patient's health-related interests the physician's primary concern and motivation and to keep both individual and group self-interest consistently secondary [3, 5]. Percival emphasizes this component when he called for physicians to treat medicine, not as a merchant guild but as a "public trust" [4, 5].

Gregory and Percival identified the clinical implications of the concept of medicine as a profession by appealing to four professional virtues [6]. The first is *self-effacement*, which obligates physicians not to be influenced by clinically irrelevant personal and social differences between physicians and their patients. The second is *self-sacrifice*, the willingness to risk individual self-interest, especially in the economic domain, in order to provide evidence-based care. The third is *compassion*, which obligates physicians to recognize when patients experience pain, distress, and suffering and to prevent and relieve patients from these. The distress and suffering of infertility can sometimes become clinically very significant. To fulfill this obligation, physicians should routinely ask their stressed patients "What can I do to help?" The fourth virtue is *integrity*, which obligates physicians to practice medicine to standards of intellectual and moral excellence. Intellectual excellence requires clinical care to have the strongest possible evidence base. Moral excellence requires putting the interests of patients first and keeping individual and group self-interest systematically secondary. Adherence to self-effacement, self-sacrifice, and compassion is essential for achieving moral excellence. There is therefore a synergistic bond among the four professional virtues.

1.3 Managing Threats to Professionally Responsible Reproductive Medical Care

Obstetrician-gynecologists can use the professional responsibility model of ethics in obstetrics and gynecology to responsibly manage threats to professionalism, which have major implications for clinical practice and are therefore neglected at the physician's ethical peril. The history of political philosophy provides context. Niccolo Machiavelli (1469–1527) contributed an eponymous adjective to the English language, Machiavellian, which connotes actions based on cunning or, worse, bad faith [7]. Threats to professionalism emerge when the appearance of professionalism masks the neglect or absence of professionalism, especially in an organization's culture. The result is organizational dysfunction, which, from the perspective of professionalism in medicine, is an ethical pathology, which can, like other pathologies, be staged. There are three stages of dysfunctional organizational cultures in reproductive medicine [8].

1.3.1 Stage 1: Cynicism

Stage 1 organizational dysfunction is a cynical organizational culture. The defining symptom of this stage is a deteriorating connection between organizational rhetoric and reality and a defensive posture of leadership in response to criticism. For example, a colleague dean may extol the virtues of excellent patient care but not meet its evidence-based standards. In such a culture, physicians committed to professionalism need to form moral enclaves that provide strength and support for efforts to confront and reform this incipient deterioration of professionalism [8].

1.3.2 Stage 2: Wonderland

Stage 2 organizational dysfunction is a wonderland culture [6]. The defining symptom of this stage is self-deceptive rhetoric. For example, a reproductive group reports only pregnancy rates and suppresses take-home baby rates and complications. Pointing this out will prompt denial and accusations of disloyalty. In such a culture, physicians committed to professionalism need to strengthen their moral enclaves and vigorously seek to resist and expose self-deception as antithetical to professionalism [8].

1.3.3 Stage 3: Kafkaesque

Stage 3 organizational dysfunction is a Kafkaesque culture. The defining symptom of this state is dissociative organizational rhetoric and reality. For example, a reproductive medicine group completely ignores take-home baby rates in touting success to new patients. The response to criticism is threats, for example, in the form of

"You might be happier elsewhere." In such a culture, physicians committed to professionalism need to further strengthen their moral enclaves against organizational assault on professional integrity. When it has become futile to change the culture, it is time to leave, to preserve one's professionalism [8].

Conclusion

The professional responsibility model of ethics in obstetrics and gynecology guides physicians in reproductive medicine by providing the ethical foundations for professionally responsible clinical practice. We have emphasized the importance of a professional organizational culture that sustains the professional virtues of self-effacement, self-sacrifice, compassion, and integrity. Sustaining these professional virtues in reproductive medicine will support physicians in preventing what professionalism prohibits: undermining professional responsibility by allowing the emergence of organizational cultures that are antithetical to the life of service to patients. We have provided a clinical ethical taxonomy of dysfunctional organizational cultures and remedies for them guided by the professional medical ethics of Drs. Gregory and Percival. Such organizational cultures would be devoid of moral worth occupied by physicians who, in the words of T.S. Eliot, "would be hollow men and stuffed men working in the dead land" [9].

References

1. Chervenak FA, McCullough LB, Brent RL. The professional responsibility model of obstetric ethics: avoiding the perils of clashing rights. Am J Obstet Gynecol. 2011;205:315.e1–5.
2. LB MC. John Gregory and the invention of professional medical ethics and the profession of medicine. Dordrecht, Netherlands: Kluwer Academic Publishers; 1998.
3. Gregory J. Lectures on the duties and qualifications of a physician. London: W. Strahan and T. Cadell, 1772. In: LB MC, editor. John Gregory's writings on medical ethics and philosophy of medicine. Dordrecht, Netherlands: Kluwer Academic Publishers; 1998. p. 161–245.
4. Percival T. Medical ethics, or a code of institutes and precepts, adapted to the professional conduct of physicians and surgeons. London: Russell and Johnson, 1803. In: Pellegrino ED, editor. Medical ethics by Thomas Percival. The classics of medicine library. Birmingham, AL: Gryphon Editions; 1985.
5. McCullough LB. The ethical concept of medicine as a profession: its origins in modern medical ethics and implications for physicians. In: Kenny N, Shelton W, editors. Lost virtue: professional character development in medical education. New York: Elsevier; 2006. p. 17–27.
6. Chervenak FA, McCullough LB. The moral foundation of medical leadership: the professional virtues of the physician as fiduciary of the patient. Am J Obstet Gynecol. 2001;184:875–80.
7. Machiavelli N. The prince: a revised translation. Adams RM, trans. New York: Norton; 1992.
8. Chervenak FA, McCullough LB. The diagnosis and management of progressive dysfunction of health care organizations. Obstet Gynecol. 2005;105:882–7.
9. Eliot T. The hollow men. In: Untermeyer L, editor. Modern American poetry. New York: Harcourt Brace & World; 1962. p. 395–6.

2 Human Reproduction: From State of the Art to Future Developments "Endometriosis Therapeutic Approaches"

Liselotte Mettler and Ibrahim Alkatout

2.1 Introduction

As the impact of endometriosis on the health of women in this more than 300 years ago first-described disease remains of high importance, one of our academy focus highlights this disease. Novel insights into the pathogenesis of endometriosis as well as the art of clinical markers and new surgical possibilities allow a better treatment of clinical symptoms: chronic pelvic pain, inflammation, dysmenorrhea, subfertility, and disturbances in reproduction. I would like to highlight the mechanism behind vascularization and immune factors in endometriosis and discuss the current pharmaceutical options for pain management and surgical excision for our patients.

Endometriosis is affecting an estimated 176 million females of reproductive age; worldwide endometriosis is considered the second most common benign female genital disease after uterine myomas [1]. It has been defined as the presence of endometrial glands and stroma outside the internal epithelial lining of the uterine cavity. Endometrial implants are typically situated in the pelvis (genital endometriosis) but can occur anywhere (extragenital endometriosis) (Fig. 2.1). Figure 2.2 reveals the histopathological picture of an endoscopic lesion.

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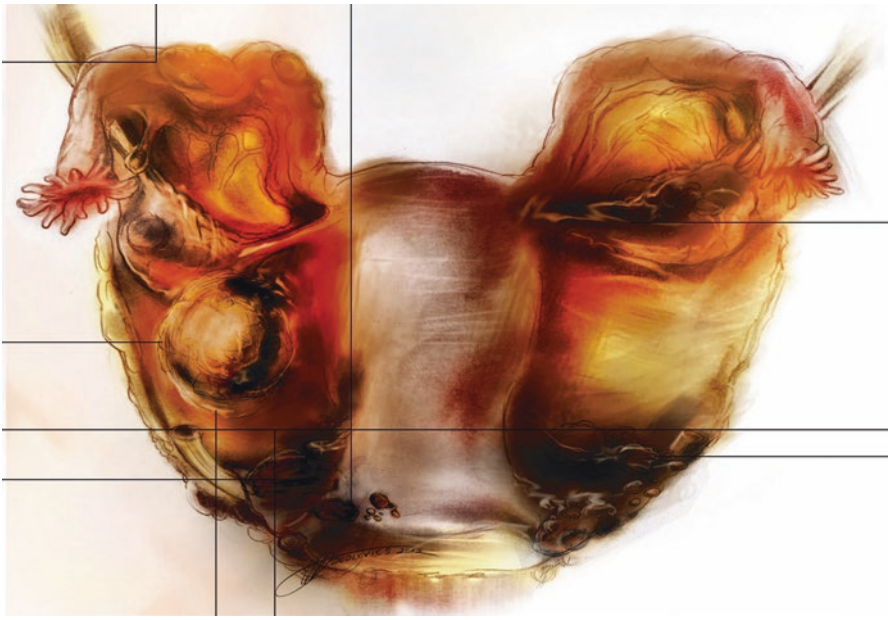

Fig. 2.1 Overview of the typical locations of endometriosis genitalis externa. Implants can be raised flame-like patches, whitish opacifications, yellow-brown discolorations, translucent blebs, or reddish irregularly shaped spots

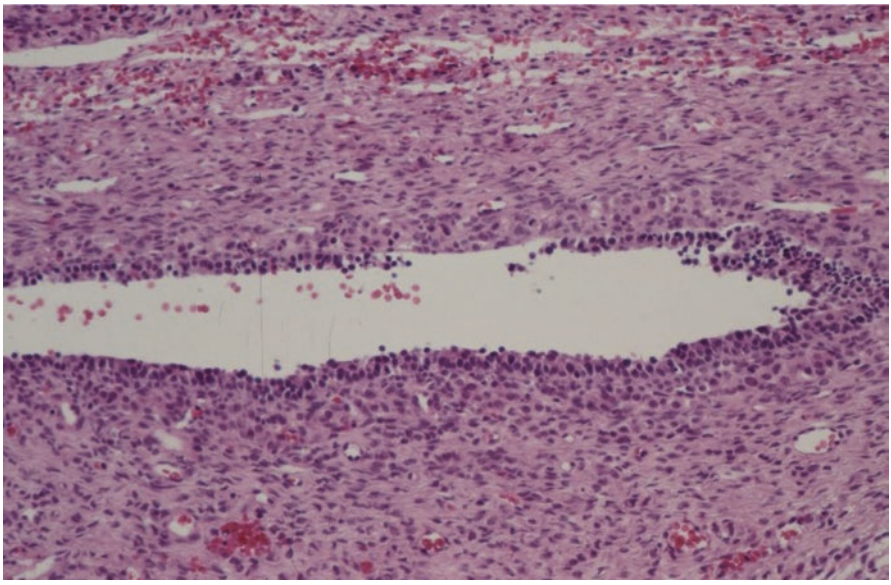

Fig. 2.2 Hematoxylin-eosin (H&E staining, EEC stage II) section of an endometriotic lesion in a 32-year-old patient on cycle day 8 showing endometriosis in the fibro-muscular stroma and an unspecific chronic fibrotic infectious reaction with some blood residuals. The subepithelial stroma tissue resembles endometrial glands and stroma. Neutrophil granulocytes or lymphocytes can hardly be found

2.2 Therapeutic Strategies

The recently established international consensus statement on the current management of endometriosis [1] with the engagement of 56 representatives of 34 national and international medical and nonmedical organizations and persons led to the assumption of endometriosis being a chronic disease with multifaceted appearances and treatment options.

1. *Medical treatment.* In the past the main strategy was the induction of a pseudopregnancy and the application of gestagens and later danazol and GnRH analogues [2]. Up to now, this theory has been regarded as the "gold standard," but it is now supplemented by a simple progesterone (dienogest—mg per day) treatment or a GnRH analogue treatment with add-back therapy [3]. To prevent side effects of the GnRH agonist, such as bone demineralization, vasomotor symptoms, and mood swings, a serum estradiol concentration of approximately 60 pg/mL is required [2, 4–6]. Every medical treatment today is well tolerable but should only be used as long as necessary. In case it is used as longtime treatment, it should reduce the number of surgical interventions and improve the quality of life.

   Inhibition of mediators. Research work has focused on inhibiting the interaction of various mediators which maintain the illness by way of inflammatory processes, vascularization, and cell proliferation. Specific aromatase inhibitors (such as letrozole, anastrozole, or exemestane) or selective COX-2 inhibitors (e.g., celecoxib, rofecoxib) are of great interest and have been studied in clinical trials [7–9]. There is no proven evidence that one medical therapy is superior to another in the treatment of the clinical symptoms of endometriosis or infertility.
2. *Surgical treatment.* As endometriosis is a progredient disease, which can cause the anatomic destruction of the reproductive organs, surgical therapy plays an important role. Laparoscopy provides the only possibility to ascertain the expected diagnosis of endometriosis. Endometriosis has a varying phenotype and can appear as raised flame-like patches, whitish opacifications, yellow-brown discolorations, translucent blebs, or reddish irregularly shaped spots (Fig. 2.1) [10, 11]. In advanced stages, pain and sterility are predominantly caused by organ damage, fibrosis, and adhesions, thus constituting a clear indication for surgical intervention. Early laparoscopy can prevent any delays in diagnosis of the disease or symptom progression. The importance of laparoscopy with biopsy and/or resection is reinforced as visual diagnosis alone can often lead to a misdiagnosis [12, 13]. Risk factors and disadvantages of laparoscopy include damage of organs adjacent to the affected areas and postoperative complications, such as adhesion formation or infection [14–18]. Symptom relief is achieved in most patients after successful ablation/resection of endometriosis and adhesiolysis. Nevertheless, the recurrence rate is as high as 40% after a 10-year follow-up [17, 19–21].
3. *Combined surgical and medical treatment.* The combined treatment involves diagnostic laparoscopy, removing all visible endometriosis foci as far as possible,

a 3- to 6-month endocrine therapy, and a subsequent second-look laparoscopy with resection of residual foci, adhesiolysis, and reconstruction of organs [9, 20–24]. Despite maximal efforts, the therapy of first choice in the management of endometriosis is still unclear [15, 25].

2.3 Material and Methods (Part I)

In the following, we focus on current treatment possibilities, pain, fertility, and the obstetrical outcome in endometriosis patients.

In a recent study, 450 endometriosis patients underwent 1 of 3 different therapeutic strategies (medication, surgical, or combined treatment) at the Kiel University Department of Obstetrics and Gynecology [26]. The evaluation aims at determining the most successful of the available endometriosis therapies.

2.3.1 Patients

Informed consent forms were completed by all patients. This study, which included operation, medical treatment, and a selected second-look operation, was approved by the Ethical Committee of the Christian-Albrechts-University Kiel, Germany (D 426/10). Each patient signed an informed consent form for the use of his specimen and clinical data.

The study comprised 450 symptomatic endometriosis patients (18–44 years of age) for whom 2 consecutive laparoscopic interventions were to be assessed. There were pain and/or infertility patients. Four hundred and ten patients from the original collective returned for a second-look laparoscopy (Fig. 2.3).

Endometriosis was diagnosed or confirmed by laparoscopy and rated according to the endoscopic endometriosis classification (EEC) introduced by Kurt Semm and Liselotte Mettler (Fig. 2.4) [27] which compares well to the r-AFS classification.

2.3.2 Exclusion Criteria

Previous surgery and hormone therapy for endometriosis were exclusion criterion, as were deep infiltrating endometriosis with bladder or rectum excision. The treatment of deep infiltrating endometriosis with big lesions affecting bowel and/or urinary tract, favorably diagnosed before surgery, was performed via extensive laparoscopic resection.

Figure 2.5 differentiates stages I, II, and III in the laparoscopic appearance.

2.3.3 Tissue Samples

Samples of ectopic endometrium ($n = 450$) were obtained from patients undergoing diagnostic hysteroscopy and laparoscopy for the treatment of endometrioma.

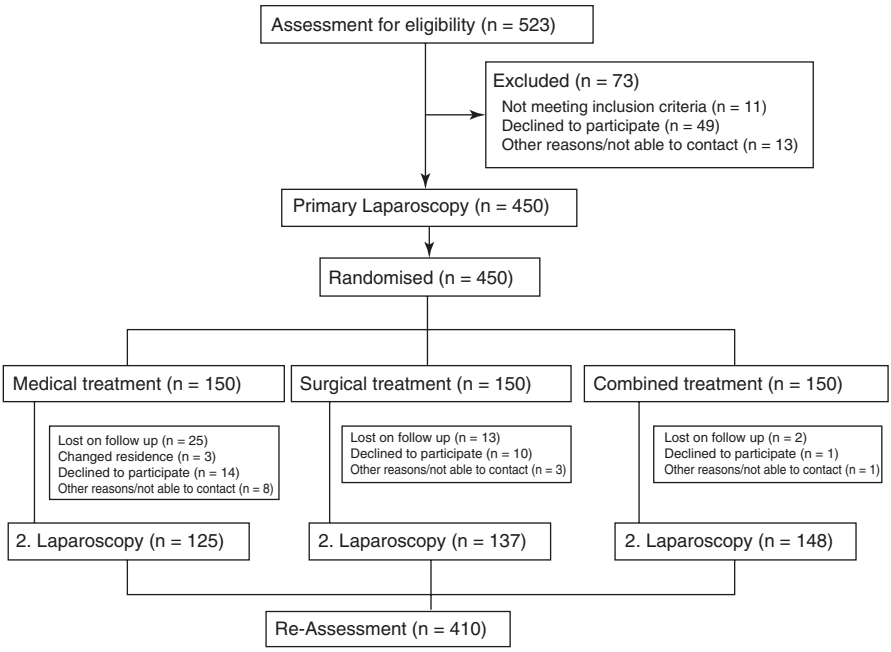

Fig. 2.3 Trial profile differentiating medical, surgical, and combined treatment of endometriosis

The patients ranged in age from 18 to 44 years and received no hormonal treatment prior to surgery. Cryostat sections were prepared and stained with hematoxylin-eosin. Histopathological assessment confirmed the site of origin, i.e., proliferative endometrium or endometrioma cyst wall, respectively.

2.3.4 Interventions

The 450 patients were randomly distributed to the following 3 treatment groups, 150 per group. Of the original 450 patients, 410 returned for the second-look pelviscopy, and their findings were assessed:

Group 1 (n = 125) underwent hormonal treatment after diagnostic laparoscopy with 3.75 mg of leuprorelin acetate depot which was injected subcutaneously in monthly intervals over 3 months. Leuprorelin acetate depot is a GnRH agonist and is commercially available in Germany as Enantone Gyn Depot.

Group 2 (n = 137) underwent surgical laparoscopy without any subsequent medical treatment. Endometriosis foci were totally excised and adhesions removed, and the normal anatomy of the reproductive organs was restored. Ureter and superficial bowel lesions were removed. For infertility patients, tubal patency was checked, and chromopertubation was performed at the second-look laparoscopy. Patients with deep infiltrating endometriosis with bladder or rectum resection were not included in the study.

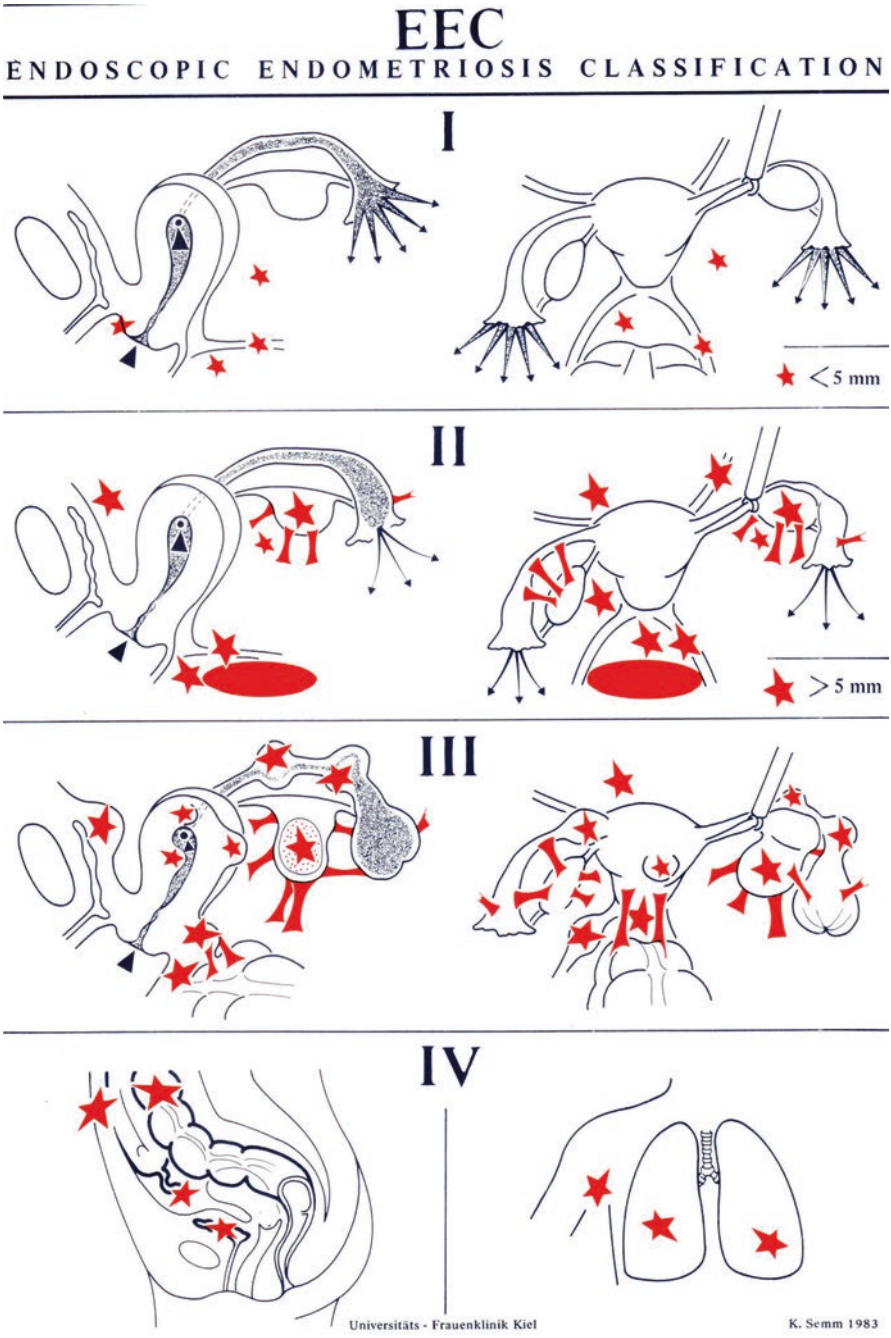

Fig. 2.4 The EEC system used to classify endometriotic lesions. In contrast to the rASRM classification, the EEC classification includes extragenital endometriosis and is divided into four stages

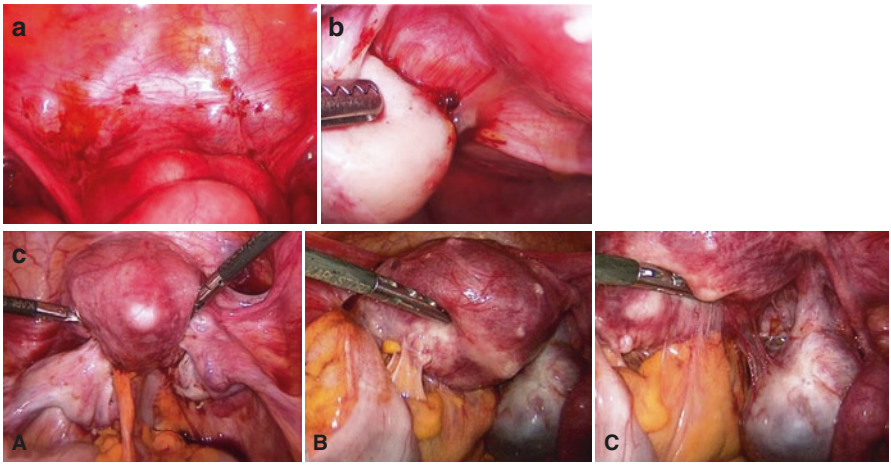

Fig. 2.5 Endoscopic image of endometriosis EEC stage I (**a**), EEC stage II (**b**), EEC stage III (**c**: A–C)

Group 3 (n = 148) underwent the same hormonal therapy as group 1 over the same time period after surgical laparoscopy. The combined or three-step therapy comprised diagnostic laparoscopy, removal of all visible endometriosis foci, a 3-month endocrine therapy with GnRH agonists (e.g., 3.75 mg of leuprorelin acetate depot), and a subsequent second-look laparoscopy 1–2 months after conclusion of the hormonal therapy with resection of residual foci and reconstructive surgery of organs.

The same team of physicians performed the primary and secondary intervention as well as the primary and secondary endometriosis staging according to the EEC [27, 28]. For groups 1 and 3, a second-look laparoscopy was performed 1–2 months after hormonal therapy and for group 2, 5–6 months after surgical endometriosis treatment. After the second-look laparoscopy, patients were monitored over a period of 2 years and completed an extensive questionnaire to determine recurrence of symptoms and pregnancy rates.

2.3.5 Maine Outcome Measures

The central issue for this study was: Which endometriosis therapy is currently the most successful technique? The success of each therapeutic strategy was assessed—independent of the original EEC stage—according to the following criteria after the second-look laparoscopy:

1. A response rate to EEC stages 0 and I of at least 75%
2. The lowest recurrence rate
3. The highest pregnancy rate

Within the framework of this study, the endometriosis therapy that fulfilled all of the criteria or at least two of them was regarded as the most successful therapy.

2.3.6 Statistical Evaluation and IRB Approval

Our results were statistically evaluated with the chi-squared test and analyzed with a significance level of $p < 0.05$ and a confidence interval of 95%. Institutional review board approval was obtained at the beginning of the study.

2.3.7 Results (Part I) for Extend of Endometriosis, Fertility, and Pain

Results in the three treatment groups were analyzed to assess the new endometriosis staging or EEC downstaging. There was no significant difference between the groups insofar as distribution of EEC stages before treatment (p 5 = 0.11). After the individual treatment, the distribution of EEC stages indicated a significant difference between the three groups ($p = 0.01$).

The shift in the CI is indicative of a higher rate of cure in patients in group 3 compared with groups 1 and 2. This was most remarkable for EEC stage 0 (Table 2.1).

At the onset of the study, in the 125 patients in group 1 (hormone therapy), disease stage was EEC I in 40%, ECC II in 38%, and EEC III in 22%. After hormone therapy, and independent of the previous EEC stage, disease stage was EEC I in 32% of patients, EEC II in 13%, and EEC III in 5%. In 50% of the patients, second-look laparoscopy showed no signs of endometriosis (EEC 0). In these patients, the disease seemed to be cured (cure rate = 50%). In the 137 patients in group 2 (surgical treatment), disease stage was EEC I in 50%, EEC II in 32%, and EEC III in 18%.

Table 2.1 Distribution of patients to EEC stages before ($p = 0.105$) and after therapy ($p = 0.010$)

Therapy methods	EEC stage							
	EEC 0	CI	EEC I	CI	EEC II	CI	EEC III	CI
Group 1 hormonal (n = 125)								
Before therapy	0	–	50 (40%)	31.3–49.1	47 (38%)	29.1–46.7	28 (22%)	15.4–30.7
After therapy	62 (50%)	40.5–58.7	40 (32%)	23.9–40.9	16 (13%)	7.4–20.0	7 (5%)	2.3–11.2
Group 2 surgical (n = 137)								
Before therapy	0	–	69 (50%)	41.7–59.0	44 (32%)	24.4–40.6	24 (18%)	11.5–24.9
After therapy	75 (55%)	46.0–63.3	20 (13%)	9.2–21.6	30 (23%)	15.3–29.8	12 (9%)	4.6–14.8
Group 3 combined (n = 148)								
Before therapy	0	–	79 (53%)	45.0–61.1	36 (24%)	17.7–32.1	33 (23%)	15.9–29.9
After therapy	89 (60%)	51.3–68.1	26 (18%)	11.8–24.7	25 (17%)	11.2–23.9	8 (5%)	2.4–10.4

CI confidence interval

At second-look laparoscopy, the disease could be downstaged to EEC I in 13% of patients, EEC II in 23%, and EEC III in 9%.

The cure rate for the exclusively surgically treated group was 55% (EEC 0). In the 148 patients in group 3 (combined treatment), disease stage was EEC I in 53%, EEC II in 24%, and EEC III in 23%. After combined surgical and hormone therapy, disease stage was EEC I in 18% of the patients, EEC II in 17%, and EEC III in 5%. With combined treatment, the cure rate was 60% (EEC 0).

The three treatment options achieved, independent of the initial EEC stage, an overall cure rate of ≥50%. With combined treatment, the cure rate was 60%, with exclusive hormone therapy was 55%, and with exclusive surgical treatment was 50%. Within the framework of the study, cure was defined as a reduction in disease stage to EEC 0. This new endometriosis downstaging was confirmed at second-look laparoscopy. The best total cure rate was achieved with combined treatment (Table 2.1).

In a second step, we differentiated light, intermediate, and advanced endometriosis and evaluated therapeutic strategies. An improvement of at least 75% to EEC stage 0 or I was defined as highly efficient. These conditions were met with the exclusive hormone therapy, with a rate of 82% (50% EEC 0 and 32% EEC I), and with the combined treatment (three-step therapy), with a response rate of 78% (60% EEC 0 and 18% EEC I) (Table 2.1).

Because endometriosis generally causes recurrent pain, we asked our study patients to complete an extensive questionnaire and report recurrent symptoms, before and at 1 year after the end of all therapeutic activities as a second outcome measure.

Results in the three treatment groups were analyzed to assess the treatment effect considering the recurrence of symptoms of endometriosis. There was no significant difference between the groups insofar as distribution of symptoms before treatment (p 5 = 0.61 for dysmenorrhea, p = 0.59 for dyspareunia, and p = 0.54 for abdominal pain).

After the individual treatment, the distribution of recurrence of symptoms highlights a general reduction in symptoms, with the greatest benefit observed in the combined treatment group.

There was a difference, statistically not significant, for dysmenorrhea between the therapeutic groups (p = 0.05) after treatment. The 95% CI demonstrated a remarkable difference in the treatment effect in all three groups. Nevertheless, the treatment effect was strongest in group 3, followed by group 2. Insofar as dyspareunia, a significant difference was noted between the three treatment groups (p = 0.007). The CIs demonstrated the biggest treatment effect in group 3, followed by group 2. Abdominal pain could not be reduced significantly (p = 0.284). Nevertheless, the CIs showed the biggest effect in group 3, followed by group 1. In group 1 (hormone therapy) at the onset of the study, 60% of the 125 patients had dysmenorrhea, 56% had dyspareunia, and 48% had abdominal pain. The group that received exclusively hormone therapy had the highest recurrence rates: dysmenorrhea in 28% of the patients, abdominal pain in 26%, and dyspareunia in 22%. In group 2 (surgical treatment), 57% of the 137 patients had dysmenorrhea, 50% had

dyspareunia, and 42% had abdominal pain. After follow-up, 20% of the women in this group reported dysmenorrhea, 15% reported dyspareunia, and 24% reported abdominal pain.

In group 3 (combined treatment), 54% of the 148 women had dysmenorrhea, 51% had dyspareunia, and 42% had abdominal pain. Patients in the combined treatment group achieved the lowest general recurrence rate and the lowest recurrence rate per symptom: 16% of the patients reported dysmenorrhea, 8% reported dyspareunia, and 17% reported abdominal pain at 1-year follow-up (Fig. 2.6). In

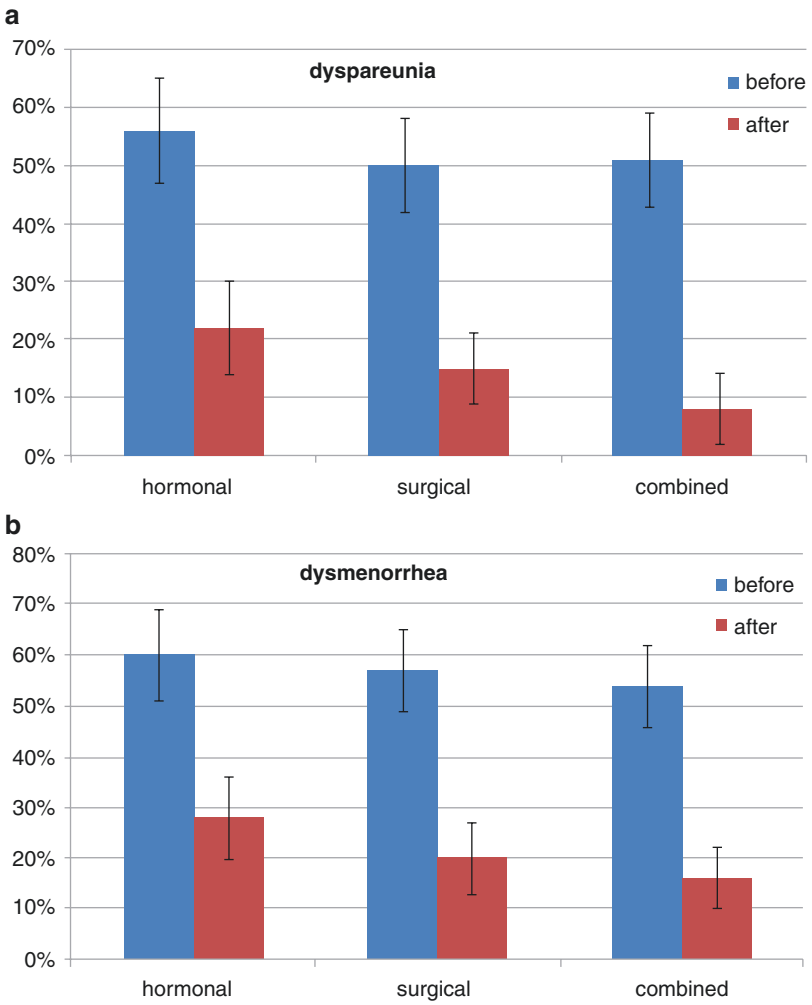

Fig. 2.6 Comparison of recurrence rates of symptoms including dyspareunia (**a**), dysmenorrhea (**b**), and abdominal pain (**c**) for each of the three treatment groups before 1 year after treatment. Therapeutic benefit is supported by the marked confidence intervals

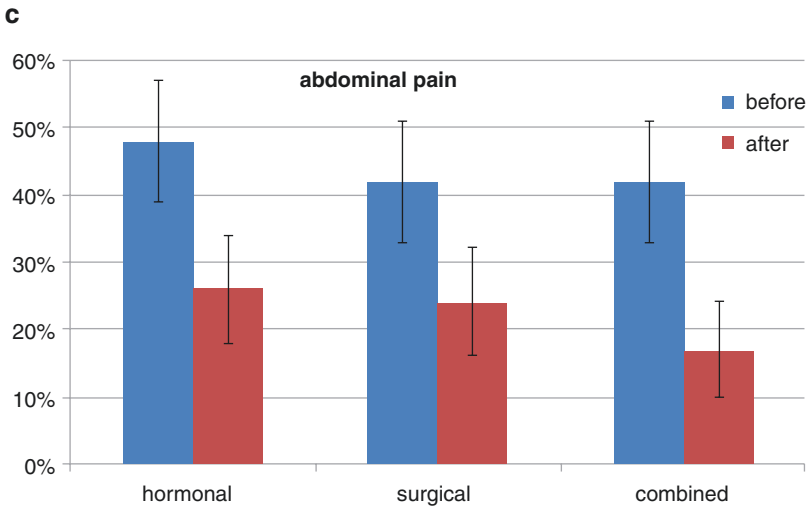

Fig. 2.6 (continued)

Table 2.2 Comparison of pregnancy rates for the three therapy methods after 2 years

Therapy methods	Pregnancies ($p = 0.251$)	Abortions ($p = 0.967$)	Extrauterine pregnancies ($p = 0.653$)	Live births ($p = 0.275$)
Group 1: hormonal ($n = 125$)	81 (65%)	10 (8%)	2 (2%)	69 (55%)
Group 2: surgical ($n = 137$)	75 (55%)	12 (9%)	1 (1%)	62 (45%)
Group 3: combined ($n = 148$)	89 (60%)	13 (9%)	3 (2%)	74 (50%)

comparison with groups 1 and 2, group 3 had significantly better results after treatment ($p < 0.001$).

The third outcome measure was the pregnancy rate. We determined an overall pregnancy rate over 2 years of 55–65% in the three treatment groups, independent of ECC stage (Table 2.2). The pregnancy rate after the exclusive surgical restoration was 55%, after combined treatment was 60%, and after exclusive hormone therapy was 65%.

There was no statistical significance ($p = 0.25$) between these results.

Of these 245 pregnancies, 41 (17%) were not carried to term (6 extrauterine pregnancies [$p = 0.65$] and 35 abortions [$p = 0.97$]). However, 205 children, including 1 set of twins, were born. There was no statistically significant difference between the three therapeutic strategies insofar as the pregnancies and their course ($p = 0.28$).

2.4 Material and Methods and Results (Part II)

2.4.1 Endometriosis and Obstetrical Outcome

The largest study to date of endometriosis in pregnant women has found that the condition is a major risk factor for premature birth [29]. This study was presented at the 25th Annual Conference of the European Society of Human Reproduction and Embryology (ESHRE) in 2009 by Henrik Falconer of the Department of Woman and Child Health, Karolinska Institute, Stockholm, Sweden. His team found that women with endometriosis had a higher risk of pregnancy complications and premature deliveries and were more likely to deliver by Cesarean section.

The researchers investigated the association between adverse pregnancy outcome, assisted reproduction technology (ART), and a previous diagnosis of endometriosis in 1,442,675 single births to Swedish women. They were 13,090 singleton births among 8922 women diagnosed with endometriosis. Compared with women without endometriosis, they had a 33% greater risk of preterm birth.

Women with endometriosis were also more likely to have difficulty in conceiving, and the use of ART was more prevalent, which in itself may be considered a risk factor for adverse pregnancy outcome, although we strongly disagree with this concept. Once conception takes place, the embryo has to prove itself and fight for survival. That certainly promotes a healthy embryo, but, of course, we agree that it is tiring to start the fight for life against possible cytokines or antibodies at that early stage.

Among women with endometriosis, 11.9% conceived after ART compared with the 1.4% of women without endometriosis who used the technique. This means that patients with endometriosis have a better chance of becoming pregnant with ART than without it.

The risk of preterm birth associated with endometriosis among women with ART was 1.24 and among women without ART 1.37.

Dr. Falconer said that endometriosis appears to be a risk factor for preterm birth, irrespective of ART. The findings of the study indicate that women with endometriosis may be considered a high-risk group and need special care during pregnancy.

Endometriosis is a chronic inflammatory disease, affecting up to 15% of all women of reproductive age, in which the endometrial cells that line the uterus are deposited in other areas. Such displacement of endometrial cells can lead to anatomical distortion and also the release of anti-inflammatory cytokines, signaling molecules used in communication between cells. Known symptoms of endometriosis include severe pelvic pain, heavy menstrual periods, and nausea.

One explanation for the interaction of endometriosis with preterm birth has been given by the group of Ivo Brosens with the enigmatic uterine junctional zone [30].

In addition to an increased risk of preterm birth, the researchers also found other differences in the pregnancies of women with endometriosis. Dr. Falconer reported that nearly twice as many women in this group were delivered by Cesarean section. The study group observed that among these women, the risk of induced preterm birth was higher than for spontaneous preterm birth. They believe that women with

endometriosis are more frequently scheduled for preterm Cesarean section, possibly due to placental complications.

Women with endometriosis were also more likely to suffer from preeclampsia, a condition that develops in the second or third trimester of pregnancy and involves the development of high blood pressure and the presence of protein in the urine. However, this is strongly contradicted by others [31].

Antepartal bleeding was also found to be more common among women with endometriosis.

As endometriosis is so strongly associated with infertility, we know that women suffering from endometriosis are of higher maternal age and have fewer children. Even after adjusting for maternal age and other confounding factors, the strong association between endometriosis and risky pregnancies still remains.

Given that endometriosis is relatively common in women of childbearing age, we advise pregnant patients with a history of previous endometriosis to observe extra care, to enable them to have a normal pregnancy and give birth to a healthy baby.

Concerning pain in pregnancy in patients with endometriosis, there are several reports on intensified pain in pregnancy, although others report that pain totally disappears during pregnancy and breastfeeding. Pregnancy appears to be a cure for these patients. However, it is a misconception to believe that pregnancy cures endometriosis. The symptoms usually recur after each pregnancy [32].

2.4.2 Discussion

2.4.2.1 General Remarks

Our team did basic research in endometriosis as for the distribution pattern of the macrophage colony-stimulating factor receptor (M-CSFR) producing cells in endometrial and endometriotic tissue. Membrane-bound M-CSF or soluble M-CSF of peritoneal macrophages may cause the survival of dystopic endometrium by direct interaction, giving rise to endometriosis [33].

We also compared in a cDNA microarray analysis a set of 940 genes expressed in endometrium and endometriosis and identified 38 genes which were differentially expressed in endometriotic implants compared to uterine endometrium [34]. Based on older extensive studies, a lot of further research is necessary [35–37]. Concerning endometriosis and fertility, we advise to use the fertility index of David Adamson [38].

The presented clinical studies comparing medical, surgical, and combined therapy and the assessment of how endometriosis can affect pregnancy and deliveries show the current needs for the treatment of endometriosis and point out some advice for future therapeutic modalities.

2.4.2.2 Three-Step Therapy of Endometriosis

In the presented study, 450 endometriosis patients, aged 18–44 years, were randomly assigned to 1 of 3 different therapeutic strategies (medical, surgical, or combined treatment) at the Kiel University Department of Obstetrics and Gynecology,

Germany. The success of each therapeutic strategy was assessed—independent of the original EEC stage [27]—according to the following criteria:

- The therapy after which the patients achieved the highest cure rate (EEC stage 0)
- A response rate to EEC stages I and 0 of 75% or higher
- The lowest recurrence rate
- The highest pregnancy rate

Within the framework of this study, the endometriosis therapy that fulfilled the majority of the criteria, or at least two of them, was regarded as the most successful therapy. The three treatment options reached an overall cure rate of 50% or higher. There was no statistically significant difference ($p > 0.05$), but with a cure rate of 60%, the combined therapy ranks first. The combined (three-step) and the exclusively hormonal therapy managed to surpass the 75% response rate with 78% and 82%, respectively. Nevertheless, the combined treatment reached the lowest recurrence rate per symptom at a statistically significant level. No statistically significant difference was recorded for the pregnancy rate which ranged between 55 and 65%, independent of the therapeutic strategy. As an overall result, we have been able to confirm the high efficacy of the combined endometriosis therapy in this study.

Medical therapy can be applied prior to surgery to decrease the size of endometriotic implants and the extent of the operation [39]. However, so far there is no clear evidence that perioperative hormonal treatment decreases the extent of operation necessary to remove endometriotic implants, delays or prevents recurrence, or increases pregnancy rates. In contrast, several trials were able to report an increased duration of pain relief and delayed recurrence rates using postoperative medical therapy [11, 39, 40]. Schweppe concluded that in all cases of active endometriosis, pelviscopic treatment alone is not sufficient [41]. Schindler demonstrated that the primary surgical intervention reduced the total r-AFS score (revised American Fertility Society) by 34%, whereas the combined therapy brought about a reduction of 66% [42].

Our study showed only a weak and statistically nonsignificant difference between the combined treatment (decrease of EEC stage by 60%) and the solitary surgical treatment (decrease of EEC stage by 55%). Regidor found a significant improvement of the r-AFS score after treatment with triptorelin (GnRH analogue). Sixty-three percent of these patients were no longer diagnosed with endometriosis, 30% presented with stage I residual endometriosis according to the AFS classification, and only 7% had stage II endometriosis [43]. It could be demonstrated that after administration of buserelin (GnRH analogue), the average AFS score went down from 17.4 ± 12.9 before therapy to 7.2 ± 8.2 after a 6-month therapy [42, 44]. Although up to 90% of patients experience some symptom relief with medical therapy, medical treatment alone neither enhances fertility, diminishes pelvic mass, nor removes adhesions [2, 11].

Similar to Schweppe and Römer [41, 45], we also determined a significantly lower recurrence rate after application of the three-step therapy. Römer reported that retrospective analyses 12–48 months after endometriosis therapy presented a

recurrence rate for hormonal and surgical treatment (three-step concept) of only 16.7%, whereas the recurrence rate for exclusively surgically treated patients was 47%.

Regidor showed in a long-term follow-up study that 70 of 112 patients (62.5%) again reported ailments and that the recurrence-free interval amounted to an average of 11 months after finishing the three-step therapy (with the GnRH analogue leuprorelin acetate) [46]. In another long-term follow-up study, Schindler established recurrent endometriosis in 62 of 112 patients (55%) after a combined surgical-hormonal therapy [2, 34]. Our recurrence rate (41%) was lower than Regidor's rate (62.5%) and Schindler's rate (55%) for the combined therapy. Zupi et al. were able to show that patients treated with GnRH agonists had a significantly higher rate of symptom reduction (pelvic pain, dysmenorrhea, and dyspareunia) than women treated with continuous estrogen-progestin oral contraceptives. Quality of life was increased by extending the GnRH treatment to include add-back therapy [4]. Other investigations comparing oral contraceptives to GnRH agonists found an equal reduction of pain [47]. Sutton 1994 and Abbott 2004 performed a second-look laparoscopy 6–12 months after the primary operation and found that 29–45% of the patients had disease progression, 22–29% disease regression, and 33–42% the disease remained static [19, 48].

Endometriosis can reduce the fecundability rate without completely preventing conception. Impaired fertility might be due to anatomic variations after adhesion formation and endometriomas [49]. An enhancement of fertility rates through ovulation suppression has not yet been proven [2]. In our study after the combined therapy, we had 89 (60%) pregnancies in 148 patients and 13 abortions and 3 extrauterine pregnancies. Sixteen (18%) of the 89 pregnancies did not lead to a live birth (13 abortions and 3 extrauterine pregnancies). Regidor reported 55 (60%) pregnancies for 91 patients for the same therapeutic strategy [46]. Nineteen (34.5%) of the 55 pregnancies were not carried to term (5 extrauterine pregnancies, 14 abortions). Our pregnancy rate was comparable to Regidor's rate, but our abortion rate was significantly lower than his. After the exclusive surgical treatment, we registered a pregnancy rate of 55%. In comparison, Marcoux et al. presented a pregnancy rate of 29% [50].

All research focusing on macroscopic or microscopic markers as well as biochemical criteria for assessing the degree of activity of endometriosis are not convincing. Essential factors for deciding the optimal endometriosis therapy are clinical symptoms, the patient's age, localization, severity, duration of the disease, recurrence rate, and activity [51, 52]. Active endometriosis foci are characterized by hypervascularization, edema, and infiltration of inflammatory cells [33]. It still needs to be determined how endometriosis activity can best be characterized using macroscopic, microscopic, and biochemical criteria [16]. Laparoscopy currently constitutes one of the most accurate methods of diagnosing endometriosis.

2.4.2.3 The Influence of Endometriosis on Obstetrical Outcome

Concerning this constellation let us pose two critical questions:

1. Endometriosis is known to interfere with conception and implantation. Is there any effect on the obstetrical outcome?
2. Do women with endometriosis need special care during pregnancy to avoid premature deliveries?

The effect on the obstetrical outcome seems to be premature delivery.

The question whether endometriosis triggers recurrent spontaneous abortions was investigated following the observation that "natural killer (NK) cell activity" is low in endometriosis patients and high in unexplained recurrent abortions. There is good evidence that endometriosis is associated with an opposite regulation of NK cell behavior [53]. From 1991 to 1995, at least nine independent groups reported a functional defect of peripheral NK cells in patients with endometriosis. However, Somigliana et al. in 1999 concluded that the relationship between NK cell activity, endometriosis, and infertility seems to be "more puzzling" than considered so far [53]. These fine implied mechanisms may still reveal interesting biological and clinical possibilities for treatment.

Several case reports deal with obstetrical emergencies arising during delivery through endometriosis. Let us discuss a few of them:

1. A 22-year-old woman with unoperated deep endometriosis of the uterosacral ligament suddenly experienced severe abdominal pain, hematuria, and intrauterine death at 31 gestational weeks. Surgical intervention revealed an active hemorrhage from the right uterine artery and urine leakage from interruption of the right ureter in the area of a laparoscopic documented, but not treated, endometriotic nodule [54].
2. An emergency exploratory laparotomy was performed on a patient 3 days postpartum. This patient had a history of previous laparoscopic treatment for deep infiltrating endometriosis before her pregnancy. Active bleeding was found at the right uterine vein, near the site of previous surgery for deep infiltrating endometriosis [55].
3. A 30-year-old woman, at 24 weeks of gestation, was admitted with acute intraabdominal bleeding. Endometriosis lesions infiltrating the lateral wall of the uterus, the right ovarian fossa, and the right cardinal ligament were found [56].
4. A case of spontaneous postpartum hemorrhage due to massive preperitoneal implants suggestive of decidualized endometriosis was reported by M. Mabrouk et al. [57].

2.5 Conclusions

2.5.1 The Three-Step Therapy

Since the identification of endometriosis as a progredient estrogen-related disease, various substances have been used to suppress ovarian steroid biosynthesis. Currently all modern therapeutic strategies aim at ovarian downregulation with

GnRH agonists or gestagens. In most cases, therapeutic approaches take into consideration not only medical but also laparoscopic and, if required, laparotomic surgical treatment of endometriosis and the combined therapy. The three-step therapy comprises surgical laparoscopy with removal of all visible endometriosis foci, a 3- to 6-month endocrine therapy and a subsequent second-look laparoscopy with resection of residual foci, adhesiolysis, and reconstructive surgery of the organs [58]. Within the framework of the present study, combined treatment was the most successful treatment for endometriosis. Comparison of the three different therapeutic strategies implicates a higher benefit for combined treatment insofar as downstaging and reduction in symptoms (disease-free period) and pregnancy rates.

Following this concept, new treatment modalities are published [59, 60] in various scientific journals including specific journals focusing on endometriosis selectively.

2.5.2 Endometriosis and Obstetrical Outcome

As endometriosis remains an enigmatic disease, there is a growing realization that the origin of major obstetrical complications and problems during pregnancy may lie in very early pregnancy events. Recent studies have focused on the implantation window, particularly in endometriosis patients. The implantation window may not only be responsible for delayed implantation but also for defective deep placentation leading to preterm labor, fetal growth restriction, and preeclampsia.

It is a myth that pregnancy can heal endometriosis as severe pain attacks due to endometriosis can also occur during pregnancy. A relation between endometriosis and abortions remains questionable.

Obstetrical emergencies based on endometriosis, such as bleeding endometriotic lesions, uterine arteries, or veins, interruption of ureter, and postpartum hemorrhage due to decidualized endometriosis, have occurred and have to be considered.

References

1. Johnson NP, Hummelshoj L. Consensus on current management of endometriosis. Hum Reprod. 2013;28(6):1552–68.
2. Hughes E, et al. Ovulation suppression for endometriosis. Cochrane Database Syst Rev. 2007;(3):CD000155.
3. Strowitzki T, et al. Dienogest is as effective as leuprolide acetate in treating the painful symptoms of endometriosis: a 24-week, randomized, multicentre, open-label trial. Hum Reprod. 25(3):633–41.
4. Zupi E, et al. Add-back therapy in the treatment of endometriosis-associated pain. Fertil Steril. 2004;82(5):1303–8.
5. Wong CL, et al. Oral contraceptive pill as treatment for primary dysmenorrhoea. Cochrane Database Syst Rev. 2009;(2):CD002120.
6. Davis L, et al. Modern combined oral contraceptives for pain associated with endometriosis. Cochrane Database Syst Rev. 2007;(3):CD001019.
7. Mousa NA, Bedaiwy MA, Casper RF. Aromatase inhibitors in the treatment of severe endometriosis. Obstet Gynecol. 2007;109(6):1421–3.

8. Ferrero S, et al. Letrozole combined with norethisterone acetate compared with norethisterone acetate alone in the treatment of pain symptoms caused by endometriosis. Hum Reprod. 2009;24(12):3033–41.
9. Mettler L, et al. Challenges of endometriosis. Eur Obstet Gynecol. 2011;6(2):83–90.
10. Olive DL, Schwartz LB. Endometriosis. N Engl J Med. 1993;328(24):1759–69.
11. Kennedy S, et al. ESHRE guideline for the diagnosis and treatment of endometriosis. Hum Reprod. 2005;20(10):2698–704.
12. Stegmann BJ, et al. Using location, color, size, and depth to characterize and identify endometriosis lesions in a cohort of 133 women. Fertil Steril. 2008;89(6):1632–6.
13. Wykes CB, Clark TJ, Khan KS. Accuracy of laparoscopy in the diagnosis of endometriosis: a systematic quantitative review. BJOG. 2004;111(11):1204–12.
14. Christensen B, Freie HM, Schindler AE. Endometriosis—diagnosis and therapy. Results of a current survey of 6,700 gynecologists. Geburtshilfe Frauenheilkd. 1995;55(12):674–9.
15. Medicine PCoASfR. Treatment of pelvic pain associated with endometriosis. Fertil Steril. 2008;90(5 Suppl):S260–9.
16. Buchweitz O, et al. The diagnostic dilemma of minimal and mild endometriosis under routine conditions. J Am Assoc Gynecol Laparosc. 2003;10(1):85–9.
17. Mettler L, Schollmeyer T, Alkatout I. Adhesions during and after surgical procedures, their prevention and impact on women's health. Women's Health (Lond Engl). 2012;8(5):495–8.
18. Alkatout I, et al. Endometriose: Medikamentöse und operative Therapieoptionen. gynäkologie + geburtshilfe. 2013;18(1):38–45.
19. Abbott J, et al. Laparoscopic excision of endometriosis: a randomized, placebo-controlled trial. Fertil Steril. 2004;82(4):878–84.
20. Healey M, Ang WC, Cheng C. Surgical treatment of endometriosis: a prospective randomized double-blinded trial comparing excision and ablation. Fertil Steril. 2010;94(7):2536–40.
21. Jacobson TZ, et al. Laparoscopic surgery for pelvic pain associated with endometriosis. Cochrane Database Syst Rev. 2001;(4):CD001300.
22. Crosignani PG, et al. Laparoscopy versus laparotomy in conservative surgical treatment for severe endometriosis. Fertil Steril. 1996;66(5):706–11.
23. Taylor E, Williams C. Surgical treatment of endometriosis: location and patterns of disease at reoperation. Fertil Steril. 2010;93(1):57–61.
24. Yeung PP Jr, Shwayder J, Pasic RP. Laparoscopic management of endometriosis: comprehensive review of best evidence. J Minim Invasive Gynecol. 2009;16(3):269–81.
25. Engemise S, Gordon C, Konje JC. Endometriosis. BMJ. 2010;340:c2168.
26. Alkatout I, et al. Combined surgical and hormone therapy for endometriosis is the most effective treatment: prospective, randomized, controlled trial. J Minim Invasive Gynecol. 2013;20(4):473–81.
27. Mettler L. Diagnostik und Therapie der Endometriose. Eur Secur. 1984;5:234–40.
28. Mettler L, Semm K. Three step medical and surgical treatment of endometriosis. Ir J Med Sci. 1983;152(Suppl 2):26–8.
29. Stephansson O, et al. Endometriosis, assisted reproduction technology, and risk of adverse pregnancy outcome. Hum Reprod. 2009;24(9):2341–7.
30. Brosens I, et al. The enigmatic uterine junctional zone: the missing link between reproductive disorders and major obstetrical disorders? Hum Reprod. 2010;25(3):569–74.
31. Hadfield RM, et al. Is there an association between endometriosis and the risk of pre-eclampsia? A population based study. Hum Reprod. 2009;24(9):2348–52.
32. Wood R. Myths and misconceptions in endometriosis.
33. Mettler L, et al. Identification of the M-CSF receptor in endometriosis by immunohistochemistry and RT-PCR. Am J Reprod Immunol. 2004;52(5):298–305.
34. Mettler L, et al. Comparison of c-DNA microarray analysis of gene expression between eutopic endometrium and ectopic endometrium (endometriosis). J Assist Reprod Genet. 2007;24(6):249–58.
35. Sampson JA. Peritoneal endometriosis due to menstrual dissemination of endometrial tissue into peritoneal cavity. Am J Obstet Gynecol. 1927;14:422–69.

36. Ebert AD, editor. Endometriose. Berlin: Walther de Gruyter Verlag; 2003.
37. Schweppe KW, et al., editors. Endometriose: Entstehung, Diagnostik, Behandlungsmöglichkeiten und Probleme in Klinik und Praxis. Heidelberg: Seminar in Gynäkologische Endokrinologie; 2012. p. 516–34.
38. Adamson GD, Pasta DJ. Endometriosis fertility index: the new, validated endometriosis staging system. Fertil Steril. 2010;94(5):1609–15.
39. Yap C, Furness S, Farquhar C. Pre and post operative medical therapy for endometriosis surgery. Cochrane Database Syst Rev. 2004;(3):CD003678.
40. Seracchioli R, et al. Post-operative use of oral contraceptive pills for prevention of anatomical relapse or symptom-recurrence after conservative surgery for endometriosis. Hum Reprod. 2009;24(11):2729–35.
41. Schweppe KW. Therapie der Endometriose unter Berücksichtigung der Aktivitätsgrade. Gynakologe. 2002;35:255–61.
42. Schindler AE, et al. Treatment of endometriosis with the GnRH agonist buserelin (Suprecur): a multicenter study. Geburtshilfe Frauenheilkd. 1994;54(10):569–73.
43. Regidor PA, et al. Treatment of endometriosis with the GnRH analog triptorelin with special reference to the effect on bone density. Zentralbl Gynakol. 1993;115(11):473–7.
44. Raitz von Frentz M, Schweppe KW. Ovarian suppression by the GnRH analog buserelin in the treatment of endometriosis. Clinical, biochemical and pelviscopic studies. Geburtshilfe Frauenheilkd. 1990;50(8):634–9.
45. Romer T, Schwesinger G. Wie durchbricht man den Teufelskreis der aktiven Endometriose? Neue Aspekte der Therapieentscheidung bei aktiver Endometriose. Zentralbl Gynakol. 1999;121(7):336–40.
46. Regidor PA, et al. Prospective randomized study comparing the GnRH-agonist leuprorelin acetate and the gestagen lynestrenol in the treatment of severe endometriosis. Gynecol Endocrinol. 2001;15(3):202–9.
47. Guzick DS, et al. Randomized trial of leuprolide versus continuous oral contraceptives in the treatment of endometriosis-associated pelvic pain. Fertil Steril. 2011;95(5):1568–73.
48. Sutton CJ, et al. Prospective, randomized, double-blind, controlled trial of laser laparoscopy in the treatment of pelvic pain associated with minimal, mild, and moderate endometriosis. Fertil Steril. 1994;62(4):696–700.
49. Cahill DJ. What is the optimal medical management of infertility and minor endometriosis? Analysis and future prospects. Hum Reprod. 2002;17(5):1135–40.
50. Marcoux S, Maheux R, Berube S. Laparoscopic surgery in infertile women with minimal or mild endometriosis. Canadian Collaborative Group on Endometriosis. N Engl J Med. 1997;337(4):217–22.
51. Barcena de Arellano ML, et al. Overexpression of nerve growth factor in peritoneal fluid from women with endometriosis may promote neurite outgrowth in endometriotic lesions. Fertil Steril. 2011;95(3):1123–6.
52. Keichel S, et al. Lymphangiogenesis in deep infiltrating endometriosis. Hum Reprod. 2011;26(10):2713–20.
53. Somigliana E, Vigano P, Vignali M. Endometriosis and unexplained recurrent spontaneous abortion: pathological states resulting from aberrant modulation of natural killer cell function? Hum Reprod Update. 1999;5(1):40–51.
54. Chiodo I, et al. Urohemoperitoneum during pregnancy with consequent fetal death in a patient with deep endometriosis. J Minim Invasive Gynecol. 2008;15(2):202–4.
55. Wada S, et al. Uterine vein rupture at delivery as a delayed consequence of laparoscopic surgery for endometriosis: a case report. J Minim Invasive Gynecol. 2009;16(4):510–2.
56. Falcao JJ, SJ WE. Endometriosis in obstetric emergency. Free Communications and Posters, P1–108, in 11 th World Congress of Endometriosis, Montpellier, France, 4–7 September 2011.
57. Mabrouk M, et al. Spontaneous postpartum hemoperitoneum in primipara with decidualized pelvic endometriosis. Free Communication and Posters, P1–112, in 11 th World Congress on Endometriosis, Montpellier, France, 4–7 September 2011.
58. Mettler L, et al. Accuracy of laparoscopic diagnosis of endometriosis. JSLS. 2003;7(1):15–8.

59. da Silva-Filho AL, Ferreira MCF, Lamaita RM, Rocha ALL, Cândido EB, Carneiro MM. Endometriosis and ovarian cancer: from molecular evidences to clinical implications. Journal of Endometr. 2016;8(3):81–6. https://doi.org/10.5301/je.5000248.
60. Bluett R, Fonseca-Kelly Z, Burke C. Efficacy of surgical intervention for the treatment of pain and improvement of quality of life in patients with endometriosis. J Endometr. 2016;8(3):101–5. https://doi.org/10.5301/je.5000246.

3 The Genetics of Polycystic Ovary Syndrome: From Genome-Wide Association to Molecular Mechanisms

Jerome F. Strauss III, Bhavi P. Modi, and Janette M. McAllister

3.1 Hyperandrogenemia and Polycystic Ovary Syndrome (PCOS)

PCOS is a common endocrine disorder reported to affect 5–7% women of reproductive age. The incidence of PCOS appears to be similar across racial/ethnic groups. Although there has been debate about the most appropriate diagnostic criteria for PCOS, hyperandrogenemia/hyperandrogenism, not explained by other causes (e.g., androgen-secreting tumors, Cushing's syndrome, late-onset congenital adrenal hyperplasia), is a hallmark of the disorder, and it is included as an essential element in all "consensus" diagnosis schemes [1, 2].

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3.2 The Phenotype of Human Theca Cells from Normal and PCOS Ovaries

Studies on freshly isolated thecal tissue from normal and PCOS ovaries or cultures of human theca cells derived from normal and PCOS women have demonstrated that PCOS theca secretes greater amounts of androgen than theca tissue or cells from regularly ovulating women [3–9]. Our success in developing conditions to propagate human theca cells isolated from individual, size-matched follicles from ovaries of normal-cycling women and women with PCOS, provided the first evidence to show that increased *CYP17A1* (P450 17α-hydroxylase) gene expression in PCOS theca cells is associated with an excess androgen production in the PCOS ovary [10, 11]. Our previous molecular characterization of PCOS and normal theca cells from multiple individuals by microarray analysis and quantitative PCR established that normal and PCOS theca cells have distinctive molecular signatures [5–7, 12–15]. Similar results have been observed with granulosa cells collected from normal and PCOS women undergoing assisted reproduction [16].

3.3 The Genetics of PCOS

There is strong evidence for genetic predisposition to PCOS in most ethnic/racial groups studied to date [17, 18]. However, despite advances in genetic technologies, very few PCOS susceptibility genes have been validated. Numerous candidate gene association studies, based on genes selected because of their putative roles in PCOS phenotypes, have been conducted. While some of these studies yielded statistically significant associations of genetic variants with PCOS, these candidate gene studies have been uniformly conducted on small sample sizes and have had limited statistical power. Moreover, few of these studies have produced sufficiently robust results, and rarely have they been replicated by different investigators studying diverse populations of women [19, 20].

A major milestone was achieved with the publication of a genome-wide association study (GWAS) by Chen and colleagues [21], who reported their findings on a Han Chinese population. Chen and colleagues identified loci on chromosome 2 (2p16.3 and 2p21) and chromosome 9 (9q33.3) that had significant associations with PCOS, conferring protection or increased risk, at levels exceeding the threshold statistical significance for genome-wide associations. A subsequent GWAS with additional subjects identified eight new putative PCOS loci on chromosomes 2p16.3, 9q22.32, 11q22.1, 12q13.2, 12q14.3, 16q21.1, 19p13.3, and 20q13.2 [22] (Table 3.1). Replication studies conducted in populations of European ancestry confirmed a number of these associations [23, 24]. Subsequently, GWAS carried out on European populations added new putative PCOS genes, bringing the total of PCOS candidates to 22 [26, 27].

While several loci identified in the GWAS, including those in or near the *FSHB*, *FSHR*, *LHCGR*, and *INSR* genes, are plausible PCOS candidates, the pathophysiological links of other loci identified in the GWAS (e.g., *C9orf3*, *YAP1*, *RAB5B*,

Table 3.1 PCOS risk loci reported to date

Locus	Gene	GWAS index SNP	Risk/other allele	Discovery *p*-value	Discovery population	Ref.
2p16.3	FSHR	rs2268361	T/C	9.89×10^{-13}	CHN	35
		rs2349415	T/C	2.35×10^{-12}	CHN	35
2p16.3	LHCGR	rs13405728	G/A	7.55×10^{-21}	CHN	34
2p21	THADA	rs13429458	C/A	1.73×10^{-23}	CHN	34
		rs12478601	T/C	3.48×10^{-23}	CHN	34
		rs12468394	A/C	1.59×10^{-20}	CHN	34
		rs7563201	G/A	3.3×10^{-10}	EUR	36
2q34	ERBB4	rs1351592	G/C	1.2×10^{-12}	EUR	36
5q31.1	RAD50	rs13164856	T/C	3.5×10^{-9}	EUR	36
8p23.1	GATA4/NEIL2	rs804279	A/T	8×10^{-10}	EUR	37
9q22.32	C9orf3	rs4385527	A/G	5.87×10^{-9}	CHN	35
		rs3802457	A/G	5.28×10^{-14}	CHN	35
		rs10993397	C/T	4.6×10^{-13}	EUR	37
9q33.3	DENND1A	rs2479106	G/A	8.12×10^{-19}	CHN	34
		rs10818854	A/G	9.4×10^{-18}	CHN	34
		rs10986105	C/A	6.9×10^{-15}	CHN	34
		rs10760321	A/G	1.4×10^{-6}	EUR	36
11p14.1	KCNA4/FSHB	rs11031006	G/A	1.9×10^{-8}	EUR	37
11q22.1	YAP1	rs1894116	G/A	1.08×10^{-22}	CHN	35
		rs11225154	A/G	7.6×10^{-11}	EUR	36
12q13.2	RAB5B/SUOX	rs705702	G/A	8.64×10^{-26}	CHN	35
12q13.2	ERBB3	rs7312770	C/T	2.1×10^{-7}	EUR	36

(continued)

Table 3.1 (continued)

Locus	Gene	GWAS index SNP	Risk/other allele	Discovery *p*-value	Discovery population	Ref.
12q14.3	HMGA2	rs2272046	C/A	1.95×10^{-21}	CHN	35
12q21.2	KRR1	rs1275468	C/T	1.9×10^{-8}	EUR	24
16q12.1	TOX3	rs4784165	G/T	3.64×10^{-11}	CHN	35
17q12	ERBB2	rs7218361	A/G	9.6×10^{-7}	EUR	36
19p13.2	INSR	rs2059807	G/A	1.09×10^{-8}	CHN	35
20q13.2	SUMO1P1/ZNF217	rs6022786	A/G	1.83×10^{-9}	CHN	35

Genetic variants associated with PCOS risk

HMGA2, *TOX3*, *SUMO1P1/ZNF217*, *THADA*, and *DENND1A*) to reproduction or ovarian function are less obvious.

The *DENND1A* locus assumed significance among these candidates as a result of several studies confirming the association of *DENND1A* SNPs with PCOS in European populations [23, 24], and its role as a guanine nucleotide exchange factor and component of clathrin-coated pits places it in pivotal position for linking signaling between plasma membrane receptors and downstream signal transduction.

3.4 *DENND1A*: A Reasonable Starting Point for Dissection of the Genetics of PCOS

The *DENND1A* gene encodes a protein named connecdenn 1, which interacts with members of the Rab family of small GTPases involved in membrane trafficking. Connecdenn 1 has a clathrin-binding domain and is thought to facilitate endocytosis (Fig. 3.1) [25].

There are at least two *DENND1A* transcripts produced as a result of alternative splicing. One of these transcripts, *DENND1A* variant 1 (V1), codes for a 1009

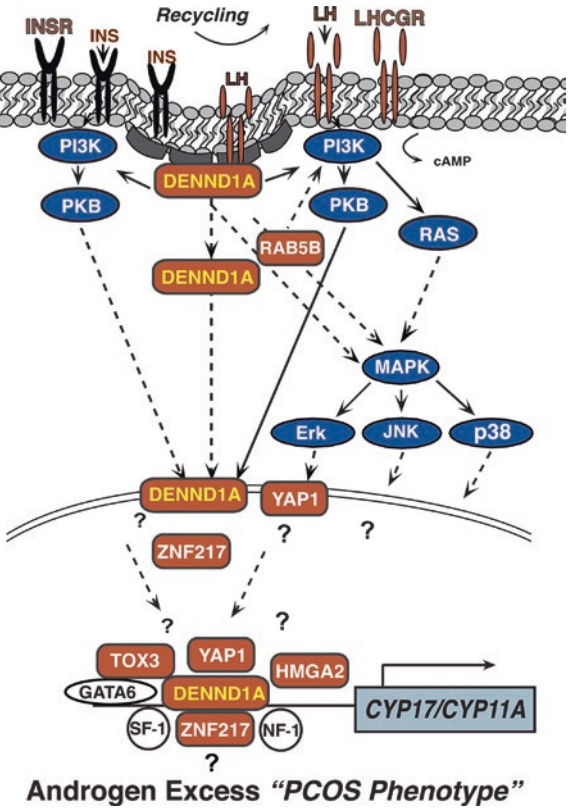

Fig. 3.1 A network of genes postulated to contribute to the hyperandrogenemia associated with PCOS based on loci identified in published GWAS and replication studies. Our preliminary observations suggest that DENND1A. V2 internalization may affect LHRH and INSR recycling and downstream signaling in conjunction with other PCOS GWAS candidates to mediate increased androgen biosynthesis as well as changes in insulin and LH-receptor sensitivity. This figure has been modified and updated from our TEM review [30]

amino acid protein with C-terminal proline-rich domain; the other, *DENND1A* variant 2 (V2), codes for a truncated 559 amino acid protein that contains the DENN domain, which serves as a guanine nucleotide exchange factor, and the clathrin-binding domain, but lacks the proline-rich domain and includes a C-terminal 33 amino acid sequence that differs from the larger V1. Interestingly, a locus encoding a Rab GTPase, RAB5B, was also identified in the GWAS noted above, suggesting a link between DENND1A and a regulator of endocytic recycling of cell surface receptors.

Up until recently, little has been known about *DENND1A* expression in cells and tissues related to reproduction with the exception that it is expressed in testes, ovarian theca cells, adrenocortical reticularis, brain, and H295 adrenal carcinoma cells, for the most part cells that synthesize androgens [28, 29]. Work from our laboratories has directly implicated DENND1A.V2 in the pathophysiology of PCOS: DENND1A.V2 mRNA and protein are overexpressed in PCOS theca cells compared to normal theca cells; overexpression of DENND1A.V2 in normal theca cells increased the abundance of CYP17A1 mRNA, augmented androgen (i.e., DHEA) production, and increased *CYP11A1* and *CYP17A1* promoter-reporter activity; and siRNA knockdown of V2 in PCOS theca cells reversed the PCOS phenotype. In addition, DENND1A.V2 mRNA is abundant in urinary exosomes of PCOS women, but not normal-cycling women. These results suggest the possibility of a noninvasive diagnostic for PCOS for early detection in prepubertal and adolescent females [29].

To further define the role of DENND1A.V2 in the pathophysiology of PCOS, we created a transgenic mouse that expresses human DENND1A.V2 under control of the CMV promoter. To our advantage, rodents do not express truncated DENND1A.V2. Although characterization of these animals is still under way, Cyp17A1 and Cyp11A1 mRNA were observed to be overexpressed in ovaries, testes, and adrenals of the DENND1A.V2 transgenic mouse (V2-Tg) compared to wild-type (WT) littermates. Plasma androstenedione, DHEA, testosterone, and progesterone were also observed to be elevated in the female V2-Tg compared to the WT mouse. One of the most striking findings of these studies was the discovery of *Cyp17a1* expression in the V2-Tg mouse adrenal cortex, and cortisol production by V2-Tg mouse adrenocortical cells in culture. *Cyp17a1* is not normally expressed in the mouse adrenal gland, and we could not detect *Cyp17a1* mRNA in wild-type adrenals, only in the V2-Tg mice. These findings implicate DENND1A.V2 in control of the pathways that govern androgen biosynthesis.

3.5 What Is the Mechanism for DENND1A.V2 Overexpression in PCOS Theca Cells?

The exact mechanism through which V2 is overexpressed in PCOS theca cells has not yet been determined. GWAS results indicate that genetic variation contributes to some extent. However, the *DENND1A* GWAS SNPs associated with PCOS in all populations are located in introns, and none are near the alternative splice sites, so on the surface it is not evident that they have functional roles in controlling or

transcript splicing or gene expression, although they could be embedded in intronic enhancer elements with the SNP alleles affecting transcription factor binding. This possibility has not been formally excluded.

We have yet to identify genetic variants in coding sequences from whole exome sequencing (WES) studies that can account for overexpression in PCOS. Also, copy number variants do not appear to be a common mechanism. A detailed examination of splicing mechanisms that generate DENND1A.V2 failed to disclose variants in the intron involved in V2 splicing (lying between exons 20 and 20A, which encodes the C-terminal V2 sequence) [28]. The SNPs in *DENND1A* identified by GWAS are located in introns and lack apparent functions. As suggested previously, overexpression of DENND1A.V2 in PCOS suggests the contribution of gain-of-function variation [29]. Thus, we sought to determine the mechanisms leading to production of DENND1A.V2 and its overexpression in PCOS by testing several possible mechanisms including examination of coding or splicing variants in the *DENND1A* gene via available WES data, investigation of copy number variations in the *DENND1A* gene, and sequencing of the intronic region between exons 20 and exon 20A [28] (see below) for identification and characterization of variations affecting alternative splicing of DENND1A.V1 and DENND1A.V2.

We have recently examined the possibility that epigenetic mechanisms contribute to the overexpression of V2 in human PCOS theca cells. Three lines of evidence suggest that these mechanisms are operative:

First, treatment of normal human theca cells with 5-azacytosine, which results in genomic DNA demethylation and increased DENND1A.V2 expression.

Second, treatment of normal theca cells with the histone deactylase inhibitor valproic acid augmented DENND1A.V2 mRNA accumulation.

Third, an examination of differentially expressed microRNAs in normal and PCOS theca cells identified that miR-125a-3p was downregulated in PCOS theca cells.

Our studies have shown that miR-125a-3p targets *DENND1A*; miR-125a-3p mimic reduces DENND1A.V2 but not DENND1A.V1 mRNA accumulation in human adrenal 295R cells, implicating that changes in abundance of this microRNA directly affect DENND1A.V2 mRNA. Collectively, these observations suggest that both genetic and epigenetic mechanisms contribute to DENND1A.V2 overexpression in PCOS.

3.6 A PCOS Genetic Network Incorporating *DENND1A*

Among the loci associated with PCOS in Han Chinese, several reside in or near genes that potentially define a network, including the *FSHR*, *LHCGR*, and *INSR*, which encode receptors that reside on the plasma membrane, and which are internalized by coated pits, where DENND1A protein is located (Fig. 3.1) [19, 22]. RAB5B is thought to be involved in endocytosis and could, therefore, be a molecule interacting with the DENN domain. YAP1, TOX3, HMGA2, and ZNF217 are all

involved in transcriptional regulation, although none of them have been specifically implicated in the expression of genes involved in steroidogenesis. However, TOX3 (transcriptional coactivator of the p300/CBP-mediated transcription complex) transactivates through cAMP response element (CRE) sites, which are present in gene-encoding steroidogenic proteins. These genes can be assembled into a signaling network beginning at the receptor level, receptor coupling, or recycling and downstream molecules that ultimately regulate gene transcription, either of steroidogenic genes directly or possibly through the upregulation of other transcription factors that directly influence steroidogenic gene promoter function (Fig. 3.1). This framework provides a road map for the identification of genetic variation/mutations that predispose to PCOS and the molecular basis for the action of the identified risk alleles [30].

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References

1. Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, et al. Positions statement: criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an Androgen Excess Society guideline. J Clin Endocrinol Metab. 2006;91:4237–45.
2. 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;19:41–7.
3. Gilling-Smith C, Storey H, Rogers V, Franks S. Evidence for a primary abnormality in theca cell steroidogenesis in the polycystic ovarian syndrome. Clin Endocrinol. 1997;47:1158–65.
4. Gilling-Smith C, Willis DS, Beard RW, Franks S. Hypersecretion of androstenedione by isolated thecal cells from polycystic ovaries. J Clin Endocrinol Metab. 1994;79:1158–65.
5. Nelson VL, Legro RS, Strauss JF 3rd, McAllister JM. Augmented androgen production is a stable steroidogenic phenotype of propagated theca cells from polycystic ovaries. Mol Endocrinol. 1999;13:946–57.
6. Nelson VL, Qin KN, Rosenfield RL, Wood JR, Penning TM, et al. The biochemical basis for increased testosterone production in theca cells propagated from patients with polycystic ovary syndrome. J Clin Endocrinol Metab. 2001;86:5925–33.
7. Wickenheisser JK, Quinn PG, Nelson VL, Legro RS, Strauss JF 3rd, et al. Differential activity of the cytochrome P450 17alpha-hydroxylase and steroidogenic acute regulatory protein gene promoters in normal and polycystic ovary syndrome theca cells. J Clin Endocrinol Metab. 2000;85:2304–11.
8. Magoffin DA. Ovarian enzyme activities in women with polycystic ovary syndrome. Fertil Steril. 2006;86(Suppl 1):S9–S11.
9. Jakimiuk AJ, Weitsman SR, Navab A, Magoffin DA. Luteinizing hormone receptor, steroidogenesis acute regulatory protein, and steroidogenic enzyme messenger ribonucleic acids are overexpressed in thecal and granulosa cells from polycystic ovaries. J Clin Endocrinol Metab. 2001;86:1318–23.
10. Wickenheisser JK, Nelson-Degrave VL, McAllister JM. Dysregulation of cytochrome P450 17alpha-hydroxylase messenger ribonucleic acid stability in theca cells isolated from women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2005;90:1720–7.

11. Nelson-Degrave VL, Wickenheisser JK, Hendricks KL, Asano T, Fujishiro M, et al. Alterations in mitogen-activated protein kinase kinase and extracellular regulated kinase signaling in theca cells contribute to excessive androgen production in polycystic ovary syndrome. Mol Endocrinol. 2005;19:379–90.
12. Wood JR, Ho CK, Nelson-Degrave VL, McAllister JM, Strauss JF 3rd. The molecular signature of polycystic ovary syndrome (PCOS) theca cells defined by gene expression profiling. J Reprod Immunol. 2004;63:51–60.
13. Wood JR, Nelson VL, Ho C, Jansen E, Wang CY, et al. The molecular phenotype of polycystic ovary syndrome (PCOS) theca cells and new candidate PCOS genes defined by microarray analysis. J Biol Chem. 2003;278:26380–90.
14. Wickenheisser JK, Nelson-DeGrave VL, Quinn PG, McAllister JM. Increased cytochrome P450 17alpha-hydroxylase promoter function in theca cells isolated from patients with polycystic ovary syndrome involves nuclear factor-1. Mol Endocrinol. 2004;18:588–605.
15. Strauss JF 3rd, Wood JR, Christenson LK, McAllister JM. Strategies to elucidate the mechanism of excessive theca cell androgen production in PCOS. Mol Cell Endocrinol. 2002;186:183–8.
16. Coskun S, Otu H, Awartani KA, Al-Alwan LA, Al-Hassan S, Al-Mayman H, Kaya N, Inan MS. Gene expression profiling of granulosa cells from PCOS patients following varying doses of human chorionic gonadotropin. J Assist Reprod Genet. 2013;30(3):341–52.
17. Legro R, Driscoll D, Strauss J III, Fox A, Dunaif A. Evidence for a genetic basis for hyperandrogenemia in polycystic ovary syndrome. Proc Natl Acad Sci U S A. 1998;95:14956–60.
18. Legro RS, Strauss JF. Molecular progress in infertility: polycystic ovary syndrome. Fertil Steril. 2002;78:569–76.
19. Strauss JF 3rd, McAllister JM, Urbanek M. Persistence pays off for PCOS gene prospectors. J Clin Endocrinol Metab. 2012;97:2286–8.
20. Ewens KG, Stewart DR, Ankener W, Urbanek M, McAllister JM, et al. Family-based analysis of candidate genes for polycystic ovary syndrome. J Clin Endocrinol Metab. 2010;95:2306–15.
21. Chen ZJ, Zhao H, He L, Shi Y, Qin 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;43:55–9.
22. Shi Y, Zhao H, Shi Y, Cao Y, Yang D, et al. Genome-wide association study identifies eight new risk loci for polycystic ovary syndrome. Nat Genet. 2012;44:1020–5.
23. Goodarzi MO, Jones MR, Li X, Chua AK, Garcia OA, et al. Replication of association of DENND1A and THADA variants with polycystic ovary syndrome in European cohorts. J Med Genet. 2012;49:90–5.
24. Welt CK, Styrkarsdottir U, Ehrmann DA, Thorleifsson G, Arason G, et al. Variants in DENND1A are associated with polycystic ovary syndrome in women of European ancestry. J Clin Endocrinol Metab. 2012;97:E1342–7.
25. Marat AL, Dokainish H, McPherson PS. DENN domain proteins: regulators of Rab GTPases. J Biol Chem. 2011;286:13791–800.
26. 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.
27. 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.
28. Tee MK, Speek ML, Legeza B, Modi B, Teves ME, McAllister JM, Strauss JF 3rd, Miller WL. Alternative splicing of DENND1A, a PCOS candidate gene, generates variant 2. Mol Cell Endocrinol. 2016;434:25–35.
29. McAllister JM, Modi B, Miller BA, Biegler J, Bruggeman R, Legro RS, Strauss JF 3rd. Overexpression of a DENND1A isoform produces a polycystic ovary syndrome theca phenotype. Proc Natl Acad Sci U S A. 2014;111(15):E1519–27.
30. 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.

Psychosomatic Aspects of Infertility

4

Klimek Rudolf

"Stress and human reproduction have a great impact on our life and function."

(J.G. Schenker 1992)

4.1 Introduction

Life is existing informational event (process, characteristic feature of living organisms) known by different words, which are particles of different human languages, the existence of which distinguishes the "spoken world of man" from the rest of the nonverbal universe and above all from all other living multicellular species. Language is used for verbal communication between people, and its function depends on the method and context of its use by a man taking also in account of the nonverbal body language, e.g., gestures, facial expressions, posture, and tone of voice. First of all, each word has executive power, because extracting from the environment any structure or process gives it a name of the actual event in the universal information field of the universe, in which the eternal natural laws exist. Information is present everywhere as the essence of order in the nature of animate or inanimate things. The most important feature of living multicellular organisms is not—as is commonly believed—life itself, but the ability of its intergenerational transmission. People notice accurately only material changes, and many philosophers also connect the emergence of life to the beginning of the existence of matter. In fact, matter and energy are two sides of the same thing as a separate part of the universe. Leonardo da Vinci has recognized movement as one of the fundamental features of life, but as it is now known, something moves in the atoms themselves, and therefore to define the

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concept of life, one shall always refer to a specific system. In relation to man, his life is distinguished by the ability of assessing the state of their health and socioeconomic environment. An intrinsic quality of human aging is the growing up loss of strength and well-being, what defined as thermodynamic entropy, which also occurs in other natural phenomena. The more disorganized the system (e.g., organism, cell, nucleus, or just cell cytoplasm) is, the greater the losses are. The information is the most important part of reality composed of matter, energy, and information because everyone knows that each effect has its cause. Only people forgot that in every cause there is precisely this image of the plan, what this cause will precipitate. Single-celled organisms are considered too be alive, which by their division form daughter cells of the same genome (genetic identity), as evidenced by the discovery of the fact that identical carriers of hereditary (genes) are associated with the structure of the nucleic acid of the cell nuclei. Unfortunately, the biochemical nucleic genomes were divided into individual nucleotides (chemical compounds), which also were called genes, although heredity determines appropriate characteristics only with the participation of the whole structures of individual cells. Scientists are still discovering new rules and laws trying to understand the existing structures and processes. The organism, starting from individual atoms (e.g., oxygen, iron, calcium) and cells, unites man's biophysical structure at the atomic level, which has, for decades, been directly tested in spatial and energetic dimensions using technology based on magnetic resonance imaging. The thermodynamic interpretation of body structure recognizes all the cells as individual entities in relation to the whole body, wherein they include not only the cellular components of body fluids but also natural tissue macrophages infiltrating the stable cells. The end of the existence of many of them is programmed even in epithelial cells of the body's surface or in the periodically renewing endometrial cells. It must be emphasized that this is always cytomolecular, i.e., the overall interaction of cells and their components. Their role is emphasized when noxious agents, organic and inorganic biophysically active proteins, or microorganisms appear on their surface. The existence of cells requires primarily biochemical compounds, molecules to be involved in their metabolism, even while denatured losing its biological properties, they retain their existing material composition. Life itself in the cells does not arise de novo, because it passed through generations existing earlier also in the zygotes of the mother and father (i.e., the grandparents), which requires the contemporary assessment of every living person. Each person is not the only clone of the zygote, because in addition to his somatic cells, reproductive cells arise to initiate the next human generation (gametes), as well as the cells forming pregnancy together with the mother, as a joint structure, i.e., embryonic membranes and the placenta. Without the latter boundary structures, there can be no successful course of pregnancy, because nothing can replace a mother's body in human development as a result of fertilization.

Therefore, as early as in the period of pregnancy, reproductive cells should be protected by taking care of gonads, due to their prime importance in the intergenerational passage of life. Prolongation of human life achieved by mankind has resulted by a longer period of old age which is among others favorable for self-organization of neoplastic cells. So far, man has not interfered so often in reproduction on a cell level. Germ cells, as any other cells of embryo, fetus, neonate, and parents, are

subject to psychoneurocybernetic laws with the second law of thermodynamics being dominant and extended with dissipative structures. Pathological states of cells may regress following neurohormonal normalization and/or immunopotentialization of their environment, which improves the results of medical treatment, especially in early stages of the disease. Many causes of infertility and the cervical cancer fall within a wide range of factors, from genetic hereditary states to psychoemotional procreative and sexual reactions. The most common—but at the same time the easiest to eliminate—factors are the constantly increasing cases of abnormal course of pregnancy and birth due to iatrogenic and medial causes.

Infertility and cancer are the most informational diseases of personal and social human lives, being the important parts of prosperity and development of mankind. Both have their own beginning related to only one cell which appears according to health states and behaviors of individual persons what enables their modern causal prevention taking into account direct relation between each other of them, e.g., infertility, abortion, preterm deliveries from one side, and cervical cancers from the other side have common etiopathogenetic roots. The lack of significant progress in combating infertility, aging, and carcinogenesis is the result of focusing only on the molecular biology as genetic, chemical, physical, and biological factors, without the use of basic knowledge, mainly that of quantum thermodynamics.

A lot of medical theories are concerned with various estimations of the same phenomena, which has created the necessity of their generalization. The desire for the uncovering of mysteries is one of the most common motivations for human action, from a simple need to satisfy one's curiosity as to one's surroundings to the philosophical indulgence in the state of being or the sense of life. Some of the most daunting secrets are those which deal with the cause of sickness, since they touch the lives of all human beings in terms of quantum thermodynamics. Due to the nature of a physician's work, and especially due to the need of obtaining clinical experience, it is unreasonable to expect physicians to closely follow the rapid scientific developments. Unfortunately, it is much easier to consult the instruction manual of many useful tools than to adapt one's thinking to the new medical age, which moved from the level of molecular biology's cellular nuclei to the level of nuclear atomic nuclei with the universal use of magnetic resonance all over the world. Thanks to the thermodynamic interpretation, the standardization of medical terminology, starting from the cellular nuclei levels to the wide range of epidemiological studies, became possible.

4.2 Informational Medicine

Medicine is one of those unique areas of human activity in which man is not only the object but also the subject of interest but primarily connects directly all the theoretical (cognitive) achievements with the art of the possible protection of life and bringing people back to health. This obliges physicians to continuously track the progress of both general knowledge and its technological use. The universe exists due to the eternal, progressively more known and understood natural laws, that is, due to information, e.g., in the late twentieth century, the newly discovered law of natural dissipative self-organization was understood and described. Fortunately the

nature of many different phenomena is regulated by the same biophysical and biochemical universal mechanisms. Man can stand in front of the mirror(s) to looking at a copy of one's character, which does not contain even a single atom of real own body, as observed objects and the environment. The existing reality consists of atoms, while the mirrored character form is from the same localized resonating atoms, but devoid of the human matter simply because it is observed only their information parts, even in the colors and their shades (Fig. 4.1). Such observable information seemingly disappears by looking away or shattering the mirror. What's more, an outside observer's information forms (the image seen in the mirror) can be stored separately in digital technique or a photograph! The image information can also be painted or even sculpted as a copy of it, but it is formed from completely different atoms.

Information not only exists in our environment when you can see it in the mirror, but it also has executive power, e.g., when the chess players convert a pawn to any piece upon it reaching the last line of the chess board in accordance with the rules laid down by the creators of the game. Therefore, these rules contain informational existence. In the same way, but completely independent from humans, work the laws of nature, which further expand human learning information. It is known that hundreds of thousands of biochemical reactions consist of metabolism of each cell, and all function in accordance with the laws of nature, but doctors would determine the internal state of cells, and even the whole body, based on only a few cells. Such a reductionism in medicine led to the fact that the unit of inheritance of traits in species—called a gene—began to be seen as simple chemical nucleotide compounds or even a single one to be assigned biochemical names as appropriate oncogenes. This time passed when it turned out that the polynucleotide genome is sufficient to determine the identity of the cell, but the inheritance effect of traits depends on the whole of the nuclear DNA (genome), as well as the entire cell, while preserving the genetic identity (identical to the nuclear genome DNA) of the zygotes, there occur more than 2500 different types of human somatic cells. In an adult organism, an enormous number of them exist—10^{14}, several thousand times that of all the people inhabiting the earth. Therefore the continuity of life on Earth is ensured not only by the continuity of information provided by genes (or DNA) but mainly by the continuity of living cells.

Information is a third consistent part of the universe, inextricable bound with its mass and energy, importantly co-existing together with both of them as an active real field. Virtual information becomes understood, widely considered as something unreal, since it is morphologically unimaginable but functionally verifiable. Each person can immediately see their own informational image by looking in a mirror in which her/his picture seen (the mirror pattern form) does not contain a single atom of his body but is only the resonant result of feedback information (Fig. 4.1). The world is characterized by spontaneous mutual informational interchange of its elementary particles and the information of their states. In this spontaneity lies the ultimate cause of the universe, which existed in the beginning before man began describing his environment and reality, to finally understand his own consciousness, his own "I" as an extra-material motivation of his life and actions. The atoms of the body of the deceased man disintegrate into separate entities, but the information about his personal life remains and exists in human experiences or the memory of them. In this view, death is a transfer into an informational eternal human life,

Fig. 4.1 Real and mirror informational pictures

which is localized in a virtual reality of each individual, experienced as his own pure information, which begun in the moment of fertilization. The problem lies in the fact that one tries to separate the informational consciousness from the existent program of the whole material-energetic development of the individual contained in the zygote. Also the universe exists due to the eternal, progressively more known and understood natural laws, that is, due to information, e.g., in the late twentieth century, the newly discovered law of natural dissipative self-organization was understood and described.

4.3 Feedback Information

The phenomenon of feedback is an example of information's action, which is found on both sides of the equation of equivalence: $E =^{i} mc^2$ of every cause-and-effect relationship in all events in nature, in which information (i) is of fundamental importance in the mutual, continuous, and spontaneous alternation of matter (m) and energy (E) of the universe, directly and/or indirectly perceived by people. Each beginning ($=^{i}$) is real new information which on the one hand belongs to its source while on the other to the newly created process or structure. What more, a man's life is distinguished by the ability for informational self-assessment of the status of personal health in a socioeconomic environment as described by the universal equation of equivalence. Humans' bodies are embedded in the unified cosmic

informational field, i.e., they are parts of the universe as a cell in their bodies is parts of them. For example, reducing the activity of biophysical and/or biochemical changes in a single cell can result in the risk of a multicellular organism's existence but can also help to control the further growth of the species in depending on the state of reproductive health, which plays a decisive role in the intergenerational transmission of human life with the participation of information. For example, the resonance is the condition of any system in which there is a sharp maximum probability for the absorption of energy or capture of particles as well as information, i.e., the power to evoke enduring images, memories, and emotions by the synchronous vibration (action) of a neighboring object or environment. Resonance occurs widely in nature in the form of generating all types of vibrations and waves at a distance, for example, mechanical, electrical, optical, chemical, electromechanical, nuclear, and electron, including informational self-assessment of the status of health in a socioeconomic environment. It is important to distinguish between living beings and the universal existence of life from the inanimate world, which is carried out by the same elementary particles of the universe. The life of any human cell ends physiologically with its division into cells of the same genetic type or as a result of dematerialization of its matter into energy needed for formation of new biological structures, among others, also to protect only its cellular form even without the possibility of autonomous neoplasm existence. Both structural and energy changes, which occur in a single cell, are in fact also a change in information of the structures that surround this cell. Prevention of human cancers and their therapy have happen according to general knowledge so that everyone could understand the neoplastic diseases and the primary significance of own life style in the formation and progress of cancer along with importance of caring about the environment inhabited by people. The thermodynamic etiology of carcinogenesis provides new treatment alternatives in the case of standard management insufficiency or failure, e.g., the suicidal cancerogenesis may be prevented by the positive effects of the hyperthermia therapy, which itself causes more damage to cancer cells than to healthy ones. The aim of zygote is to uphold the species, but the cancer cell exclusively grows in own multicellular organism due to increasing dissipation of matter, information, and energy by which it kills neighboring cells according to universal formula of reality $E =^{i} mc^2$. The division of neoplastic cells is dependent upon the existing and emerging vessels in their environment determined by the number and the quality of blood and lymphatic vessels, whose endothelial cells perform an essential informational role in the cellular metabolism.

The discovery of cause of cancer in 1977 was based on the model of cervical cancer to explain to people the resonant image not only as mental, intellectual, cognitive entity according to the scientific concept of cognitive resonance, because, first of all, it is a kind of resonance connection, like resonance in electrical engineering, radio technology, or physics. It is based on the fact that there are two objects (systems), which are synchronized. Activation of one of them causes the reaction of the second and vice versa. This is a feedback loop via various fields, through different interactions, sometimes having the character of forces or patterns, sometimes having the character of, for example, electromagnetic fields. Resonance is a synchronization of a source with its reactive object. In humans, there are many areas of the psychological structure, which are ready to resonate with various things. The word

becomes a causative agent only if there is an agreement between the person who gave that word or other information and the person who accepted it and used it in an appropriate way. There is also a reverse relationship—material phenomena, for example, pain, evoke specific information response, which is linked with certain type of changes at the molecular level or even the sub-molecular one.

The feedback loop is a very special type of informational system, the return of part of the output to the point of input for monitoring or self-regulation (input $=^{i}$ output). The human mind, confronted with many very different problems, generally can cope with them by focusing on certain fragments, usually the most visible ones. Somewhere there is a mechanism of cause and effect, locked in a form of feedback, and this informational feedback really stabilizes and regulates it, e.g., somewhere within the system, there is small means and it in reality determines what will happen. For example, physicists have described the universe, the movement of the planets, or the formation of the universe focused on these visible elements. They can see the stars and are interested in where they come from. In contrast, suddenly it turns out that somewhere there is dark matter, which is only a small fragment of reality, while the rest escapes the observation and somewhere out there is the element that is responsible for order of the universe. The cybernetic approach relying on information explores the most basic elements of a causative mechanism, which allow interact with the real causes of what, on the surface, man sees as waves. For example, each body cell performs additional work using its own internal structures (mitochondria), and thus the organism is kept stable with the optimum body temperature being 36.6 °C. Heat is a potent measure of effect on the level of biophysical repair reactions of the body and has been used for centuries to treat many diseases, including cancer without harming healthy tissue, but strengthening the fight against cancer development processes already in precancerous cells. For this the cell uses only the intracellular structures, which are not necessary to maintain their own metabolism but are producing substances for entire organism. Their mass can be turn on energy necessary to the cell metabolism and ultimately even to self-organize nuclear DNA and sustain cellular live with a new genome under these new conditions. Therefore, when the ambient temperature of the body increases again by a few degrees, the new tumor cells must die in the absence of their potential energy sources, which still are the other normal cells own the organism.

4.4 Biocybernetic Conquest of Human Diseases

Thermodynamic evaluation of cell metabolism has allowed separating etiology from the analysis of identifiable pathogenic changes underlying disease symptoms and ailments. Prevention of human cancers and their therapy have happen according to general, not only medical knowledge so that everyone could understand the diseases and the primary significance of own life style in their formation and progress along with importance of caring about the environment inhabited by people. What more, the new informational disease (informatonosis) lies in the source of harmful information that simultaneously relates to both an individual and a whole social group. For example, cancer cells, compared to healthy cells of the body, can produce enzymes or hormones like such healthy cells, having the same or altered structure and/or function. They can also cease such production or synthesize completely

new biochemicals. These four options allow imagining a large number of antigens of cancer cells, enabling to produce drugs against them. Therefore, the coexistence of symbiotic microorganisms should be used in the production of immunopotentializing vaccines, because they not only interact with labile human cells (e.g., macrophages, leukocytes, lymphocytes, and erythrocytes) but, first and foremost for the sake of their own existence, they eliminate from their environment any pathogenic organisms. For example, the lactovaginal vaccine is used not only in the prevention of infertility but also in the treatment of cancer, particularly in precancerous states of the cervix.

The lactovaginal vaccine provides a long-lasting protection against new inflammations via interaction of symbiotic bacteria. *Lactobacillus vaginalis* directly competes with pathological human bacteria, viruses, and parasites, as is evidenced by the high percentage of healings from infections or even viral inflammations, not only of intraepithelial cervical cancer in women after beginning sexual intercourse. The cure lies in restoring the full recovery mechanisms of defense and repair of the organism, one of which is of course the immune system. Thus it is not only the presence of pathogenic flora but also the elevated pH and the lack of these rods that indicate an unfavorable environment for epithelial cells, thereby deteriorating the conditions for the existence of the other cervical cells and promoting the growth of a more efficient cell clone. The lactovaginal vaccine affects the environment of the vagina and cervix through strengthening the local and body-wide repair mechanisms without contraindications to its use, regardless of the age of the woman. It complements any type of cancer therapy and all chronic or recurrent inflammations of the reproductive organs, especially ones with elevated vaginal pH values and/or lactic acid deficiency.

For many years, neogenesis was seen in terms of the role of oncogenes, until the proponents of this theory convinced themselves that the unit of hereditary traits consists not only of nuclear DNA. The same goes for the infectious etiology of neoplasm, advertised by individuals believing only in their own ideas, despite tried and tested medical rules. For example, 50% of all recurring miscarriages still surprise obstetricians, who not only do not use enzymatic monitoring (with the help of oxytocinase), which has been known for 50 years, but also do not examine the essential hypothalamo-hypophyseal-adrenal axis. They use excessive gonadotropins, but do not underestimate the meaning of adrenocorticotrophin (ACTH), or even block it with synthetic steroid drugs without determining the ACTH levels in the blood of the mother. Moreover, they justify the excessive number of induced and operative births by the accumulation of pathology in their obstetrics units, which they often cause themselves, setting the date of birth based solely on the date of the last period. Neglecting giving birth in the correct moment can be compared to the harmfulness of thalidomide in the early pregnancy, with the difference that the cause of injury to the child is not from a specific drug with a proven harmfulness but due to lack of knowledge or conscious misinformation.

The human being functions not only as a separate whole, but simultaneously always in interaction with its environment not only in a material-energetic sense, but also in a psychological and emotional one. Every human must do work, (1) internal, in order to sustain the internal state of the organism so as to function and evolve, and (2) external, when supplied with the basic nourishment and excreting the products

of transforming matter and energy; and additionally he works to benefit his social surrounding (as productive and creative work). One should recall the rule which states that the sum of the created entropies in the system and in its environment must always be positive, meaning the reduction of entropy in the system (neoplasm) must be accompanied by its rise in the environment (organism of cancer patient). Dissipathogenic human states are diagnosed by clinical symptoms and disorders as well as using nucleomagnetic imaging, taking into account pathological factors in the past (obstetric hemorrhage, infections, shortened lactation, drug addiction, etc.) and/or the hereditary and socioeconomic factors.

4.5 Carcinogenesis According to the Model of Cervical Cancer

Each neoplastic disease possesses a variety of forms as well as a unique identity of the neoplasm as its own sufficing cause. This needs for an early detection, as well as an etiological treatment of preneoplastic states which can be ever more precisely detected with new methods based on psychoneuroimmunology. For example, cryosurgery, laser therapy, and radical electrocautery are all effective in eradicating cervical cancer, but not the dissipathogenic state (cancerogenic) of tissue cells. Consequently this local ablative therapy must be followed by medical restoration of the body's defense mechanism to prevent the recurrence of the disease. Only neuro-, immuno-, or thermotherapy as a mean of treating the whole body can alone prevent and cure the dissipathogenic states, as a final cause of carcinogenesis of any part of the body. Also primary prophylaxis of neoplasms requires that not only the dissipathogenic state of cells be prevented but also their tissue surrounding be normalized to head off the risk of the self-organization of neoplastic forms of life.

The basic component of the universe is information about the degree of order of each system, called entropy: the bigger it is, the greater the chaos (disorder) and thus less physical fitness, e.g., the kind of feeling of increasing ease of fatigue as a person grows older. The entropy production is associated with any biochemical conversion of thousands of proteins that are found in human cells that could exist and exchange metabolites with the environment and in particular to produce the hormones, enzymes, antibodies, etc. for the entire organism. The second law of thermodynamics states that the sum of the increment (d) over time (t) of the entropy (S) simultaneously in a cell (c) and its surroundings (o) must be positive ($dS_c + dS_o > 0$). Cell metabolism is disturbed, or even disappears, if any change reduces its current production of entropy. Therefore for the continued existence of the entire system (cell), it is necessary to increase production of entropy, or the new source is needed. The cell, by reducing its additional production of metabolites for the use of the whole body, can generate entropy sources only with the new genome, using the self-organization of cancer's matter and energy of these cellular organelles that participated in the additional production of the substances for the benefit of the whole system. In place of the existing inefficient cells, there appears a disposable biological system (cancer) with increased dispersion (dissipation) of entropy in the environment (Fig. 4.2).

The relationship of the individual as a human in society can be compared to the cells in the body. If the cell is not supplied with blood, is not supplied with oxygen,

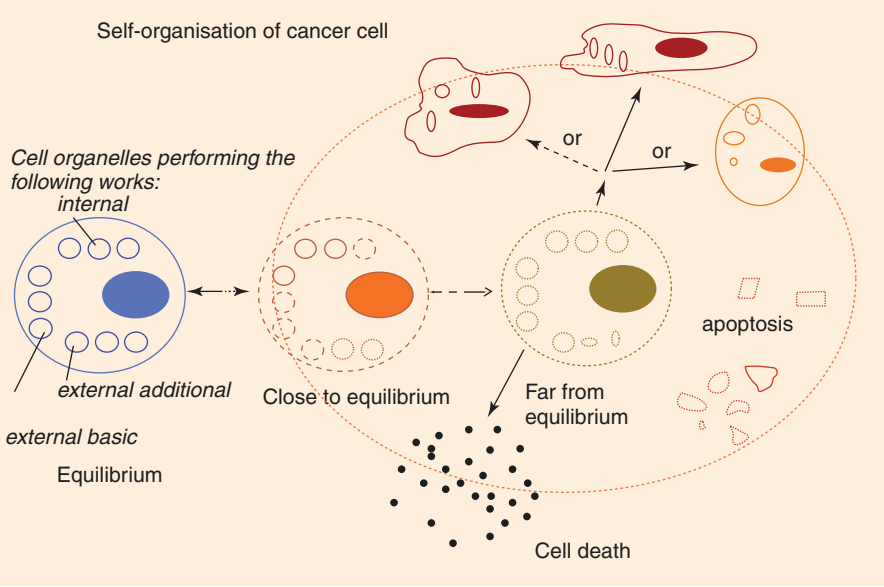


Fig. 4.2 Thermodynamic branch of cell's inner states

and does not give off carbon dioxide, it begins to choke and stops working, and what is essential is that for its existence, it is more important than to produce hormones and enzymes for the other cells. There is a law in nature, where a cell being far from equilibrium may reorganize itself into a new more efficient system in a body in such a bad condition. The tumor cell, under these conditions, can last and divide into daughter cells, if it is efficient enough that it can take care of vascularization. Therefore, people need to know that cancer arises from their own cells because the cancer cell cares primarily about itself. It works according to the laws of nature where in order to survive in these wrong conditions, it must harm the environment, which does not care for it. New dissipative structure is responsible for the signs and symptoms of diseases popularly called cancer. In the fight with it, health is the protection, and the defense—proper nutrition and care for the environment and taking advantage of the biological and economic heritage—determines the outcome of the fight against cancer. Medicine, through operations, electromagnetic radiation, hyperthermia, chemotherapy, and hormone therapy, is only responsible for about 20% of treatment effects.

Preventing cancer lies in strengthening the body's defenses and the continuing improvement of health education and regular control and prevention studies. For example, the human papilloma virus (HPV) uses, for its propagation, natural exfoliating epithelial cells of the body. Once the virus has infected a cell and begins replicating itself, also new capsid subunits are synthesized according to genetic information of the virus. The viruses themselves controlled by the human often change their protein capsid (sheath structure of their nucleic acid) to penetrate inside them. They spread outside the body of the patient, which eliminates the infection of 80–90% of

cases before the disease fully develops. The recently produced prophylactic vaccine against HPV is deprived of nucleic component of this virus, but it is just information arising from the similarity of its composition to viral protein capsid. Following subcutaneous injection, it only provides information which exceeded the epidermal barrier before the existence of the infectious disease, which in turn may initiate an immune response throughout the body, leading even to death. Not the nucleic virus but the misinformation about its alleged existence is the cause of this pathology.

A healthy body, through systemic mechanisms of repair and defense, every day eliminates cancer cells appearing at the informational level of carcinogenesis through the literally causal power of information in response to a sudden increase in local matter and energy dissipation. At the same time, doctors provide services to people suffering from diseases or disasters or affected by the socioeconomic development. The scope of the means for this purpose is huge, ranging from single words and/or gestures, and ending with the use of space equipment, or the use of even fire, radioactive radiation, a knife (scalpel), or even poisons. In this perspective, medicine obliges doctors to continuously track the progress of general knowledge and its technological use. Unfortunately, it is much easier to go into detailed knowledge and understanding of reality rather than to use the necessary generalizations, as is apparent in, e.g., the lack of full public understanding of the theory of relativity and statistical evaluation of reality, not to mention the significance of commonly used terms, such as time, entropy, information, life, etc.

4.6 Causal ACTH Therapy of Recurrent Abortions and Premature Deliveries

During pregnancy, there is increase in the production of hormones and enzymes of the placenta, the function of which has an essential meaning in the mutual mother-fetus neuro-immuno-endocrine relationship. This applies especially to the synthesis of isooxytocinases (cystine-beta-aminopeptidase, CAP_1, and isocystine-beta-aminopeptidase—CAP_2), which decompose hypothalamic hormones. Any damage to the placenta (partial separation, calcification, vascular clots) or only hypoxia leads to a decrease of the concentration of these enzymes in the mother's blood, which automatically results in the increase of not only oxytocin and vasopressin but also of corticotropin-releasing hormone (CRH) and gonadotropin-releasing hormone (GnRH). On the basis of the rate of change in the levels of CAP_1 and CAP_2 in the mother's blood, one can determine when the death of the fetus has or—much more importantly—could occur or if it is in danger of miscarriage or premature birth. An important part is played also by the endocrine glands themselves, in which the biophysical processes are of great importance, since they are related both to atomic level of metabolism and purely physical blood flow and concentration of its components. One may observe underestimation of the dominance of neurohormonal hypothalamus-pituitary-adrenal axis over an analogical axis ended with gonads, which are related to adrenal glands by metabolism of steroid hormones. Excessive use of steroid hormones not only inhibits gonadal steroidogenesis but also blocks hypothalamic stimulation of endocrine glands, i.e., gonads and adrenal glands. For instance,

the use of contraceptive pills for several months excludes a cyclic activity of gonads, whose role is to prepare a potential mother not only to get pregnant but also to a proper development of pregnancy and fetus, e.g., in case of recurrent miscarriages, necessary adrenal stimulation is rarely used. What more, a correlation between serial administration of corticotropin and the mass, the maturity, and the fetal age of the newborn has been repeatedly shown to exist. A low concentration of ACTH in the mother's blood decides on the necessity of a substitution treatment. The fall of ACTH concentration is a natural occurrence only before birth in pregnancies brought to term physiologically, while at an earlier time, it signals an endangerment of the pregnancy due to a miscarriage or premature birth.

Long-acting adrenocorticotropin (ACTH-depot) is recommended also in cases of multiple gestations, premature rupture of membranes, or fetal and maternal complications, i.e., diabetes hypertension or infection. The therapy (0.5 mg dose/week) is safe and can be used multiply during all trimesters of pregnancy. Low concentration of maternal blood ACTH and insufficient increase of oxytocinases provide the effective hormonal treatment before the clinical symptoms of abortion become evident. Indications for the treatment are also neurohormonal hypothalamic post-pregnancy syndrome, habitual miscarriages, a premature childbirth, shortened or nonexistent lactation after previous childbirths, long-term usage of anti-conception pills (especially during maturation years), as well as cytological or colposcopically determined precancerous cervical states. Special group for the treatment are pregnant women who underwent infertility treatment, of which 67% show clinical and laboratory indication for its implementation. The level of ACTH below 5 pg/mL is an indication for a continued substitution therapy with ACTH-depot, because the hypothalamic-pituitary-adrenal axis is more significant for the viability of the fetus than the hypothalamic-pituitary-gonad axis.

The role of ACTH in creating a tolerance for the embryo becomes apparent in a slight decrease of prepregnancy level of this hormone in women with 14.1 ± 7 pg/mL to 12 ± 6 pg/mL and a return to them in the second trimester (15.4 ± 5 pg/mL) and to increase in the third trimester to the highest pre-birth levels of 23 ± 10 pg/mL, which, in contrast to oxytocinases, sharply decrease already during delivery. Also levels of $CAP_1 < 0.8$ μmol/L/min and $CAP_2 < 1.4$ μmol/L/min in an early pregnancy are an indication for beginning the therapy with single 0.5 mg doses of ACTH-depot, while levels of both these enzymes ≤4 μmol/L/min in the third trimester require their continued weekly use. A low prenatal concentration of oxytocinases on the order of 3 ± 1 μmol/L/min unambiguously points to an insufficiency in the production of neurohormones, while a substitutive therapy with ACTH-depot causes a normalization of the prenatal concentration of oxytocinases (7.8 and 8.1 μmol/L/min). The higher is the concentration of enzymes at the end of the pregnancy, the higher the neurosecretive and immunological capacity of the mother with the corresponding increase in fetal age, mass, length, maturity, and the postnatal adaptation of the infant to the values characterizing the infants of the control groups which did not need a substitutive adrenocortical therapy. The application of ACTH-depot results in the disappearance of symptoms of a premature birth without the need for tocolysis and leads to the decrease of breathing disorders in infants. Newborn's maturity index shows a high positive correlation with fetus age, mass, and length. Iatrogenic and social factors predisposing all diseases in women result from main of

obstetrical causes: (1) the increase in the number of operative deliveries and prematurely due to lack of correct understanding of the relative duration of pregnancy, (2) failure to conduct the measuring of the blood levels of oxytocinases (CAP_1 and CAP_2) as the most stable enzymes regulating the neuroimmunological state of pregnancy, (3) use of dexamethasone and betamethasone instead of adrenocorticotropin for preventing miscarriages and prematurity of neonates, and (4) discounting the incidence of the hypothalamic neurohormonal insufficiency syndromes as the large causes of the pathological course of pregnancies.

4.7 Individual Date of Human Birth

Pregnancy as a process of transmitting life to the next generation in relation to man applies not only to biology but also to the psycho-emotional processes so often more powerful than ecological phenomena. Man himself decides about procreation even without the necessity of informing the sexual partner, especially if the possible pregnancy is a threat to his personal socioeconomic status. Unfortunately, more and more people are delaying the possibility of pregnancy, thus deliberately limiting the primary process in which biology precisely determines the optimum time for conception and only allows psychological motivation. The vast majority of people simply ignore the fundamental task pertaining to the maintenance of the species of *Homo sapiens*, and only infertile people know the value of pregnancy as the most precious gift of life (Fig. 4.3).

Man matures in the mother's womb and is born at any time of day and year with a naturally large range of the duration of pregnancy (259–302 days), weight gain (2600–4000 g), growth (48–60 cm), and maturity (6–12 technical quanta). According to the existing equivalence of energy, information, and mass, these different features combine the same ratio of increase of their value relative to the lapse of time between their two consecutive obstetrical ultrasonographic measurements. This allows one to forecast the state of the newborn and the date of his birth with an accuracy of ±3 days instead of ±3 weeks in the currently poorly programmed ultrasound machines (declaration of birth). The obstetric calendar scale falsifies reality, because it considers a premature birth to occur only before the 37th week, which happens with the same frequency during later weeks. Fetuses of the same mass (3400 g) and height (54 cm) can mature slowly (A), regularly (B), or quickly (C) in different calendar time, correspondingly until 42, 40, or 37 weeks, e.g., child B born on the 281st day ($40^1/_7$ weeks) is carried longer relative to child C but shorter to child A despite the same maturity real time. By performing two ultrasonographic measurements of the fetal size 2 weeks apart, already 2 or 3 months before individual birth term, one can determine the date of the expected delivery with a precision of ±3 days. It can be confirmed with an oxytocin test for uterine sensitivity and/or with the fall of oxytocinasemia in the last days of the pregnancy in order to assist with actual premature births also after the 37th week of pregnancy or not induce them and through this increase the prematurity of children through a too-early intervention. An enzymatic monitoring of the pregnancy prevents not only premature births but also a possible loss of child by using ACTH-depot therapy due to low or diminishing concentrations of oxytocinases in the mother's blood if there is no direct method of determining the ACTH levels.

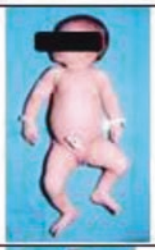
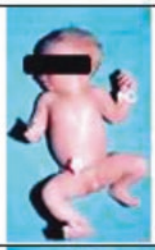
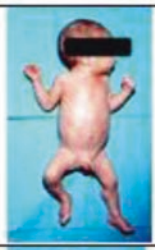
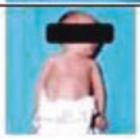
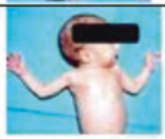
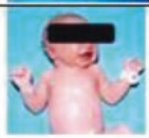
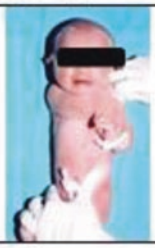
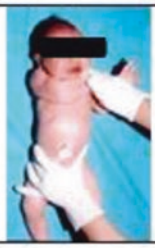
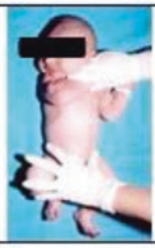
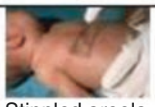
Points	0	1	2
Posture			
Angle forearm to arm	 100°-180°	 90°-100°	 <90°
Pulling an elbow to the body			
Lanugo	Thinning	Single bald areas	Mostly bald
Plantar creases	Only on anterior sole	On anterior 2/3 sole	Cover entire sole
Breast	 Stippled areola bud<3 mm	 Raised areola bud 3-4 mm	 Full areola bud >4 mm

Fig. 4.3 Maturity index of child 0–12 points

Premature births occur at the latest at the end of the 42nd week of pregnancy, contrary to a popular lie that it can only affect pregnancy before 37 weeks of their duration. Obstetricians determine the beginning of the pregnancy on the basis of the last period reported by the woman with an accuracy of a few days. As a result, the number of iatrogenic preterm births increases, with a documented causal link to cervical cancer. Preterm birth means a not fully mature fetus born a week or more before the real date of the individual term. Relying on statistics and not on individual clinical data has led for the need to combat "obstetric terrorism" because of frequent interruptions by cesarean section the natural fetal human development without the necessary medical indication. After all, this is one of the most iatrogenic factors, namely, while done before the last week of normal pregnancy, in which rapid maturing occurs: the lungs and heart adapt to rapid changes in blood circulation and

respiration of the child outside the mother's system, which has a decisive influence on the child's life. The tragic consequences are also neglected by doctors when informing patients about the possibility of using proven and already existing measures to prevent an excessive number of induced deliveries, prematurity, obstetric hemorrhage, and morbidity after pregnancy, lack of or reduced lactation, and especially the issuing of textbooks and monographs which knowingly omit a description of effective medical procedures, and the recommendations contained therein often contain biased advertising. These problems take on a new dimension in connection with describing informatonosis as a new disease that is easiest to detect in doctors due to their vocation for expressing their pernicious notions. This purely informational disease affects people with an intact sanity and differs from schizophrenia in that it is not characterized by delusions, hallucinations, or distraction. Therefore infonotics, unlike schizophrenics, may be subject to criminal prosecution for an intellectual crime, i.e., through their own words and harmful actions against socially acceptable values and principles. In contrast, effective treatment is the verbal communication of truth about the natural reality in which the word has executive power.

Conclusions

The introduction of purely informational diseases is one of the greatest discoveries of twenty-first-century medicine. Lies, telling untruths, or concealment is an adequate cause of many diseases, because they destroy motivated human behavior. For example, man instead of the exchange of products and services for money (coins) unfortunately began to use only information of their values or their very own price. Thus money has become the informational basis of business and joined such concepts as power, fame, authority, awareness, love, and so on, but through the life and work of individuals, money indirectly still is associated with substantive information and energy of human reality, because in social life if one does not know what is going on, it is usually due to money and power. Between the sender of information, the recipient of information, the source of information, and the place where this information is used, there must exist a kind of resonance, so that the information can function already in the unique intrauterine human development stage, later in his upbringing, education, and finally in regard to their own existence in society. A special gift is pregnancy, not only due to the biological transmission of the cells of the human life but also due to the support of the psycho-emotional health of the family, which takes place in optimum condition of the woman's health. Procreation provides the emergence of the family as the most valuable supporting structure in the existence of the species. Infertility or carcinogenesis is a state of the living body where it is not in its natural inner balance, in accordance with its age. Nothing in nature is free. Physicians should not only fight existing cancer cells, but they should fight them as they arise and not look for present cancer to treat it but look for people who are likely to develop cancer. In every living human, there are around a hundred mutated cancer cells created every day. If the immune system is in a bad state, any one of these cells can survive and hide for a while, and when once again the immune forces are impaired, they can begin to grow. Cancer is the defense of a single cell against its nonexistence. The time between the first cancer cell and the first clinical symptoms lies at 10–15 years. The fight against cancer must be shifted to the first causal stage of information.

Medicine which is focused only on pathology does not fulfill the requirements of the modern perinatology, which points to the necessity of using the entire human knowledge and does not allow to change physiological events into pathological ones. Modern medical means as ultrasonography devices, cardiotocographs, or neonatological incubators from the technical point of view stems from the greatest advances of quantum mechanics, theory of relativity, and biocybernetics. Unfortunately, their use in obstetrics sometimes paradoxically leads to iatrogenic morbidity and mortality due to lack of understanding of fetal maturation and relativity of calendar pregnancy duration through unnecessary labor induction or cesarean section at a time improper for individual pregnancy. Labor at an improper time is a common obstetrical error, especially 1 week before true individual term as consequence of its iatrogenic induction. By means of the existent ultrasonography devices on the basis of two measurements within ≥2 weeks, the obstetricians not only assess the current maturity, mass, length, and gestational age of the child but also predict those values in the perinatal period to bring the percentage of premature birth down to the natural limit of 2.5% of all deliveries. Currently, 10–18% of labors are induced prematurely only because the calendar time of pregnancy duration has exceeded 287 or 294 days from the date of the last menstrual period, which additionally is given by the mothers accurate to several days, anyway. After reprogramming of ultrasonography biometry as well as revitalizing of enzymatic monitoring of pregnancies, there is a moral imperative to evaluate fetal maturity of newborn infants immediately after their deliveries. Therefore, to bring out the role of obstetricians there in, one should permanently introduce two other clinical criteria: distribution of birth in the range of 6-week norm of occurrence in humans and ratio of premature infants to the mature ones at the consecutive gestational age. Such individual evaluation of each delivery ought to be performed directly in obstetrical ward not only by routine assessment of the adaptation of the newborn in Apgar scale but also of its fetal maturity according to new index of fetal maturity, what is particularly important in the case of instrumental deliveries.

In 1847 I.P. Semmelweis in his historic publication described *The Etiology, Concept, and Prophylaxis of Childbed Fever* remarked about the lack of medical students washing their hands. It was not until the late nineteenth century that the introduction of his discovery to obstetrics led to a steep fall in deaths from puerperal fever. Nowadays, it is time to transform pure morphological and biochemical medical views into a more thermodynamic interpretation of cause-effect relationships. It has taken 20 years to achieve recognition of neurohormonal background of cervical cancer and 30 years to understand the thermodynamic cause of cancerogenesis. Thirty years ago, has been introduced immunotherapy of cervical intraepithelial neoplasia, but the first of all, preventing cancer depends on strengthening the immune system, a constant improvement in better health education, and regular control/prophylactic examinations. In fighting diseases, the defenses are proper eating habits and taking care of the environment in such a way as to use one's biological-economic heredity, which is essential in the battle with cancer. Medicine, through surgery, radio-, chemo-, thermo-, and hormonal therapy, is responsible only for about 20% of the treatment. Therefore each person needs knowledge and also skill in practical application of life force

of science and current and correct information. That is why self-education in medicine became the most important guarantee of a professional level, and each professional has two duties: competence and supplying information.

In its primary form, information manifests itself as consciousness, the "I" (ego) in the existence of every person. In reality it is contained in each cause as a program for a caused effect. There are tragic consequences of doctors' neglect of informing the patient about the methods of preventing an excessive number of operative births, prematurity, blood loss, and illnesses after the pathological pregnancy, a missing or shortened lactation, infertility, etc., due to an informational lack of knowledge. These problems acquire a new importance in connection with the description of informatonosis as a new disease of social life which can be found in doctors when diagnosed with a conscious distancing from their professional calling and proclaiming harmful views. This illness pertains to persons with an intact understanding to their own words and actions, but not to a socially accepted hierarchy of values and rules, which makes it different from schizophrenia and can therefore fall under criminal law.

The withdrawal of objective monitoring methods of a pregnancy and birth prognosis from clinical practice led to an excessive rise in iatrogenic cesarean sections even in clinics which have been using them successfully for decades, including also oncological prophylactics. There are many factors that contribute to cancer disease, among them viruses and infections, accounting only for 10–15% of all cancers while tobacco smoking and diet ca 60%. The self-organization of the body's cells existing at lethal risk prolongs only the cellular form of living matter with a new genome to protect life itself. Cancer can develop only in the host's organism, and owing to that, they perish together, because the sum of entropy production in both of them must always grow according to the second thermodynamic law. It is enough to understand the difference between emerging neoplasm's cells and the neoplastic illnesses created by them, of which society knows only the last phase, without understanding the role of the patient in their emergence, as well as the lack of understanding by many doctors of the biophysical factors in life and death, the alternatives to which are neoplasms.

Information has a significant impact on the lives and health of people already in the unique intrauterine human development stage, later in his upbringing, education, and finally, in regard to their own existence in society due to the support of the psycho-emotional health of the family, which takes place in optimum condition of the woman's health. Man consciously may limit procreation, which in many cases has already led to the real threat of denationalization of most developed societies. Information govern not only the distribution of matter and energy but also the informational reality as perceived by people as love, fear, hatred, jealousy, self-awareness, etc. Each of these feelings has a beginning and its conditions, as well as all the events and their perceived causes and sources. In every civilization, verbal information not only applies to an event which informs but is in itself, as, for example, a lie, always evil in contrast to promoting the good of truth, even if it sometimes reveals the negative aspects of social life. In the informational twenty-first century, it is essential to elevate the public awareness of medical knowledge, since it is the public that uses modern medicine, and as such it should be well informed as it is responsible for understanding the results of the

commercialization of medical benefits. People must be convinced that not only the doctors can win the battle but primarily the patient and his organism. It is essential that the doctor and the patient share an intimate relationship in all aspects of medical knowledge, from technology to psycho-medicine.

References

1. Cosmi EV, Klimek R, Di Renzo GC, Kulakov V, Kurjak A, Maeda K, Mandruzzato GP, van Geijn HP, Wladimiroff J. Prognosis of birth term: recommendations on current practice and overview of new developments. Arch Perinatal Med. 1997;3(2):31–50.
2. Jasiczek D, Klimek R, Štencl J, Tadeusiewicz R, Tholt M. Obstetrical prevention of human cancers. Neuroencrinol Lett. 2012;33(2):118–23.
3. Jasiczek D, Kaim I, Czajkowski K. Historic discovery of natural thermodynamic cause of cancer. Neurocrinol Lett. 2012;33(4):101–11.
4. Jasiczek D, Klimek R. Informatonosis—an information disease affecting the society. Int J Prenat Perinat Psychol Med. 2013;25:22–31.
5. Khroutski KS. Forming an evolutionary vector to the Aristotelian pole of scientific organicism (Biocosmology). Biocosmology-Neo-Aristotelism. 2013;3(1):28–52.
6. Khroutski KS. Reinstating Aristotle's Comprehensive Organokosmology and the genuine language of his organicist naturalism archetype. Biocosmology-Neo-Aristotelism. 2016;6(3–4):394–413.
7. Klimek M. The effectiveness of adrenocorticotropin repeated doses in high risk pregnancies. Fetal Diagn Ther. 2006;21:528–31.
8. Klimek M, Klimek R, Mazanek-Mościcka M. Preterm birth as an indicator of cancer risk for the mother. It J Gynacol Obstet. 2002;3:73–7.
9. Klimek R. Monitoring of pregnancy and prediction of birth date. New York, London: The Parthenon Publishing Group; 1994.
10. Klimek R. Threefold material informational energetic reality: $E \overset{i}{\rightleftarrows} MC^2$. Biocosmology-Neo-Aristotelism. 2014;4(4):393–402.
11. Klimek R. Prevention o iatrogenic cervical cancer. Open J Obstet Gynecol. 2015;5:775–83.
12. Klimek R, editor. Psychoneurocybernetic conquest of carcinogenesis and cancers. New York: Nova Science Publishers; 2015.
13. Klimek R. Chapter 4: Causal prevention of cervical carcinogenesis. In: Recent advances in cervical cancer: Avid Science; 2016.
14. Klimek R, Bręborowicz GH, Chazan B, Czajkowski K, Dębski R, Dubcak J, Fedor-Freybergh P, Hajek Z, Kamiński K, Klimek M, Krzysiek J, Lauterbach R, Lukacin S, Malarewicz A, Maly Z, Marianowski L, Oleszczuk J, Pisarski T, Reroń A, Roztocil A, Sajdak S, Skręt A, Stencl J, Szymański W, Wilczyński J, Unzeitig V, Zdebski Z, editors. Declaration of the Childbirth in XXI century. Arch Perinatal Med. 2001;7(3):8–14.
15. Klimek R, Czajkowski K, Kojs Z, Szymański W, Śpiewankiewicz B. Psychoneurocybernetic etiopathogenesis of cancers. Curr Gynecol Oncol. 2013;11(3):202–9.
16. Klimek R, Jasiczek D, Stencl J. Explained cause of cancer. Cracow: Polish Academy of Science; 2013.
17. Kurjak A, Chervenak FA, editors. Controversies on the beginning of human life. New Delhi: Jitendar; 2008.
18. Schenker JG. Stress and human reproduction. In: Klimek R, editor. Pre- Peri-Natal Psycho-Medicine. Cracow, Poland: DReAM; 1992. p. 77–82.
19. Schenker JG. Ethical dilemma in perinatal medicine. New Delhi: Jaypee Brothers Medical Pub.; 2010.
20. Tadeusiewicz R. Place and role of intelligent systems in computer science. Comput Methods Mater Sci. 2010;10(4):193–206.
21. Tadeusiewicz R. Biocybernetics links medicine and technology. In: Klimek R, editor. Psychoneurocybernetic conquest of carcinogenesis and cancers. New York: Nova Science Publishers; 2015. p. 77–97.

Thyroid Diseases and Female Infertility

5

Petrache Vartej and Ioana Vartej

5.1 Introduction

Normal thyroid function is crucial for human reproduction. Thyroid diseases—hypothyroidism and hyperthyroidism—in women of reproductive age are associated with a broad spectrum of disorders, from menstrual irregularities and infertility to pregnancy loss. The effects of thyroid hormones on female reproduction have been extensively studied and are well documented; an important amount of evidence from animal and human studies is available, supporting the role of thyroid hormones on ovarian, endometrial, and placental physiology. Treatment of thyroid diseases can successfully restore menstrual function and fertility, reducing the likelihood of further procedures of assisted reproduction technology. Consistently negative association exists between thyroid autoimmunity without thyroid dysfunction and infertility and early miscarriage.

5.2 Epidemiology of Thyroid Diseases

Thyroid diseases are common endocrine disorders. Estimates of the prevalence of thyroid disorders are mainly from iodine replete areas—in the US National Health and Nutrition Examination Survey (NHANES III), the prevalence of hypothyroidism was 4.6% (0.3 overt and 4.3% subclinical) and the prevalence of hyperthyroidism 1.3% (0.5 overt and 0.7% subclinical) [1]. The incidence of thyroid disorders in

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adults in a community followed for 20 years (The Whickam Study) was for hypothyroidism in women 350/100,000/year and in men 60/100,000/year while for hyperthyroidism in women 80/100,000/year and in men 8/100,000/year. The most striking features of the epidemiological studies were the high prevalence of hypothyroidism, the marked female preponderance, and the increasing occurrence with advancing age [2]. Worldwide, the most common cause of hypothyroidism is iodine deficiency. In areas of adequate iodine intake, autoimmunity is the main cause of hypothyroidism. Autoimmune thyroid disease (AITD) refers to the interrelated conditions: Hashimoto thyroiditis (goitrous or atrophic), hyperthyroid Graves' disease, and postpartum thyroiditis.

In women of reproductive age, the prevalence of hypothyroidism is 2–4%. In this age group, AITD is the most frequent cause of hypothyroidism. In iodine replete areas, the incidence of autoimmune hypothyroidism in women was 498/100,000/year and in men 2/100,000/year while for autoimmune hyperthyroidism in women 99/100,000/year and in men 0.7/100,000/year [3]. The US NHANES III reported the presence of TPO-AB in 13% and Tg-Ab in 11.5% of the population. AITD is the most common autoimmune disorder, affecting 5–20% of women in the childbearing period. Although thyroid autoimmunity predisposes to development of hypothyroidism, the majority of women with AITD do not have thyroid dysfunction.

5.3 Physiological Effects of Thyroid Hormones on the Female Reproductive System: Molecular Basis and Mechanism of Action

Thyroid hormones play an important role in reproductive function both through direct effects on the female reproductive organs and indirectly by interacting with sex steroids and sex hormone-binding protein. Thyroid hormone synthesis and release into circulation are regulated in a negative feedback manner by the pituitary hormone thyroid-stimulating hormone (TSH); hypothyroidism results in increased TSH levels, and hyperthyroidism produces suppression of TSH levels. Thyroid hormones are produced by the follicular cells of the thyroid gland, from the precursor protein thyroglobulin; iodination of the tyrosine residues of thyroglobulin by thyroid peroxidase produces the thyroid hormones T4 (thyroxine or tetraiodothyronine) with four iodine atoms and T3 (triiodothyronine) with three iodine atoms per molecule. The predominant physiological thyroid production is the biologically inactive T4, with a smaller amount of bioactive T3—in a ratio 14/1 to 20/1 [4]. The circulating thyroid hormones are bound to transport proteins (thyroxine-binding globulin TBG, transthyretin TTR, and albumin), with only a small fraction (0.1%) being free and transferred across the membranes in target cells by an active transporting mechanisms involving monocarboxylate transporters (MCT), L-type amino acid transporters (LAT), and organic anion transporting polypeptides [5]. The prohormone T4 is converted to the active T3 form by iodothyronine deiodinases—three distinct enzymes (DIO1, DIO2, DIO3) with specific tissue expression. T3 acts through nuclear thyroid hormone receptors alpha (THRA) and beta (THRB),

expressed in a tissue-specific manner. Thyroid hormone receptors regulate gene expression by binding to hormone response elements [6].

The role of thyroid hormone in reproduction and early pregnancy is supported by the evidence that almost all factors essential for thyroid hormone action, such as THRA and THRB, thyroid hormone transporters, and deiodinases, are expressed in the oocytes, cumulus cells, granulosa and stromal cells, endometrium, placenta, and early embryo, indicating the bioavailability of thyroid hormones in these tissues and their dynamic local regulation.

5.4 Effects on Ovarian Follicles

Thyroid hormones T3 and T4 are detected in the follicular fluid, with positive correlation between serum and follicular fluid levels [7]. Both thyroid hormone receptor isoforms THRA and THRB are expressed in human oocytes, with an increased expression during follicular growth—indicating a direct effect on folliculogenesis and ovulation [8]. The growth of rat preantral follicles is stimulated by thyroid hormones. T3 in combination with FSH enhances granulosa cell proliferation and inhibits granulosa cell apoptosis by the PI3K/Akt pathway [9]. Thyroid hormones play a role in the production of ovarian steroid hormones. T3 acts as a biological amplifier of the stimulatory action of FSH on granulosa cell function, also increasing LH receptors and progesterone secretion by the granulosa cells. T3 stimulates the proliferation of granulosa cells and hCG-induced c AMP in these cells [10]. Abnormal thyroid hormone levels may therefore produce alterations in ovulation

5.5 Effects on the Endometrium

Thyroid hormones have direct effects on the endometrium. Thyroid hormone receptors THRA and THRB are detected in the glandular endometrium, with variable expression during the phases of the menstrual cycle, increasing in the secretory phase and decreasing subsequently. Deiodinases DIO2 and DIO3 are present in the human endometrium throughout the menstrual cycle with lower expression in the mid-secretory phase and an inverse relationship with serum progesterone levels [11]. DIO2 activity in the stromal cells is regulated by progesterone; DIO2 produces intracellular T3 that may influence the uterine response to implantation [12].

5.6 Effects on Fertilization

Data of effects of thyroid hormones on fertilization and embryo quality derive from studies of assisted reproduction technologies ART. The positive effect of thyroid hormones on fertilization was evidenced by improvement of the number of high quality embryos and birth rate in women with subclinical hypothyroidism undergoing ART treated with L-thyroxine compared with non-treated women [13].

5.7 Effects on Implantation and Placentation

A potential endocrine, paracrine, and intracrine role of the thyroid hormones on embryo implantation and trophoblasts has been discussed. Thyroid hormone receptors THRA and THRB, TSH receptors, thyroid hormone transporters, and deiodinases are widely expressed in the fetomaternal unit during implantation and placentation. Thyroid hormones act either directly or by modulating the production and activity of various cell adhesion molecules and cytokines. Furthermore, the expression of genes involved in thyroid hormone production—thyroglobulin, thyroid peroxidase, and NIS sodium iodide symporter—in endometrial and syncytiotrophoblast cells could support a local production of thyroid hormones. It is unknown if the relatively low expression levels also mean that there is a functional effect, but we could speculate that alteration of factors that might regulate the uterine production of thyroid hormones could modify the local effects of thyroid hormones, even in the presence of normal systemic hormone levels [14]. Thyroid hormones stimulate the secretion of progesterone and human placental lactogen in trophoblast cells. Progesterone is responsible for the optimal endometrial lining and embryo implantation, as well as for the local immune tolerance preventing the rejection of the fetal allograft. Human placental lactogen is involved in fetal glucose supply by reducing maternal insulin sensitivity and increasing lipolysis, playing also a role in embryonic growth. T3 increases the expression of matrix metalloproteinases MMP-2 and MMP-3, fetal fibronectin, and integrin α5β1 in cultured early placental extravillous trophoblasts, suggesting a role in the invasive potential of trophoblasts. Also, thyroid hormones modulate the inflammatory cytokines involved in implantation—upregulating leukemia inhibitory factor (LIF) expression in endometrial cell cultures [15].

The placental tissue has all the equipment for transporting thyroid hormones—T3 membrane transporters are localized in the syncytiotrophoblast, in order to supply the thyroid hormones to the embryo. A highly sensitive regulation of thyroid hormones in the placenta is possible due to the expression of all three types of deiodinase.

Further studies are needed to better characterize the molecular mechanisms of thyroid hormones involved in female reproduction and the implications associated with thyroid dysfunction that could help resolve infertility in thyroid diseases.

Pathophysiology of reproductive abnormalities in thyroid disorders—hormonal changes.

Both primary hypothyroidism and hyperthyroidism in women of reproductive age produce variable degrees of gonadal dysfunction leading to ovulation disturbances and menstrual cycle irregularities.

The hormonal changes in women with in hypo- and hyperthyroidism are summarized in Table 5.1.

Changes in SHBG (sex hormone-binding globulin) due to the stimulating effects of thyroid hormones on hepatic production of SHBG, altered estrogen

Table 5.1 Hormonal abnormalities in women with thyroid dysfunction

	Hypothyroidism	Hyperthyroidism
SHBG	Decreased	Increased
E2	Decreased Decreased metabolic Clearance rate	Increased D\decreased metabolic clearance rate
LH	Reduced response to GnRH	Increased Increased response to GnRH
FSH	Reduced response to GnRH	Increased Increased response to GnRH
PRL	Increased	Unmodified
Progesterone	Decreased/unmodified	Decreased/unmodified
Testosterone	Decreased	Increased
Δ4-androstenedione	Decreased	Increased
Testosterone to E2 conversion	Increased	Increased

and androgen metabolism, and modified response of gonadotropins to GnRH are frequently observed in hypo- and hyperthyroidism. By influencing peripheral estradiol metabolism, abnormal thyroid hormone has profound effects on regulation of the hypothalamic-pituitary-ovarian axis, inadequate ovulation, and corpus luteum formation, on the proliferation and maturation of endometrial tissue, and consequently on implantation and early development of the blastocyst. In hypothyroidism SHBG serum level is reduced leading to decreased level of total estradiol and increased level of free estradiol, while in hyperthyroidism increased SHBG leads to an increase of circulating total estradiol, with normal or reduced free estradiol. The metabolic clearing rate of estradiol is reduced in both hypo- and hyperthyroidism [16].

Alterations in steroid metabolism resolve with the restoration of the euthyroid state. Testosterone and androstenedione plasma levels increase, and the production rates of testosterone and androstenedione are significantly elevated in hyperthyroid women. The conversion ratio of androstenedione to estrone, as well as of testosterone to estradiol, is increased in hyperthyroid women. LH levels are significantly higher in hyperthyroid women than in normal women, with an exaggerated response to GnRH. FSH levels may be increased. Serum LH levels decrease to normal after a few weeks of treatment with antithyroid drugs. Hyperthyroxinemia increases the gonadotrophin response to GnRH, and baseline gonadotrophin concentrations are also frequently elevated [17]. Hypothyroid women, beside a blunted or delayed LH response to GnRH and an abnormal pulsatile release of LH, can also present hyperprolactinemia due to increased hypothalamic TRH that stimulates both TSH and PRL secretion and explain the high frequency of ovulatory dysfunction and infertility in women with hypothyroidism. Galactorrhea may also occur; all these hormonal modifications usually disappear after restoration of normal thyroid hormone levels; thyroxine administration in hypothyroid women increases the chance of spontaneous pregnancy [18].

5.8 Menstrual Abnormalities in Thyroid Dysfunction

Changes in menstrual cycle length and blood flow are common in women with altered thyroid function; approximately three times higher than in the normal population, oligomenorrhea and menorrhagia are the most common menstrual abnormalities. Amenorrhea was already reported by von Basedow in 1840. Since then, amenorrhea has been frequently described, as well as a number of other changes, including oligomenorrhea, hypomenorrhea, polymenorrhea, menorrhagia, and anovulation.

There is a striking difference between earlier and recent studies reporting the prevalence of menstrual irregularities in hypo- and hyperthyroid women, with earlier studies showing a higher prevalence. This can be explained by the delayed diagnosis of hypothyroidism in earlier studies, with a more severe clinical picture. The prevalence of menstrual abnormalities in hyperthyroidism was described in earlier studies up to 60%, while the recent studies found irregular cycles in approximately only 20% [19].

Similar patterns are observed for hypothyroidism in women of reproductive age—early studies showing up to 80% prevalence of menstrual disorders [20] and recent studies about 20%. Menorrhagia and polymenorrhea are frequently described in hypothyroid women, probably due to estrogen breakthrough bleeding secondary to anovulation and defects in hemostasis factors associated with hypothyroidism (decreased levels of factors VII, VIII, IX, and XI) [21].

5.9 Fertility in Women with Thyroid Dysfunction

Few studies about fertility in women with hyperthyroidism are available, mainly uncontrolled and cohort studies, making difficult to assess the impact of hyperthyroidism on female infertility. The prevalence of infertility in hyperthyroid women was estimated 2–5% [22]. Most hyperthyroid women remain ovulatory according to results of endometrial biopsies [20]. What we can affirm is that women of reproductive age and increased thyroid hormone levels should be treated appropriately restoring euthyroidism, avoiding radioiodine especially if pregnancy or ART procedure is planned.

Studies that examined the incidence of infertility in hypothyroid women are also scarce, most of them uncontrolled, retrospective, cross-sectional, or in selected hypothyroid patients (therefore biased) attending infertility clinics. Ideally, this should be studied prospectively and with an age-matched control group. The estimated prevalence of overt hypothyroidism in infertile women from different available studies was 2–6% [23, 24].

Subclinical hypothyroidism (SCH) is a challenging aspect for female infertility. The classic definition of SCH is a TSH level greater than the upper limit of normal range (4.5–5.0 mIU/L) with normal free thyroxine (FT4) levels. Controversies persist in the appropriate upper limit for serum TSH in women attempting pregnancy and the decision to treat, some authors proposing a value of 2.5 mIU/L and the

initiation of therapy with L-thyroxine. TSH upper reference values in pregnancy have already been modified because human chorionic gonadotropin (hCG) can bind to the TSH receptor and influence TSH values: 2.5 mIU/L is the recommended in the first trimester, 3 mIU/L in the second, and 3.5 mIU/L in the third. Studies investigating the association between SCH and female infertility are based on different upper serum TSH cutoffs, and the data are limited and highly variable due to study design, poorly controlled, prospective, or retrospective, selection criteria, and type of infertility studied. Overall, the prevalence of SCH in women with infertility ranged from 1 to 4%, reporting also as high as 30% [25], while some authors suggested that the prevalence of SCH is similar in infertile women and the general female population [26]. The main trend in these studies is that SCH is higher in women with ovulatory dysfunction than in other causes of infertility.

5.10 Autoimmune Thyroid Disease AITD and Female Infertility

The immunological markers of AITD are the circulating serum antibodies TPO-Ab (thyroid peroxidase antibodies), Tg-Ab (thyroglobulin antibodies), and TSHR-Ab (TSH-receptor antibodies). The last 10 years brought considerable progress in understanding the multifactorial etiology of autoimmune thyroid disease (AITD)—a combination between genetic susceptibility (HLA-DR alleles, CTLA-4 polymorphism) and environmental factors (iodine intake, radiation, smoking, selenium and vitamin D deficit, drugs, toxins, microorganisms). AITD can be associated with other organ-specific and non-organ-specific autoimmune diseases (autoimmune polyglandular syndromes I, II, and III), suggesting a shared immunogenetic background [27].

AITD has a strong female preponderance, 5–10 times higher than in men. This can be explained, at least in part, by a combination of genetic factors, estrogen-related effects, chromosome X abnormalities, and fetal microchimerism. Autoimmunity is abnormally high in practically all X-linked disorders. The X chromosome contains the largest number of immune-related genes of the human genome, and the long arm (Xq) controls functional ovarian reserve and autoimmunity. The FMR1 gene (fragile X mental retardation 1-ovarian function) is involved in ovarian recruitment and reserve, and its mapping at Xq27.3 occupies the crossroads between ovarian function and autoimmunity. X chromosome might constitute the common trait of the susceptibility to autoimmune diseases [28].

Many studies have investigated the relationship between AITD and female infertility. However, the interpretation of the data is rather difficult because of the heterogeneity of the sample size or geographic origins with variable iodine intake, uncontrolled retrospective design of many studies, difference in assays used to measure thyroid antibodies, selection biases, and causes of infertility. Overall, the majority of the studies showed an increased prevalence of AITD among women with infertility; the relative risk of AITD for female infertility ranges from 1.2 to 3.8 [25], while only a few studies did not show any difference in AITD prevalence in infertile women compared with fertile controls [26].

AITD is especially prevalent among women with polycystic ovary syndrome (PCOS) and endometriosis. A strong association between thyroid autoimmunity and PCOS—two of the most common endocrine diseases in women—has been described; the prevalence of AITD in PCOS patients is 2.2–3.5 times higher than in controls [29–33]. Women with PCOS and AITD showed a higher risk of clomiphene citrate resistance (OR of 7.7) compared to controls; elevated TPO-Ab levels were associated with poor treatment response in infertile PCOS women [34].

AITD and PCOS frequently occur together. A shared immunogenetic background has been incriminated; lower levels of TGFβ were found in AITD as well as in PCOS women. *FBN3* gene polymorphism seems to be the most plausible candidate due to their influence on TGFβ activity-key regulator of immune tolerance by stimulating regulatory T cells (Tregs) which are known to inhibit excessive immune response [35]. Vitamin D deficiency is often seen in autoimmune disease and PCOS. Sex hormone imbalances with high estradiol to progesterone ratio due to anovulatory cycles in PCOS women could trigger an exacerbate immune response. The role of sex hormones in the pathogenesis of autoimmunity has been studied; estradiol decreases the activity of T suppressor cells, increases the activity of B cells, and increases the secretion of Th2 cytokine IL6 and the formation of antibodies; progesterone decreases the synthesis of IL6 and the peripheral antibody production [36].

A positive association between AITD and endometriosis has also been described in various studies; the relative risk of AITD was 2–3.5 in women with endometriosis as a cause of infertility [24]. The prevalence of endometriosis reaches 25–44% among women with AITD versus 9–14% among controls [26]. Other study could not confirm the association [37]. Endometriosis is associated with immunological changes: autoantibodies to endometrial antigens, complement deposits, decline in the concentration of natural killer cells, and cytotoxic effects on autologous endometrium [38].

AITD with normal thyroid function is associated with greater ART failure and miscarriage after controlled ovarian hyperstimulation (COH). It is important to mention the major impact of COH on thyroid function. The marked increase in serum estradiol and TBG levels with a decrease of free T4 levels—changes that occur more rapid and pronounced after COH—can produce a significant burden on the thyroid, especially in women with AITD, leading to more severe thyroid dysfunction than observed with spontaneous pregnancy. During the first weeks of pregnancy after COH in women with AITD, the TSH values are higher, and FT4 values are lower compared with women without AITD [39].

A first study that described the negative impact of AITD on ART outcomes showed lower pregnancy rates per cycle in women with thyroid antibodies (10.8%) compared with controls (25%), with no differences in the numbers of oocytes retrieved, fertilization rate, or number of transferred embryos [40]. Studies that followed showed controversial results but overall indicate a significant increased risk of miscarriage in euthyroid women with thyroid autoimmunity following ART. Most of the studies reported no difference in clinical pregnancy and delivery rates [41–43], while another study showed lower oocyte fertilization and percentage of grade

A embryos in AITD than in women with negative thyroid antibodies [44]. A recent meta-analysis of the impact of thyroid autoimmunity on ART outcomes involving 12 cohort studies of women showed higher miscarriage rates and lower live birth rate in thyroid antibody-positive women than in women without antibodies. The number of oocytes retrieved and rates of fertilization and implantation did not differ between antibody-positive and antibody-negative women, and clinical pregnancy rates were similar [45]. A randomized study of L-thyroxine therapy impact in women with AITD undergoing ART and throughout pregnancy [46] revealed that therapy in TPO-Ab-positive women did not affect the pregnancy and delivery rate. The study also evidenced a twofold increase in the risk of miscarriage in AITP patients compared with controls; miscarriage rate was reduced to 33% in treated TPO-Ab-positive women, compared with 52% in untreated controls. Recently, a prospective cohort study of women with a history of miscarriage showed that TSH levels greater than 2.5 mIU/L or positive thyroid antibodies were not associated with impaired fecundity, pregnancy loss, or live birth rates [47].

The pathophysiological mechanisms associating AITD and female infertility remain hypothetical until today; there are no clear data that indicate a causal relationship between thyroid antibodies and reproduction failure. The current knowledge on thyroid antibodies and their impact on female fertility are extrapolated mainly from studies in ART settings, examining oocyte retrieval, fertilization rates, embryo quality, miscarriage, and pregnancy rates. Serum thyroid antibodies can pass the blood-follicle barrier; TPO-Ab and Tg-Ab have been identified in the follicular fluid and correlate with serum levels; this could negatively influence the quality of the maturating oocyte, as lower fertilization rates, grade A embryos, and pregnancy rates were observed in AITD women undergoing ART compared with controls [44]. Antithyroid antibodies may react with antigens in the zona pellucida altering its functional role. Human anti-zona pellucida antibodies recognize antigens within murine thyroid tissue; zona pellucida and thyroid tissue seem to share similar antigens [48]. From a practical point of view, intracytoplasmic sperm injection ICSI (which requires no interaction between the sperm cell and the zona pellucida) could be the preferred assisted reproductive method in infertile women with thyroid autoimmunity; this finding must be confirmed through randomized controlled studies.

Evidence for the influence of TPO-Ab on embryo quality is inconsistent among studies, showing either no differences in oocytes retrieved, fertilization rate, and embryo grades between AITD women and controls [40, 49] or a lower fertilization, implantation, and pregnancy rate following ART in women with antithyroid antibody than in controls [50].

No studies are available on the direct effect of thyroid antibodies on the endometrium. TPO and Tg are expressed in the endometrium, and it can be only hypothesized that the endometrium is a potential target for the action of TPO-Ab and Tg-Ab. It is also worth mentioning that TPO-Ab diffuse through the placental barrier, with good evidence of this during the third trimester, without excluding a transfer at earlier pregnancy stages and speculating an interaction at the maternal-fetal interface [51].

Three main hypotheses have been proposed for the association between AITD and infertility, a combination of TSH-dependent and TSH-independent mechanisms. A subtle thyroid hormone deficit due to chronic lymphocytic thyroiditis produced by thyroid antibodies could be incriminated. Although in euthyroid range, TSH levels in AITD infertile women are slightly increased compared to controls. A second hypothesis is that TPO-Ab and Tg-Ab reflect a general autoimmune imbalance (both humoral and innate immunity) that could be responsible for the rejection of the embryo. A third hypothesis is the age factor—the prevalence of AITD increases with age and older women have a higher risk of infertility and miscarriage, but this has not been confirmed in recent meta-analyses [52].

5.11 Recommendations and Guidelines for Women with Infertility and Thyroid Diseases/AITD

Women with infertility—especially with PCOS and endometriosis—should be screened for AITD and thyroid dysfunction, because of the increased prevalence of AITD in this category of patients. Treatment of thyroid diseases and restoration of euthyroidism can improve fertility.

Universal screening of healthy women for thyroid dysfunction and thyroid antibodies before pregnancy is not recommended. TPO-Ab and Tg-Ab measurement should be considered when evaluating women with infertility and particularly miscarriage (spontaneous or recurrent). Selected thyroid screening in women seeking pregnancy is recommended for women over 35 years, infertility, history of miscarriage, history of autoimmune disease, family history of AITD, clinical signs of thyroid dysfunction or goiter, and living in areas of iodine deficiency.

The Endocrine Society, the American Thyroid Association (ATA), and the American Association of Clinical Endocrinologists (AACE) have elaborated guidelines on treating subtle, subclinical thyroid dysfunctions in women of childbearing age. L-thyroxine therapy should be initiated in women who are pregnant or planning a pregnancy, including ART, if TPO-Ab positive and TSH greater than 2.5 mIU/L. Therapy should be considered in women planning a pregnancy including ART or in the first trimester of pregnancy if the TSH level is between 2.5 mIU/L and the upper reference range. Treatment with L-thyroxine should be considered in women with normal serum TSH levels when they are pregnant or planning a pregnancy, including ART, if they have or have had positive levels of serum TPO-Ab, particularly when there is a history of miscarriage or past history of hypothyroidism. The upper limit of TSH of 2.5 mIU/L is therefore proposed as a cutoff before ART, independent of thyroid antibody status. Serum TSH levels should be maintained below 2.5 mIU/L before starting the COH procedure, and monitor thyroid function tests closely thereafter [53, 54].

The American Society for Reproductive Medicine recommends that if TSH levels prior to pregnancy are between 2.5 and 4 mIU/L, either monitor levels and treat when TSH >4 mIU/L or treat with levothyroxine to maintain TSH <2.5 mIU/L. Due to the inconsistent data of the impact of subclinical hypothyroidism on fertility outcomes, L-thyroxine therapy for TSH levels greater than 2.5 mIU/L remains controversial [55].

References

1. Hollowell JG, Staehling NW, Flanders WD, et al. Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). J Clin Endocrinol Metab. 2002;87:489–99.
2. Vanderpump MP, Tunbridge WM, French JM. The incidence of thyroid disorders in the community: a twenty-year follow-up of the Whickham Survey. Clin Endocrinol (Oxf). 1995;43(1):55–68.
3. Mc Grogan A, Seaman HE, Wright JW, DeVries C. The incidence of autoimmune thyroid disease: a systematic review of the literature. Clin Endocrinol (Oxf). 2008;69(5):687–96.
4. Chiamolera MI, Wondisford FE. Minireview: thyrotropin-releasing hormone and the thyroid hormone feedback mechanism. Endocrinology. 2009;150:1091–6.
5. Karapanou O, Papadimitriou A. Thyroid hormone transporters in the human. Hormones. 2011;10:270–9.
6. Lazar MA. Thyroid hormone receptors: multiple forms, multiple possibilities. Endocr Rev. 1993;14:184–93.
7. Wakim AN, Marrero MA, Polizotto SL, et al. Thyroid hormones in human follicular fluid and thyroid hormone receptors in human granulosa cells. Fertil Steril. 1993;59:1187–90.
8. Zhang SS, Carrillo AJ, Darling DS. Expression of multiple thyroid hormone receptor mRNAs in human oocytes, cumulus cells, and granulosa cells. Mol Hum Reprod. 1997;3:555–62.
9. Wakim AN, Polizotto SL, Burholt DR. Augmentation by thyroxine of human granulosa cell gonadotrophin-induced steroidogenesis. Hum Reprod. 1995;10:2845–8.
10. Maruo T, Hiramatsu S, Otani T, Hayashi M, Mochizuki M. Increase in the expression of thyroid hormone receptors in porcine granulosa cells early in follicular maturation. Acta Endocrinol (Copenh). 1992;127:152–60.
11. Aghajanova L, Stavreus-Evers A, Lindeberg M, et al. Thyroid-stimulating hormone receptor and thyroid hormone receptors are involved in human endometrial physiology. Fertil Steril. 2011;95:230–7.
12. Catalano RD, Critchley HO, Heikinheimo O, et al. Mifepristone induced progesterone withdrawal reveals novel regulatory pathways in human endometrium. Mol Hum Reprod. 2007;13:641–54.
13. Kim CH, Ahn JW, Kang SP, et al. Effect of levothyroxine treatment on in vitro fertilization and pregnancy outcome in infertile women with subclinical hypothyroidism undergoing in vitro fertilization/intracytoplasmic sperm injection. Fertil Steril. 2011;95:1650–4.
14. Colicchia M, Campagnolo L, Baldini E, et al. Molecular basis of thyrotropin and thyroid hormone action during implantation and early development. Hum Reprod Update. 2014;20(6):884–904.
15. Oki NM. Effects of 3,5,3′-triiodothyronine on the invasive potential and the expression of integrins and matrix metalloproteinases in cultured early placental extravillous trophoblasts. J Clin Endocrinol Metab. 2004;89:5213–21.
16. Redmond GP. Thyroid dysfunction and women's reproductive health. Thyroid. 2004;14(Suppl 1):S5–S15.
17. Pontikides N, Kaltsas TH, Krassas GE. The hypothalamic-pituitary-gonadal axis in hyperthyroid female patients before and after treatment. J Endocrinol Invest. 1990;13(2 Suppl):203.
18. Krassas GE, Poppe K, Glinoer D. Thyroid function and human reproductive health. Endocr Rev. 2010;31:702–55.
19. Krassas GE, Pontikides N, Kaltsas T. Menstrual disturbances in thyrotoxicosis. Clin Endocrinol (Oxf). 1994;40:641–4.
20. Goldsmith RE, Sturgis SH, Lerman J, Stanbury JB. The menstrual pattern in thyroid disease. J Clin Endocrinol Metab. 1952;12:846–55.
21. Krassas GE. Thyroid disease, menstrual function and fertility. Thyroid Int. 2000;1:1–15.
22. Vissenberg R, Manders VD, Mastenbroek S, et al. Pathophysiological aspects of thyroid hormone disorders/thyroid peroxidase autoantibodies and reproduction. Hum Reprod Update. 2015;21(3):378–87.

23. Arojoki M, Jokimaa V, Juuti A, et al. Hypothyroidism among infertile women in Finland. Gynecol Endocrinol. 2000;14:127–31.
24. Poppe K, Glinoer D, Van Steirteghem A, et al. Thyroid dysfunction and autoimmunity in infertile women. Thyroid. 2002;12:997–1001.
25. Poppe K, Velkeniers B, Glinoer D. Thyroid disease and female reproduction. Clin Endocrinol. 2007;66(3):309–21.
26. Abalovich M, Mitelberg L, Allami C, et al. Subclinical hypothyroidism and thyroid autoimmunity in women with infertility. Gynecol Endocrinol. 2007;23:279–83.
27. Effraimidis G, Wiersinga WM. Autoimmune thyroid disease: old and new players. Eur J Endocrinol. 2014;170:R241–52.
28. Gleicher N, Kushnir VA, Weghofer A, Barad D. How the *FMR1* gene became relevant to female fertility and reproductive medicine. Front Genet. 2014;5:284.
29. Janssen OE, Mehlmauer N, Hahn S, et al. High prevalence of autoimmune thyroiditis in patients with polycystic ovary syndrome. Eur J Endocrinol. 2004;150:363–9.
30. Garelli S, Masiero S, Plebani M, et al. High prevalence of chronic thyroiditis in patients with polycystic ovary syndrome. Eur J Obstet Gynecol. 2013;169(2):248–51.
31. Kachuei M, Jafari F, Kachuei A, Keshteli AH. Prevalence of autoimmune thyroiditis in patients with polycystic ovary syndrome. Arch Gynecol Obstet. 2012;285:853–6.
32. Al-Saab R, Haddad S. Detection of thyroid autoimmunity markers in euthyroid women with polycystic ovary syndrome: a case-control study from Syria. Int J Endocrinol Metab. 2014;12(3):e17954.
33. Novais Jde S, Benetti-Pinto CL, Garmes HM. Polycystic ovary syndrome and chronic autoimmune thyroiditis. Gynecol Endocrinol. 2015;31(1):48–51.
34. Ott J, Aust S, Kurz C, et al. Elevated antithyroid peroxidase antibodies indicating Hashimoto's thyroiditis are associated with the treatment response in infertile women with polycystic ovary syndrome. Fertil Steril. 2010;94(7):2895–7.
35. Gleicher N, Barad D, Weghofer A. Functional autoantibodies, a new paradigm in autoimmunity? Autoimmun Rev. 2007;7:42–5.
36. Pennel L, Galligan CL, Fish EN. Sex affects immunity. J Autoimmun. 2012;38:J282–91.
37. Petta CA, Arruda MS, Zantut-Wittmann DE, et al. Thyroid autoimmunity and thyroid dysfunction in women with endometriosis. Hum Reprod. 2007;22:2693–7.
38. Matarese G, De Placido G, Nikas Y, Alviggi C. Pathogenesis of endometriosis: natural immunity dysfunction or autoimmune disease? Trends Mol Med. 2003;9(5):223–8.
39. Poppe K, Glinoer D, Tournaye H, et al. Impact of ovarian hyperstimulation on thyroid function in women with and without thyroid autoimmunity. J Clin Endocrinol Metab. 2004;89:3808–12.
40. Geva E, Vardinon N, Lessing JB, et al. Organ-specific autoantibodies are possible markers for reproductive failure: a prospective study in an *in vitro* fertilization-embryo transfer programme. Hum Reprod. 1996;11(8):1627–31.
41. Negro R, Formoso G, Coppola L, et al. Euthyroid women with autoimmune disease undergoing assisted reproduction technologies: the role of autoimmunity and thyroid function. J Endocrinol Invest. 2007;30(1):3–8.
42. Toulis KA, Goulis DG, Venetis CA, et al. Risk of spontaneous miscarriage in euthyroid women with thyroid autoimmunity undergoing IVF: a meta-analysis. Eur J Endocrinol. 2010;162:643–52.
43. Poppe K, Glinoer D, Tournaye H, et al. Assisted reproduction and thyroid autoimmunity: an unfortunate combination? J Clin Endocrinol Metab. 2003;88(9):4149–52.
44. Monteleone P, Parrini D, Faviana P, et al. Female infertility related to thyroid autoimmunity: the ovarian follicle hypothesis. Am J Reprod Immunol. 2011;66:108–14.
45. Busnelli A, Paffoni A, Fedele L, Somigliana E. The impact of thyroid autoimmunity on IVF/ICSI outcome: a systematic review and meta-analysis. Hum Rep Update. 2016;22(6):775–90.
46. Negro R, Mangieri T, Coppola L. Levothyroxine treatment in thyroid peroxidase antibody-positive women undergoing assisted reproduction technologies: a prospective study. Hum Reprod. 2005;20:1529–33.

47. Plowden TC, Schisterman EF, Sjaarda LA, et al. Subclinical hypothyroidism and thyroid autoimmunity are not associated with fecundity, pregnancy loss or live birth. J Clin Endocrinol Metab. 2016;101(6):2358–65.
48. Kelkar RL, Mejerji PK, Kadam SS, et al. Circulating auto-antibodies against the zona pellucida and thyroid microsomal antigen in women with premature ovarian failure. J Reprod Immunol. 2005;66:53–67.
49. Kilic S, Tasdemir N. The effect of anti-thyroid antibodies on endometrial volume, embryo grade and IVF outcome. Gynecol Endocrinol. 2008;24:649–55.
50. Zhong YP, Ying Y, Wu HT. Relationship between antithyroid antibody and pregnancy outcome following *in vitro* fertilization and embryo transfer. Int J Med Sci. 2012;9:121–5.
51. Seror J, Amand G, Guibourdenche J, et al. Anti-TPO antibodies diffusion through the placental barrier during pregnancy. PLoS One. 2014;9:e8464.
52. Thangaratinam S, Tan A, Knox E, et al. Association between thyroid autoantibodies and miscarriage and preterm birth: meta-analysis of evidence. BMJ. 2011;342:d2616.
53. De Groot L, Abalovich M, Alexander EK, et al. Management of thyroid dysfunction dyring pregnancy and postpartum. J Clin Endocrinol Metab. 2012;97(8):2543–65.
54. Garber JR, Cobin RH, Gharib H, et al. Clinical practice guidelines for hypothyroidism in adults: cosponsored by the American Association of Clinical Endocrinologists and the American Thyroid Association. Endocr Pract. 2012;18:988–1028.
55. Practice Committee of the American Society for Reproductive Medicine. Subclinical hypothyroidism in the infertile female population: a guideline. Fertil Steril. 2015;104:545–53.

Fertility and Longevity

6

Neri Laufer

The association between natural fertility and infertility and the effect of late-age conceptions on longevity are important because one of the most significant demographic trends of the last 30 years is a delay in the age of first births. It is therefore crucial to assess a woman's potential for extended fertility and to evaluate the health consequences of having children at a later age.

Delayed childbearing and reduced fertility, coupled with extended life expectancy, have led to an increasingly aging population. Worldwide, the number of older individuals is expected to surpass the number of children aged 0–5 years, by 2020. The total fertility rate (TFR)—the average number of children per woman—stayed constant at 5 children per woman in the 1950s and 1960s, declined to 2.55 in 2005, and is expected to be below 2.0 in 2050. In Europe the TFR is already below 2.0. These changes result from increased educational opportunities available to women, longer time devoted to education until the attainment of personal goals, more employment opportunities, higher wages and standard of living, and delayed marriage and age at first pregnancy. While the median age at first birth was 24–25 in the 1970s, it had risen to 28–29 in 2000. For example, 42% of high-achieving women in corporate America (companies with 5000 or more employees) are still childless after age 40, 49% of women who earn $100,000 or more are childless after age 40, and overall, 20% of women between the ages of 40 and 44 are childless [1].

Delayed childbearing is accompanied by reduced fertility. Infertility rates increase from 6% among women <24 years of age, rising to more than 30% among women in the 35–39 year age group, and exceeds 50% among women aged 40–44. This observed age-dependent decrease in fertility stems from ovarian aging and a decline in oocyte quantity and quality [2–4]. Two possible markers for this decline

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are spontaneous abortions and aneuploidy. Older women encounter a marked decrease in fertility in conjunction with a strikingly increased risk of spontaneous abortion (SAB), from 10% at 25–29 years of age to over 50% for those older than 45 years. In addition, the majority of embryos obtained from women approaching 40 years who undergo IVF are chromosomally abnormal [5], meiotic nondisjunction due to failure of normal microtubule apparatus development being the most plausible explanation [6]. Meiotic nondisjunction might be caused by deterioration of the spindle microtubular apparatus, thereby causing errors in chromosome segregation. Several mechanisms have been proposed to explain the effects on oocyte quality and aneuploidy, of which the most important are age-related mitochondrial dysfunction [7, 8] and increased cellular oxidative stress [9, 10].

In populations not practicing contraception, the mean maternal age at the birth of last child is 43 years. The effect of age is further accentuated in women undergoing IVF. In a single-center study of women aged 40–47 undergoing IVF, we demonstrated that the overall delivery rate was 15% for this age group and no children were born to women undergoing this treatment over the age of 45 [11] (Fig. 6.1). This observation was further corroborated by a 2014 USA national report [12].

Contrary to this general phenomenon, we observed that the delivery room registries in Jerusalem contain from time to time patients who conceive naturally and deliver babies after the age of 45. We sought to characterize the reproductive history of these women who are a select population in which ovarian senescence is extremely deferred. They may serve as a unique model to characterize the association between fertility and the aging process.

We searched delivery ward registries of four major hospitals in Jerusalem for the years 1995–2000, for women aged ≥45 years at the time of their most recent delivery. Individual medical charts and complete history of the outcomes of all the

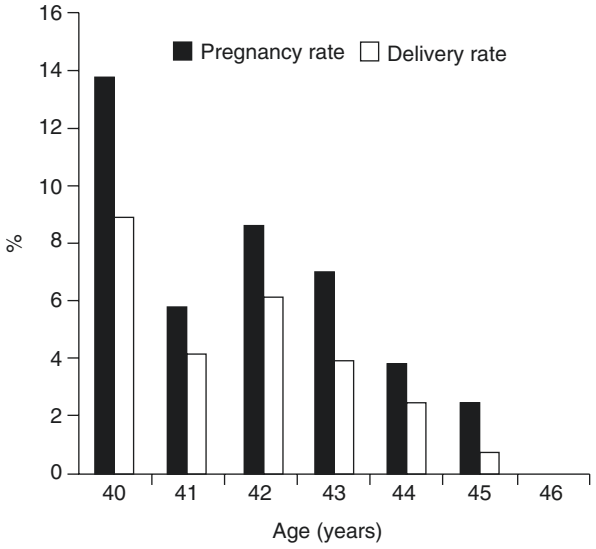

Fig. 6.1 Pregnancy and delivery rates per initiated cycle according to age (Tsafrir et al. [11])

patients' pregnancies (deliveries and spontaneous abortions) documented in the patient file were recorded. From a cohort of 104,659 deliveries, 258 gravidae ≥45 years old at the time of the index delivery were identified; 49 women who were found to have undergone fertility treatments were excluded, leaving a study group of 209 (0.2%). The group ranged in age from 45 to 49 (mean 45.7), gravidity ranged from 1 to 26 (mean 11.5), and parity ranged from 2 to 20. Over half of the study group had ≥12 deliveries: 80% were grandmultiparas (>6 deliveries), of whom grand-grand multiparas were the largest group (46%). The study population was found to belong almost exclusively to ultra-Orthodox Jewish sects in Jerusalem [13]. Like the Hutterites, this community is relatively isolated, contraception is not used, and there is a strong societal pressure to reproduce for as long as possible.

Rates of spontaneous abortion over the course of these women's childbearing years were found to be much lower than background population rates (Fig. 6.2). While multiparity in itself did not affect SAB rates, this measure was remarkably low in our exceptionally fertile group as compared with the rates published for a general population. This unique group of grandmultiparous women might possess a genetic propensity that delays the normal rate of ovarian and oocyte senescence [13].

In order to investigate whether women who conceive and deliver after 45 years of age have a distinct gene expression profile, we identified from our delivery room records a study group of eight Ashkenazi Jewish women who conceived spontaneously and delivered over the age of 45, who were more than 6 months from their last delivery, and as controls, six Ashkenazi Jewish women aged 45 at the time of the study, who delivered their last child before the age of 30 and did not try to conceive again [14]. Women were asked to attend our clinic on days 2–7 of their cycles, when blood samples were collected. Gene expression profiling was performed on an Affymetrix HG-U133A oligonucleotide microarray (15,000 genes). We found that 671 genes (4.9%) exhibited statistically significant ($p < 0.05$) differences between women conceiving spontaneously after the age of 45 and those who did not

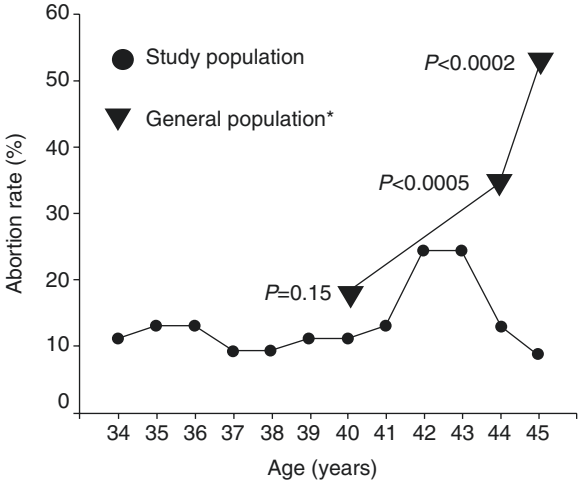

Fig. 6.2 Comparison of spontaneous early (first trimester) abortion rates between the study population (closed circles) and a general population (closed triangles). *Data from Warburton et al. [2]

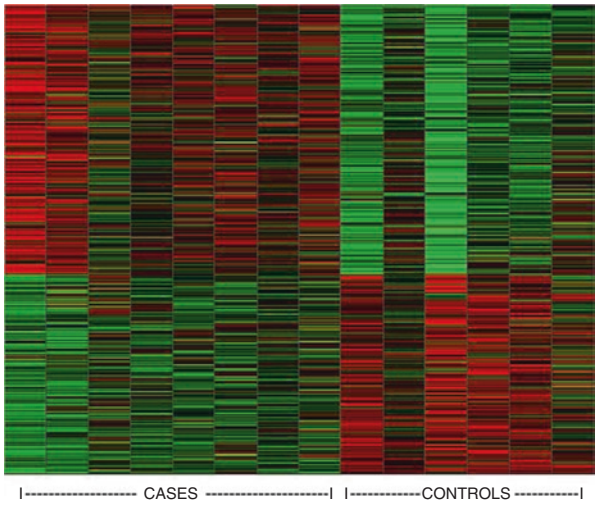

Fig. 6.3 Each column represents the gene expression of one woman, and each row represents a single gene. Red squares represent higher than median level gene expression (upregulation), and green squares represent lower than median level gene expression (downregulation) [15]

(Fig. 6.3). Of the 671 genes, 383 displayed increased transcript levels in the studied group, and 288 displayed decreased levels. Of particular interest to our study were those genes known to be involved in apoptosis and associated both with ovarian function and general aging. SPIN-2 is a novel nuclear protein that functions to regulate cell cycle progression and is proapoptotic. Its expression is decreased, and it differed most significantly between the study and control groups. BCL2L1 protein was shown to suppress apoptosis induced by p53, the main tumor suppressor gene. The mode of p53 suppression is similar to other BCL2 family members. Its expression was shown to be increased in the study group. IGF1R and SERPINB2, two proteins considered proapoptotic genes (death-promoting factors), show a decrease in their expression. IGF1R (insulin-like growth factor 1 receptor) plays a key role in the apoptosis pathway and in progress of human malignancies. Downregulation of the IGF-1 transcript is associated with an extension of life span in mice and humans. Likewise, SERPINB2 (also known as PAI-2) is correlated with cell senescence. It was found that PAI-2 was markedly overexpressed in senescent cells [15].

The major categories of genes that were differentially enriched include genes involved in apoptotic/anti-apoptotic processes and the ubiquitin-cycle pathway. These pathways are associated with fertility and aging and may therefore serve as a potential explanation for the exceptional preservation of fertility and fecundity in the studied group. Women conceiving after the age of 45 years have a unique gene expression profile. Understanding the cellular and molecular mechanisms that activate and execute programmed cell death in the female germ line has implications for the therapeutic management of infertility, sustaining ovarian function into old age and perhaps the aging process itself [15].

Several theories have been proposed to explain the connection between fertility and longevity. The trade-off theory posits that reproduction comes at the expense of life span. As stated by Westendorp and Kirkwood "There may be a trade-off between reproductive success and longevity because resources invested in longevity

assurance may be at the expense of reproduction. The number of progeny was negatively correlated with longevity" [16].

The antagonistic pleiotropy theory, on the other hand, hypothesizes that the same set of genes controls reproduction and aging: genes responsible for increased fitness in the younger fertile organism may contribute to decreased fitness later in life [17]. The psychosocial theory proposes that the characteristics which vary among populations, such as family support, nutritional intake, and quality of medical care, affect maternal health in general and reproductive health in particular and impact longevity [18].

The rejuvenating effect theory, which we explored, suggests that the physiological changes of pregnancy have a regenerative impact on mothers and may help to overcome the negative effects of aging. Falick Michaeli et al. reviewed the regenerative effects of pregnancy on several organ systems, including the liver, CNS, heart, fetal microchimerism, and longevity [19].

In the liver, we found that liver regeneration proceeded differently in pregnant and nonpregnant mice, demonstrating that a physiologic condition (i.e., pregnancy) causes a switch from proliferation-based liver regeneration to a regeneration process mediated by cell growth (Fig. 6.4). The cell growth-mediated regeneration seen in pregnant mice is relatively resistant to the detrimental effects of aging. In addition, we showed that the hypertrophy mode can be induced with bpV(phen) administration, which improved the regenerative capacity and survival rate of old mice, through pharmacologic means [20].

In the central nervous system, the Pregnancy in Multiple Sclerosis (PRIMS) study [21, 22], a large prospective study, demonstrated a lower relapse rate during pregnancy, particularly in the third trimester, relative to the rate in the previous year. Experiments in mice showed significantly more newly generated oligodendrocytes in an induced injury site in pregnant mice as opposed to virgin controls, as well as a significant increase in the number of remyelinated axons [23]. Prolactin and embryo-secreted preimplantation factor (PIF) have been shown to significantly increase the number of dividing oligodendrocyte precursor cells, resulting in the generation of new oligodendrocytes, and enhance remyelination, respectively, in murine models [19, 23, 24].

Studies investigating the effect of pregnancy on heart regeneration have shown that pregnancy protects against cardiac ischemic injury. In mouse and rat models, pregnancy initiates upregulation of cardiac progenitor cells in the affected sites. Placenta-derived factors could potentially be used for clinical cardiac cell therapy [19].

Fetal cells can be detected in the maternal circulation; there is some evidence of a contribution of persistent fetal cells to maternal tissue repair. These several observed rejuvenating effects, found in various maternal organs, are probably not due to a single common pathway but rather to specific mechanisms in different tissues [19].

In order to examine the effect of late-age pregnancies on maternal long-term survival, we conducted a large cohort study controlling for and assessing the influence of parity on this outcome [15]. The study cohort was created using three sources of information: general population censuses, birth records, and death

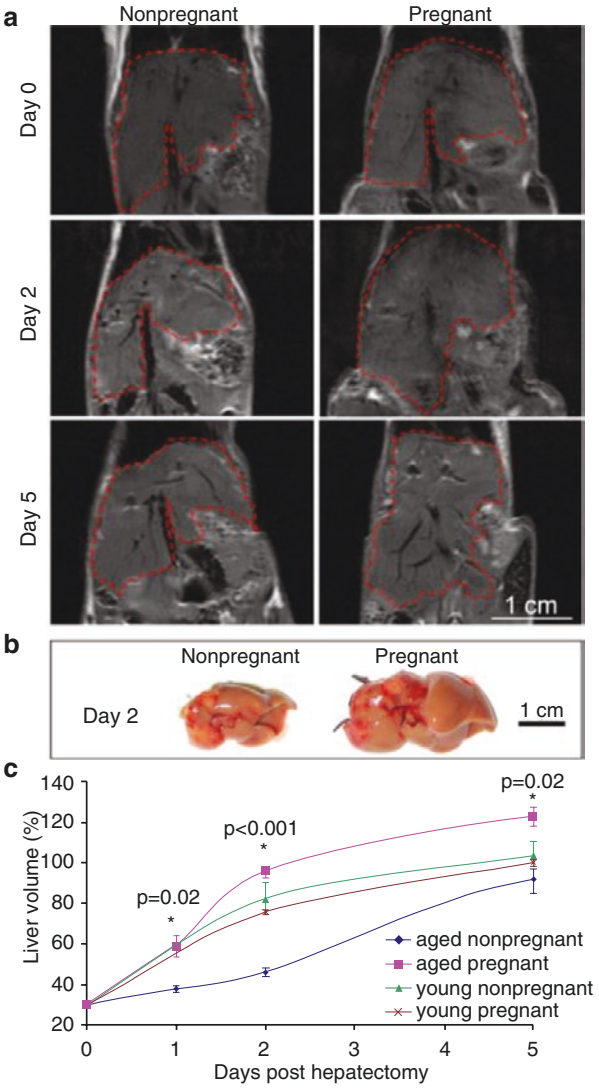

Fig 6.4 Liver regeneration in pregnant vs. nonpregnant mice (Gielchinsky et al., G&D 2010, [20])

registries. The Israel Central Bureau of Statistics (ICBS) maintains a data file containing records for all live births in Israel from 1969 through 2008. During the study period, the ICBS performed three censuses: in 1972, 1983, and 1995. The census files were linked to the reproductive data set to retrieve information on parity and maternal age at first and last birth. These files were also linked to death records (January 1972 through December 2009) and national delivery registry.

Only women who reported having children and who had at least one birth record were included as parous women in the study cohort. Never married women were excluded from the study because their health risks and behaviors differ from those

of ever married women, and they represented a small fraction (~7%) of the total study population.

The population study included 245,845 married women from three national censuses (4,398,029 person-years). The average follow-up period was 18 years, with a maximum follow-up of 36 years. During the follow-up period, 14,019 (6%) women died. The majority of women were Jewish (86%). Ninety-three percent of women (n = 228,733) had their first child before 35 years of age, whereas less than 0.1% of women (n = 115) had their first child at 45 years of age or older. For maternal age at last birth, 72% of women (n = 176,357) had their last child before 35 years of age, whereas 0.05% (n = 1234) had their last child at 45 years of age or older. High parity (6+ children) was more prevalent among women who had children at older ages (>45 years, 52% vs. <35 years, 12%). The age-adjusted mortality rates demonstrated a U-shaped pattern: lowest for women with 2–5 children (1246 died; 2.8 deaths per 1000 women) compared to childless women (1150 died; 4.9 deaths per at older ages or women with 6+ children (2727 died; 4.6 deaths per 1000 women).

The mortality rates for parous women decreased linearly with increasing age at last birth ($P < 0.001$). Age-adjusted mortality rates for women whose last birth was before 35 years of age were 3.4 deaths per 1000 vs. 1.6 deaths per 1000 for women whose last birth was at ≥45 years of age (Fig. 6.5).

This study highlights an important factor related to maternal longevity, namely, the protective effect of late maternal births, even among the high-risk groups of low and high parity women. Late parity has a beneficial effect on maternal life span. Multiparity is associated with higher mortality (if the last child was born before the age of 35), but not in women with extended fertility [15].

In conclusion, women conceiving spontaneously and giving birth after 45 years of age are a unique and rare anthropological—biological model to study delayed ovarian senescence. Extended fertility and longevity seem to be connected. Studies of these populations may shed light on the rejuvenating potential of pregnancy and the genetic propensities associated with prolonged fertility and longevity.

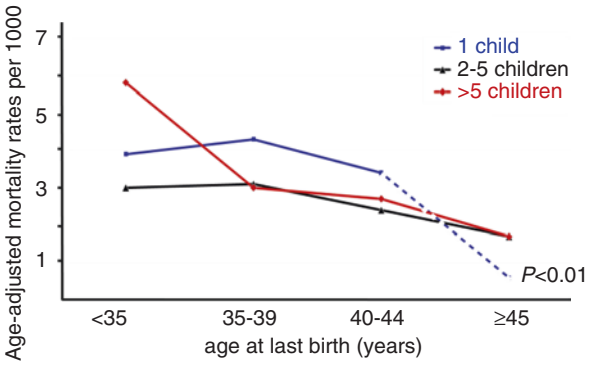

Fig. 6.5 Late parity reduced mortality in all parity groups (Jaffe et al. [15])

References

1. Hewlett SA. Creating a life: professional women and the quest for children. New York: Miramax Books; 2002.
2. Warburton D, Kline J, Stein Z, Strobino B. Cytogenetic abnormalities in spontaneous abortions of recognized conceptions. In: Porter IH, Willey A, editors. Perinatal genetics: diagnosis and treatment. New York: Academic Press; 1986. p. 133–45.
3. Vaskivuo TE, Anttonen M, Herva R, Billig H, Dorland M, te Velde ER, et al. Survival of human ovarian follicles from fetal to adult life: apoptosis, apoptosis-related proteins, and transcription factor GATA-4. J Clin Endocrinol Metab. 2001;86:3421–9.
4. 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.
5. Gianaroli L, Magli MC, Ferraretti AP, Munne S. Preimplantation diagnosis for aneuploidies in patients undergoing in vitro fertilization with a poor prognosis: identification of the categories for which it should be proposed. Fertil Steril. 1999;72:837–44.
6. te Velde ER, Pearson PL. The variability of female reproductive ageing. Hum Reprod Update. 2002;8:141–54.
7. Keefe DL, Niven-Fairchild T, Powell S, Buradagunta S. Mitochondrial deoxyribonucleic acid deletions in oocytes and reproductive aging in women. Fertil Steril. 1995;64:577–83.
8. Tilly JL. Commuting the death sentence: how oocytes strive to survive. Nat Rev Mol Cell Biol. 2001;2:838–48.
9. Perez GI, Tilly JL. Cumulus cells are required for the increased apoptotic potential in oocytes of aged mice. Hum Reprod. 1997;12:2781–3.
10. Tarin JJ. Aetiology of age-associated aneuploidy: a mechanism based on the 'free radical theory of ageing'. Hum Reprod. 1995;10:1563–5.
11. Tsafrir A, Simon A, Revel A, Reubinoff B, Lewin A, Laufer N. Retrospective analysis of 1217 IVF cycles in women aged 40 years and older. Reprod Biomed Online. 2007;14:348–55.
12. Centers for Disease Control and Prevention ASfRM, Society for Assisted Reproductive Technology. In: 2014 assisted reproductive technology national summary report, USA. Atlanta: US Department of Health and Human Services; 2016.
13. Laufer N, Simon A, Samueloff A, Yaffe H, Milwidsky A, Gielchinsky Y. Successful spontaneous pregnancies in women older than 45 years. Fertil Steril. 2004;81:1328–32.
14. Gielchinsky Y, Bogoch Y, Rechavi G, Jacob-Hirsch J, Amariglio N, Shveiky D, et al. Gene expression in women conceiving spontaneously over the age of 45 years. Fertil Steril. 2008;89:1641–50.
15. Jaffe D, Kogan L, Manor O, Gielchinsky Y, Dior U, Laufer N. Influence of late-age births on maternal longevity. Ann Epidemiol. 2015;25:387–91.
16. Westendorp RG, Kirkwood TB. Human longevity at the cost of reproductive success. Nature. 1998;396:743–6.
17. Williams PD, Day T. Antagonistic pleiotropy, mortality source interactions, and the evolutionary theory of senescence. Evolution. 2003;57:1478–88.
18. Ross CE, Mirowsky J. Family relationships, social support and subjective life expectancy. J Health Soc Behav. 2002;43:469–89.
19. Falick Michaeli T, Bergman Y, Gielchinsky Y. Rejuvenating effect of pregnancy on the mother. Fertil Steril. 2015;103:1125–8.
20. Gielchinsky Y, Laufer N, Weitman E, Abramovitch R, Granot Z, Bergman Y, et al. Pregnancy restores the regenerative capacity of the aged liver via activation of an mTORC1-controlled hyperplasia/hypertrophy switch. Genes Dev. 2010;24:543–8.
21. Vukusic S, Hutchinson M, Hours M, Moreau T, Cortinovis-Tourniaire P, Adeleine P, et al. Pregnancy and multiple sclerosis (the PRIMS study): clinical predictors of post-partum relapse. Brain. 2004;127:1353–60.

22. Vukusic S, Confavreux C. Pregnancy and multiple sclerosis: the children of PRIMS. Clin Neurol Neurosurg. 2006;108:266–70.
23. Gregg C, Shikar V, Larsen P, Mak G, Chojnacki A, Yong VW, et al. White matter plasticity and enhanced remyelination in the maternal CNS. J Neurosci. 2007;27:1812–23.
24. Paidas MJ, Annunziato J, Romano M, Weiss L, Or R, Barnea ER. Pregnancy and multiple sclerosis (MS): a beneficial association. Possible therapeutic application of embryo-specific pre-implantation factor (PIF*). Am J Reprod Immunol. 2012;68:456–64.

Improving IVF Results: How Far Can We Tamper with Human Biology?

7

Pasquale Patrizio and Sherman Silber

7.1 Introduction

The use of assisted reproductive technology (ART) procedures to treat infertile couples has significantly increased in the United States since its inception in the late 1970s. According to the Society for Assisted Reproductive Technology (SART), a total of 87,089 fresh, non-donor, in vitro fertilization (IVF) cycles were performed in 2013, and it is projected that IVF utilization rates will continue to climb [1]. However, despite significant advancements in the field, the process of human reproduction remains inefficient. Previous work analyzed the number of embryos transferred compared to the number of live births between 1995 and 2001 and showed that the overwhelming majority of embryos produced during IVF cycles (about 85%) and chosen for transfer failed to result in a live born infant [2]. Recently, the same analysis, but for the years 2004 and 2013, demonstrated that out of the total number of embryos replaced (1,808,082), the total number of live born infants was 358,214, for an overall (across all ages and across the 10 years) "embryo wastage" rate of 80% [3]. Similarly, notwithstanding multiple oocytes are retrieved for IVF, the overall live birth rates per oocyte during ART are low (5–10%) and have not changed significantly since the start of IVF almost 40 years ago [4–6].

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7.2 How to Decrease the "Embryo Wastage" Rates

The transfer of fewer embryos is certainly one mechanism to reduce the embryo wastage rates. Examining the trend in mean number of embryos transferred over the last 20 years, the reduction in the mean number of embryos transferred is striking. In fact, in 1995 the overall mean number of embryos transferred during IVF was 3.9 [2] and has steadily decreased from an average of 2.75 in 2004 to 2.04 in 2013, and this trend, seen across all age groups, was significant [3]. Despite the reduction in the mean number of embryos replaced, the transfers resulting in a live birth have significantly increased each year across all age groups with the exception of the group of women age greater than 42. In 2004, the overall embryo wastage rate, meaning the number of embryos that did not lead to a live birth, was 83%, which decreased to 76.5% in 2013, and this trend was statistically significant ($p < 0.001$).

When age groups were analyzed individually, "embryo wastage" rates decreased ($p < 0.05$) across all age groups, and it was more pronounced in the younger women, particularly for the group of women under the age of 35, where "embryo wastage" decreased from 76.1% in 2004 to 65.2% in 2013 ($p < 0.001$) [3]. In women over the age of 42, the "embryo wastage" rate only marginally decreased and remained relatively high from 2004 to 2013 (98.0% to 97.2%, respectively), and in this age group, there was also the smallest change in the mean number of embryos transferred (3.3 in 2004 to 2.8 in 2013). Data analysis further showed that the average number of embryos transferred per year, averaged across all age groups, positively correlated with the "embryo wastage" rate (Spearman coefficient = 0.988, $p < 0.001$). This illustrates that as the number of embryos transferred decreased, the percentage of non-implanting embryos also decreased without having an impact on the pregnancy rates. This pattern has been consistent since 1995 and is further proof that only a few embryos, if any, are competent for live birth per cohort in each ART cycle [2, 3, 7]. In other words, it is possible to decrease the wastage rate, but this is not due to an improved oocyte or embryo biology but merely to a reduction in the mean number of embryos transferred (i.e., a smaller denominator in the equation of total live births divided by the total number of embryos transferred).

7.3 Biological Questions and Embryo Selection

Many unanswered biological questions remain: (1) Why so many human oocytes and embryos retrieved and produced during IVF do not result in a live birth? (2) Why most menstrual cycles in the human do not yield a competent oocyte or a viable embryo? (3) Why the use of IVF has not changed this biological law of nature?

Over the years, it has become apparent that IVF can maximize a reproductive cycle but there is a biological limit imposed on the fraction of human eggs retrieved after stimulation, to the percent of human eggs that can produce a live baby [8]. In fact, even with the application of the currently best methods available in clinical practice or in the embryology laboratories, not every IVF cycle (like not every menstrual cycle in nature [9, 10]) will yield a competent oocyte/embryo for live birth.

We can only strive to maximize pregnancy and live birth rates per transfer by identifying whether in that specific reproductive cycle there is or there is not an embryo of high potential for a birth. However, this is just a matter of selection, not true "improvement." In fact, no selection method will ever increase live birth rates per started cycle (due to the intrinsic biological limits of human reproduction) [8].

An approach slowly gaining more acceptance is for all the embryo transfers to be at the blastocyst stage of growth, which means growing the embryos in vitro up to day 5 or 6 of development before transfer. If the embryos arrest before day 5 or day 6, given today's greatly improved laboratory conditions, it probably means that, in that particular cycle, they were not viable and not destined to become a live birth [8]. The recent Cochrane literature supports improved pregnancy rates per transfer with blastocyst as opposed to cycle day 3 transfers [11]. If we were to do all transfers at the blastocyst stage, we would improve the live birth rates when calculated per transfer (since there will be fewer, unnecessary, transfers), although no patients should be deceived into thinking it will improve their overall chance for pregnancy.

There are a number of other methods being promoted for the assessment of embryo viability and competence for a live birth. However, they are invasive, not completely reliable, costly, and likely result in the non-transfer/discard of some viable embryos. If one proposes PGS (preimplantation genetic screening) for all blastocysts, there is now sufficient data to demonstrate that PGS is neither sensitive nor specific enough to select all euploid embryos, and there is accumulating data to demonstrate that this could ironically even lower live birth rates [12–18]. Indeed offering sophisticated embryo PGS testing (via array CGH or SNP or qPCR or next-generation sequencing—NGS) has been shown be impacted by high rates of trophectoderm embryo mosaicism [12–15], making the diagnostic accuracy very challenging with the risk of discarding some normal embryos that were incorrectly diagnosed as abnormal. Therefore, the overall pregnancy rate might be decreased rather than increased [15–17]. Recently the use of PGS has been shown to be ineffective also in improving pregnancy rates on intent to treat analysis (IVF with PGS versus expectant management) in patients with recurrent pregnancy loss [18].

Another method is the selection of embryos by time-lapse imaging of morphokinetic and morphological parameters, based on the assumption that a continuous observation of embryo's growth can be predictive of embryos with the highest capacity to implant. However, this methodology comes with vastly increased costs and the risk of deselecting embryos still able to produce live births. One study in fact has reported the birth of healthy children after transfer of blastocysts originating from embryos with abnormal morphokinetic cleavage patterns that should have been not transferred if the time-lapse indications were needed. These authors concluded that only the transfer of viable embryos after 5–6 days of cultured (blastocysts) provides optimal embryo selection [19]. Likewise, another very recent randomized control trial, comparing time-lapse-selected embryos versus those selected by morphology alone, showed that the addition of time-lapse morphokinetic data did not improve clinical reproductive outcomes [20, 21]. But again, all these methods are only selection, and not true improvement of oocytes or embryos biological quality [8].

Therefore, the easiest, noninvasive, and least expensive way to increase the pregnancy rate and live baby rate per transfer without lowering the live baby rate per patient is the adoption of exclusive blastocyst-stage transfers (day 5 or 6 embryos). Blastocyst transfers are not associated with any likelihood of discarding normal embryos that were wrongly diagnosed as abnormal (as with PGS because of trophoblast mosaicism, or judged abnormal as per morphokinetic parameters). By utilizing only blastocyst-stage embryos for transfer, the live baby pregnancy rates per transfer across all ages will quickly surge (since the same number of pregnancies will be calculated out of a smaller denominator), and also the implantation rates will increase since fewer embryos will be transferred [8].

There will be some cases with no transfers, and so clinicians may worry, what will be the patient reaction to not having a transfer? With proper counseling, patients will be thankful for knowing early in the process that the cycle was not successful because there were no transferable embryos, if none developed to the blastocyst stage. This will save unnecessary emotional stress, reduce "false hopes," avoid unnecessary supplementation of progesterone, reduce costs, and allow patients to move sooner to another cycle or to alternative plans. A question that cannot be answered at this time with unequivocal certainty is whether some patients may still benefit of day 3 embryo transfers instead of day 5, on the assumption that the laboratory conditions might impair the further development of an embryo. If there is any doubt in the physician's mind for a particular group of patients (particularly those with three or fewer embryos), then they could opt for a cycle day 3 transfer at the first cycle of IVF, and if it fails, then blastocyst culture would be used for selection in a subsequent cycle. So as long as viable embryos are not mistakenly discarded as with the current imprecise trophectoderm biopsy results and morphokinetic parameters, the shorter time to pregnancy will be of benefit.

In summary, despite today's greatly improved laboratory conditions and the individualization of stimulation protocols, the process of IVF is still inefficient with low live birth rates per embryos produced and per oocytes retrieved. This is because the majority of human oocytes harvested and the majority of embryos produced are chromosomally or genetically abnormal. The ability to confidently identify gametes and embryos with the greatest reproductive potential would not improve overall live baby rate, but it would improve the success rate per transfer and lessen the agony of waiting for what turns out to be a negative pregnancy test.

Of course, better than improved embryo selection is to actually improve live baby rates per oocyte and per stimulated cycle. A carefully conducted "big data" multivariate regression analysis has ironically linked high FSH overdosage in ovarian stimulation to lower success [22]. Tampering too much with nature in this case actually has lowered IVF success. So perhaps the best advance now for IVF is to take a step backward rather than forward, recognizing the inherited deficiency of the human oocyte and just maximizing natural selection accuracy.

A recent paper [23] noted that the intrinsic fertility of the oocyte as ascertained by natural cycle IVF with single-embryo transfer does not decline until the age of 34 years. In fact, the fecundity rate of about 25% is maintained until this time. This work also showed that during natural cycles the intrinsic fertility of the oocyte,

assessed by live baby per oocyte collected, is much higher (five times higher) than when there is ovarian stimulation strongly arguing in favor of considering a much lower stimulation for IVF cycles to reduce oocyte wastage [23].

References

1. Society for Assisted Reproductive Technologies (SART). www.sart.org. 2015.
2. Kovalevsky G, Patrizio P. High rates of embryo wastage with use of assisted reproductive technology: a look at the trends between 1995 and 2001 in the United States. Fertil Steril. 2005;84:325–30.
3. Ghazal S, Patrizio P. Embryo wastage rates remain high in assisted reproductive technology (ART): a look at the trends from 2004-2013 in the United States. JARG. 2017;34(2):159–66.
4. Patrizio P, Bianchi V, Lalioti MD, Gerasimova T, Sakkas D. High rate of biological loss in assisted reproduction: it is in the seed, not in the soil. Reprod Biomed Online. 2007;14:92–5.
5. Patrizio P, Sakkas D. From oocyte to baby: a clinical evaluation of the biological efficiency of in vitro fertilization. Fertil Steril. 2009;91:1061–6.
6. Martin JR, Bromer JG, Sakkas D, Patrizio P. Live babies born per oocyte retrieved in a subpopulation of oocyte donors with repetitive reproductive success. Fertil Steril. 2010;94:2064–8.
7. Doherty L, Martin JR, Kayisli U, Sakkas D, Patrizio P. Fresh transfer outcome predicts the success of a subsequent frozen transfer utilizing blastocysts of the same cohort. Reprod Biomed Online. 2014;28(2):204–8.
8. Patrizio P, Silber S. Improving IVF: is there a limit to our ability to manipulate human biology? JARG. 2017;34(1):7–9.
9. Hertig TA. Human trophoblast: normal and abnormal. Am J Clin Pathol. 1967;47(3):249–68.
10. Opitz JM, Fitzgerald JM, Reynolds JF, Lewin SO, Daniel A, Ekblom SL, Phillips S. The Montana fetal genetic pathology program and a review of prenatal death in human. Am J Med Genet Suppl. 1987;3:93–112.
11. Glujovsky D, Blake D, Farquhar C, Bardach A. Cleavage stage versus blastocyst stage embryo transfer in assisted reproductive technology. Cochrane Database Syst Rev. 2012;(7):CD002118.
12. Greco E, Minasi MG, Fiorentino F. Healthy babies after intrauterine transfer of mosaic aneuploid blastocysts. N Engl J Med. 2015;373:2089–90.
13. Orvieto R, Shuly Y, Brengauz M, Feldman B. Should pre-implantation genetic screening be implemented to routine clinical practice? Gynecol Endocrinol. 2016;32(6):506–8. (e-pub Feb 12, 2016).
14. Scott RT, Galliano D. The challenge of embryonic mosaicism in preimplantation genetic screening. Fertil Steril. 105(5):1150–2. (e-pub Feb.10, 2016).
15. Gleicher N, Vidali A, Braverman J, Kushnir VA, Barad DH, Hudson C, et al. Accuracy of preimplantation genetic screening (PGS) is compromised by degree of mosaicism of human embryos. Reprod Biol Endocrinol. 2016;14(1):54.
16. Kang HJ, Melnick AP, Stewart JD, Xu K, Rosenwaks Z. Pre-implantation genetic screening: who benefits? Fertil Steril. 2016;106(93):597–602.
17. Orvieto R, Gleicher N. Should pre implantation genetic screening (PGS) be implemented to routine IVF practice? J Assist Reprod Genet. 2016;33(11):1445–8. (e pub Sept.15).
18. Murugappan G, Shahine LK, Perfetto CO, Hickok LR, Lathi RB. Intent to treat analysis of in vitro fertilization and preimplantation genetic screening versus expectant management in patients with recurrent pregnancy loss. Hum Reprod. 2016;31:1668–74.
19. Stecher A, Vanderzwalmen P, Zintz M, Wirleitner B, Schuff M, Spitzer D, Zech NH. Transfer of blastocysts with deviant morphological and morphokinetic parameters at early stages of in-vitro development: a case series. Reprod Biomed Online. 2014;28(4):424–35.
20. Goldman LR, Goldberg J, Falcone T, Austin C, Desai N. Does the addition of time-lapse morphokinetics in the selection of embryos for transfer improve pregnancy rates? A randomized controlled trial. Fertil Steril. 2016;105(2):275–85.

21. Wu YG, Lazzaroni-Tealdi E, Wang Q, Zhang L, Barad DH, Kushnir VA, et al. Different effectiveness of closed embryo culture system with time-lapse imaging in comparison to standard manual embryology in good and poor prognosis patients: a prospectively randomized pilot study. Reprod Biol Endocrinol. 2016;14(1):49–54.
22. Baker VL, Brown MB, Luke B, Smith GW, Ireland JJ. Gonadotropin dose is negatively correlated with live birth rate: analysis of more than 650,000 assisted reproductive technology cycles. Fertil Steril. 2015;104:1145–52 e5.
23. Silber S, Kato K, Aoyama N, Yabuuchi A, Skaletsky H, Fan Y, Liao C, et al. Intrinsic fertility of human oocytes. Fertil Steril. 2017;107(5):1232–7.

Ovarian Hyperstimulation Syndrome (OHSS): Pathogenesis and Prevention

8

Lina Dauod and Joseph G. Schenker

8.1 Introduction

Ovarian hyperstimulation syndrome (OHSS) is the least prevalent, albeit the most serious complication of ovulation induction. OHSS is a syndrome in which induction of ovulation results in a wide spectrum of clinical and laboratory symptoms and signs. At one end of the spectrum, there is only chemical evidence of ovarian hyperstimulation with an increased production of steroids; at the other end of the spectrum are massive ovarian enlargement, ascites, pleural effusion, hemoconcentration, oliguria, electrolyte imbalance, and hypercoagulability, a life-threatening derangement in hemostasis.

Ovarian hyperstimulation syndrome (OHSS) is characterized by massive transudation of protein-rich fluid (mainly albumin) from the vascular space into the peritoneal pleural and to a lesser extent to the pericardial cavities. The intensity of the syndrome is related to the degree of the follicular response in the ovaries to the ovulation-inducing agents. OHSS is still a threat to every patient undergoing ovulation induction

There are two types of severe OHSS:

1. Early which occurs in response to hCG trigger within 5–7 days of ovulation
2. Late which is caused by the rising hCG hormone levels produced by the placenta in conception cycles

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8.2 Classification of OHSS

Schenker and Weinstein [1] divided OHSS into three main categories—mild, moderate, and severe.

8.2.1 Mild Hyperstimulation

Chemical hyperstimulation is a very common accompaniment of ovulation induction. The mild form of OHSS presents a sensation of abdominal heaviness, tension, swelling, and pain. The physical findings are bilateral ovarian enlargement by multiple follicular and corpus luteum cysts; the ovaries may be up to 6 cm in diameter. In recent years mild hyperstimulation has become more common with induction of superovulation in ovulatory women participating in the various kinds of assisted reproduction programs. Occasionally the cyst may rupture or undergo torsion. This often presents a difficult problem in the differential diagnosis between a ruptured cyst, torsion of adnexa, and an ectopic pregnancy.

8.2.2 Moderate Hyperstimulation

In cases of moderate hyperstimulation, the abdominal discomfort is more pronounced. Gastrointestinal symptoms, such as nausea, vomiting, and (less frequently) diarrhea, are present. There is some weight gain and an increase in abdominal circumference. The ovaries are enlarged up to 12 cm in diameter, and some ascitic fluid is detected by ultrasonography.

8.2.3 Severe OHSS

Severe ovarian hyperstimulation syndrome is one of the most important complications of ovarian stimulation with severe morbidity and is still a life threat to every patient undergoing ovarian induction. Despite the fact that only few mortality cases have been reported, we believe that more cases of maternal mortality may exist, which have gone unpublished likely due to malpractice issues. The clinical manifestations may include pleural effusion, pericardial effusion, hypovolemia, impairment of renal function, electrolyte imbalance, disturbance in liver function, thromboembolic phenomena, shock, tension ascites, and adult respiratory distress syndrome (ARDS). The condition of a patient with severe OHSS improves within several days when she is correctly treated and when conception does not occur. The large ovarian cysts gradually subside after the abrupt appearance of clinical symptoms of hyperstimulation. The presence of ascites is a major sign of the capillary leak phenomenon present in OHSS. There is a direct connection between the intensity of capillary permeability and the severity of OHSS, as has been shown in our experimental model [1, 2]. Intraperitoneal pressure exceeds the normal intraluminal pressure of

the abdominal vena cava, the inferior vena cava is compressed, and blood flow in the interior vena cava is reduced. During these pathological changes, there is reduced preload to the heart, leading to decreased cardiac output and impairment of renal and respiratory function.

The most serious complication associated with OHSS is thrombotic phenomena as shown in Schenker and Mor review of 140 cases of OHSS on both arterial and venous side [3]. Thromboembolic events on arterial side were the common cause of death. The mechanism of thromboembolism in the course of ovarian hyperstimulation syndrome mainly the arterial one was explained by us as result of increased permeability of blood vessels in response to excessive vasoactive substances of ovarian origin and vasoconstrictive effects of some other agents of ovarian origin, which lead to hemoconcentration and hypovolemia with resultant arterial hypotension, increasing the risk of OHSS. Hormones play an increased role in both arterial and venous thromboembolism. Gonadotropin administration, increased supraphysiological concentrations of 17β-estradiol following ovulation induction, may be a risk factor especially in patients with inherited thrombophilias [3]. Arterial events are predominantly cerebrovascular accidents, usually occurring concurrently with the onset of OHSS, which could be life threatening, especially when occurring in sites such as cerebral and carotid arteries. Venous thromboses occur several weeks later and are mostly reported in unusual yet specific sites such as large veins of the upper extremities and neck [4, 5]. Prophylactic anticoagulant therapy is indicated to high-risk patients. Prophylaxis should be initiated in patients with thrombophilia and who develop moderate–severe OHSS [3].

Hepatocellular and cholestatic changes have been noted with and without conception. Several factors may account for these changes including increased estrogen levels and increased vascular permeability.

Severe OHSS is characterized by an extraparenchymal restrictive type of pulmonary dysfunction, attributed to intraabdominal or pleural fluid accumulation, which limits descent of the diaphragm and expansion of the thoracic cage. This may induce uncoordinated lung ventilation and atelectasis with subsequent ventilation-perfusion mismatch and hypoxemia. The clinical picture may deteriorate further because of pulmonary infection, pulmonary thromboembolism, or ARDS, all of which have distinct clinical, radiographic, and blood gas characteristics [6].

Hypoalbuminemia, a well-established feature of OHSS, is caused by leakage of albumin to the third space we determined globulin concentrations in the plasma and ascitic fluid of patients with severe OHSS from the time of admission until convalescence. Our studies demonstrated severe OHSS are at increased risk for infection due to leakage of gamma globulins from intravascular space [7].

8.3 Incidence

The incidence and severity of OHSS vary between different types of fertility treatment, with treatments involving greater degrees of ovarian stimulation being associated with a higher incidence. In cycles of In vitro fertilization (IVF), mild OHSS affects around one third of cycles, while the incidence of moderate and severe OHSS ranges from 3.1 to 8% [8].

8.4 Pathogenesis of OHSS

There is a continuous effort to find the exact factors responsible for the increased vascular permeability which was shown in our experimental model: such as histamine, serotonin, prostaglandins, prolactin, and variety of other substances that were implicated in the past. However, only scant data support an important role for any of these factors.

The following factors were studied:

Histamine—It was found in animal model that ovarian hyperstimulation syndrome could be blocked in rabbits by administration of antihistaminic preparations. In animals treated with antihistamine, a more rapid regression of the hyperstimulated ovaries was observed than in a control group. Although these animal studies had promising results, later studies demonstrated no difference in histamine levels between rabbits, in whom OHSS was induced, and controls [9].

Estrogens—Abnormally high levels of various steroids, estrogens in particular, are found in ascitic fluid and serum in cases of OHSS following hMG-hCG ovulation induction. Thus, it is not a surprise that estrogens were implicated as a possible triggering factor that eventually increases capillary permeability. On the other hand, it is known that the administration of high doses of estrogens do not, by themselves, produce clinical hyperstimulation. Moreover, Meirow et al. [10] concluded that estradiol measurements alone are not sufficient to alert the physician to the possible development of OHSS.

Prostaglandins—Experiments in animal models set out to determine whether prostaglandins are the "active substances" playing a role in the development of this syndrome. It was demonstrated in early experiments on an animal model that indomethacin, a blocker of prostaglandin synthesis, can prevent the fluid shift associated with the ascites, pleural effusion, and hypovolemia seen in this syndrome [2]. Moreover, other animal studies showed that in the presence of OHSS, ascites formation is not effectively suppressed by indomethacin, and, in the clinical setting, indomethacin was used as a therapeutic measure in cases of severe OHSS with variable results. Therefore, the role of prostaglandins in triggering the pathological processes of OHSS was not proved.

Renin–angiotensin system—Increased vascularity as well as increased capillary permeability at the time of ovulation is an important part of the angiogenic response in the follicle. The angiogenic properties of human follicular fluid combined with high plasma renin-like activity, angiotensin II-like immunoreactivity, and angiotensin-converting enzyme (ACE) raised the hypothesis on the possible involvement of renin–angiotensin system in the pathogenesis of OHSS through new vessel formation and increased capillary permeability. The involvement of a locally activated renin–angiotensin–aldosterone cascade has been implicated as a possible cause of the severe form of the syndrome through neovascularization and increased capillary permeability rate through endothelial cells in vitro. We studied plasma renin activity and aldosterone in patients with ovarian hyperstimulation and demonstrated: the pattern of plasma renin activity in hMG hyperstimulated cycles is characterized by a mid-luteal peak which declines to normal in the late luteal phase in non-conceptual cycles, whereas a sustained elevation of plasma renin activity occurs in

conceptual cycles. A direct correlation between the magnitude of plasma renin activity and the severity of OHSS was established [11].

According to this concept, the increased capillary permeability present in OHSS is due to the involvement of the renin–angiotensin system and the synthesis of prostaglandins in the ovaries. However, although the renin–angiotensin system may explain some of the characteristics in OHSS such as vasoconstriction as well as several other signs, it is not clear whether this system triggers the cascade leading to OHSS or merely is a secondary reactional feature.

Vascular endothelial growth factor—It was found by us and others that VEGF is responsible for the significant increase in the capillary permeability in OHSS [12]. VEGF, also known as vascular permeability factor (VPF), can provoke extravascular fluid accumulation, hemoconcentration, and elevated plasma concentration of von Willebrand factor, all known complications of OHSS. VEGF is a potent vasoactive protein with a remarkable permeability enhancing capacity that is approximately 1000 times that of histamine. Elevated levels of VEGF were found in the serum of patients who developed severe OHSS. Follicular fluid VEGF was found to be 100-fold greater than serum or peritoneal fluid 36 h after hCG administration.

Abramov et al. [12] investigated the role of VEGF in OHSS. Samples of therapeutic paracentesis were collected from severe OHSS patients. They found that VEGF is the major capillary permeability factor in OHSS ascites, since adding specific antibodies against VEGF (rhVEGF) was able to neutralize 70% of capillary permeability activity. Several other evidence for the key role of VEGF in the pathogenesis of OHSS was also found. High concentration of this substance was found in ascites from OHSS patients. Lately, dynamic changes of VEGF levels in the ascitic fluid of patients with severe OHSS were reported [12]. Moreover, it was found that VEGF is HCG trigger for OHHS.

8.5 Human Chorionic Gonadotropin

Severe OHSS is depended on either exogenous administration of hCG or endogenous pregnancy-derived hCG. It is administered during ovarian stimulation for both triggering ovulation and for luteal support. It is well known that hCG administration is critical for the development of OHSS. This iatrogenic syndrome cannot be totally prevented by GnRH substitution for hCG and inducing endogenous LH surge.

8.6 Treatment

Mild OHSS usually does not require any active form of therapy other than observation and maintenance of hydration by the oral route. Moderate-grade OHSS requires close observation and, in most instances, hospitalization, since patients may rapidly undergo a change of status, particularly when conception occurs, and it may become severe with subsequent complications; thus, vigilant observation is required. Patients with severe OHSS require immediate hospitalization and treatment.

During hospitalization, meticulous monitoring of hemodynamic stability is required by restoration of the depleted intravascular volume. Large-volume crystalloid infusion is recommended. However, these patients must be closely monitored, as this can result in sequestration of fluid in the third space. Since no treatable single causative mechanism has been found for this syndrome, therapy has remained conservative and supportive, aimed at refilling the arteriolar vasculature, mobilizing fluids from the third space back to the intravascular tree, maintaining circulatory hemodynamics, and preventing hemoconcentration. A rare but life-threatening risk for patients with severe hypovolemia involves arterial venous thromboembolism; therefore, prophylactic anticoagulation is warranted in cases of severe OHSS from the time of diagnosis through the first trimester of pregnancy [13].

We have previously shown that severe OHSS is characterized by leakage of albumin (with a molecular weight of 69 kDa) as well as IgG and IgA (with molecular weights of 150 and 180 kDa, respectively) to the abdominal cavity. Since IgM, which has a molecular weight of approximately 900 kDa, did not leak at all and since IgA leaked much less than IgG and albumin, we suggested that molecular human albumin, however, is considered the most "physiologic" solution for this purpose and is probably the most common one used. Its popularity may be attributed to publications that reported a benefit of prophylactic administration of human albumin before and immediately after oocyte retrieval in women at high risk for severe OHSS. However, some recent reports could not reproduce these results and found no significant benefit of human albumin therapy in prevention of severe OHSS.

We compared human albumin with 6% hydroxyethyl starch, a powerful, high-molecular-weight colloid currently used to treat other states of intravascular volume depletion, such as burns and hemorrhagic or septic shock. It was proved that 6% hydroxyethyl starch has advantage over albumin [14].

Tension ascites with oliguria calls for paracentesis. Impending renal failure and unrelenting hemoconcentration require intensive care and possibly dopamine drip. Heparin should be added for thromboembolic phenomena, whereas surgical intervention should be reserved for ovarian torsion, rupture of cysts, or ectopic (heterotopic) gestation. Therapeutic termination of an existing pregnancy may be lifesaving when all other measures have failed, making both patient and physician face extremely difficult decision with vast psychological consequences for the patient.

The majority of studies examining the treatment of OHSS are retrospective cohort studies that mostly evaluated both volume expanders and surgical interventions. There are more robust data supporting surgical intervention, such as paracentesis and culdocentesis, than fluid management [13].

8.7 Prevention

The key to the primary prevention of OHSS during ovarian stimulation is individual approach recognizing risk of the patient to develop OHHS. Several measures can he employed to prevent OHSS [15]. There are, however, numerous reasons why even

with the most careful and painstaking preventative measures, OHSS cannot be eliminated.

Monitoring of induction of ovulation is the most reliable method in the prevention of OHSS. Measurable parameters, which more or less accurately reflect follicular maturation, are used to monitor ovulation induction since direct observation is impossible.

Clinical evaluation is important, and such methods as cervical scoring may he used as adjuvant methods of evaluation. Determining the cervical score reflects indirectly the total estrogen activity.

Serum estrogen values have established their effectiveness in monitoring induction of ovulation.

Estrogen monitoring has effectively reduced OHSS with clinical symptoms necessitating hospitalization [4]. Higher levels of 17 B-estradiol are reached in induced cycles to achieve the optimal pregnancy rate. However, we and others have observed OHSS with peak plasma estradiol levels of >2000 pg/mL, HCG should be withheld then.

An additional factor that may serve as a warning sign is the slope of rise of the plasma estradiol level. If values are more than doubling during 2 or 3 days (steep slope), then this should be regarded as a serious warning sign and HCG withheld in that cycle. In assisted reproductive programmers, HCG should be withheld when estradiol levels are >3000 pg/mL.

We, along with others, have demonstrated that there is a linear correlation between the follicular diameter and estradiol levels in plasma in normal ovulatory cycles. However in induced cycles, where there is more than one dominant follicle and several maturing follicles, there is a poor statistical correlation between the ultrasonographic ovarian morphology and the plasma estradiol level.

In assisted reproductive of ovarian stimulation, the following manipulations and interventions during the treatment cycle have been used:

(a) Withholding HCG—canceling of cycle—As OHSS is associated with hCG, terminating the ovulation cycle by canceling the hCG trigger in the presence of several risk factors for OHSS is the most effective technique to prevent OHSS. hCG induces the production of VEGF, the primary mediator of OHSS. It is usually reserved for patients at high risk of OHSS and those with total loss of cycle control. Canceling a cycle has an economic and psychological effect on the patient.
(b) Rescue of overstimulated cycles—coasting—Withholding exogenous gonadotropins and postponing the hCG trigger until a patient's E2 level has declined. Coasting leads to the selective regression of the pool of immature follicles, thereby reducing the functioning granulosa cell mass available for luteinization and resulting in a decline in vasoactive substances involved in the pathogenesis of OHSS, including VEGF. Coasting has been shown to reduce the incidence of OHSS in high-risk patients but affecting cycle outcome. Coasting results in lower pregnancy rates.

(c) Aspiration of follicles—Has a protective effect with a decline in hormonal levels being noted. This may account for the reduced incidence of OHSS, but this approach does not offer complete protection against the development of OHSS.
(d) Albumin—Potential benefit of intravenous (IV) albumin at the time of oocyte retrieval to prevent OHSS was reported. An early Cochrane review clearly showed a benefit associated with the administration of IV albumin at the time of oocyte retrieval in patients at high risk of OHSS, with no effect on pregnancy rate. Recent studies found that while there was no statistical benefit regarding the rate of OHSS, it may reduce pregnancy rates [12].
(e) Dopamine agonist—A therapeutic strategy to prevent OHSS development is to use a dopamine agonist in order to benefit from its established action of inhibiting phosphorylation of VEGFR2 and preventing increased vascular permeability. It reduces the early onset of OHSS, without causing any effect in pregnancy, implantation, and miscarriage rate [16].
(f) GnRH agonist triggering—GnRHa triggering minimizes the risk of OHSS and secures the appropriate maturation of oocytes. The GnRHa triggering is possible only when using a GnRH antagonist protocol and requires modified luteal support in order to be as efficient as hCG triggering. The complete eradication of OHSS has made the GnRHa triggering concept the protocol of choice in IVF with fresh embryo transfer. It was demonstrated that if luteal support is with intramuscular progesterone and estradiol patches, the delivery rate is comparable to that seen after hCG triggering [17].
(g) Freezing all the oocytes or embryos—Since pregnancy will increase the severity of OHSS, it is often prudent to postpone embryo transfer to a subsequent cycle in patients that are at high risk for OHSS.
(h) Aspirin-platelet cyclooxygenase-1 (COX-1) inhibitor—There is fair evidence that it reduces OHSS: A large RCT included initiation of 3154 IVF cycles, for which gonadotropin-releasing hormone agonist was used in 2425 cycles, 1503 cycles randomly selected for low-dose aspirin treatment starting from the first day of controlled ovarian hyperstimulation compared with no treatment in the remaining 922 cycles [18]. Result showed only two (0.25%) cases of severe or critical OHSS developing in the 780 high-risk patients treated with 100 mg aspirin, as compared to 43 patients (8.4%) of the 412 who did not receive aspirin; there was no difference in pregnancy outcomes in the two groups [19].
(i) Metformin—The use of this insulin-sensitizing agent increased clinical pregnancy rates and decreased the risk of OHSS in PCOS patients [18]. By improving intraovarian hyperandrogenism, it is theorized that metformin can affect the ovarian response by reducing the number of non-periovulatory follicles and thereby reduce estradiol secretion. A recent meta-analysis that included 12 studies (1516 participants) showed no significant differences between metformin and placebo groups for rates of pregnancy (risk ratio [RR] 1.11, 95% CI 0.92–1.33), live birth (RR 1.12, 0.92–1.36), spontaneous abortion (RR 1.00, 0.60–1.67), or multiple pregnancy (RR 0.96, 0.47–1.96). However, OHSS rate was significantly lower among patients who received metformin than among

those who received placebo (RR 0.44, 0.26–0.77). Regarding the use of metformin in lean PCOS patients, some studies show that it does not decrease the risk of OHSS.

(j) Calcium—Increasing calcium levels has an inhibitory role on adenylyl cyclase resulting in cAMP inhibition and thus renin inhibition as well, which lowers VEGF production and thus lowers the risk of OHSS. Administration of 10 mL of 10% calcium gluconate on the day of the oocyte retrieval and days 1, 2, and 3 after oocyte retrieval associated with a reduction on moderate and severe OHSS without compromising the pregnancy rate [18].

(k) Luteal support phase—Progesterone appears to provide the best method of providing luteal phase support, as it is associated with higher rates of live birth or ongoing pregnancy than placebo and lower rates of OHSS than hCG. According to a meta-analysis of 94 RCTs, there was no evidence of a difference between progesterone and hCG regimens (hCG regimens included hCG alone and hCG with progesterone) in live birth or ongoing pregnancy rates (OR 0.95, 95% CI 0.65–1.38, 5 RCTs, 833 women, I2 = 0%, low-quality evidence). Progesterone was associated with lower OHSS rates than hCG regimens (OR 0.46, 95% CI 0.30–0.71, 5 RCTs, 1293 women, I2 = 48%) [18].

Conclusions

OHSS is characterized by massive transudation of protein-rich fluid (mainly albumin) from the vascular space into the peritoneal pleural and to a lesser extent to the pericardial cavities. The intensity of the syndrome is related to the degree of the follicular response in the ovaries to the ovulation-inducing agents. Ovarian hyperstimulation syndrome is still a threat to every patient undergoing ovulation induction. The pathophysiology of ovarian hyperstimulation syndrome is of extreme importance in the face of the increased use of ovulation induction agents as well as the development of sophisticated assisted reproductive techniques. In this manuscript we reviewed the factors that are probably responsible for this syndrome. The correlation found between plasma cytokine activities and the severity of OHSS suggests that plasma cytokines may be involved in the pathogenesis of OHSS and may serve as a mean for monitoring the syndrome during the acute phase and throughout convalescence. The interactions between cytokine and non-cytokine mediators of the syndrome, such as the renin-angiotensin system and vascular endothelial growth factor (VEGF), await further clarification. However, it seems that a combination of immune and nonimmune mechanisms may allow profound understanding of this enigmatic disorder.

VEGF, endothelin-1, renin-angiotensin system, and cytokines probably play a pivotal role in the increased capillary permeability which explains most of the symptoms and signs of OHSS; however the puzzle is not complete, and many questions are still open. Awareness of possible mechanisms and factors in the pathophysiology of OHSS will hopefully provide opportunities to design specific treatment regimens effective for both prevention and treatment of this potentially fatal iatrogenic condition.

References

1. Schenker JG, Polishuk WZ. The role of prostaglandins in ovarian hyperstimulation syndrome. Eur J Obstet Gynecol Reprod Biol. 1976;6:47–52.
2. Polishuk WZ, Schenker JG. Ovarian overstimulation syndrome. Fertil Steril. 1969;20:433–50.
3. Mor YS, Schenker JG. Ovarian hyperstimulation syndrome and thrombotic events. Am J Reprod Immunol. 2014;72:541–8.
4. Schenker JG, Weinstein D. Ovarian hyperstimulation syndrome: a current survey. Fertil Steril. 1978;30:255–68.
5. Sachar P, Rajamani K. Young ischemic stroke in association with ovarian hyperstimulation syndrome. J Stroke Cerebrovasc Dis. 2016;25:e134–40.
6. Abramov Y, Elchalal U, Schenker JG. Pulmonary manifestations of severe ovarian hyperstimulation syndrome: a multicenter study. Fertil Steril. 1999;71:I645–1651.
7. Abramov Y, Naparstek Y, Elchalal U, Lewin A, Schechter E, Schenker JG. Plasma immunoglobulins in patients with severe ovarian hyperstimulation syndrome. Fertil Steril. 1999;71:102–5.
8. Mathur RS, Drakely AJ, Raine-Fenning NJ, Evabuomwan IO, Hamoda H. The management of ovarian hyperstimulation syndrome. Royal College Obstet Gynecol. 2016;1–22.
9. Erlik Y, Naot M, Friedman M, Ben-David E, Paldi E. Histamine levels in ovarian hyperstimulation syndrome. Obstet Gynecol. 1979;53:580–2.
10. Meirow D, Schenker JG, Rosler A. Ovarian hyperstimulation syndrome with low estradiol in non classic 17alpha-hydroxylase, 17,20-lyase deficiency: what is the role of estrogens? Hum Reprod. 1996;11:2119–21.
11. Navot D, Margalioth EJ, Laufer N, Birkenfeld A, Relou A, Rosler A, Schenker JG. Direct correlation between plasma renin activity and severity of ovarian hyperstimulation syndrome. Fertil Steril. 1987;48:57–61.
12. Abramov Y, Barak V, Nisman B, Schenker JG. Vascular endothelial growth factor plasma levels correlate to the clinical picture in severe ovarian hyperstimulation syndrome. Fertil Steril. 1997;67:261–4.
13. Kasum M, Danolić D, Orešković S, Ježek D, Beketić-Orešković L, Pekez M. Thrombosis following ovarian hyperstimulation syndrome. Gynecol Endocrinol. 2014;30:764–8.
14. Abramov Y, Fatum M, Abrahamov D, Schenker JG. Hydroxyethylstarch versus human albumin for the treatment of severe ovarian hyperstimulation syndrome: a preliminary report. Fertil Steril. 2001;75:1228–30.
15. Schenker JG. Prevention and treatment of ovarian hyperstimulation. Hum Reprod. 1993;8:653–9.
16. Youssef MA, van Wely M, Hassan MA, Al-Inany HG, Mochtar M, Khattab S, van der Veen F. Can dopamine agonists reduce the incidence and severity of OHSS in IVF/ICSI treatment cycles? A systematic review and meta-analysis. Hum Reprod Update. 2010;16:459–66.
17. Kol S, Itskovitz-Eldor J. Gonadotropin-releasing hormone agonist trigger: the way to eliminate ovarian hyperstimulation syndrome—a 20-year experience. Semin Reprod Med. 2010;28:500–5.
18. Mathur RS, Tan BK. British Fertility Society Policy and Practice Committee: prevention of ovarian hyperstimulation syndrome. Hum Fertil (Camb). 2014;17(4):257–68.
19. Nelson SM. Prevention and management of ovarian hyperstimulation syndrome. Thromb Res. 2017;Suppl 1:S61–4.

Future of Imaging in Human Reproduction

9

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9.1 Introduction

If performed by trained gynecologist and/or reproductive endocrinologist, three-dimensional transvaginal ultrasound (3D TVUS) offers numerous advantages over two-dimensional (2D) TVUS such as multiplanar views, visualization of the coronal plane, and accurate volume measurements. Tomographic ultrasound and surface rendering improve the assessment of the uterine structural abnormalities and adnexal masses. Volume storage enables reconstruction and manipulation of the stored volumes after the patient leaves the office and application of telemedicine and e-consultation. This seems to be advantageous for patients scheduled for serial ovarian monitoring and infertility treatment. 3D power Doppler US and virtual organ computer-aided analysis (VOCAL) enable assessment of the vascularity and function of the pelvic structures. This chapter reviews current applications and future directions for 3D TVUS in human reproduction.

9.2 Congenital Uterine Anomalies

Congenital uterine anomalies are commonly associated with infertility and adverse pregnancy outcomes. Hysteroscopic metroplasty has improved the unfavorable obstetric prognosis and poor reproductive outcome of surgically correctable uterine anomalies, such as septate uterus [1]. In our study, out of 310 patients undergoing septum removal, 225 (72.6%) achieved pregnancy. 3D TVUS is proposed as the first-line technique for the assessment of uterine cavity and detection of congenital

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uterine anomalies due to its ability to obtain planar reformatted sections through the uterus and simultaneous assessment of the fundal indentation. Numerous studies have confirmed a high degree of concordance between 3D TVUS and previously set gold standards (MRI and combined hysteroscopy and laparoscopy) for detection and differentiation between different types of uterine anomalies [1–7].

9.3 Acquired Uterine Abnormalities

Intracavitary lesions such as endometrial polyps, intrauterine adhesions, and masses distorting uterine cavity such as submucous fibroids may adversely affect implantation and diminish fertility outcomes [8, 9]. The removal of the endometrial polyps, adhesions, and submucous fibroids was shown to improve the fertility outcomes. Meta-analysis by Kroon et al. reported 95% sensitivity and 88% specificity of saline infusion sonohysterography (SIS) in the evaluation of the uterine cavity [10]. SIS should be performed in the follicular phase to avoid thickened endometrium hampering the accurate detection of endometrial polyps [10]. SIS followed by aspiration biopsy under direct sonographic visualization is reported to be superior compared to blind endometrial biopsy in both peri- and postmenopausal patients with abnormal uterine bleeding [11]. Another study by Moschos et al. compared SIS and SIS-guided endometrial sampling for benign and malignant endometrial conditions. They reported that saline infusion sonohysterography endometrial sampling (SISES) significantly improves the specificity of SIS impression [12].

SIS is superior to transvaginal sonography and comparable to hysteroscopy for detection of uterine pathology. The sensitivity for detection of submucous myomas and polypoid lesions is close to 100%. The specificity and positive predictive value (PPV) of SIS for detection of endometrial hyperplasia are 100%, but sensitivity is reported to be slightly lower (87%) [13]. Grimbizis et al. proposed that SIS should be performed for complete diagnostic workup in patients with suspected intracavitary lesions (endometrial polyps and submucous myomas) [14]. 3D multislice SIS is an excellent tool to precisely diagnose, localize, and measure intracavitary lesions (endometrial polyps, submucous and intracavitary fibroids) [15].

9.4 The Significance of the Junctional Zone

The JZ is a highly specialized and dynamic inner myometrium. It goes through various changes with age and hormonal levels [16]. The remodeling of spiral arteries, which are embedded within the inner myometrium of the uterus, is an important step in the placentation [17]. Lack of endovascular trophoblastic invasion and remodeling is associated with various adverse obstetrical outcomes like preterm labor, preeclampsia, and fetal growth restriction [17]. The JZ may be disrupted in patients with endometriosis and adenomyosis, which in turn may account for the

increased risk of adverse pregnancy outcome. It is well documented that endometriosis is associated with infertility, lower fecundity, and lower live birth rates. Mechanisms associated with these associations have been debated for many decades and include distorted pelvic anatomy and function, endocrine and peritoneal abnormalities, and abnormal endometrial receptivity secondary to alterations of the humoral and cellular immunity factors.

More recently Puente et al. reported that the incidence of adenomyosis is as high as 24% among infertile patients [18]. Adenomyosis is an important risk factor for spontaneous preterm delivery and preterm premature rupture of membranes (PPROM) [19]. Women with adenomyosis have 28% lower clinical pregnancy rate with IVF/ICSI compared to those without adenomyosis [20]. Fernando et al. and Stephansson et al. demonstrated endometriosis as a major risk factor for preterm delivery [21, 22]. Pelvic endometriosis is also strongly associated with junctional zone (JZ) thickening, especially in severe stages [23, 24].

Interestingly, Maubon et al. reported thickened JZ has a negative predictive factor for embryo implantation among IVF patients. Implantation failure rate was 96% for patients with average JZ thickness greater than 7 mm and maximal JZ greater than 10 mm versus 37% in the control group [25]. Therefore, assessment of the JZ with high-resolution TVUS and MRI is recommended for identifying and predicting obstetrical complications in high-risk patients [26]. The accuracy of 3D TVUS when at least two ultrasound features of adenomyosis (JZ maximum thickness $\geq$8 mm, myometrial asymmetry and hypoechoic striations) are present is as high as 90% [27].

Propagated myometrial contractions in the nonpregnant uterus originate from the JZ as seen with high-resolution US and cine MRI [26]. Uterine contractions are noted to be higher in patients with endometriosis [28]. High-frequency uterine contractions on the day of embryo transfer (ET) are found to be associated with lower implantation and ongoing pregnancy rates [29]. Higher progesterone concentration was associated with decreased frequency of uterine contractions, which supports the uterine relaxing property of progesterone [29]. To counteract uterine contractions, various tocolytic agents have been tested to improve the implantation rate [30–32]. Fanchin et al. used progesterone and noted that the group which received progesterone immediately after oocyte retrieval showed a significant decline in uterine contractions at the time of ET and had higher implantation and pregnancy rates compared to the control group, which received progesterone 2 days after retrieval [30]. Moon et al. reported significant improvement in the implantation rate with the use of NSAIDS (piroxicam) in the randomized controlled study involving 180 patients [31]. Moraloglu et al. studied the use of oxytocin/vasopressin V1a antagonist (atosiban). The treatment group, which received atosiban 30 min prior to ET and continued for 2 h after ET, had significant increase in implantation and pregnancy rates [32]. Prospective studies should be conducted to assess the effect of different medications on the frequency, direction, and amplitude of the uterine contractions, their effects on implantation and pregnancy rates, and strategies to optimize them.

9.5 Integrated Endometrial Receptivity Score

The assessment of endometrial thickness, volume, pattern, and vascularity has been in the focus of interest since introduction of US in human reproduction. 3D and color or power Doppler US allows detailed assessment of the endometrium and its vascularization [33]. Until now there is no general consensus on the importance of endometrial measurements, morphology, and perfusion for prediction of implantation and pregnancy rates in assisted reproduction techniques (ART). Embryo implantation is considered as the critical step of ART requiring receptive endometrium, normal and functional embryo at the blastocyst development stage, and a synchronized dialogue between maternal and embryonic tissue [34]. The implantation window is the spatially and temporally restricted phase when endometrium is receptive to blastocyst and begins 6–10 days after the LH surge and lasts for about 48 h [34].

In the study by Kupesic et al. no pregnancy was achieved in patients with endometrial volume of <2 or >8 mL [35]. Interestingly, in the group of patients with endometrial volume ranging from 2 to 7 mL, no relationship was apparent in terms of pregnancy rate. In terms of morphology, hyperechogenic endometrial texture was predictive of implantation, while no pregnancy occurred in patients with multilayered (triple line) endometrial pattern on the day of ET. Simultaneous assessment of flow resistance and subendometrial vessel density contributed to better assessment of the endometrial receptivity [35]. Significantly higher flow index (FI) values (13.2 ± 2.2) were obtained by 3D power Doppler US in women who achieved a pregnancy than in those who did not, and no pregnancy occurred when the FI was <11.0. Strikingly Ng et al. reported no correlation between vascularization index changes in the endometrial and subendometrial blood flow on the days of HCG and ET [36]. Same authors studied the endometrial and subendometrial blood flow parameters measured by 3D power Doppler US and reported that measurement of these vascularization indices at onetime point during IVF treatment is not a good predictor of IVF/ET outcome [37]. Contrary to that, Kim et al. reported that 3D power Doppler US was a useful and effective method for assessing endometrial blood flow in IVF/ET cycles. Pregnant group had higher endometrial vascularization index (VI), FI, and vascularization flow index (VFI) scores than nonpregnant group [38]. Recently El-Zenneni et al. reported that women with unexplained infertility had lower EG-VEGF score, lower endometrial thickness, and higher subendometrial flow resistance index (RI) compared to age-matched fertile group [39]. These findings suggest poor angiogenesis in preimplantation phase among women with unexplained infertility. At present there is no consensus for routine use of 2D color Doppler and/or 3D power Doppler US in ART setting, neither there is standardization for the endometrial volume and vascularity assessment. The results and outcomes of the ultrasound studies depend upon population, timing of ultrasound examination, equipment type, and setting.

Integrated endometrial receptivity score combining endometrial thickness, endometrial surface area and volume, texture, endometrial contractility, JZ, and

endometrial vascularity may assist in better prediction of pregnancy rates in ART [38, 39]. However, this process requires expertise, is time-consuming, and has high interobserver variability. It is expected that automated volume analysis consisting of quantification of endometrial thickness, volume and vascularity, determination of JZ regularity and thickness, and assessment of uterine contractions frequency, direction, and amplitude may increase the objectivity and reduce interobserver variability and time required by the sonographer.

9.6 Automated Follicle Count

Success of infertility treatment greatly depends on the ovarian response to gonadotropin induction. Accurate prediction of the ovarian responsiveness prior to ovulation induction is helpful in individualization of the dosage of gonadotropin and patient counseling. Although there is no consensus on how the ovarian reserve measurements should be performed, clinical studies suggest that a small ovarian volume and decreased number of antral follicles are suggestive of poor ovarian response, while large ovarian volume and increased follicle count indicate favorable ovarian response [40–42].

3D TVUS provides accurate volume measurements and helps in detecting surface irregularity [43]. Sonography-based automated volume calculation (SonoAVC) is a 3D US software, which identifies and measures antral follicle count (AFC) in a given ovarian volume. SonoAVC can also be used for the assessment of the follicular growth. Initial studies found that the follicular volume measured by SonoAVC is more accurate and correlates well with the volume of the follicular aspirate. Similarly, follicular diameter measurements correlate well with the manual 2D US measurements. Removal of hypoechogenic regions that are not follicles by post processing helps in quality control measures and teaching [44]. Deb et al. assessed the reliability of automated measurements of total follicle count using SonoAVC, 2D US, and manual 3D US techniques [45]. 3D US measurements were quicker than 2D US for AFC but has led to lower count of the follicles. The authors reported best intra- and interobserver reliability of total AFC with SonoAVC post processing of manual 3D US multiplanar view and 2D US real-time equivalent. The mean total AFC was lower with SonoAVC, but the results were improved with post processing, most likely due to color-coding and avoiding the recounting [46]. In a recent review article on the value of automated follicle measurements in IVF/ICSI, Vandekerckhove et al. validated same results [47]. SonoAVC showed lower inter- and intraobserver variability and excellent accuracy when compared to 2D manual follicle measurements. Quick volume acquisition and opportunity of offline assessment are the main advantages of this tool [47]. Murtinger et al. reported that improved monitoring of ovulation induction using 3D TVUS automated volume count may result in greater oocyte retrieval and increased oocyte fertilization [48].

9.7 Polycystic Ovarian Morphology

Diagnosis of polycystic ovarian syndrome (PCOS) relies on clinical, endocrinological, and morphological criteria assessed by US. Increased ovarian volume (>10 cm^3) and stromal area, together with follicular excess (more than 10–12 follicles measuring 2–9 mm in diameter), are the main diagnostic criteria for PCOS [49]. Recently some authors have suggested that ovarian volume for PCOS should be reduced to 6.4–7 cm^3 and that the change in ovarian volume with age should also be considered [50]. Kelsey et al. developed a normative model for ovarian volume assessment. In their publication normal ovarian volume at 2 years is 0.7 mL (95% confidence interval 0.4–1.1 mL) and peaks to 7.7 mL at 20 years of age (95% confidence interval 6.5–9.2), followed by decline to 2.8 mL (95% confidence interval 2.7–2.9) at the menopause [51]. Based on this information, using the same ovarian volume >10 cm^3, threshold for androgen excess and polycystic ovary syndrome (AE-PCOS). Society recommended the threshold for increased AFC or follicle number per ovary (FNPO) to 25 [49]. The FNPO threshold of 26 proposed by Lujan et al. achieved the best compromise between sensitivity and specificity (85% and 94%, respectively), indicating that this threshold may be conveniently used to distinguish normal from polycystic ovarian morphology (PCOM) in most populations [52]. Future 3D TVUS and SonoAVC studies are required to confirm the validity of these tools for evaluation of PCOM.

9.8 Intraovarian Vascularity

3D color and power Doppler US has made feasible the noninvasive assessment of ovarian stromal and follicular blood flow. Engman et al. [53] and Kupesic et al. [54] found a decrease in ovarian response to ovulation induction and lower pregnancy rates in patients with diminished ovarian blood flow, irrespective of the patient's age. Intensity of ovarian stromal perfusion seems to be predictive of increased delivery of gonadotropins to target cell for stimulation of follicular growth.

9.9 Fallopian Tube Patency

For decades tubal patency was assessed by laparoscopy with chromopertubation and X-ray hysterosalpingography (HSG). More recently 2D and 3D hysterosalpingo-contrast-sonographies (HyCoSy) were introduced as relatively cheap, less invasive, and widely accepted alternatives that successfully overcome the disadvantages of former gold standards (such as invasiveness, use of iodinated contrast media, exposure to X-rays, etc.). 2D HyCoSy is a real-time examination that is highly observer dependent and requires experienced operator for accurate results. Due to fallopian tubes' tortuosity, complete visualization of tubal morphology and patency is impossible using 2D B-mode US. In contrast, color and power Doppler

US is sensitive to flow and can identify the passage of color signals along the tube, as well as free spillage from the fimbrial end of the tube [55]. Kupesic and Plavsic pointed out that addition of color and power Doppler to 2D and 3D HyCoSy significantly contributes to diagnostic precision. Using automated 3D volume rendering, the time of investigation and amount of contrast media were significantly reduced leading to decreased patient's discomfort [55]. 3D HyCoSy enables offline reconstruction of planes of interest and surface rendering, as well as application of telemedicine and teleconsultation. The vivid images obtained with this technique are useful for teaching purposes [56].

In a recent meta-analysis by Wang et al., 3D and 4D HyCoSy are reported as accurate tests for tubal patency. The pooled estimate of sensitivity and specificity was reported to be 0.92 and 0.91, respectively [57]. The use of 3D volume acquisition with automated 3D coded contrast imaging enables visualization of the uterine cavity and fallopian tubes in the coronal plane, making the evaluation relatively easy and less operator dependent [58]. With above reported results from various prospective studies and meta-analysis, HyCoSy has become a widely utilized tool in the hands of reproductive endocrinologist when tubal patency is in question.

9.10 Cul-De-Sac Assessment

As a part of comprehensive ultrasound examination, cul-de-sac should be evaluated for free fluid, masses, and signs of deep infiltrating endometriosis (DIE). The presurgical diagnosis of DIE is critical since bowel involvement often requires interdisciplinary approach. The diagnosis of endometriosis in the absence of endometrioma has been challenging with ultrasound. Sonographic characteristics of endometriosis bowel implants were studied and found to be solid, focal, and tubular lesions with slightly irregular margins and thinner section at one end, resembling a comet [59]. Recently high-resolution TVUS and assessment of the sliding organ sign demonstrated high accuracy for prediction of DIE of the rectum in patients with symptoms of endometriosis [8, 9]. Meta-analysis by Hudelist et al. reported the pooled estimate of sensitivity and specificity as 91% and 98%, respectively, of TVUS for presurgical detection of bowel endometriosis [8]. Negative uterine sliding sign, commonly associated with DIE and uterosacral adhesions, is observed as lack of the movement of the rectum against the posterior uterine wall and posterior vaginal fornix in the midsagittal plane by TVUS transducer following the external pressure on the uterus by the examiner's hand on the patient's abdomen [23]. Multicenter prospective observational study by Hudelist et al. reported 85% sensitivity and 96% specificity of the negative uterine sliding sign [9]. Similarly, Reid et al. demonstrated 93% diagnostic accuracy, 84% sensitivity, and 97% specificity of the negative sliding organ sign for obliteration of pouch of Douglas [60]. More recently Guerriero et al. reported superior diagnostic accuracy of 2D TVUS and good reproducibility of 3D TVUS for presurgical diagnosis of DIE involving uterosacral ligaments, rectovaginal septum, vagina, and bladder [61].

9.11 The Value of Comprehensive Preimplantation Ultrasound Scan

A comprehensive assessment of reproductive anatomy and function using 3D US and 3D color/power Doppler US as one-step evaluation should be optimally utilized before infertility treatment [62]. Systematic approach for 3D US examination consists of a detailed examination of the uterine shape, size, and contour; evaluation of the endometrial thickness, volume, pattern, and vascularity; and assessment of the JZ regularity, echogenicity, and thickness (Fig. 9.1). Uterine anatomy is explored in the coronal plane by simultaneous visualization of the uterine cavity, the external surface of the fundus and cervix. SIS is recommended for patients with increased endometrial volume, abnormal endometrial pattern, and irregular uterine cavity shape suggestive of the Müllerian duct anomalies or acquired intracavitary abnormalities. Myometrial lesions should be recognized, and proper dimensions and locations should be ascertained. Ovarian dimensions and volume are measured and the AFC is recorded. Adnexa are carefully assessed for masses, endometriosis, and dilated tubes. Color power Doppler US may be applied to evaluate vascularity of the ovaries and pelvic lesions. HyCoSy should be optimally utilized for the assessment of tubal patency. Accessibility and mobility of the ovaries should be checked in real time for better planning of the ultrasound-guided oocyte retrieval. The cul-de-sac is assessed for the presence of free fluid or masses.

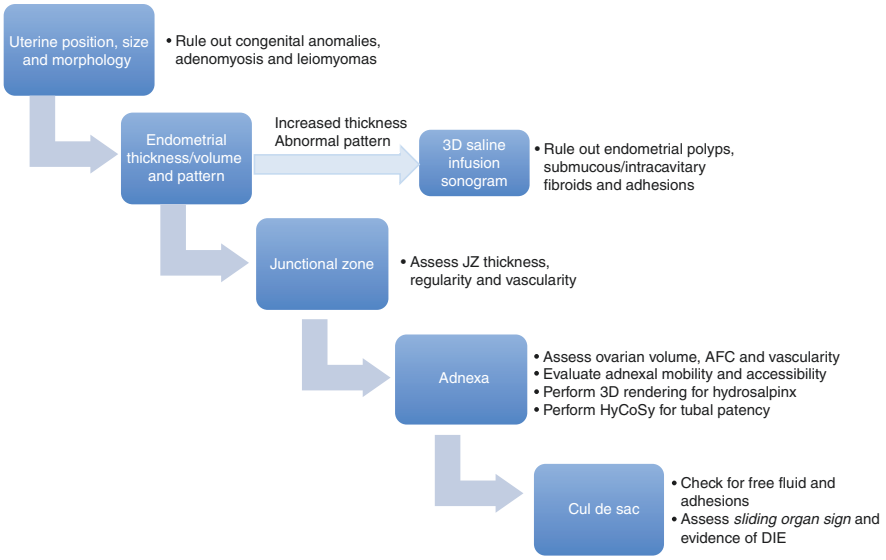

Fig. 9.1 Algorithm for comprehensive 3D TVUS exam for infertility workup

Conclusion

In today's clinical practice, there is no integrated 3D US system to optimize ultrasound imaging in reproductive medicine. Currently an expert must designate the ovarian and endometrial volumes for accurate measurements, which is both subjective and time-consuming. Automated 3D US and power Doppler angiography may objectively and precisely measure the ovarian and endometrial volumes and quantify the ovarian and endometrial perfusion. We propose the development of 3D US robotic imaging and creation of an automatic mathematic algorithm to extract uterine anomalies from the coronal plane provided in a multiplanar view for detection and classification of congenital uterine anomalies.

Application of automated scoring system and 3D power Doppler angiography may contribute to objective assessment and better differentiation of adnexal masses.

In the future, robotic US may also assist and guide physicians during assisted reproductive techniques such as HyCoSy, real-time oocyte aspiration, and US-guided embryo transfer. Telerobotic ultrasound technology may revolutionize the way we are practicing medicine by enabling experts to perform ultrasound anytime and anywhere. By reaching patients in underserved and rural areas, this technology could change the way we practice reproductive medicine. Future investigation and collaboration with specialists in medical robotics and robotic-assisted imaging are needed to develop a robotic 3D US system and test its application in reproductive medicine setting.

References

1. Homer HA, Li T-C, Cooke ID. The septate uterus: a review of management and reproductive outcome. Fertil Steril. 2000;73(1):1–14.
2. Kupešić S, Kurjak A, Skenderovic S, Bjelos D. Screening for uterine abnormalities by three-dimensional ultrasound improves perinatal outcome. J Perinat Med. 2002;30(1):9–17.
3. Ergenoglu AM, Sahin C, Simsek D, Akdemir A, Yeniel A, Yerli H, et al. Comparison of three-dimensional ultrasound and magnetic resonance imaging diagnosis in surgically proven Müllerian duct anomaly cases. Eur J Obstet Gynecol Reprod Biol. 2016;197:22–6.
4. Graupera B, Pascual M, Hereter L, Browne J, Úbeda B, Rodríguez I, et al. Accuracy of three-dimensional ultrasound compared with magnetic resonance imaging in diagnosis of Müllerian duct anomalies using ESHRE-ESGE consensus on the classification of congenital anomalies of the female genital tract. Ultrasound Obstet Gynecol. 2015;46(5):616–22.
5. Bermejo C, Martinez Ten P, Cantarero R, Diaz D, Perez Pedregosa J, Barron E, et al. Three-dimensional ultrasound in the diagnosis of Mullerian duct anomalies and concordance with magnetic resonance imaging. Ultrasound Obstet Gynecol [Internet]. 2010;35(5):593–601. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20052665.
6. Ludwin A, Ludwin I, Kudla M, Pitynski K, Banas T, Jach R, et al. Diagnostic accuracy of three-dimensional sonohysterography compared with office hysteroscopy and its interrater/intrarater agreement in uterine cavity assessment after hysteroscopic metroplasty. Fertil Steril [Internet]. 2014;101(5):1392–1399.e4. https://doi.org/10.1016/j.fertnstert.2014.01.039.
7. Moini A, Mohammadi S, Hosseini R, Eslami B, Ahmadi F. Accuracy of 3-dimensional sonography for diagnosis and classification of congenital uterine anomalies. J Ultrasound Med [Internet]. 2013;32(6):923–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23716512.

8. Hudelist G, English J, Thomas AE, Tinelli A, Singer CF, Keckstein J. Diagnostic accuracy of transvaginal ultrasound for non-invasive diagnosis of bowel endometriosis: systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2011;37(3):257–63.
9. Hudelist G, Fritzer N, Staettner S, Tammaa A, Tinelli A, Sparic R, et al. Uterine sliding sign: a simple sonographic predictor for presence of deep infiltrating endometriosis of the rectum. Ultrasound Obstet Gynecol. 2013;41(6):692–5.
10. de Kroon CD, de Bock GH, Dieben SW, Jansen FW. Saline contrast hysterosonography in abnormal uterine bleeding: a systematic review and meta-analysis. BJOG. 2003;110(10):938–47.
11. Moschos E, Ashfaq R, McIntire DD, Liriano B, Twickler DM. Saline-infusion sonography endometrial sampling compared with endometrial biopsy in diagnosing endometrial pathology. Obstet Gynecol. 2009;113(4):881–7.
12. Moschos E, Bailey AA, Twickler DM. Comparison of saline infusion sonography (SIS) versus SIS-guided endometrial sampling in the diagnosis of endometrial pathology. J Clin Ultrasound. 2016;44(7):416–22.
13. Bingol B, Gunenc Z, Gedikbasi A, Guner H, Tasdemir S, Tiras B. Comparison of diagnostic accuracy of saline infusion sonohysterography, transvaginal sonography and hysteroscopy. J Obstet Gynaecol [Internet]. 2011;31(1):54–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21280995.
14. Grimbizis GF, Tsolakidis D, Mikos T, Anagnostou E, Asimakopoulos E, Stamatopoulos P, et al. A prospective comparison of transvaginal ultrasound, saline infusion sonohysterography, and diagnostic hysteroscopy in the evaluation of endometrial pathology. Fertil Steril. 2010;94(7):2720–5.
15. Jurisic A, Garalejic E, Jurisic Z, Arsic B, Rudic J, Hadzovic M. Transvaginal 3D multislice saline infusion sonohysterography [Internet]. Reprod Biomed Online. 2011;22:S103. Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed10&NEWS=N&AN=70389772.
16. Brosens JJ, de Souza NM, Barker FG. Uterine junctional zone: function and disease. Lancet. 1995;346(8974):558–60.
17. Brosens I, Pijnenborg R, Benagiano G. Defective myometrial spiral artery remodelling as a cause of major obstetrical syndromes in endometriosis and adenomyosis. Placenta. 2013;34(2):100–5.
18. Puente JM, Fabris A, Patel J, Patel A, Cerrillo M, Requena A, et al. Adenomyosis in infertile women: prevalence and the role of 3D ultrasound as a marker of severity of the disease. Reprod Biol Endocrinol. 2016;14:60.
19. Juang C-M, Chou P, Yen M-S, Twu N-F, Horng H-C, Hsu W-L. Adenomyosis and risk of preterm delivery. BJOG [Internet]. 2007;114(2):165–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17169011.
20. Vercellini P, Consonni D, Dridi D, Bracco B, Frattaruolo MP, Somigliana E. Uterine adenomyosis and in vitro fertilization outcome: a systematic review and meta-analysis. Hum Reprod. 2014;29(5):964–77.
21. Fernando S, Breheny S, Jaques AM, Halliday JL, Baker G, Healy D. Preterm birth, ovarian endometriomata, and assisted reproduction technologies. Fertil Steril. 2009;91(2):325–30.
22. Stephansson O, Kieler H, Granath F, Falconer H. Endometriosis, assisted reproduction technology, and risk of adverse pregnancy outcome. Hum Reprod [Internet]. 2009;24(9):2341–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19439428.
23. Kunz G, Herbertz M, Beil D, Huppert P, Leyendecker G. Adenomyosis as a disorder of the early and late human reproductive period. Reprod Biomed Online [Internet]. 2007;15(6):681–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18062865.
24. Landi S, Mereu L, Pontrelli G, Stepniewska A, Romano L, Tateo S, et al. The influence of adenomyosis in patients laparoscopically treated for deep endometriosis. J Minim Invasive Gynecol. 2008;15(5):566–70.
25. Maubon A, Faury A, Kapella M, Pouquet M, Piver P. Uterine junctional zone at magnetic resonance imaging: a predictor of in vitro fertilization implantation failure. J Obstet Gynaecol Res. 2010;36(3):611–8.

26. Brosens I, Derwig I, Brosens J, Fusi L, Benagiano G, Pijnenborg R. The enigmatic uterine junctional zone: the missing link between reproductive disorders and major obstetrical disorders? Hum Reprod. 2010;25:569–74.
27. Luciano DE, Exacoustos C, Albrecht L, LaMonica R, Proffer A, Zupi E, et al. Three-dimensional ultrasound in diagnosis of adenomyosis: histologic correlation with ultrasound targeted biopsies of the uterus. J Minim Invasive Gynecol. 2013;20(6):803–10.
28. Bulletti C, De Ziegler D, Polli V, Del Ferro E, Palini S, Flamigni C. Characteristics of uterine contractility during menses in women with mild to moderate endometriosis. Fertil Steril. 2002;77(6):1156–61.
29. Fanchin R, Righini C, Olivennes F, Taylor S, De Ziegler D, Frydman R. Uterine contractions at the time of embryo transfer alter pregnancy rates after in-vitro fertilization. Hum Reprod. 1998;13(7):1968–74.
30. Fanchin R, Ayoubi JM, Righini C, Olivennes F, Schönauer LM, Frydman R. Uterine contractility decreases at the time of blastocyst transfers. Hum Reprod. 2001;16(6):1115–9.
31. Moon HS, Park SH, Lee JO, Kim KS, Joo BS. Treatment with piroxicam before embryo transfer increases the pregnancy rate after in vitro fertilization and embryo transfer. Fertil Steril. 2004;82(4):816–20.
32. Moraloglu O, Tonguc E, Var T, Zeyrek T, Batioglu S. Treatment with oxytocin antagonists before embryo transfer may increase implantation rates after IVF. Reprod Biomed Online. 2010;21(3):338–43.
33. Kupesic Plavsic S. New imaging diagnostics. In: Simon C, Giudice L, editors. The endometrial factor. Boca Raton, FL: CRC Press; 2017. p. 15–36.
34. Simón C, Martín JC, Pellicer A. Paracrine regulators of implantation. Baillieres Best Pract Res Clin Obstet Gynaecol. 2000;14:815–26.
35. Kupesic S, Bekavac I, Bjelos D, Kurjak A. Assessment of endometrial receptivity by transvaginal color Doppler and three-dimensional power Doppler ultrasonography in patients undergoing in vitro fertilization procedures. J Ultrasound Med [Internet]. 2001;20(2):125–34. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11211132.
36. Ng EH, Chan CC, Tang OS, Yeung WS, Ho PC. Changes in endometrial and subendometrial blood flow in IVF. Reprod Biomed Online [Internet]. 2009;18(2):269–75. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=19192349.
37. Ng EHY, Chan CCW, Tang OS, Yeung WSB, Ho PC. The role of endometrial and subendometrial blood flows measured by three-dimensional power Doppler ultrasound in the prediction of pregnancy during IVF treatment. Hum Reprod. 2006;21(1):164–70.
38. Kim A, Jung H, Choi WJ, Hong SN, Kim HY. Detection of endometrial and subendometrial vasculature on the day of embryo transfer and prediction of pregnancy during fresh in vitro fertilization cycles. Taiwan J Obstet Gynecol. 2014;53(3):360–5.
39. El-Zenneni H, Moustafa R, Abdel-Hafeez M, El-Salally H, Abdel-Kader A, Elnaggar A. Assessment of uterine, subendometrial blood flows and endometrial gland vascular endothelial growth factor (EG-VEGF) in women with unexplained infertility. Middle East Fertil Soc J. 2015;20(2):119–26.
40. Bhal PS, Pugh ND, Gregory L, O'Brien S, Shaw RW. Perifollicular vascularity as a potential variable affecting outcome in stimulated intrauterine insemination treatment cycles: a study using transvaginal power Doppler. Hum Reprod. 2001;16(8):1682–9.
41. Robson SJ, Barry M, Norman RJ. Power Doppler assessment of follicle vascularity at the time of oocyte retrieval in in vitro fertilization cycles. Fertil Steril. 2008;90(6):2179–82.
42. Raine-Fenning N, Jayaprakasan K, Clewes J, Joergner I, Dehghani Bonaki S, Chamberlain S, et al. SonoAVC: a novel method of automatic volume calculation. Ultrasound Obstet Gynecol. 2008;31(6):691–6.
43. Ata B, Tulandi T. Ultrasound automated volume calculation in reproduction and in pregnancy. Fertil Steril. 2011;95(7):2163–70.
44. Ata B, Seyhan A, Reinblatt SL, Shalom-Paz E, Krishnamurthy S, Tan SL. Comparison of automated and manual follicle monitoring in an unrestricted population of 100 women undergoing controlled ovarian stimulation for IVF. Hum Reprod. 2011;26(1):127–33.

45. Deb S, Campbell BK, Clewes JS, Raine-Fenning NJ. Quantitative analysis of antral follicle number and size: a comparison of two-dimensional and automated three-dimensional ultrasound techniques. Ultrasound Obstet Gynecol. 2010;35(3):354–60.
46. Deb S, Jayaprakasan K, Campbell BK, Clewes JS, Johnson IR, Raine-Fenning NJ. Intraobserver and interobserver reliability of automated antral follicle counts made using three-dimensional ultrasound and SonoAVC. Ultrasound Obstet Gynecol. 2009;33(4):477–83.
47. Vandekerckhove F, Bracke V, De Sutter P. The value of automated follicle volume measurements in IVF/ICSI. Front Surg. 2014;1:18.
48. Murtinger M, Aburumieh A, Rubner P, Eichel V, Zech MH, Zech NH. Improved monitoring of ovarian stimulation using 3D transvaginal ultrasound plus automated volume count. Reprod Biomed Online. 2009;19(5):695–9.
49. Dewailly D, Lujan ME, Carmina E, Cedars MI, Laven J, Norman RJ, et al. Definition and significance of polycystic ovarian morphology: a task force report from the androgen excess and polycystic ovary syndrome society. Hum Reprod Update. 2014;20(3):334–52.
50. Dewailly D, Gronier H, Poncelet E, Robin G, Leroy M, Pigny P, et al. Diagnosis of polycystic ovary syndrome (PCOS): revisiting the threshold values of follicle count on ultrasound and of the serum AMH level for the definition of polycystic ovaries. Hum Reprod. 2011;26(11):3123–9.
51. Kelsey TW, Dodwell SK, Wilkinson AG, Greve T, Andersen CY, Anderson RA, et al. Ovarian volume throughout life: a validated normative model. PLoS One. 2013;8(9):e71465.
52. 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.
53. Engmann L, Sladkevicius P, Agrawal R, Bekir JS, Campbell S, Tan SL. Value of ovarian stromal blood flow velocity measurement after pituitary suppression in the prediction of ovarian responsiveness and outcome of in vitro fertilization treatment. Fertil Steril. 1999;71(1):22–9.
54. Kupesic S, Kurjak A. Predictors of IVF outcome by three-dimensional ultrasound. Hum Reprod [Internet]. 2002;17(4):950–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11925388.
55. Kupesic S, Plavsic BM. 2D and 3D hysterosalpingo-contrast-sonography in the assessment of uterine cavity and tubal patency. Eur J Obstet Gynecol Reprod Biol. 2007;133(1):64–9.
56. Jiangxiu Y, Mingjin C, Weixiang L, Zhifeng D, Yinong X. Diagnostic efficacy of 3-D hysterosalpingo-contrast sonography in the detection of tubal occlusion: systematic meta-analysis. J Obstet Gynaecol Res. 2015;41(9):1418–25.
57. Wang Y, Qian L. Three- or four-dimensional hysterosalpingo contrast sonography for diagnosing tubal patency in infertile females: a systematic review with meta-analysis. Br J Radiol. 2016;89(1063):20151013. https://doi.org/10.1259/bjr.20151013.
58. Exacoustos C, Di Giovanni A, Szabolcs B, Romeo V, Romanini ME, Luciano D, et al. Automated three-dimensional coded contrast imaging hysterosalpingo-contrast sonography: feasibility in office tubal patency testing. Ultrasound Obstet Gynecol. 2013;41(3):328–35.
59. Benacerraf BR, Groszmann Y, Hornstein MD, Bromley B. Deep infiltrating endometriosis of the bowel wall: the comet sign. J Ultrasound Med [Internet]. 2015;34(3):537–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25715375.
60. Reid S, Lu C, Casikar I, Reid G, Abbott J, Cario G, et al. Prediction of pouch of Douglas obliteration in women with suspected endometriosis using a new real-time dynamic transvaginal ultrasound technique: the sliding sign. Ultrasound Obstet Gynecol. 2013;41(6):685–91.
61. Guerriero S, Ajossa S, Minguez JA, Jurado M, Mais V, Melis GB, et al. Accuracy of transvaginal ultrasound for diagnosis of deep endometriosis in uterosacral ligaments, rectovaginal septum, vagina and bladder: systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2015;46(5):534–45.
62. Arya S, Kupesic Plavsic S. Preimplantation 3D ultrasound: current uses and challenges. J Perinat Med. 2017;45:745.

New Challenges ofEchography inReproduction

10

Sonal Panchal and Asim Kurjak

10.1 Introduction

Over the past few decades, there have been vast and unimagined advances in the assisted human reproductive technologies (ART). These include the inventions of new molecules, advancements in laboratory technologies and also advancements in the monitoring technologies that include blood investigations and ultrasound. In the last few decades, there have been major breakthroughs in the ultrasound technologies. These include colour Doppler, power Doppler, spectral Doppler and volume ultrasound. Volume ultrasound consists of 3D and 4D ultrasound with understanding of multiplanar anatomy, various rendering technologies and several automated volume calculation softwares. These collectively play a very important role in monitoring and understanding of the hormonal changes occurring during the treatment cycle and also in better assessment of the follicular maturity and endometrial receptivity, ultimately to improve the results of ART.

The discussion on how these technologies and softwares can be used for advantage of assisted reproductive technologies can be divided under the following heads:

1. Doppler—what is its role?
   It is known that the menstrual cycle is a very finely balanced orchestra of several hormones. These hormonal changes reflect instantaneously as vascular changes followed by morphological changes. Optimally, one can understand the

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hormonal status of the female at that time. Doppler plays a very important role to understand the hormonal changes occurring during menstrual cycle and plan treatment protocol [1–3].

2. 3D ultrasound and 3D power Doppler (3D PD) ultrasound—what is the role?
   There are several literature evidences that have proved the superiority of 3D ultrasound over B-mode ultrasound for the assessment of polycystic ovaries and also for assessment follicle maturity and endometrial receptivity. 3D ultrasound gives information about the entire volume, whereas 3D power Doppler gives qualitative information about the global vascularity in the volume. This definitely means a more in-depth information about the anatomy and blood supply of the organ/lesion assessed.
3. Volume calculation softwares
   These special softwares for automated volume calculation have already proved their efficacy over the manual calculation methods. These include calculation of the volume as well as quantitative assessment of the global vascular parameters in the selected volume. The later can give the most in-depth information on the global blood supply of the structure or lesion assessed.
   But it will be convenient and more easy to understand if the discussion progresses through the different phases of menstrual cycle. The menstrual cycle can be divided mainly into proliferative and secretory phases and then further each phase into early and late part. To make it a little more convenient, we shall divide our discussion into three phases:

1. Early proliferative (menstrual) phase
2. Preovulatory phase
3. Secretory phase

10.2 Early Proliferative (Menstrual) Phase

Early proliferative phase evaluation is started on day 2–3 of the cycle. This is considered a baseline phase of the menstrual cycle. It is at this phase of the cycle that oestrogen and progesterone, the important steroids of the entire hormonal orchestra, are at their lowest levels. It is at this stage that LH (luteinizing hormone) is also low, though variates with pulses, and the FSH (follicle-stimulating hormone) levels start rising. As is known, the latter two hormones are produced by pituitary and are controlled by feedback mechanisms depending on level of oestrogen in this phase of the cycle. Oestrogen is produced from the granulosa cells of the growing follicle. FSH is the hormone that stimulates the growth of the follicle and production of oestrogen from the granulosa cells. But FSH acts on granulosa cells under the influence of LH only. The aim of the ultrasound scan done at this stage is therefore to evaluate the effect of oestrogen, progesterone, LH and FSH in the ovaries and on the endometrium. This acts as a guide to decide stimulation protocols for ART. It is also interesting to understand here that androgen also has a crucial role to play in this early phase of the cycle as the recruitment of follicles from preantral to antral is a function

of androgen. Till follicle size of 6mm, the follicular growth is androgen dependent.

The decision on the stimulation protocols is chiefly dependent on ovarian reserve and response. The ovarian reserve can be correlated with the number of antral follicles in the ovary and the ovarian volume, whereas the ovarian response (sensitivity of the ovary to the stimulating drugs) relates to the ovarian stromal blood supply.

10.2.1 Ovarian Reserve andtheAntral Follicle Count

Jarvela et al. have shown in their study that the number of retrieved oocytes is correlated only with the antral follicle count and ovarian volume and not with vascularization indices [4]. Multivariate analysis confirmed that AFC represents the most useful ovarian response marker to COS in all study groups, as the number of mature oocytes retrieved increased with increasing AFC [5]. Antral follicle count (AFC) and ovarian volume showed significant correlation with AMH [6]. AFC also correlates well with the age, FSH and inhibin B [7]. Though anti-Mullerian hormone (AMH) has gained a marked popularity as the most reliable marker for assessment of the ovarian reserve so far, studies have proved that whether for hyperresponse or poor response, AFC is as reliable a marker as AMH [8–10]. This can be also explained because AMH is produced from the granulosa cells of preantral and small antral follicles and therefore correlates well with the antral follicle pool. Androgen can also be correlated to antral follicle count. Making it simple, androgen is the cause and AMH is the effect of antral follicle count.

AFC and ovarian volume provide direct measurements of ovarian reserve, while AMH, inhibin B and oestradiol are released from growing follicles, and so their levels reflect the size of developing follicle cohort [11]. FSH is controlled by negative feedback of inhibin B, and so high FSH represents small cohort size [11].

More exact value of AFC was acquired when counted by 3D US. Automated 3D ultrasound measures do provide reliable information on follicle number and size and can be used to individually identify each follicle [12]. It has also been shown that intraobserver and interobserver reliability of automated antral follicle counts made using three-dimensional ultrasound and SonoAVC a preferred method [13].

The number of follicles >12 mm on the day of oocyte retrieval is correlated significantly with AFC counted by 3D US rather than 2D US. Rendering on inversion mode is the most convenient method for counting antral follicles when they are multiple [14]. Inversion mode rendering is actually visualizing the fluid-filled structures as solid structures, making their presence more evident (Fig. 10.1). The inversion mode has been further sophisticated by colour coding of these follicles, and this software on 3D ultrasound is named as SonoAVC (sonographic automated volume calculation). Due to colour coding, the risk of overcounting the follicles, especially when the number of follicles is more, is excluded (Fig. 10.2).

SonoAVC measured less number of follicles on AFC, though it took significantly less time to measure the size and record the number of antral follicles (132 ± 56.23s vs. 324.47 ± 162.22s) [15]. Moreover this software also calculates the size of each

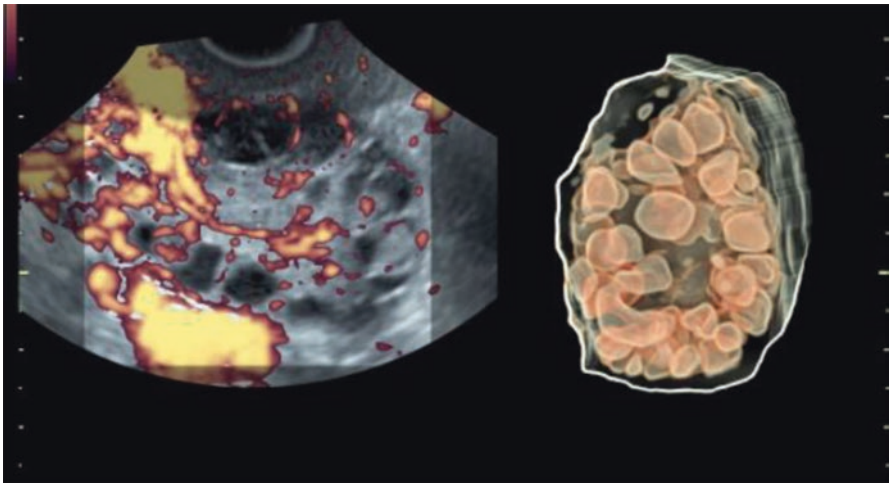


Fig. 10.1 Antral follicles seen on 3D ultrasound volume of the ovary rendered on inversion mode

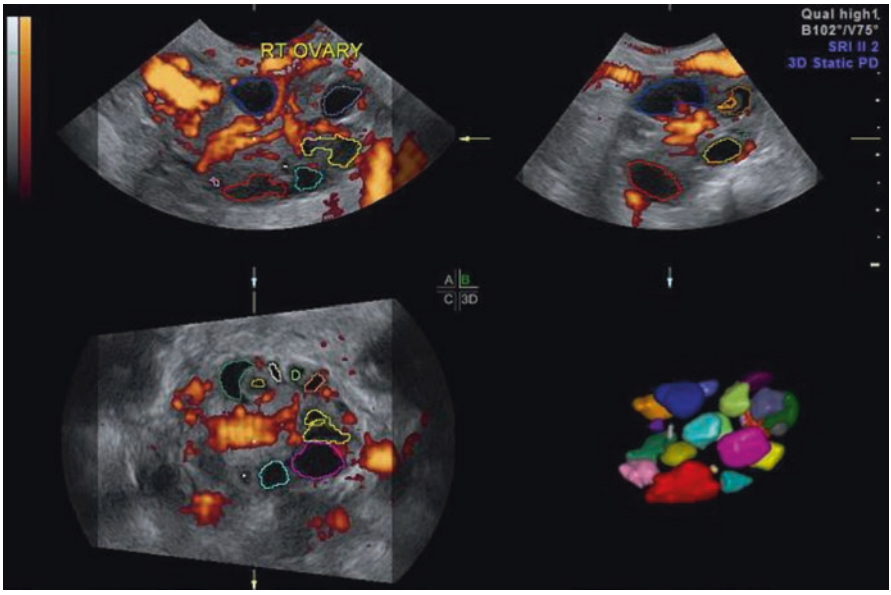
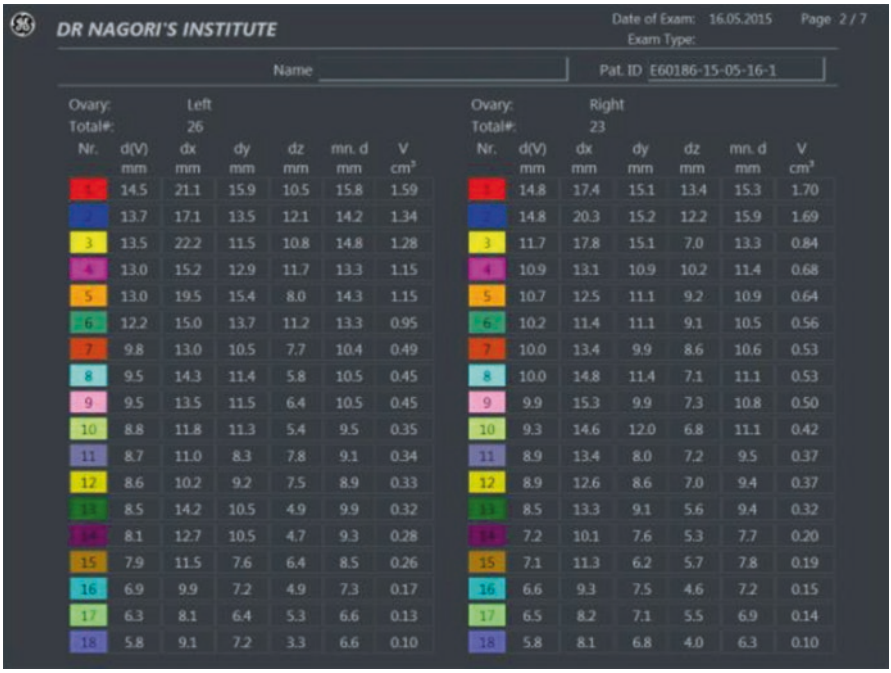


Fig. 10.2 3D ultrasound volume of the ovary showing sectional planes and the lower right image shows colour-coded follicles on SonoAVC

follicle as mean diameter and volume (Fig. 10.3). Knowing the follicle size and estimating the number of follicles of >6 mm in size are important as it is these follicles that will respond to gonadotrophin stimulation. This information is more clinically applicable in IUI cycles.



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Date of Exam: 16.05.2015 Page 2 / 7
Exam Type:

Name

Pat. ID E60186-15-05-16-1

Ovary: Left Total#: 26							Ovary: Right Total#: 23						
Nr.	d(V) mm	dx mm	dy mm	dz mm	mn. d mm	V cm^3	Nr.	d(V) mm	dx mm	dy mm	dz mm	mn. d mm	V cm^3
1	14.5	21.1	15.9	10.5	15.8	1.59	1	14.8	17.4	15.1	13.4	15.3	1.70
2	13.7	17.1	13.5	12.1	14.2	1.34	2	14.8	20.3	15.2	12.2	15.9	1.69
3	13.5	22.2	11.5	10.8	14.8	1.28	3	11.7	17.8	15.1	7.0	13.3	0.84
4	13.0	15.2	12.9	11.7	13.3	1.15	4	10.9	13.1	10.9	10.2	11.4	0.68
5	13.0	19.5	15.4	8.0	14.3	1.15	5	10.7	12.5	11.1	9.2	10.9	0.64
6	12.2	15.0	13.7	11.2	13.3	0.95	6	10.2	11.4	11.1	9.1	10.5	0.56
7	9.8	13.0	10.5	7.7	10.4	0.49	7	10.0	13.4	9.9	8.6	10.6	0.53
8	9.5	14.3	11.4	5.8	10.5	0.45	8	10.0	14.8	11.4	7.1	11.1	0.53
9	9.5	13.5	11.5	6.4	10.5	0.45	9	9.9	15.3	9.9	7.3	10.8	0.50
10	8.8	11.8	11.3	5.4	9.5	0.35	10	9.3	14.6	12.0	6.8	11.1	0.42
11	8.7	11.0	8.3	7.8	9.1	0.34	11	8.9	13.4	8.0	7.2	9.5	0.37
12	8.6	10.2	9.2	7.5	8.9	0.33	12	8.9	12.6	8.6	7.0	9.4	0.37
13	8.5	14.2	10.5	4.9	9.9	0.32	13	8.5	13.3	9.1	5.6	9.4	0.32
14	8.1	12.7	10.5	4.7	9.3	0.28	14	7.2	10.1	7.6	5.3	7.7	0.20
15	7.9	11.5	7.6	6.4	8.5	0.26	15	7.1	11.3	6.2	5.7	7.8	0.19
16	6.9	9.9	7.2	4.9	7.3	0.17	16	6.6	9.3	7.5	4.6	7.2	0.15
17	6.3	8.1	6.4	5.3	6.6	0.13	17	6.5	8.2	7.1	5.5	6.9	0.14
18	5.8	9.1	7.2	3.3	6.6	0.10	18	5.8	8.1	6.8	4.0	6.3	0.10

Fig. 10.3 Report sheet of SonoAVC showing follicles in the left and right ovary with diameters and volume of each follicle

AFC can be used as the only one parameter to decide the ovarian reserve. A meta-analysis has shown that AFC is a better predictor for poor response compared to ovarian volume [16]. Another study has also shown that 3D ultrasound has shown no significant difference between the volume of low responders and controls on baseline scan [17]. So, 3D ultrasound is the modality of choice for assessment of the ovarian reserve for both AFC and ovarian volume, though AFC being clinically more relevant.

10.2.2 The Second Important Assessment Is That oftheOvarian Response

Ovarian stromal blood flow predicts ovarian responsiveness and hence provides a non-invasive and cost-effective prognostic factor of IVF outcome [18] (Fig. 10.4). It is fairly simple to understand that if the blood flow to the ovary is abundant, the gonadotrophins that are used for ovulation induction will reach the ovary in large amount, and therefore total drugs required to be put into patient's system will be less and vice versa. This has also been proved in a study that measurement of ovarian stromal flow in early follicular phase is related to subsequent ovarian response in

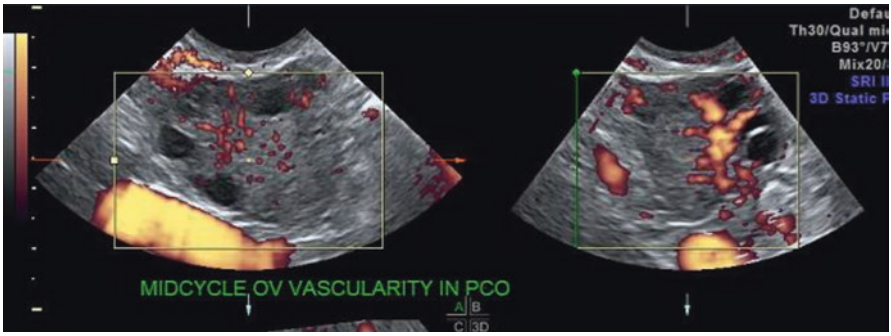

Fig. 10.4 Power Doppler showing flow in the ovarian stroma

IVF treatment [19]. One more study has also proved that ovarian stromal peak systolic velocity (PSV) after pituitary suppression is predictive of ovarian responsiveness and outcome of IVF treatment [20].

This means that calculating ovarian stromal PSV can be a guide to decision of stimulation protocol. The total flow in any part of the body is a function of resistance and velocity, and we have documented in a study that ovaries that had low resistance and/or high PSV are required lower doses of gonadotrophins for ovulation induction as compared to those with high resistance and/or low PSV which required higher doses of gonadotrophins for ovulation induction [21]. Doppler of the ovaries therefore is an important investigation for deciding the stimulation protocols for ART.

But it is also known that when ovarian stromal resistance and velocity are assessed by Doppler, it is a random assessment of one or few vessels. It does not assess the abundance of the blood flows in the ovaries. The exact estimation of the ovarian flow can therefore only be achieved by 3D power Doppler which can assess the abundance of flow and can also estimate the average intensity of flow (velocity) in the entire ovary (Fig. 10.5). An ultrasound-based study on prediction of ovarian response in 2007 by Merce et al. [13] evaluated ovarian volume, AFC and 3D power Doppler indices VI (vascularisation index), FI (flow index) and VFI (vascularity flow index) for ovarian response and mentioned that 3D power Doppler indices made the assessment of ovarian response to stimulation protocols easier [22] (Fig. 10.6). VI is an index that indicates the abundance of flow in the selected volume, FI is an index that assesses the average intensity of flow in a selected volume and VFI is an index derived as a composite value of the previous two which indicates the perfusion in the selected volume. Kupesic has also shown correlation in the ovarian stromal flow index and number of mature oocytes retrieved in an IVF cycles and pregnancy rates (<11, low responder; 11–14, good; >15, risk of OHSS) [23].

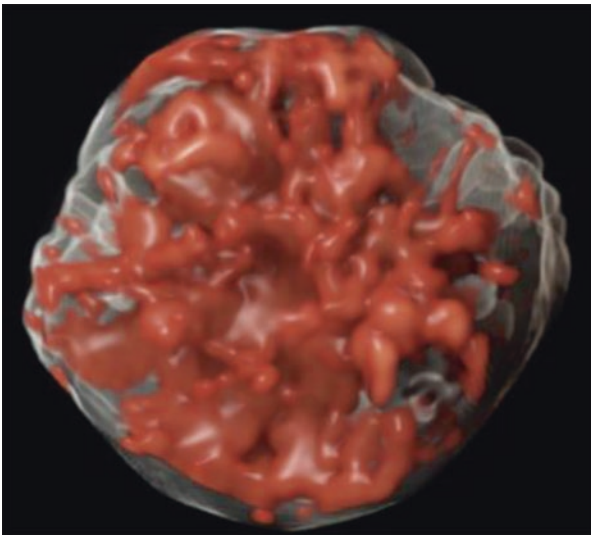

Fig. 10.5 3D power Doppler of the ovary rendered in glass body mode showing vascularity of the ovary

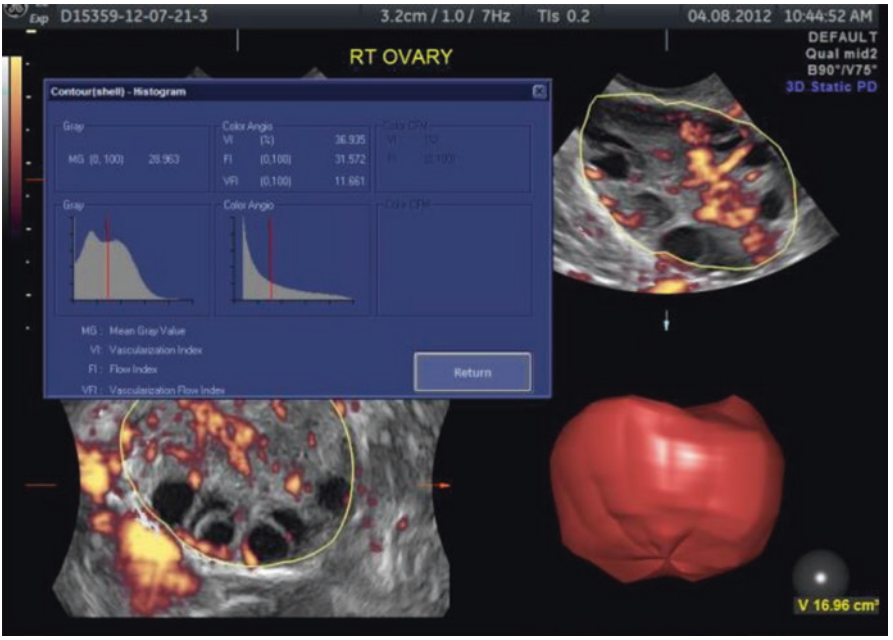

Fig. 10.6 3D power Doppler acquired ovarian volume shows volume calculated with VOCAL in the lower right image. The blue box shows the volume histogram with VI, FI and VFI values in the middle graph, whereas the graph on the left side shows the mean grey value of the ovary

10.2.3 Is Evaluation oftheUterus withAny oftheModalities Like Doppler or 3D Ultrasound ofAny Importance at theBaseline Scan?

Subendometrial flow on day 2 if present indicates a low receptive endometrium for implantation (Fig. 10.7). 3D power Doppler for subendometrial vascularization on the first day of ovarian stimulation showed significantly lower VI and VFI in conception cycles [24]. This can be explained by the simple fact that, at this phase of the cycle, the two steroids responsible for endometrial vascularity, oestrogen and progesterone, are both at their baseline levels, and therefore the endometrial vascularity should also be low. Instead if it is more or is documented, it may most likely be due to some pathology that may cause hyperaemia, like inflammation. If inflammation is present evidently, the endometrial receptivity will be low.

It is also found to be importance to assess the uterine cavity for any abnormalities that may either interfere with implantation or lead to abortion. These include Mullerian duct abnormalities and also the endometrial pathologies, e.g. polyps, synechiae, etc. For both groups 3D ultrasound has been proved to be the modality of choice (Fig. 10.8).

B-mode ultrasound provides only a limited view of the uterine fundus and therefore cannot reliably differentiate between arcuate, bicornuate and subseptate uteri [25]. Three- dimensional ultrasound overcomes those limitations by providing a coronal view of the uterus, which can rarely be seen by conventional two-dimensional ultrasound [26]. According to Caliskan et al., 3D transvaginal ultrasound's, if

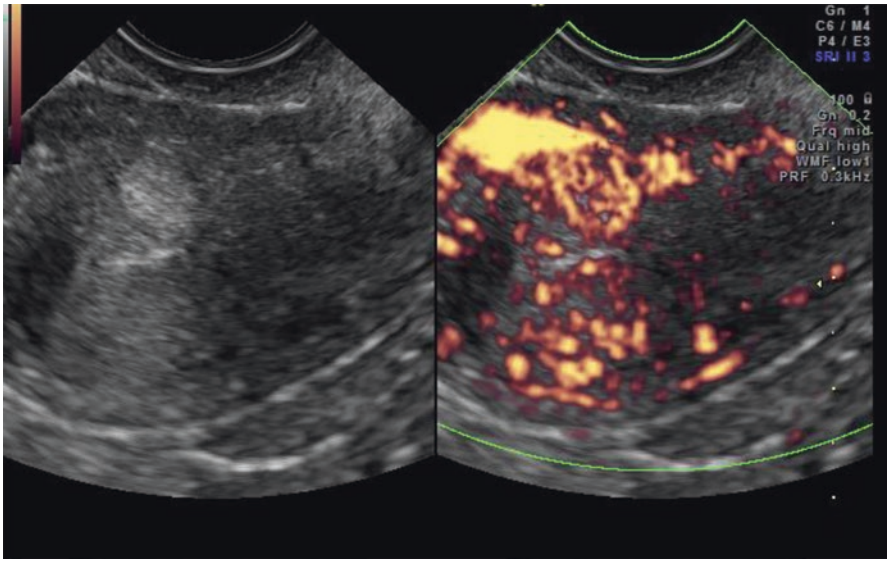

Fig. 10.7 Thin endometrium on day 3 of the cycle shows subendometrial vascularity on power Doppler, indicating poor endometrial receptivity. Though in this case myometrial heterogenecity and vascularity is also seen anteriorly suggestive of adenomyosis

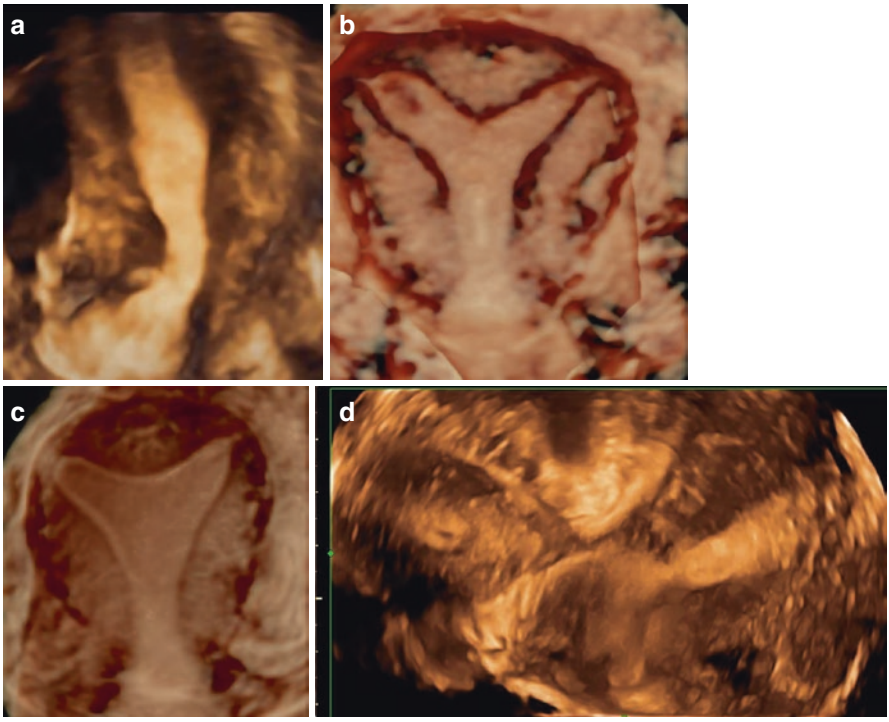

Fig. 10.8 (**a**) Unicornuate uterus, (**b**) subseptate uterus, (**c**) arcuate uterus, (**d**) uterus didelphys. All rendered on 3D ultrasound

done in luteal phase, when the endometrium is bright echogenic, accuracy for diagnosis of congenital uterine anomalies reaches that of MRI [27, 28]. Transvaginal 3D ultrasound seems to have replaced endoscopy as the gold standard technique for diagnosis of septate uterus [29] (Fig. 10.9).

For the assessment of the endometrial lesions like polyps, a B-mode transvaginal ultrasound scan has been widely used with fair results (Fig. 10.10). When the diagnosis is in doubt, saline infusion sonohysterography especially with 3D ultrasound is thought to be the modality of choice (Fig. 10.11). Though hysteroscopy is the gold standard, sonohysterography is the second most reliable modality and the most preferred one being minimally invasive for diagnosis of all endometrial and subendometrial pathologies, viz. polyps, synechiae, subendometrial fibroids, etc. [30, 31] (Fig. 10.12). Doppler is also an important modality when one has to differentiate between a polyp and a subendometrial fibroid. These can be confidently differentiated by their distinct vascular patterns. Polyps typically show a single feeding vessel, whereas fibroids typically have a peripheral vascular ring [32] (Fig. 10.13a, b). In case of polyps and endometrial fibroids, 3D also can be a useful tool to assess the compromise of the endometrial cavity (Fig. 10.13c, d).

Though not at the baseline scan (because of the menstrual bleeding), assessment of the tubal patency is very important before initiating ART like intrauterine

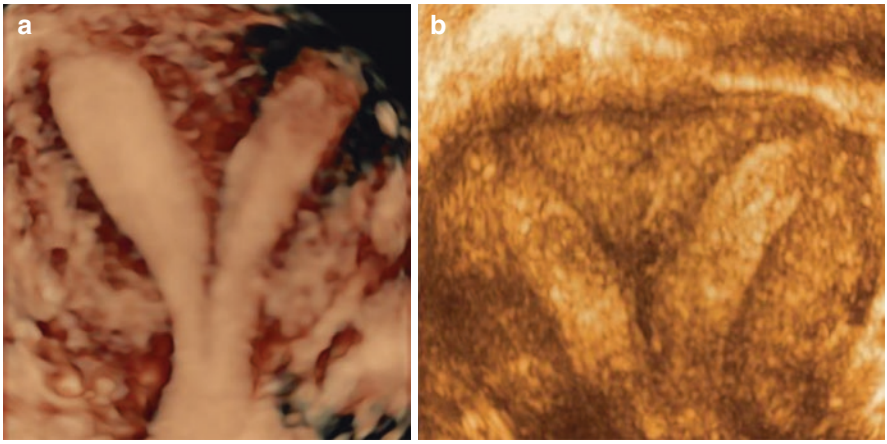

Fig. 10.9 (**a**, **b**) Uteri with septa seen in both images rendered on 3D ultrasound

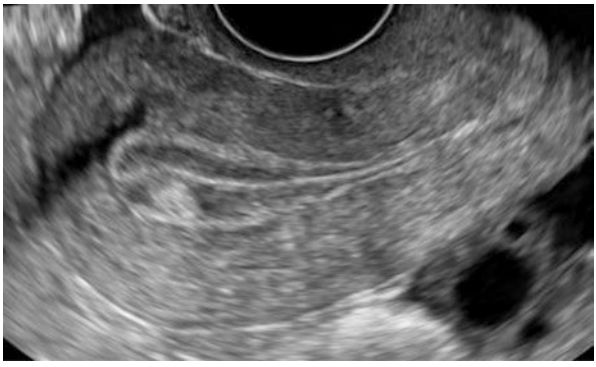

Fig. 10.10 B-mode ultrasound showing polyp arising from the posterior wall

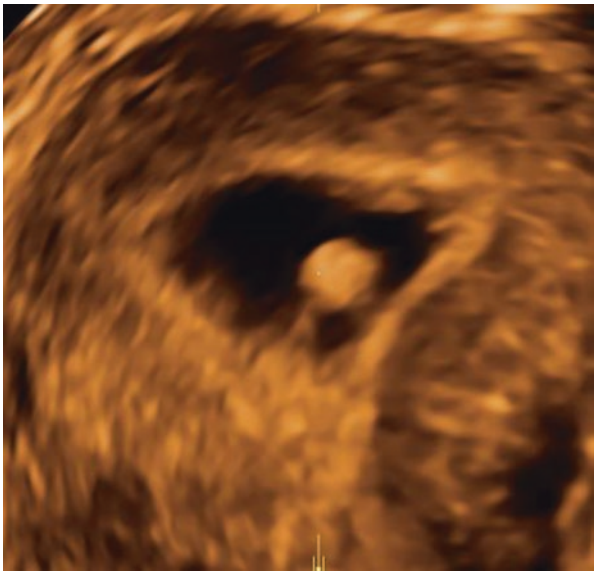

Fig. 10.11 3D sonohysterography showing endometrial polyp

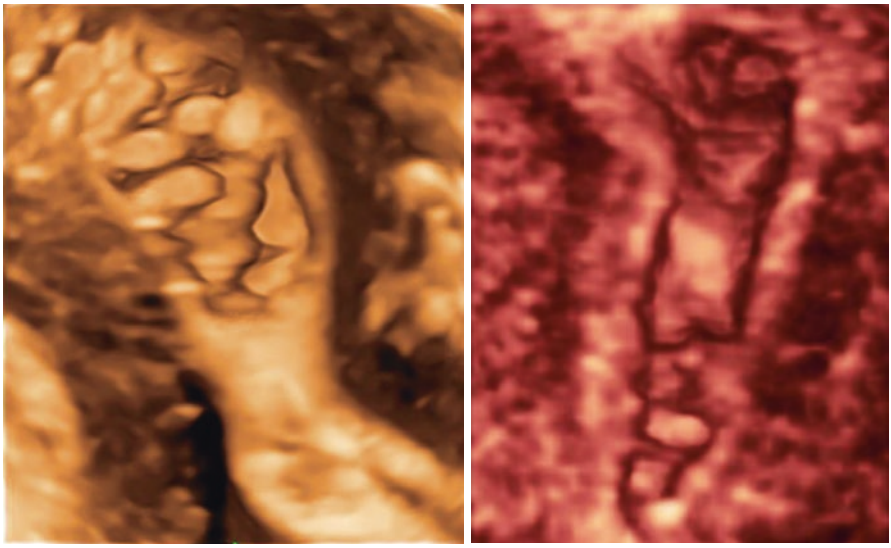

Fig. 10.12 Two images of 3D sonohysterography showing multiple polyps in the left image and synechiae in the lower half of the uterus in the right image

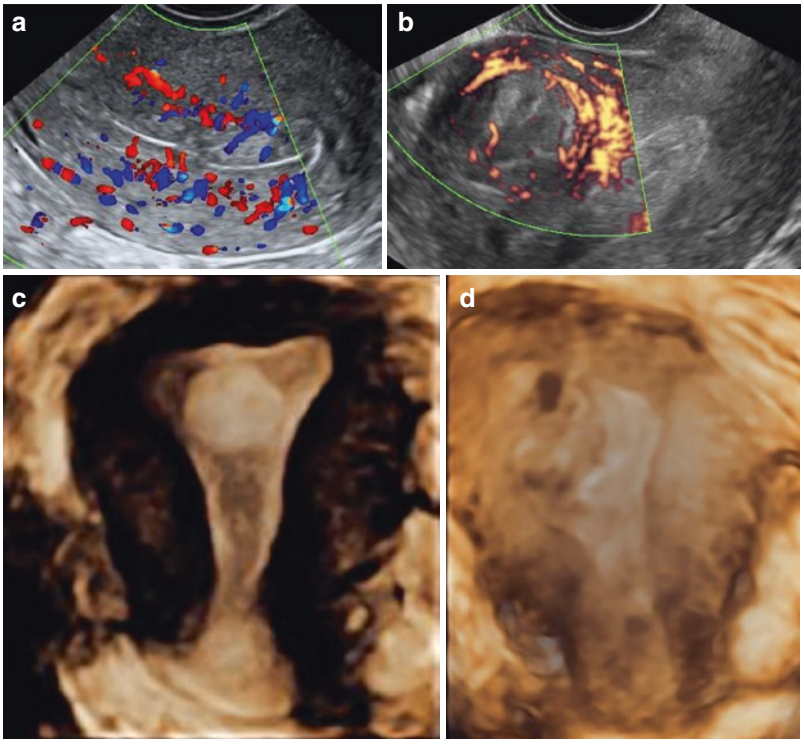

Fig. 10.13 (**a**) Colour Doppler image of the endometrium showing polyp with single feeding vessel, (**b**) power Doppler image of the uterus showing anterior myometrial fibroid with typical peripheral vascularity, (**c**) 3D ultrasound rendered image of polyp, (**d**) 3D ultrasound image of the submucous fibroid

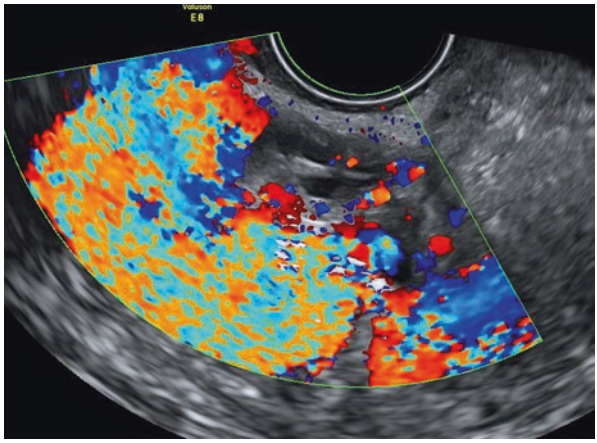

Fig. 10.14 Colour Doppler image showing the colour-filled box as passage of saline from the fimbrial end of the tube suggesting the patency

insemination (IUI). Tubal evaluation by ultrasound can be done using saline and is named as saline infusion salpingography (SIS). The passage of saline from the fimbrial end of the tube can be observed on B-mode ultrasound to confirm the patency of the tubes. Using colour Doppler for the same has proved to be more accurate [33] (Fig. 10.14).

In a study by Peters and Coulam of 129 infertile patients, Doppler SIS showed complete agreement with X-ray HSG in 81% cases. When compared with the gold standard test of tubal assessment, chromopertubation, Doppler SIS and saline infusion sonosalpingography (SIS) showed agreement with chromopertubation findings in 86% of cases, while only 75% of cases for X-ray HSG agreed with chromopertubation [34]. In a small study by Kupesic et al., in 91.548% of cases, agreement was seen between findings of colour Doppler sonosalpingography and that of chromopertubation [35]. Correlation of colour Doppler sonosalpingography and X-ray HSG with chromopertubation was 81% versus 60%, respectively, in one study [36].

Though this investigation had a good correlation with other conventionally used modalities for tubal patency assessment, the tubal lumen assessment was made possible only by positive contrast salpingography, named as HyCoSy (hystero-contrast-salpingography). Adding 3D ultrasound to HyCoSy is considered to be one of the best imaging modalities for assessment of the tubes as it gives information about the tubal patency, lumen condition and also the condition of the fimbria (Fig. 10.15). Automated 3D volume acquisition permits visualization of the tubes in the coronal view and of the tubal course in 3D space and should allow less experienced operators to evaluate tubal patency status relatively easily [37].

Large studies have reported that 3D HyCoSy is highly accurate with 100% sensitivity, 67% specificity, 89% PPV and 100% NPV for tubal patency and concordance rate with laparoscopy of 91% [38].

In a study by Kupesic et al., 3D HyCoSy (sensitivity, specificity, PPV and NPV of 97.9%, 100%, 97.9% and 100%, respectively) was found to be marginally superior to 2D HyCoSy (sensitivity, specificity, PPV and NPV of 93.6%, 97.3%, 98.2% and 97.3%, respectively) for tubal assessment [39].

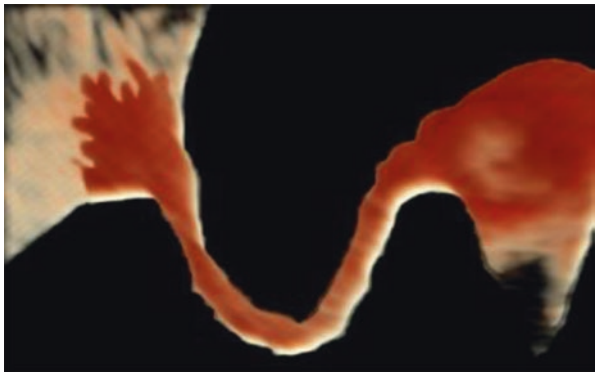

Fig. 10.15 3D HyCoSy image showing the endometrial cavity, right tubal lumen, fimbrial end and spill of contrast

10.3 Preovulatory Phase

It has been well known that when the follicle diameter is 16–18 mm, it is considered as a mature follicle and is a time for hCG in ART cycles. The accuracy of 3D US measurement of follicular volume compared to the standard 2D techniques by comparing the volume of individual follicles is estimated by both methods with the corresponding follicular aspirates: using the formula of ellipse, the limit of agreement between aspirates and calculated volume was +3.47 to −2.42 as compared to +0.96 to −0.43 when calculated by 3D US using VOCAL [40] (Fig. 10.16).

Increase in perifollicular vascularity of dominant follicle in theca layer starts developing as early as 8th day of the cycle. This indicates granulosa cell proliferation due to development of FSH receptors and therefore the rising oestrogen levels from these granulosa cells. Oestrogen is reflected as conversion of the endometrium to multilayered followed by development of endometrial vascularity. Fall in perifollicular RI starts 2 days before ovulation, reaches nadir at ovulation, remains low for 4 days and then with gradual rise, reaches 0.5 in midluteal phase [41].

When functionally mature, on colour Doppler, the follicle shows blood vessels covering most part of the follicular circumference.

On pulse Doppler these blood vessels show an RI of 0.4–0.48 and PSV of >10 cm/s [42] (Fig. 10.17). It has been quoted in a study by Nargund et al. that embryos produced by fertilization of the ova obtained from the follicles which had a perifollicular PSV of <10 cm/s are less likely to be grade I embryos and also have higher chance of chromosomal malformations. When functionally mature, on colour Doppler, the follicle shows blood vessels covering at least 3/4 of the follicular circumference [43, 44].

It is important to assess the follicular vascularity and to decide the time of ovulation trigger based on the follicular blood supply because if the follicular blood supply is inadequate, it means the oocyte may be hypoxic and if the oocyte is hypoxic, there is a very high chance of chromosomal abnormalities in the oocyte and therefore may lead to non-conception or abortion or embryo with chromosomal abnormality [43, 44].

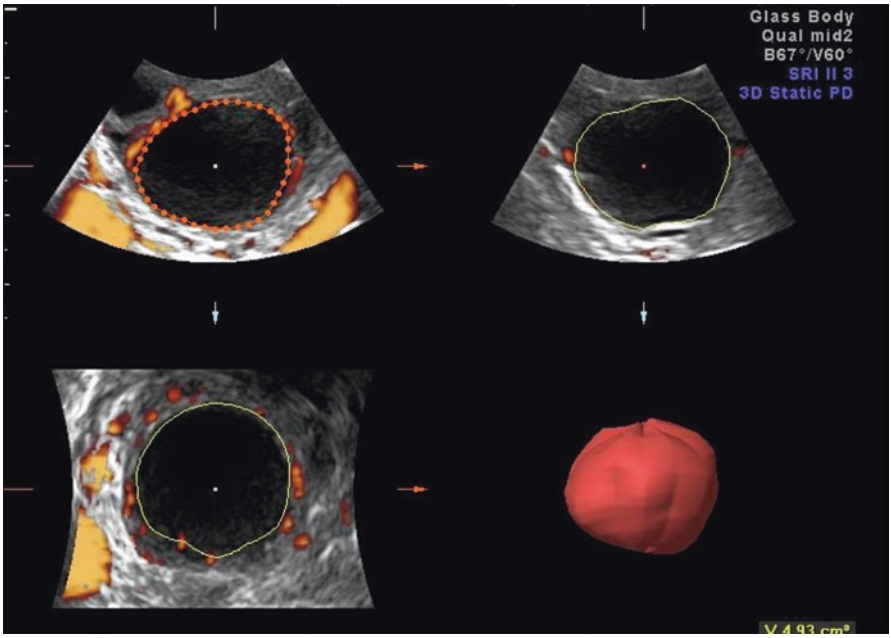

Fig. 10.16 3D ultrasound acquired volume of the follicle with VOCAL calculated volume of the follicle

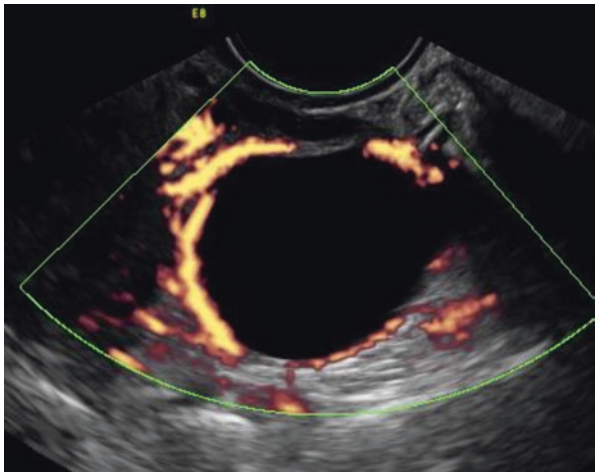

Fig. 10.17 Power Doppler showing blood vessels surrounding the follicular wall

It has also been shown that follicles with more uniform perifollicular vascular network are more likely to produce pregnancy [45].

Uniformity of the follicular vascularity can be established by 3D PD (Fig. 10.18). It allows to assess the global vascularity subjectively. The objective assessment can be assessed by volume histogram that assesses the 3D power Doppler indices VI, FI

and VFI (Fig. 10.19). In our study [34], we have found perifollicular VI of between 6 and 20 and perifollicular FI >35 as most optimum. 68.4% of patients conceived when the VI was between 6 and 18 and 50% when it was between 18 and 20. However the pregnancy rates were <25% when VI was <6 and only 7.4% when VI was >20. It was only 7.4% of patients with FI <27 who conceived, whereas beyond 27, the conception rates rose consistently. It was 50% with FI between 27 and 35,

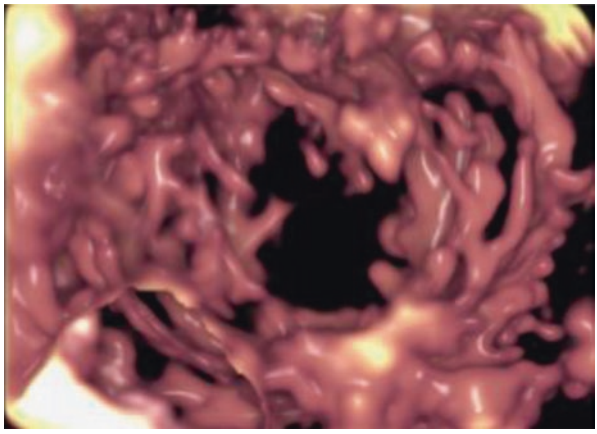

Fig. 10.18 3D power Doppler showing blood vessels surrounding the follicle

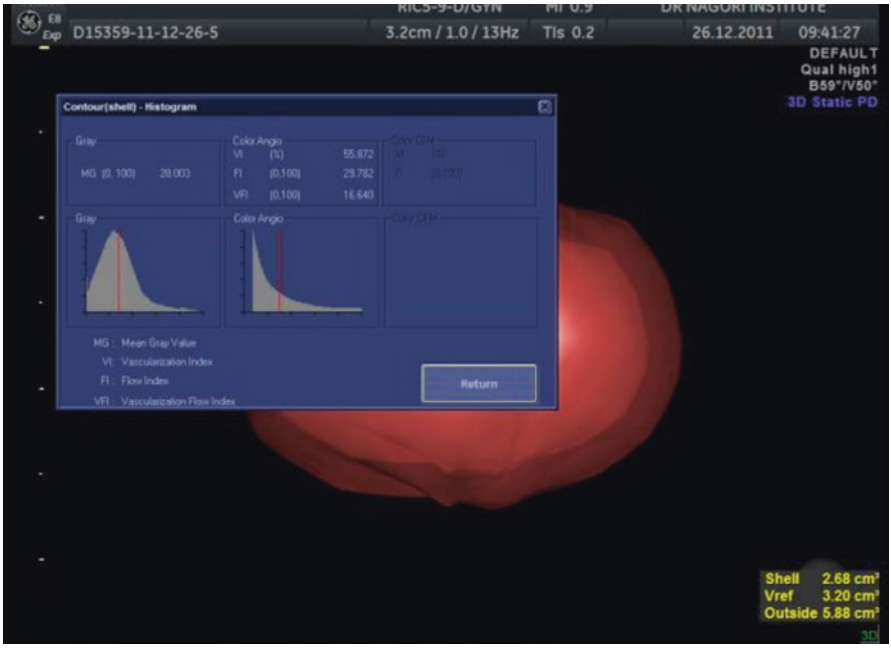

Fig. 10.19 Volume histogram showing VI, FI and VFI values of the perifollicular blood supply

70% when FI was between 35 and 43 and almost all patients had conceived when FI was >43 [46]. A study by Kupesic and Kurjak shows that when the ratio of follicular volume to blood flow index (FV/FI) is between 0.4 and 0.6, the pregnancy rates are 39%; if >0.6, it is 52%; and when <0.4, it is only 21% [23]. Cumulative experience of different studies therefore indicates that assessment of the global vascularity of the follicles can be a useful guide to decide quality of the follicle and therefore anticipate the chances of conception.

Feichtinger et al. in their study have shown presence of cumulus in follicles >15 mm by 3D US [47]. Poehl et al. also showed in their study that appearance of the intrafollicular cumulus structures by 3D US was correlated with the recovery rate of the mature oocytes [48] (Fig. 10.20). These studies have produced sufficient evidence to prove that the advances in ultrasound like Doppler, 3D and 3D PD are very useful modalities to assess the follicular quality.

Vascular changes at the time of impending ovulation include increased vascularity of the inner wall of the follicle and a coincident surge in blood velocity just prior to eruption [49].

Thus following the blood flows of the follicle, the resistance and velocities and the 3D assessment allows to follow the follicular maturity and quality and thus helps to decide the time of follicular maturity, more precise time of trigger and IUI.

10.3.1 For theAssessment oftheEndometrial Receptivity

Endometrial receptivity studies usually are based on assessment of endometrial thickness and morphology. Triple line or a multilayered endometrium is considered to be a favourable sign of endometrial receptivity. But there are several reports by different groups that agree on the fact that implantation rates can be more correlated to the vascularity of the endometrium rather than the thickness and morphology of the

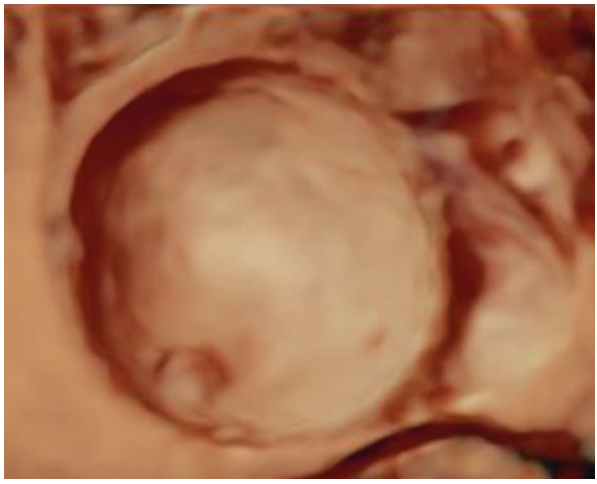

Fig. 10.20 3D ultrasound rendered image of the follicle showing cumulus

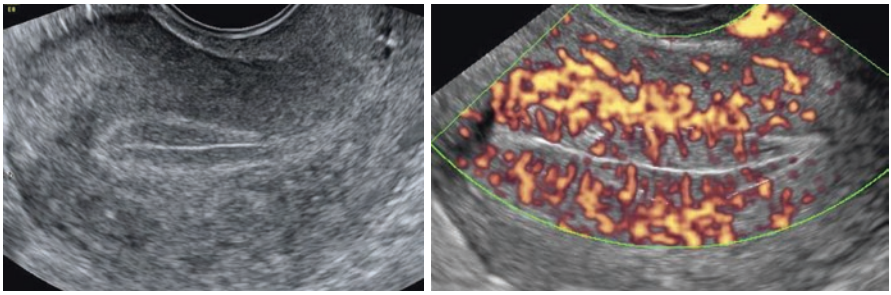

Fig. 10.21 B-mode ultrasound showing a multilayered endometrium and power Doppler showing intra-endometrial blood vessels

endometrium (Fig. 10.21). The study also showed that absence of flow in the endometrial and subendometrial zones on the day of hCG indicates total failure of implantation [50]. Another study has also shown that segmental uterine artery perfusion demonstrates significant correlation with hormonal and histological markers of uterine receptivity, reaching the highest sensitivity for subendometrial blood flow [23].

Endometrial vascularity has been classified by Applebaum et al. into four zones (Fig. 10.22). When blood vessels reach the endometrial junction, it is zone 1 vascularity, zone 2 when the blood vessels reach the outer hyperechoic line, zone 3 when the blood vessels reach the hypoechoic intervening area and zone 4 when the blood vessels reach the central line [51]. Studies have shown that when endometrial vascularity reaches zone 3–4, the chances of conception are almost double as compared to when it is present in zone 1–2 only [52].

Not only the conception rate are more, even the abortion rate are significantly low when the vascularity is present in zone 3 and 4 [53].

Vascularity in	Zone 1	Zone 2	Zone 3	Zone 4
% of patients	6.69%	20.73%	58%	14.47%
+βhCG	19%	21.87%	39.77%	70.14%
Gest. sac	9.6%	14.58%	36.8%	68.65%
Abortions	50%	23.8%	5.6%	1.5%

This vascularity should cover at least 5 mm^2 area of the endometrium. Below this cut-off, the pregnancy rates are extremely low [54]. Those women with an intra-endometrial power Doppler area (EPDA) <5 mm^2 achieved a significantly lower pregnancy rate (23.5% versus 47.5%, $P = 0.021$) and implantation rate (8.1% versus 20.2%, $P = 0.003$) than those with an EPDA >5 mm^2 [54].

Apart from the vascularity studies by Doppler, volume studies by 3D ultrasound and 3D power Doppler are of importance for evaluation of the endometrial receptivity. Endometrium being a pear-shaped structure, B-mode ultrasound cannot assess its volume correctly, when the standard volume calculation equation is used assessing three orthogonal diameters. 3D US can be a more reliable modality for assessment of the endometrial volume especially when VOCAL software is used for

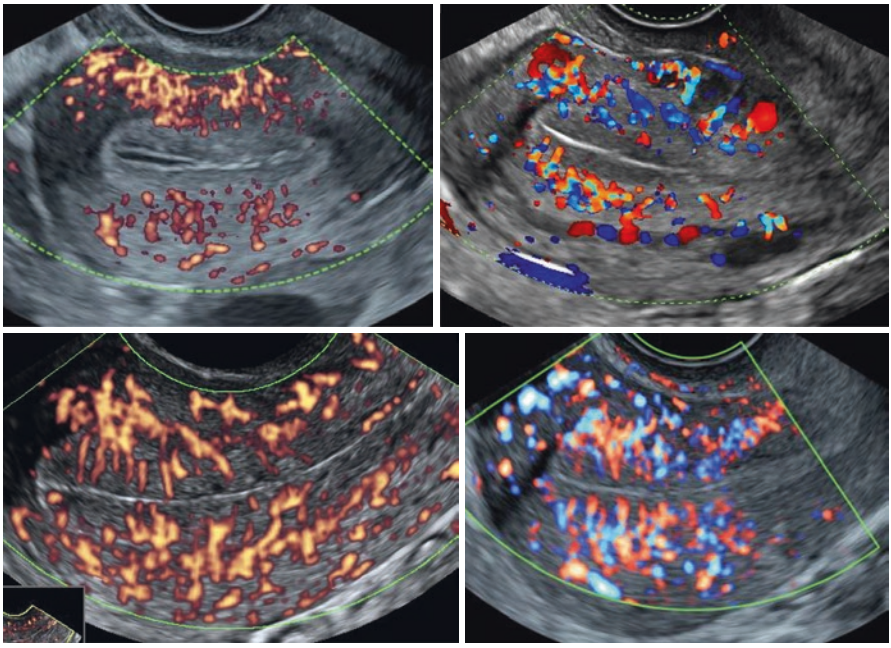

Fig. 10.22 Doppler showing endometrial blood flow zone 1–4 from the upper left to clockwise

volume calculation. It has been shown in some studies that no matter what is the thickness of the endometrium, the volume is more important. Endometrial volume by 3D US volume calculation of the endometrium may help to correlate the cycle outcome with quantitative parameter rather than endometrial thickness [55]. For endometrial volume the inter CC definition of internal os and interobserver variation was 0.82 and intra CC (intraobserver variation) was 0.90, the chief source of error being definition of endometrial margins when the endometrial volume was assessed by 3D US and the software VOCAL [56] (Fig. 10.23). Study by Kupesic et al. also shows no pregnancy when endometrial volume was <2 mL or when exceeded 8 mL.

The 3D power Doppler ultrasound is a more comprehensive and sensitive method for evaluating endometrial receptivity (Fig. 10.24).

If the endometrial volume is <3 mL, the chances of implantation and conception are extremely low, and this has been shown in this study by Merce et al. There is no pregnancy when endometrial volume is <3 mL and VI <10. Exceptionally better pregnancy rates are achieved with endometrial volume >7 mL and subendometrial VI between 10% and 35% [57]. Ng et al. in their study concluded that a number of embryos were replaced and endometrial VFI were the only two predictive factors for pregnancy [58]. Wu et al. reported that endometrial VFI was more reliable than VI and FI and best prediction rate was achieved by VFI cut-off value of >0.24 [59].

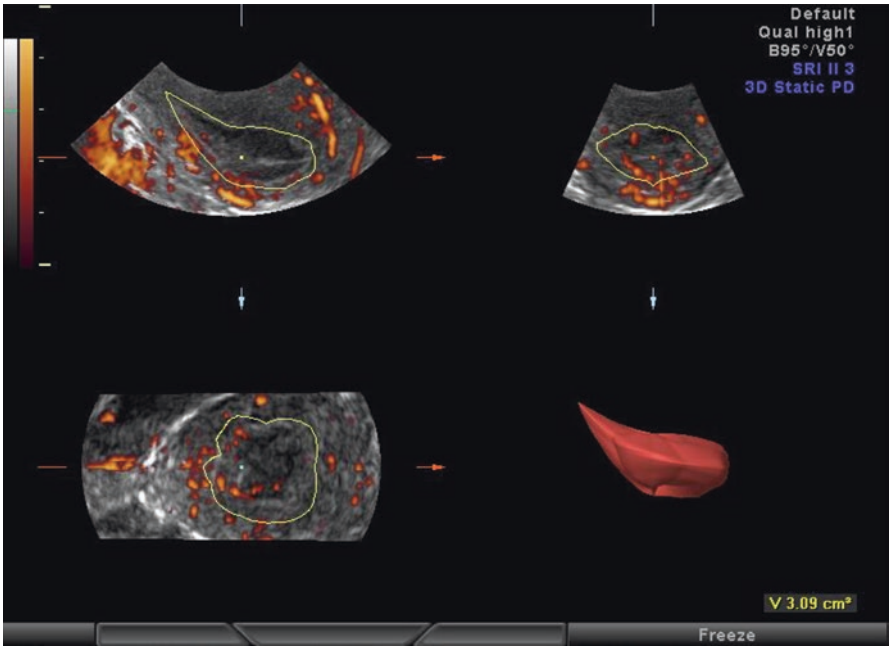

Fig. 10.23 3D ultrasound acquired and VOCAL calculated volume of endometrium

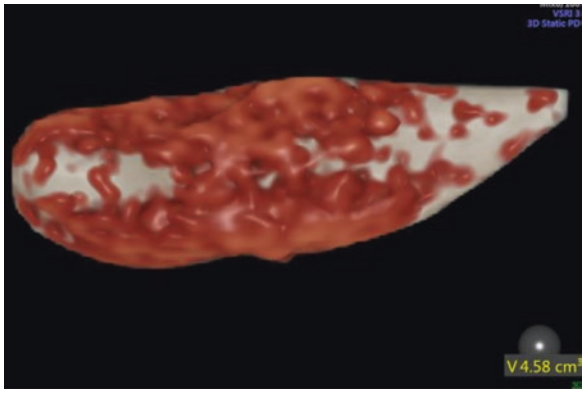

Fig. 10.24 3D PD acquired VOCAL calculated volume of the endometrium showing global vascularity of the endometrium

A scoring system reported by Kupesic et al. [28], for uterine receptivity, done on the day of embryo transfer, shows that subendometrial FI <11 was a cut-off limit. No pregnancies occurred when it was <11, and the conception group showed its values of 13.2 ± 2.2.

The evidence is sufficient to prove that the endometrial volume assessment by 3D ultrasound is a more reliable parameter for endometrial receptivity than endometrial thickness. Moreover the above-mentioned studies have also shown that when the endometrial vascularity on the day of hCG is more, the chances of conception are more, and this can be assessed by Doppler but better assessed by 3D PD.

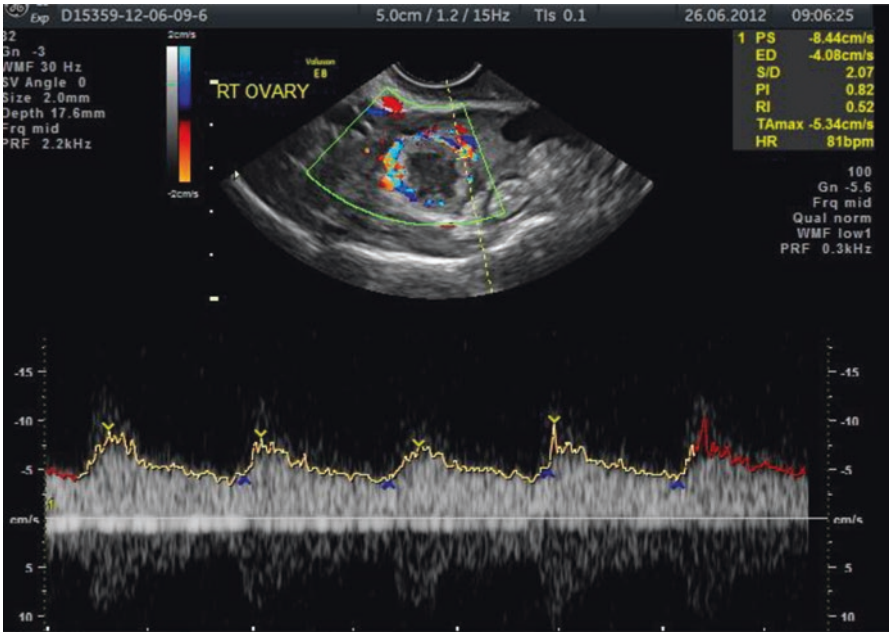

Fig. 10.25 Colour and pulse Doppler of the corpus luteum showing low resistance flow

10.3.2 Luteal Phase Assessment

A clear correlation between RI of corpus luteum and plasma progesterone levels has been seen in natural cycle. RI of the corpus luteum can therefore be used as an adjunct to plasma progesterone assay as an index of luteal function [60] (Fig. 10.25).

Segmental uterine and ovarian artery perfusion demonstrates a significant correlation with histological and hormonal markers of uterine receptivity and may aid assessment of luteal phase defect. In infertile women, the uterine artery pulsatility indices in the midluteal phase of an unstimulated cycles correlates inversely with the endometrial thickness suggesting a direct effect of uterine perfusion on endometrial growth [61] (Fig. 10.26).

Endometrial volume, VI, FI and VFI in the midluteal phase, as well as VI in early pregnancy, can be considered as predictive factors for recurrent miscarriage [62, 63].

10.4 For Diagnosis ofPCOS

PCOS is a complex endocrine condition in which ovulatory dysfunction and androgen excess are cardinal features [64].

According to the commonly used Rotterdam criteria for ultrasound diagnosis of PCOS, the ovarian volume and number of antral follicles are considered important;

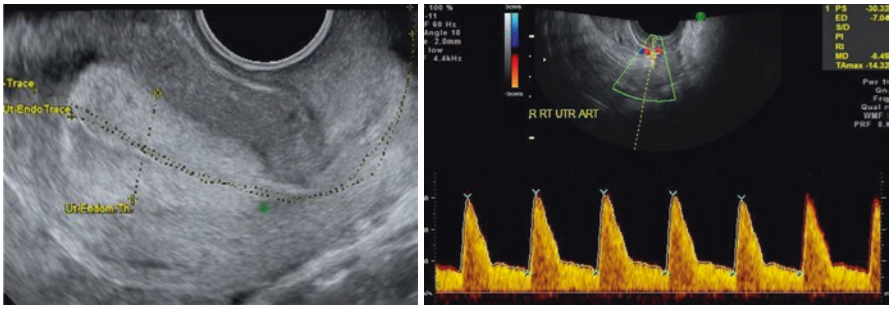

Fig. 10.26 B-mode image of the thick hyperechoic secretory endometrium and spectral Doppler of the low resistance uterine artery in normal luteal phase of the menstrual cycle

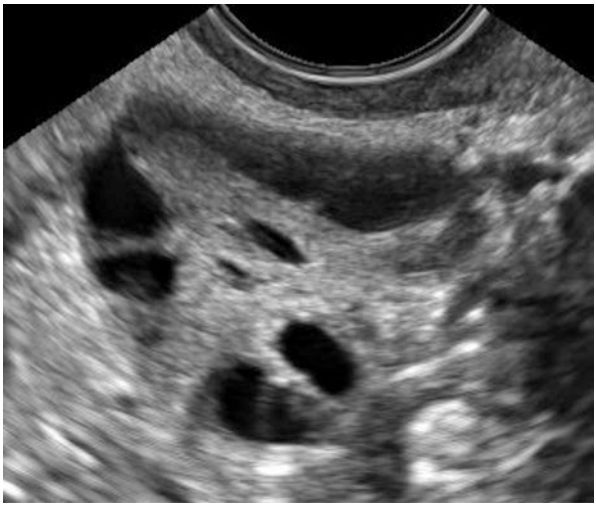

Fig. 10.27 Hyperechoic ovarian stroma typical of polycystic ovaries

it is actually the stromal abundance which is the most consistent feature of PCOS. PCOM (size and follicle number) though is consistently found in PCOS patients, it may be seen in 25% of normal controls also [65].

Polycystic ovarian morphology (stromal abundance) has been found to be a better discriminator than ovarian volume between polycystic ovarian syndrome and control women [66] (Fig. 10.27). To identify women with milder form of PCOS, further information, particularly about the ovarian stroma and the degree of vascularization, is required [67].

Three-dimensional (3D) ultrasound has the potential to address these points and improve the sensitivity and specificity of ultrasound in the diagnosis of PCOS [68].

3D ultrasound not only permits improved spatial awareness and volumetric and quantitative vascular assessment but also provides a more objective tool to examine stromal echogenicity through the assessment of the mean greyness (MG) of the ovary [69].

Mean stromal echogenecity or total ovarian echogenecity as measured by histogram is not different in controls and PCOS. But stromal index (stromal echogenecity/total ovarian echogenecity) was significantly higher in PCOS than controls [70].

Both total ovarian volume and stromal volume during the early follicular phase are significantly higher in women with PCOS. Stromal volume was positively correlated with serum androstenedione concentrations in patients with polycystic ovarian syndrome [71] (Fig. 10.28).

Statistically significant relationship was reported between ovarian volume and stroma echogenicity with serum LH and testosterone concentrations [72, 73].

Though 2D power Doppler indices were not higher in PCOS than in controls, 3D ultrasound clearly showed higher AFC (median 16.3 vs. 5.5 per ovary), ovarian volume (12.56 vs. 5.6 mL), stromal volume (10.79 vs. 4.69 mL) and stromal vascularization (VI 3.85% vs. 2.79%, VFI 1.27 vs. 0.85) [74] (Fig. 10.29).

10.5 To Conclude

The evidence from various studies on patients undergoing ART, using various ultrasound modalities, like Doppler, 3D ultrasound and 3D PD, clearly proves the significance and importance of these modalities for diagnosis of uterine and tubal

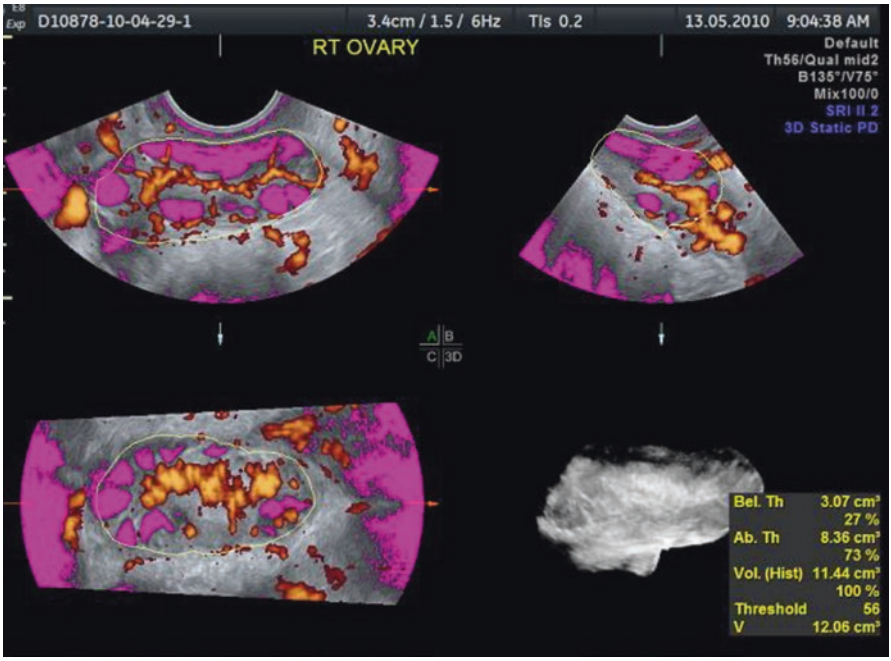

Fig. 10.28 3D ultrasound of the ovary with threshold volume used to calculate the stromal volume. On the right lower corner, in the calculation box, the figure that says above threshold indicates the stromal volume

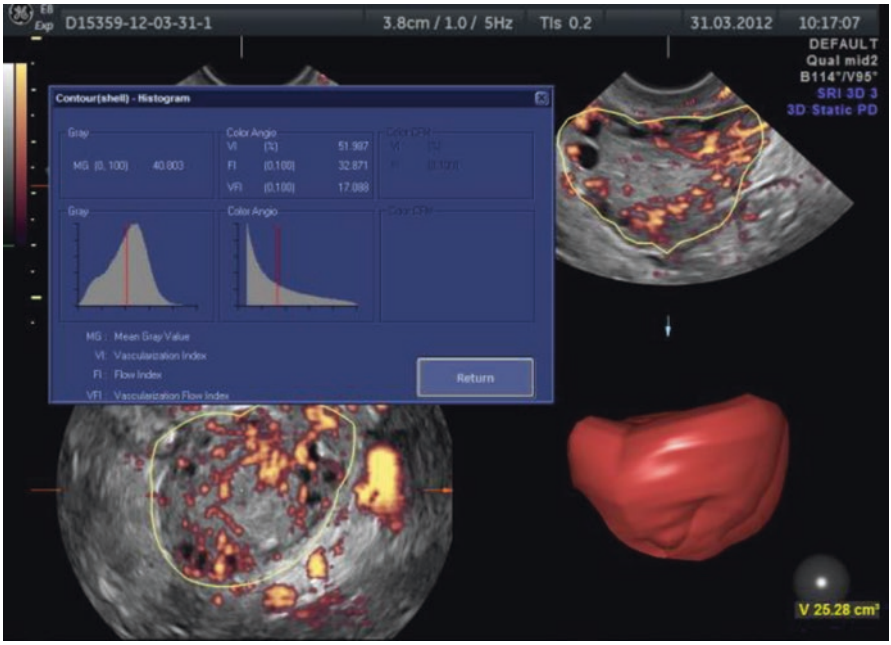

Fig. 10.29 3D power Doppler of the polycystic ovary with volume histogram showing 3D power Doppler indices VI, FI and VFI of the ovary

pathologies that may come in the way of fertility, for evaluation of ovaries to diagnose polycystic ovaries and to decide the stimulation protocols and the time of ovulation trigger and IUI depending on the parameters that suggest follicular maturity and endometrial receptivity and also for assessment of luteal phase.

References

1. Golan A, Herman A, Soffer Y, Bukovsky I, Ron-El R. Ultrasonic control without hormone determination for ovulation induction in in-vitro fertilization/embryo-transfer with gonadotropin-releasing hormone analogue and human menopausal gonadotropin. Hum Reprod. 1994;9:1631–3.
2. Shoham Z, DiCarlo C, Pater A, Conway GS, Jacobs HS. Is it possible to run a successful ovulation induction program based solely on ultrasound monitoring? The importance of endometrial measurements. Fertil Steril. 1991;56:836–4.
3. Tan SL. Simplification of IVF therapy. Curr Opin Obstet Gynecol. 1994;6:111–40.
4. Järvelä IY, Sladkevicius P, Kelly S, Ojha K, Campbell S, Nagrund G. Quantification of ovarian power Doppler signal with three-dimensional ultrasonography to predict response during in vitro fertilization. Obstet Gynecol. 2003;102(4):816–22.
5. Wahad SA, Alalaf SK, Al-Shawaf T, Al-Tawil NG. Ovarian reserve markers and assisted reproductive technique (ART) outcomes in women with advanced endometriosis. Reprod Biol Endocrinol. 2014;12:120.
6. Chen M-J, Yang W-S, Chen C-l, Wu M-Y, Yang Y-S, Ho H-N. The relationship between anti-Müllerian hormone, androgen and insulin resistance on the number of antral follicles in women with polycystic ovary syndrome. Hum Reprod. 2008;23(4):952–7.

7. Kline J, Kinney A, Kelly A, Ruess ML, Levin B. Predictors of antral follicle count during the reproductive years. Hum Reprod. 2005;20:2179–89.
8. van Disseldorp J, Lambalk CB, Kwee J, Looman CW, Eijkemans MJ, Fauser BC, Broekmans FJ. Comparison of inter- and intra-cycle variability of anti-Mullerian hormone and antral follicle counts. Hum Reprod. 2010;25(1):221–7.
9. Panchal S, Nagori CB. Comparison of AFC and AMH for assessment of ovarian reserve. JHRS. 2012;5(3):274–8.
10. Broer SL, Mol BW, Hendriks D, Broekmans SJ. The role of antimullerian hormone in prediction of outcome after IVF: comparison with the antral follicle count. Fertil Steril. 2009;91(3):705–14.
11. te Velde ER. Advances in fertility studies and reproductive medicine. IFFS. 2007;306.
12. Rainne-Fenning NJ. What is in a number? The polycystic ovary revisited. Hum Reprod. 2011;26(11):3118–22.
13. Deb S, Jayaprakasan K, Campbell BK, Clewes JS, Johnson IR, Raine-Fenning NJ. Ultrasound Obstet Gynecol. 2009;33(4):477–483(7).
14. Ng EH, Chan CC, Yeung WS, Ho PC. Effect of age on ovarian stromal flow measured by three-dimensional ultrasound with power Doppler in Chinese women with proven fertility. Hum Reprod. 2004;19:2132–7.
15. Deb S, Campbell BK, Clewes JS, Rainne-Fenning NJ. Quantitative analysis of AFC and size: a comparison of 2D & automated three-dimensional ultrasound techniques. Ultrasound Obstet Gynecol. 2010;35:354–60.
16. Hendriks DJ, Kwee J, Mol BW, te Velde ER, Broekmans FJ. Ultrasonography as a tool for the prediction of outcome in IVF patients: a comparative meta-analysis of ovarian volume and antral follicle count. Fertil Steril. 2007;87(4):764–75.
17. Pellicer A, Ardiles G, Neuspiller F, Remohi J, Simon C, Bonilla-Musoles F. Evaluation of ovarian reserve in young low responders with normal basal levels of follicle-stimulating hormone using three-dimensional ultrasonography. Fertil Steril. 1998;70(4):671–5.
18. Arora A, Gainder S, Dhaliwal L, Suri V. Clinical significance of ovarian stromal blood flow in assessment of ovarian response in stimulated cycle for in vitro fertilization. Int J Reprod Contracept Obstet Gynecol. 2015;4(5):1380–3.
19. Zaidi J, Barber J, Kyei-Mensah A, et al. Relationship of ovarian stromal blood flow at baseline ultrasound to subsequent follicular response in an in vitro fertilization program. Obstet Gynecol. 1996;88:779–84.
20. Engmann L, Saldkevicius P, Agrawal R, Bekir JS, Campbell S, Tan S. Value of ovarian stromal blood flow velocity measurement after pituitary suppression in the prediction of ovarian responsiveness and outcome of in vitro fertilization treatment. Fertil Steril. 1999;71(1):22–9.
21. Panchal S, Nagori C. Baseline scan and ultrasound diagnosis of PCOS: Donald School. J Ultrasound Obstet Gynecol. 2012;6(3):290–9.
22. Merce LT, Barco MJ, Bau S, Troyano JM. Prediction of ovarian response and IVF/ICSI outcome by three-dimensional ultrasonography and power Doppler angiography. Eur J Obstet Gynecol Reprod Biol. 2007;132(1):93–100.
23. Kupesic S, Kurjak A. Predictors of in vitro fertilization outcome by three-dimensional ultrasound. Hum Reprod. 2002;17(4):950–5.
24. Schild RL, Holthaus S, d'Alquen J, Fimmers R, Dorn C, van Der Ven H, Hansmann M. Quantitative assessment of subendometrial blood flow by three-dimensional-ultrasound is an important predictive factor of implantation in an in-vitro fertilization programme. Hum Reprod. 2000;15:89–94.
25. Pellerito JS, McCarthy SM, Doyle MB, Glickman MG, DeCherney AH. Diagnosis of uterine anomalies: relative accuracy of MR imaging, endovaginal sonography and hysterosalpingography. Radiology. 1992;183:795–800.
26. Jurkovic D, Geipel A, Gruboeck K, Jauniaux E, Natucci R, Campbell S. Three-dimensional ultrasound for the assessment of uterine anatomy and detection of congenital anomalies: comparison with hysterosalpingography and two-dimensional ultrasonography. Ultrasound Obstet Gynecol. 1995;5:233–7.

27. Troiano RN, Mc Carthy SM. Mullerian duct anomalies: imaging and clinical issues. Radiology. 2004;233(1):19–34.
28. Deutch TD, Abuhamad AZ. The role of 3-dimensional ultrasonography and magnetic resonance imaging in the diagnosis of mullerian duct anomalies; a review of the literature. J Ultrasound Med. 2008;27(3):413–23.
29. Bermejo C, Martinez TP, Cantarero R, Diaz D, Pérez Pedregosa J, Barròn E, Labrador E, Ruiz Lòpez L. Three-dimensional ultrasound in the diagnosis of Mullerian duct anomalies and concordance with magnetic resonance imaging. Ultrasound Obstet Gynecol. 2010;35:593–601.
30. Parsons AK, Lense JJ. Sonohysterography for endometrial abnormalities: preliminary results. J Clin Ultrasound. 1993;21:87–95.
31. Syrop CH, Sahakian V. Transvaginal sonographic detection of endometrial polyps with fluid contrast augmentation. Obstet Gynecol. 1992;79:1041–3.
32. Cil AP, Tulunay G, Kose MF, Haberal A. Ultrasound Obstet Gynecol. 2010;35:233–7.
33. Kalogirou D, Antoniou G, Botsis D, et al. Is colour Doppler necessary in the evaluation of tubal patency by hysterosalpingo-contrast sonography. Clin Exp Obstet Gynecol. 1997;24(2):101–3.
34. Deichert U, van de Sandt M. Transvaginal hysterosalpingo-contrast sonography(Hy-Co-Sy). The assessment of tubal patency and uterine abnormalities by contrast enhanced sonography. Adv Echo-Contrast. 1993;2:55–8.
35. Kupesic S, Kurjak A. Gynecological vaginal sonographic interventional procedures—what does colour add? Gynecol Perinatol. 1994;3:57–60.
36. Stern J, Peters AJ, Coulam CB. Colour Doppler ultrasonography assessment of tubal patency: a comparison study with traditional techniques. Fertil Steril. 1992;58(5):897–900.
37. Sladkevicius P, Ojha K, Campbell S, et al. Three-dimensional power Doppler imaging in the assessment of fallopian tube patency. Ultrasound Obstet Gynecol. 2000;16(7):644–7.
38. Exacoustos C, Di Giovanni A, Szabolcs B, Romeo V, Romanini ME, Luciano D, Zupi E, Arduini D. Automated three-dimensional coded contrast imaging hysterosalpingo-contrast sonography: feasibility in office tubal patency testing. Ultrasound Obstet Gynecol. 2013;41(3):328–35.
39. Chan CC, Ng EH, Tang OS, et al. Comparison of three-dimensional hysteron-contrast-sonography and diagnostic laparoscopy with chromopertubation in the assessment of tubal patency for the investigation of subfertility. Acta Obstet Gynecol Scand. 2005;84(9):909–13.
40. Kyei-Mensah A, Zaidi J, Pittrof R, Shaker A, Campbell S, Tan SL. Transvaginal three-dimensional ultrasound reproducibility of ovarian and endometrial volume measurements. Fertil Steril. 1996;66:718–22.
41. Jokubkeine L, Sladkevicius P, rovas L, Valentine L. Assessment of changes in volume and vascularity of ovaries during the normal menstrual cycle using three-dimensional power Doppler ultrasound. Hum Reprod. 2006;21(10):2661–8.
42. Kupesic S, Kurjak A. Uterine and ovarian perfusion during the periovulatory period assessed by transvaginal colour Doppler. Fertil Steril. 1993;60(3):439–43.
43. Nargund G, Doyle PE, Bourne TH, et al. Ultrasound-derived indices of follicular blood flow before HCG administration and prediction of oocyte recovery and preimplantation embryo quality. Hum Reprod. 1996;11:2512–7.
44. Nargund G, Bourne TH, Doyle PE, et al. Association between ultrasound indices of follicular blood flow, oocyte recovery and preimplantation embryo quality. Hum Reprod. 1996;11:109–13.
45. Vlaisavljevic V, Reljic M, Gavric Lovrec V, Zarula D, Sergent N. Measurement of perifollicular blood flow of the dominant preovulatory follicle, using three-dimensional power Doppler. Ultrasound Obstet Gynecol. 2003;22(5):520–6.
46. Panchal SY, Nagori CB. Can 3D PD be a better tool for assessing the pre HCG follicle and endometrium? A randomized study of 500 cases. Presented at 16th World Congress on Ultrasound in Obstetrics and Gynecology, 2006, London. J Ultrasound Obstet Gynecol. 2006;28(4):504.
47. Feichtinger W. Transvaginal three-dimensional imaging for evaluation and treatment of infertility. In: Merz E, editor. 3D ultrasound in obstetrics and gyneacology. Philadelphia: Lipincott Williams & Wilkins; 1998. p. 37–43.

48. Poehl M, Hohlagschwandtner M, Doerner V, Dillinger B, Feichtinger W. Cumulus assessment by three-dimensional ultrasound for in vitro fertilization. Ultrasound Obstet Gynecol. 2000;16(3):251.
49. Bourne TH, Jurkovic D, Waterson J, et al. Intrafollicular blood flow during human ovulation. Ultrasound Obstet Gynecol. 1991;1:53–9.
50. Zaidi J, Campbell S, Pittrof R, Tan SL. Endometrial thickness, morphology, vascular penetration and velocimetry in predicting implantation in an in vitro fertilization program. Ultrasound Obstet Gynecol. 1995;6:191–8.
51. Applebaum M. The 'steel' or 'teflon' endometrium—ultrasound visualization of endometrial vascularity in IVF patients and outcome. Presented at The third World Congress of Ultrasound in Obstetrics and Gynecology. Ultrasound Obstet Gynecol. 1993;3(Suppl 2):10.
52. Chein LW, et al. Assessment of uterine receptivity by the endometrial-subendometrial blood flow distribution pattern in women undergoing IVF-ET. Fertil Steril. 2002;78:245–51.
53. Nagori CB, Panchal S. Endometrial vascularity: its relation to implantation rates. Int J Infertil Fetal Med. 2012;3(2):48–50.
54. Yang J-H, et al. Association of endometrial blood flow as determined by a modified colour Doppler technique with subsequent outcome of IVF. Hum Reprod. 1999;14:1606–10.
55. Raga F, Bonilla-Musoles F, Casan EM, Klein O, Bonilla F. Assessment of endometrial volume by three-dimensional ultrasound prior to embryo transfer: clues to endometrial receptivity. Hum Reprod. 1999;14:2851–4.
56. Kyei-Mensah A, Zaidi J, Pittrof R, Shaker A, Campbell S, Tan SL. Transvaginal three-dimensional ultrasound: accuracy of follicular volume measurements. Fertil Steril. 1996;65:371–6.
57. Kupesic S, Bekavac I, Bjelos D, Kurjak A. Assessment of endometrial receptivity by transvaginal colour Doppler and three-dimensional power Doppler ultrasonography in patients undergoing in vitro fertilization procedures. J Ultrasound Med. 2001;20:125–34.
58. Merce LT, et al. 2D and 3D power Doppler Ultrasound study of endometrium as implantation marker, Text book of transvaginal Sonography. p. 241–242.
59. Ng EH, Chan CC, Tang OS, Yeung WS, Ho PC. Relationship between uterine blood flow and endometrial and subendometrial blood flows during stimulated and natural cycles. Fertil Steril. 2006;85(3):721–7.
60. Wu HM, Chiang CH, Huang HY, Chao AS, Wang HS, Soong YK. Detection of subendometrial vascularization flow index by three-dimensional ultrasound may be useful for predicting pregnancy rate for patients undergoing in vitro fertilization-embryo transfer. Fertil Steril. 2003;79(3):507–11.
61. Glock JL, Brumsted JR. Color flow pulsed Doppler ultrasound in diagnosing luteal phase defect. Fertil Steril. 1995;64:500–4.
62. Steer CV, Tan SL, Mason BA, Campbell S. Midluteal phase vaginal color Doppler assessment of uterine artery impedance in a subfertile population. Fertil Steril. 1994;61:53–8.
63. Kupesic S. The first three weeks assessed by transvaginal color Doppler. J Perinat Med. 1996;24:301–17.
64. Tan S-Y, Hang F, Purvarshi G, Li M-Q, Meng D-H, Huang L-L. Decreased endometrial vascularity and receptivity in unexplained recurrent miscarriage patients during midluteal and early pregnancy phase Taiwanese. J Obstet Gynecol. 2015;54(5):522–6.
65. Aziz R, Carmina E, Dewailly D, Diamanti-Kandaraakis E, Escobar-Morreale HF, Futterweit W, et al. The androgen excess and PCOS society criteria for polycystic ovary syndrome: the complete task force report. Fertil Steril. 2009;91:456–88.
66. Polson DW, Adams J, Wadsworth J, Franks S. Polycystic ovaries—a common finding in normal women. Lancet. 1988;1:870–2.
67. Legro RS, Chiu P, Kunselman AR, Bentley CM, Dodson WC, Dunaif A. Polycystic ovaries are common in women with hyperandrogenic chronic anovulation but do not predict metabolic or reproductive genotype. J Clin Endocrinol Metab. 2005;90(5):2571–9.
68. Lam P-M, Raine-Fenning N. The role of three-dimensional ultrasonography in polycystic ovary syndrome. Hum Reprod. 2006;21(9):2209–15.

69. Raine-Fenning NJ, Campbell BK, Clewes JS, Johnson IR. The interobserver reliability of ovarian volume measurement is improved with three-dimensional ultrasound, but dependent upon technique. J Phys Conf Ser. 2004;1:181–6.
70. Jarvela IY, Mason HD, Sladkevicius R, et al. Characterization of normal and polycystic ovaries using three-dimensional power Doppler ultrasonography. J Assist Reprod Genet. 2002;19:582–90.
71. Buckett WM, Bouzayen R, Watkin KL, Tulandi T, Tan SL. Ovarian stromal echogenicity in women with normal and polycystic ovaries. Hum Reprod. 1999;14:618–21.
72. Kyei-Mensah AA, et al. Relationship of ovarian stromal volume to serum androgen concentrations in patients with PCOS. Hum Reprod. 1998;13:1437–41.
73. Pache TD. Association between ovarian changes assessed by transvaginal sonography and clinical and endocrine signs of polycystic ovary syndrome. Fertil Steril. 1993;59:544–9.
74. Puzigaca Z, Prelevic GM, Stretenovic Z, Balint-Peric L. Ovarian enlargement as a possible marker of androgen activity in polycystic ovary syndrome. Gynecol Endocrinol. 1991;5:167–74.

Present Role of Hormonal Contraception in Family Planning

11

Giuseppe Benagiano, Carlo Bastianelli, Manuela Farris, and Ivo Brosens

11.1 Introduction

In the field of reproduction, humans have a characteristic that is basically unique in the animal kingdom: their females are accessible to the male throughout their menstrual cycle and even during menstruation, a phenomenon that has induced some religions to create a specific prohibition to engage in coital activity when the woman is "impure" (read: menstruated).

It is for this reason that, since time immemorial, humans have tried to "avoid," rather than "seek," conception. As a consequence, the availability of modern, effective methods of preventing pregnancy has represented a true social revolution, or—in more precise terms—the "first reproductive revolution" in the history of humanity.

It was a revolution because the basic reproductive strategy of the gender *homo* has not differed from the beginning to the twentieth century from that utilized by big apes for which sexual activity is fundamentally for reproductive purposes. Indeed, conceptive sexuality makes sense, because sexual behavior is costly to both sexes. To save energies to be dedicated to food gathering, hunting, and defense was—in the early days—an imperative and a winning option [1].

Although the classic theory of conceptive sex among primates remains valid, infecund copulations are today well documented among them, particularly among chimpanzees [2–6].

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Indeed, in view of the phylogenetic proximity of humans to the great apes, this variety in sexual behavior is—in evolutionary terms—fascinating: we can probably name it an evolutionary prologue to the great variety observed in the sexual behavior of humans. The behavior of the great apes suggests that among humans, sexual activity began to lose its exclusive reproductive meaning early in the evolution of the gender and that, as non-conceptive sexual activity began to take predominance, external manifestations of an impending ovulation so prevalent in primates (e.g. genital swelling) began to disappear. For sure, humans must have practiced non-conceptive sex from the outset, judging from the unique features developed by the females of gender *homo*.

Probably, non-conceptive sexual activity conditioned human evolution, including creating the basis for the trend toward monogamy. Indeed, with a female who is accessible every day of the year, there is less impetus to "look around" for other receptive females [7].

Like any revolution, modern contraception, while offering for the first time the prospect of effective family planning, has also witnessed, at least at the beginning, suspicion, occasionally bordering on hostility. This seems especially true in the case of human sexuality that—in spite of its unique characteristics—has always been exercised with the same modalities.

Here we will discuss the role, after some 60 years of ever-increasing utilization, of hormonal contraception in the lives of women of the II millennium. The starting point will be the different meaning that family planning (F-P) has taken today in industrialized and in developing countries. This difference will appear clear when considering that in the former fertility is almost always at, or below, replacement level, whereas in the latter—with the notable exception of China—it is still well above it [8].

11.2 Adapting Contraception to the Needs of Women

Following the introduction of the first combined oral hormonal contraceptive (COC) [9], a long-acting, progestin-only preparation consisting of 3-monthly injections of a depo-formulation containing 150 mg of micronized medroxyprogesterone acetate (DMPA) was also marketed as Depo-Provera© [10]. This was followed by the introduction of a 2-monthly injection of 200 mg of norethisterone enanthate (NET-EN), marketed as Noristerat© [11]. With the addition of estradiol cypionate, DMPA was also utilized to create a monthly injectable combined hormonal contraceptive (Cycloprovera©, Cyclofem©) [12]; another monthly injectable was developed combining NET-EN plus either estradiol valerate or cypionate [13].

Then in the mid-60s, a new oral contraceptive was marketed, the so-called minipill, consisting of the continuous administration of a low-dose progestin initially acting without blocking ovulation [14]. Subsequently, using a more potent progestin (desogestrel, DSG), a new, more effective "minipill" called Cerazette© was marketed [15].

To help women requiring long-term, reversible contraception, several generations of progestin-releasing subcutaneous implants with an approved duration up to 5 years have also been developed [16–18].

While these advancements took place, substantial evolution occurred in the field of COCs: the daily dosage of the estrogen (ethinyl estradiol, EE) was reduced from more than 50 μg to 20 [19] and even 15 μg [20]. This required modification of the classic 21–7 regimen into a 24–4 one. In addition, biphasic, triphasic, and even tetraphasic regimens have been marketed [21–23]. Several generations of progestins have been tested and successfully utilized [24–28], and C_{21} progestins banned in the 1970s on the basis of irrelevant findings in beagle dogs [29] have now been reutilized [30, 31].

Other developments in the field of combined hormonal contraception are the application of the subdermal [32] and vaginal [33] routes of administration, as well as oral regimens aimed at decreasing the number of withdrawal bleedings over a 1-year period [34–36]. Within COC, EE is now being replaced in new preparations by natural estrogens like estradiol (E_2) [37], its valeric ester [38], or estetrol [39].

Finally, the intrauterine route has been successfully utilized for long-term hormonal contraception. The first intrauterine system (IUS) ever developed, named Progestasert®, released daily 65 μg progesterone and exerted good contraceptive activity without inhibiting ovulation [40]. It was withdrawn from the market following an epidemiological report showing that failure in women bearing the system caused a disproportionate percentage of extrauterine pregnancies [41].

This was followed by the development of another IUS releasing 20 μg levonorgestrel (LNG) called Mirena® [42, 43], with a strong direct action on the endometrium. It has a recommended duration of use of 5 years; however, data from randomized trials of contraceptive efficacy show that this dose is effective for up to 7 years [44]. Important noncontraceptive benefits have been observed with the LNG-IUS; among them, the best known is a marked reduction in menstrual blood loss.

In deciding about family planning, the large variety of options of reversible contraception aims at offering women the type of method that suits best their needs. Indeed, adapting technology to individual requirements is the new frontier of family planning; today, when prescribing contraception, it is mandatory to provide information on the risks as well as the benefits of the various existing preparations and modalities so that each woman can choose the product most suitable for her, medically, socially, and ethically [45].

11.3 Contraception and Family Planning in the Industrialized World

Whereas the world has been defined “the global village,” major differences still remain between the wealthy industrialized countries and the often poorer developing ones. These seem especially important when dealing with sexual mores. In many Western countries, sex by “non-sanctioned unions” is accepted, and, especially among young people, avoiding pregnancy precedes F-P.

In addition, women and men living in a developed country can avail themselves of every existing method. Under the circumstances, as already mentioned, a careful analysis of the needs of individuals is mandatory before a method is selected. Caregivers should never forget that no contraceptive modality can be considered best suited for all women. In addition, while they often advice young, mostly healthy women, in reality, they do care for a large variety of subjects, sometimes having very different indications and contraindications. This is why correct knowledge of the major characteristics of a method, first and foremost, its effectiveness and how this may vary depending on compliance, becomes absolutely necessary.

In this respect, in 2008, a full review was carried out of all randomized controlled trials comparing strategies for communication with users of the effectiveness of contraceptives in preventing pregnancy. The results indicated that for presenting data on risk of pregnancy, "categories" may be better than "numbers" and "audiovisual aids" better than "oral presentations" [46].

Proper counseling can substantially lower the proportion of unintended pregnancies, defined as pregnancies occurring earlier than desired or not ever wanted in the future [47]. These are still too high, in spite of widespread use of contraception, although it has been estimated that between 1995 and 2008, the unintended pregnancy rate fell by 29% in developed regions [48]. Yet, in 2010 in the USA, 36% or more of pregnancies in every state were unintended, and in 28 states plus the District of Columbia, more than half of all pregnancies were unintended [49]. Recent information, however, shows that after a period of minimal change [50], unintended pregnancy rates in the USA declined substantially between 2008 and 2011 [51].

Unfortunately, occurrence of an unintended pregnancy is influenced by sociodemographic factors, with large disparities existing in terms of relationship status, income, and education [50].

11.3.1 Use in Adolescence

In Western countries, adolescents often have an irregular lifestyle and difficulties in assessing risk of unintended pregnancy and consequently run a high risk of contraceptive failure and unintended pregnancies [52]. At the same time, a greater awareness is developing among adolescent and young adult women of the need to prevent unwanted pregnancies. For instance, it has been shown that, even after adjusting for all behavioral health characteristics, women complaining of depression had significantly lower probability of having chosen an effective method of contraception (adjusted OR = 0.56, 95% CI: 0.36–0.87) [53].

For decades, long-acting methods of contraception (whether hormonal or not) have been considered all but contraindicated for adolescents, for fear that they may interfere with the maturation of the hypothalamic-pituitary-ovarian axis and even cause infertility. Today, on the other hand, in view of difficulty in compliance with oral contraception by young people, these methods should be offered because of their much better practical effectiveness [54, 55].

Indeed, in 2011, a study in the USA evaluated the association of age and preference for a long-acting method among two groups of adolescents: 147 aged 14–17 and 840 aged 18–20 [56]. Overall, after proper counseling, in these two groups, 62% chose a long-acting method. This proportion rose to 69% among the 14–17-year-olds. Worth of mention is the fact that in the younger group, when cost barriers were eliminated, 63% selected a subcutaneous hormone-releasing implant, indicating a preference over intrauterine systems.

Nonetheless, COCs remain an option, especially when their therapeutic effect may be exploited (see, e.g., in cases with hyperandrogenism, dysmenorrhea, or premenstrual tension) [45].

In addition, the maturation of the uterus for reproduction may require cyclic menstruations during early adolescence. A recent, large epidemiologic study has demonstrated that the risk of great obstetrical syndromes (GOS) including preeclampsia, fetal growth restriction, and preterm delivery is significantly elevated in the 13- to 15-year-old adolescent and decreases with aging [57].

Menstruation is widely viewed as serving no purpose other than to reinitiate the endometrial cycle in the absence of pregnancy. Yet, spontaneous decidualization followed by menstrual shedding is confined to a few species, including humans, where placenta formation involves deep trophoblast invasion with remodeling of the spiral arteries in the endometrium and inner myometrium. Defective deep placentation has been associated with the occurrence of GOS [58]. It is therefore not illogical to speculate that after menarche, cyclic menstruation with decidualization progressively confers protection against GOS in case of pregnancy. For this reason, a period of cyclic menstruations has been proposed to avoid the risk of GOS in adolescents with polycystic ovary syndrome (PCOS) [59]. That is why, it is not considered appropriate to administer hormonal contraception until at least 1–2 years after menarche and then to prescribe a COC containing a low dose of EE or a natural estrogen that interferes less with the development of secondary sexual characteristics.

The question arises whether the use of progestin-only oral pills or progestin long-acting reversible contraception (pLARC) in young adolescents affects the risk of abnormal uterine bleeding, as well as the risk of GOS in case of early pregnancy. Abnormal uterine bleeding accompanying pLARC accounts for most discontinuations of these safe and highly effective agents, thereby contributing to unwanted pregnancies and abortions. The endometrium of pLARC users displays progestin inhibition of defective perivascular decidualization in spiral arteries leading to distended fragile vessels prone to bleeding [60]. Therefore, the fundamental issue of whether the use of progestin-only contraception interferes with the natural process of menstruation preconditioning decreasing the risk of GOS remains to be addressed by appropriate investigations.

11.3.2 Use in Adulthood

During adulthood, personalization of hormonal contraception should consider a number of parameters, including body mass index (BMI), characteristics of menstrual bleeding, and, last but not least, the presence of specific pathological

conditions, such as PCOS and endometriosis [45]. It is among adult women that new, low-dose formulations and the new combinations containing natural estrogens find a precise indication. Indeed, they can effectively reduce menstrual flow (a reduction by almost 90% has been observed in users of a combination of E_2 and dienogest [61]). In addition, in these women, they can also exert a consistent positive impact on work productivity and activities of daily living, producing a reduction in the woman's financial burden [62].

Some 15 years ago, a Cochrane review concluded that, while there was some evidence that COCs with medium dose EE and first/second generation progestogens produced some benefit in alleviating primary dysmenorrhea, all studies included much higher doses of hormones than those commonly prescribed today [63]. Subsequent investigations, however, have shown that also modern, lower dose COCs can significantly reduce both the occurrence and severity of dysmenorrhea [64, 65].

When dealing with adult women, one neglected area concerns psychological aspects of contraception. Research carried out in the 1960s and 1970s, when COC contained much higher doses of EE found that use of oral contraception seemed to increase depressive symptoms [66]. More recently, however, no association was found between use of low-dose COCs and frequency of depressive symptoms [67].

At the same time, depressive symptoms influence behavior, resulting in contraceptive behaviors that may lead to unintended pregnancy [68, 69].

11.3.3 Use in Perimenopause

It is well-known that fertility gradually declines with age, particularly after age 35. In her 30s, each month, a woman has a 20% chance of getting pregnant. This probability decreases to less than 5% per cycle in women ≥40 [70]. Nonetheless, in many women in their fifth decade, fertility may be maintained; the latest report of the Centers for Disease Control and Prevention of the USA [71] indicates that following in vitro fertilization, in women aged 41–42, miscarriage rate averages 33%, raising to 45% in those aged 43–44 and to 60% thereafter. These statistics, however, should not be interpreted as meaning that women in their 40s do not need effective contraception to avoid unwanted pregnancies. On the contrary, they are intended to show that during the perimenopausal period, there is a residual fertility potential which is, if pregnancy occurs, unfortunately associated to an increasing incidence of maternal and fetal complications.

Some 15 years ago, an epidemiological investigation in the UK compared pregnancy outcome in a large cohort of women aged at delivery: 18–34 years (n = 336,462), 35–40 years (n = 41,327), and >40 years (n = 7331). The study found that pregnant women aged 35–40 years were at increased risk of gestational diabetes [OR 2.63–99% confidence interval (CI) 2.40–2.89], placenta previa [1.93 (1.58–2.35)], breech presentation [1.37 (1.28–1.47)], operative vaginal delivery [1.5 (1.43–1.57)], elective Caesarean section [1.77 (1.68–1.87)], emergency Caesarean section [1.59 (1.52–1.67)], postpartum hemorrhage [1.14 (1.09–1.19)], delivery before 32 weeks gestation [1.41 (1.24–1.61)], birthweight below the fifth centile

[1.28 (1.20–1.36)], and stillbirth [1.41 (1.17–1.70)]. These risks further increased in women aged >40 years [72]. More recently, a study of 361 women aged 43 years or more at the time of delivery (mean age, 44.6 years) was compared to with 361 women aged 25–35 years (mean age, 31.0 years). It was found that older women have significantly higher risks of Caesarean and preterm delivery [73].

Hormonal contraception in perimenopause has also specific noncontraceptive benefits: first, they can correct the relative hyperestrogenism caused by an imbalance between estrogens and progesterone production; as such, they can regularize menstrual cycles that often become irregular and may increase in quantity. Relative hyperestrogenism is also associated with an increased risk of endometrial carcinoma [74]. In addition, combined oral pills can significantly reduce the number and severity of hot flashes [75]; the same effect can be obtained with a brief, low-dose estrogen supplementation, combined with the insertion of the LNG-releasing IUS [76].

11.4 Contraception and Family Planning in the Developing World

It is a dire reality that many women in developing countries cannot avail themselves of the full spectrum of methods at the disposal of the more fortunate women of the West. Although the international community should make every effort to close this inequity gap, at present F-P continues to have a different meaning in the poorer countries of Africa and Asia.

In these settings F-P, through effective contraception, literally saves lives by reducing the number of pregnancies, especially those that are at greater risk for maternal, neonatal, and child survival. These are pregnancies in very young (<18 years) and older (>34 years) women, high parities, and short inter-pregnancy intervals, as well as pregnancies that would end up in an unsafe abortion.

Indeed, it has been estimated that in countries of low and middle income, a 10% increase in contraceptive use can reduce fertility by 0.6 births per woman; in addition, it can decrease by 5% the proportion of births to women with four or more children, reduce by 1.5% births to women >35 years, and lower by 3.5% birth intervals of less than 2 years [77].

The reason for such an important effect is that in developing countries, unintended pregnancies carry more serious consequences for women and their families. This is a huge phenomenon: it has been estimated that of the 208 million pregnancies that occurred in 2008, 41% were unintended, although they had fallen by 29% since 1995. Among developing countries, the highest rates were found in Eastern and Middle Africa and the lowest in Eastern Asia [48].

11.4.1 Family Planning and Maternal Mortality

For more than 30 years, data have been accumulating in support of the concept that successful family planning can substantially contribute to decreasing maternal mortality rate (MMR). Already in 1984, an attempt was made to estimate changes in

MMR that might result from altering maternal age, birth order, and birth spacing. It was shown that if childbearing would be limited to 20–39 years, the MMR would be reduced by 11% [78]. It was also shown that F-P influences MMR because it reduces the proportion of pregnancies in high-risk women: it is primarily the reduction in the number of births that produces a beneficial effect [79]. Although per se family planning cannot reduce risks during a pregnancy, combining a general fertility reduction, especially for high-risk groups, with proper spacing might effectively address about half of all maternal deaths in the developing world [80].

This approach gained momentum soon after the United Nations issued their solemn Millennium Declaration [81] and launched the Millennium Development Goals (MDGs) [82], because the family planning community realized that effective contraception would be essential to meet the challenge posed by Goal 5, "Improve Maternal Health"; with its specific Target 6, "Reduce by three-quarters, between 1990 and 2015, the maternal mortality ratio." In other words, effective spacing and avoiding great multiparity were seen as primary preventive measures to decrease maternal deaths. To stress this point, in 2010, the journal *Contraception* carried an editorial entitled "Family planning: the essential link to achieving all eight Millennium Development Goals" [83]. In this editorial, Willard Cates showed how family planning contributes to the achievement of several MDG (and now to the new Sustainable Development Goals, launched in 2015 [84]): It helps in the fight to "end poverty and hunger," since F-P generates wealth. It contributes to attain "universal education," because it prolongs the time for education (unintended pregnancy is a major obstacle to school attendance). It is an important tool to achieve "gender equality," since it empowers women. As it will be detailed below, it favors "child health" by saving infant lives through spacing and planned births, therefore limiting unintended births. It improves "maternal health" by decreasing unintended pregnancies and their negative effects. When the appropriate methods are selected, it helps to "combat HIV/AIDS." It contributes to "environmental sustainability" by preventing environmental degradation fueled by per capita consumption, the technology used to produce what is consumed and population growth. It fosters "global partnerships" by contributing to strong collaboration among international agencies, governmental ministries, national and international NGOs, and local community groups.

A second editorial appeared in the same journal the following year. Entitled "Reducing Maternal Mortality: A Global Imperative" [85], it mentioned five interventions, one being "make contraception accessible and affordable." In other words, in the developing world, F-P is seen today as a fundamental primary preventive measure to save the life of women. In this context, an investigation published in 2011 tried to analyze reasons for the overall decline in MMR observed between 1990 and 2008 in three South Asian countries (India, Pakistan, and Bangladesh). Investigators decomposed results into two categories: reduction attributable to fertility decline and reduction attributable to safe motherhood programs [86]. The study indicated that fertility decline has made a substantial contribution to the reduction of MMR; in addition, it suggested that continuing fertility decline up to the target date to achieve the MDGs (2015) would represent a major contributor to the achievement of Goal 5.

An analysis of the global situation over the period 1990–2008 indicates that fertility decline was probably responsible for the observed stable birthrate in spite of a 42% increase in the number of women aged 15–49. The analysis indicates that fertility decline brought about by F-P alone averted approximately 1.7 million deaths [87].

Extracting information from the UN World Contraceptive Use and the UN World Population Prospects databases for 2010, the number has been estimated of maternal deaths occurring in 2008 in 172 countries and those averted by contraceptive use for that year. Whereas some 342,000 women died of maternal causes in 2008, contraceptive use averted some 272,000 maternal deaths, amounting to a 44% reduction. Satisfying the unmet need for contraception could have prevented another 104,000 maternal deaths on that year, a 29% reduction [88].

The role of hormonal contraception in satisfying the F-P needs of women in Africa and Asia can be better understood when considering that less than a decade ago, it has been estimated that if 20% of the 17.6 million women in sub-Saharan Africa using oral or injectable contraceptives and desiring long-term protection against unwanted pregnancies switched to subcutaneous long-acting hormonal implants, over 1.8 million unintended pregnancies could be averted over a 5-year period [89].

Therefore, it seems abundantly clear that pLARC methods, with their duration of use exceeding 5 years and a very high effectiveness, can play a crucial role in meeting the needs of women in the developing world. Poor patterns of short-term hormonal contraceptive use (high discontinuation rates and incorrect use) contribute significantly to the problem of unintended pregnancy in sub-Saharan Africa. More availability and widespread use of highly effective methods, such as contraceptive implants, will improve reproductive health in the region. In support of this idea, a study conducted among adolescent girls aged 10–19 years in 12 secondary schools in Nigeria observed an acceptability rate for LARCs as high as 95.8% [90].

A specific area where pLARC may be of invaluable help is the care of women trapped in conflicts. This is a huge problem when considering that an estimated 43 million women of reproductive age have experienced the effects of conflict in 2012 [91]. These women suffer from a sort of “double jeopardy”: they are especially vulnerable because of the emergency situation and the ensuing risk of violence and rape and often are unable to obtain F-P services. In these situations, LARCs seem especially indicated. Indeed, a study promoted by the CARE Organization in Africa and Asia with refugees, internally displaced persons, and conflict-affected populations shows that these methods represented 61% of new modern method users [91].

11.4.2 Family Planning and Infant Mortality

The concept that family planning plays an important role in improving child survival has been officially promoted by the United Nations for some 25 years [92]. The already-mentioned study of 1984 [77] indicated that if childbearing occurred

only during the "prime" age of 20–34, infant and child mortality rates would fall by about 5%. In addition, a universal adoption of an "ideal" spacing pattern of at least 2 years apart may reduce infant mortality by about 10% and child mortality by about 21%.

Ten years ago, the importance of F-P for infant health and survival was analyzed systematically using all studies published from 1966 to the beginning of 2006 [93]. The metanalysis examined the association between birth spacing and relative risk of adverse perinatal outcomes and included more than 11 million pregnancies. When comparing inter-pregnancy intervals of 18–23 months with intervals shorter than 6 months, the latter were associated with increased risks of preterm birth, low birth weight, and small for gestational age. Intervals of 6–17 months and longer than 59 months were also associated with a significantly greater risk for these adverse perinatal outcomes.

The true existence of a relationship between infant mortality risk and the preceding birth interval has been challenged by a study evaluating possible confounding factors. Results indicate that full adjustment for these factors does not substantially change the risk ratio for short birth intervals. Therefore, there is substantive evidence that neonatal, infant, and child mortality are strongly and significantly related to the preceding birth intervals [94].

A number of recent investigations in developing countries have further documented the impact of F-P in reducing high-risk births due to younger and older maternal age, short birth intervals, and high parity [95–99]. Specifically, countries with the fastest progress in improving modern contraceptive prevalence rate experienced the greatest declines in all three parameters; 63% of the increase was due to family planning program efforts [100].

The most important hypothesis to explain the mechanisms by which short intervals between pregnancies can affect perinatal health is maternal nutritional depletion in low-income countries caused by the close succession of pregnancies and periods of lactation [77].

Conclusions

In public health, contraception occupies a central position both in the industrialized and in the developing world. Its role is different when uncontrolled population growth occurs than when fertility is below replacement level. In the former, it allows women to avoid unintended pregnancies and literally saves lives. In the latter, it gives women a tool to achieve their wanted family size, but other measures are also necessary to encourage fertility.

Today, hormonal contraception plays an ever-increasing role in the global armamentarium of F-P methods, especially after the successful introduction of highly effective pLARC methods. These can be used by women of all ages and in the foreseeable future their role is bound to increase.

References

1. Wrangham R, Peterson D. Demonic males: apes and the origin of human violence. London: Mariner Books; 1996.
2. Mitchell G. Behavioral sex differences in non-human primates. New York: Van Nostrand-Reinhold; 1979.
3. de Waal FMB. Tension regulation and non-reproductive functions of sex in captive bonobos (Pan Panicus). Nat Geogr Res. 1987;3:318–35.
4. Dumbar RIM. Primate social system. London: Croom & Helm; 1988.
5. Manson JH, Perry S, Parish AM. Non-conceptive sexual behavior in bonobos and capuchins. Internat J Primatol. 1997;18:767–86.
6. Dixson A. Primate sexuality. In: Whelehan P, Bolin A, editors. The international encyclopedia of human sexuality. Hoboken: Wiley-Blackwell; 2015.
7. Benagiano G, Bastianelli C, Farris M. Contraception: a social revolution. Eur J Contracept Reprod Health Care. 2007;12:3–12.
8. United Nations, Population Division. World fertility Data 2015. http://www.un.org/en/development/desa/population/publications/dataset/fertility/wfd2015.shtml.
9. Pincus G, Rock J, Garcia CR, Rice Wray E, Paniagua M, Rodriguez I. Fertility control with oral medication. Am J Obstet Gynecol. 1958;75:1333–46.
10. Zañartu J, Rice-Wray E, Goldzieher JW. Fertility control with long-acting injectable steroids. A preliminary report. Obstet Gynecol. 1966;28:513–5.
11. Zañartu J, Navarro C. Fertility inhibition by an injectable progestogen acting for 3 months. A clinical survey of 130 fertile women treated with norethisterone enanthate. Obstet Gynecol. 1968;31:627–33.
12. Fotherby K, Benagiano G, Toppozada HK, Abdel-Rahman A, Navaroli F, Arce B, Ramos-Cordero R, Gual C, Landgren BM, Johannisson E. A preliminary pharmacological trial of the monthly injectable contraceptive cycloprovera. Contraception. 1982;25:261–72.
13. Baweja R, Bhattacharya SK, Choudhury SD, Krishna U, Manuel M, Phillips FS, Datey S, Kumar S, Mehta S, Saxena NC, The Indian Council of Medical Research. Task force on hormonal contraception: phase II randomized clinical trial with norethisterone oenanthate 50 mg alone and in combination with 5 mg or 2.5 mg of either estradiol valerate or cypionate as a monthly injectable contraceptive. Contraception. 1985;32:383–94.
14. Martinez-Manautou J, Giner-Velasquez J, Cortes-Gallegos V, Aznar R, Rojas B, Guitterez-Najar A, Rudel HW. Daily progestogen for contraception: a clinical study. Br Med J. 1967;2(5554):730–2.
15. Benagiano G, Primiero FM. The new minipill with 75μg of desogestrel. In: challenges on Women's health and diseases: gynecologic and reproductive issues. Ann N Y Acad Sci. 2003;997:163–73.
16. Coutinho E, da Silva AR, Mattos CE, Nielsen NC, Osler M, Wiese J. Contraception with long acting subdermal implants: I. An effective and acceptable modality in international clinical trials. Contraception. 1978;18:315–33.
17. Sivin I, Viegas O, Campodonico I, Diaz S, Pavez M, Wan L, Koetsawang S, Kiriwat O, Anant MP, Holma P, el din Abdalla K, Stern J. Clinical performance of a new two-rod levonorgestrel contraceptive implant: a three-year randomized study with Norplant implants as controls. Contraception. 1997;55:73–80.
18. Díaz S, Pavez M, Moo-Young AJ, Bardin CW, Croxatto HB. Clinical trial with 3-keto-desogestrel subdermal implants. Contraception. 1991;44:393–408.
19. Endrikat J, Müller U, Düsterberg B. A twelve-month comparative clinical investigation of two low-dose oral contraceptives containing 20 micrograms ethinyl-estradiol/75 micrograms

gestodene and 30 micrograms ethinyl-estradiol/75 micrograms gestodene, with respect to efficacy, cycle control, and tolerance. Contraception. 1997;55:131–7.
20. Sullivan H, Furniss H, Spona J, Elstein M. Effect of 21-day and 24-day oral contraceptive regimens containing gestodene (60 microg) and ethinyl estradiol (15 microg) on ovarian activity. Fertil Steril. 1999;72:115–20.
21. Jung-Hoffmann C, Storch A, Kuhl H. Serum concentrations of ethinylestradiol, 3-ketodesogestrel, SHBG, CBG and gonadotropins during treatment with a biphasic oral contraceptive containing desogestrel. Horm Res. 1992;38:184–9.
22. Gaspard UJ, Deville JL, Dubois M. Clinical experience with a triphasic oral contraceptive ('Trinordiol') in young women. Curr Med Res Opin. 1983;8:395–404.
23. Nahum GG, Parke S, Wildt L, Palacios S, Roemer T, Bitzer J. Efficacy and tolerability of a new oral contraceptive containing estradiol and dienogest. Obstet Gynecol. 2008;111(Suppl 4):15S.
24. Stickland J, Tolowinska IY, Anthony F, et al. The suppression of ovarian function by the new oral contraceptive, Femodene. Contraception. 1987;35:447–56.
25. De Jager EA. New progestagen for oral contraception. Contracept Deliv Syst. 1982;3:11–5.
26. Phillips A, Hahn DW, McGuire JL. Preclinical evaluation of norgestimate, a progestin with minimal androgenic activity. Am J Obstet Gynecol. 1992;167:1191–6.
27. Foster RH, Wilde MI. Dienogest. Drugs. 1998;56:825–33.
28. Endrikat JS, Milchev NP, Kapamadzija A, Georgievska J, Gerlinger C, Schmidt W, Froze S. Bleeding pattern, tolerance and patient satisfaction with a drospirenone-containing oral contraceptive evaluated in 3488 women in Europe, the Middle East and Canada. Contraception. 2009;79:428–32.
29. Owen LN, Briggs MH. Contraceptive steroid toxicology in the Beagle dog and its relevance to human carcinogenicity. Curr Med Res Opin. 1976;4:309–29.
30. Zahradnik HP, Goldberg J, Andreas JO. Efficacy and safety of the new antiandrogenic oral contraceptive Belara. Contraception. 1998;57:103–9.
31. Coutinho EM. One year contraception with a single subdermal implant containing nomegestrol acetate (Uniplant). Contraception. 1993;47:97–105.
32. Audet MC, Moreau M, Koltun WD, Waldbaum AS, Shangold G, Fisher AC, Creasy GW, ORTHO EVRA/EVRA 004 Study Group. Evaluation of contraceptive efficacy and cycle control of a transdermal contraceptive patch vs an oral contraceptive: a randomized controlled trial. J Am Med Assoc. 2001;285:2347–54.
33. Newton J, Szontagh F, Lebech P, Rowe P. Ovarian function with a novel combined contraceptive vaginal ring. Hum Reprod. 2002;17:2594–9.
34. Lin K, Banhart K. The clinical rationale for menses-free contraception. J Women's Health. 2007;16:1171–80.
35. Sulak PJ. Continuous oral contraception: changing times. Best Pract Res Clin Obstet Gynaecol. 2007;20:1–20.
36. Benagiano G, Carrara S, Filippi V. Safety, efficacy and patient satisfaction with continuous daily administration of levonorgestrel/ethinyl estradiol oral contraceptives. Patient Prefer Adherence. 2009;3:131–43.
37. Duijkers IJ, Klipping C, Grob P, Korver T. Effects of a monophasic combined oral contraceptive containing nomegestrol acetate and 17 beta-oestradiol on ovarian function in comparison to a monophasic combined oral contraceptive containing drospirenone and ethinylestradiol. Eur J Contracept Reprod Health Care. 2010;15:314–25.
38. Whalen KL, Rose R. Estradiol valerate/dienogest: a novel oral contraceptive. Ann Pharmacother. 2011;45:1256–61.
39. Duijkers IJ, Klipping C, Zimmerman Y, Appels N, Jost M, Maillard C, Mawet M, Foidart JM, Coelingh Bennink HJ. Inhibition of ovulation by administration of estetrol in combination with drospirenone or levonorgestrel: results of a phase II dose-finding pilot study. Eur J Contracept Reprod Health Care. 2015;20:476–89.
40. Pharriss BB, Erickson R, Bashaw J, Hoff S, Place VA, Zaffaroni A. Progestasert: a uterine therapeutic system for long-term contraception: I. Philosophy and clinical efficacy. Fertil Steril. 1974;25:915–21.

41. Snowden R. The progestasert and ectopic pregnancy. Br Med J. 1977;2(6102):1600–1.
42. Nilsson C-G, Johansson EDB, Luukkainen TA. D-norgestrel-releasing IUD. Contraception. 1976;13:503–14.
43. Luukkainen T. Levonorgestrel-releasing intrauterine device. Ann N Y Acad Sci. 1991;626: 43–9.
44. Faculty of Family-Planning and Reproductive Healthcare. FFPRHC Guidance. The levonorgestrel-releasing system (LNG-IUS) in contraception and reproductive health. J Fam Plann Reprod Health Care. 2004;30:99–109.
45. De Leo V, Musacchio MC, Cappelli V, Piomboni P, Morgante G. Hormonal contraceptives: pharmacology tailored to women's health. Hum Reprod Update. 2016;22:634–46.
46. Lopez LM, Steiner M, Grimes DA, Schulz KF. Strategies for communication effectiveness. Cochrane Database Syst Rev. 2008;(4):CD006964.
47. Mosher WD, Jones J, Abma JC. Intended and unintended births in the United States: 1982-2010. Natl Health Stat Rep. 2012;55:1–28.
48. Singh S, Sedgh G, Hussain R. Unintended pregnancy: worldwide levels, trends, and outcomes. Stud Fam Plan. 2010;41:241–50.
49. Kost K. Unintended pregnancy rates at the state level: estimates for 2010 and trends since 2002. New York: Guttmacher Institute, 2015. http://www.guttmacher.org/pubs/StateUP10.pdf.
50. Finer LB, Zolna MR. Shifts in intended and unintended pregnancies in the United States, 2001-2008. Am J Public Health. 2014;104(Suppl 1):S43–8.
51. Finer LB, Zolna MR. Declines in unintended pregnancy in the United States, 2008-2011. N Engl J Med. 2016;374(9):843–52.
52. Brynhildsen J. Combined hormonal contraceptives: prescribing patterns, compliance, and benefits *versus* risks. Ther Adv Drug Saf. 2014t;5:201–13.
53. Garbers S, Correa N, Tobier N, Blust S, Chiasson MA. Association between symptoms of depression and contraceptive method choices among low-income women at urban reproductive health centers. Matern Child Health J. 2010;14:102–9.
54. Winner B, Peipert J, Zhao Q, Buckel C, Madden T, Allsworth J, Secura GM. Effectiveness of long-acting reversible contraception. N Engl J Med. 2012;366:1998–2007.
55. Committee on Adolescent Health Care Long-Acting Reversible Contraception Working Group, The American College of Obstetricians and Gynecologists. Committee opinion no. 539: adolescents and long-acting reversible contraception: implants and intrauterine devices. Obstet Gynecol. 2012;120:983–8.
56. Mestad R, Secura G, Allsworth JE, Tessa Madden T, Zhao Q, Peipert JF. Acceptance of long-acting reversible contraceptive methods by adolescent participants in the Contraceptive CHOICE Project. Contraception. 2011;84:493–8.
57. Leppälahti S, Gissler M, Mentula M, Heikinheimo O. Is teenage pregnancy an obstetric risk in a welfare society? A population-based study in Finland, from 2006 to 2011. BMJ Open. 2013;3:e003225.
58. Brosens I, Pijnenborg R, Vercruysse L, Romero R. The "Great Obstetrical Syndromes" are associated with disorders of deep placentation. Am J Obstet Gynecol. 2011;204:193–201.
59. Brosens I, Benagiano G. Menstrual preconditioning for the prevention of major obstetrical syndromes in polycystic ovary syndrome. Am J Obstet Gynecol. 2015;213:488–93.
60. Schatz F, Guzeloglu-Kayisli O, Arlier S, Kayisli UA, Lockwood CJ. The role of decidual cells in uterine hemostasis, menstruation, inflammation, adverse pregnancy outcomes and abnormal uterine bleeding. Hum Reprod Update. 2016;22:497–515.
61. Jensen JT, Parke S, Mellinger U, Machlitt A, Fraser IS. Effective treatment of heavy menstrual bleeding with estradiol valerate and dienogest: a randomized controlled trial. Obstet Gynecol. 2011;117:777–87.
62. Wasiak R, Filonenko A, Vanness DJ, Wittrup-Jensen KU, Stull DE, Siak S, Fraser I. Impact of estradiol-valerate/dienogest on work productivity and activities of daily living in European and Australian women with heavy menstrual bleeding. Int J Womens Health. 2012;4:271–8.
63. Proctor ML, Roberts H, Farquhar CM. Combined oral contraceptive pill (OCP) as treatment for primary dysmenorrhoea. Cochrane Database Syst Rev. 2001;(4):CD002120.

64. Milsom I, Sundell G, Andersch B. The influence of different combined oral contraceptives on the prevalence and severity of dysmenorrhea. Contraception. 1990;42:497–506.
65. Harada T, Momoeda M, Taketani Y, Hoshiai H, Terakawa N. Low-dose oral contraceptive pill for dysmenorrhea associated with endometriosis: a placebo-controlled, double-blind, randomized trial. Fertil Steril. 2008;90:1583–8.
66. Parry BL, Rush AJ. Oral contraceptives and depressive symptomatology: biologic mechanisms. Compr Psychiatry. 1979;20:347–58.
67. Steinberg JR, Rubin LR. Psychological aspects of contraception, unintended pregnancy, and abortion. Policy Insights Behav Brain Sci. 2014;1:239–47.
68. Hall KS, Kusunoki Y, Gatny H, Barber J. The risk of unintended pregnancy among young women with mental health symptoms. Soc Sci Med. 2014;100:62–71.
69. Hall KS, White KO, Rickert VI, Reame N, Westhoff C. Influence of depressed mood and psychological stress symptoms on perceived oral contraceptive side effects and discontinuation in young minority women. Contraception. 2012;86:518–25.
70. American Society for Reproductive Medicine. Age and fertility. A guide for patients. Patients information series. Birmingham: American Society for Reproductive Medicine; 2012. p. 4.
71. Centers for Disease Control and Prevention, American Society for Reproductive Medicine, Society for Assisted Reproductive Technology. Assisted reproductive technology fertility clinic success rates report. US Dept of Health and Human Services: Atlanta; 2012. p. 2014.
72. Jolly M, Sebire N, Harris J, Robinson S, Regan L. The risks associated with pregnancy in women aged 35 years or older. Hum Reprod. 2000;15:2433–7.
73. Vincent-Rohfritsch A, Le Ray C, Anselem O, Cabrol D, Goffinet F. [Pregnancy in women aged 43 years or older: maternal and perinatal risks] [Article in French]. J Gynecol Obstet Biol Reprod (Paris). 2012;41:468–75.
74. Bokhman JV. Two pathogenetic types of endometrial carcinoma. Gynecol Oncol. 1983;15:10–7.
75. Kaunitz AM. Oral contraceptive use in perimenopause. Am J Obstet Gynecol. 2001;185(2 Suppl):S32–7.
76. Santoro N, Teal S, Gavito C, Cano S, Chosich J, Sheeder J. Use of a levonorgestrel-containing intrauterine system with supplemental estrogen improves symptoms in perimenopausal women: a pilot study. Menopause. 2015;22:1301–7.
77. Cleland J, Conde-Agudelo A, Peterson H, Ross J, Tsui A. Contraception and health. Lancet. 2012;380(9837):149–56.
78. Trussell J, Pebley A. The potential impact of changes in fertility on infant, child and maternal mortality. Stud Fam Plan. 1984;15:267–80.
79. Fortney J. The importance of family planning in reducing maternal mortality. Stud Fam Plan. 1987;18:109–14.
80. Winikoff B, Sullivan M. Assessing the role of family planning in reducing maternal mortality. Stud Fam Plan. 1987;18:128–43.
81. United Nations General Assembly. The United Nations Millennium Declaration Resolution 55/2 adopted by the General Assembly on 8 September 2000. http://www.un.org/millennium/declaration/ares552e.htm.
82. United Nations. The Millennium Project. http://www.unmillenniumproject.org/goals/.
83. Cates W Jr. Family planning: the essential link to achieving all eight Millennium Development Goals. Contraception. 2010;81:460–1.
84. United Nations: Sustainable Development Goals; 17 goals to transform our world. United Nations, 2015. http://www.un.org/sustainabledevelopment/sustainable-developmentgoals/.
85. Coeytaux F, Bingham D, Langer A. Reducing maternal mortality: a global imperative. Contraception. 2011;83:95–8.
86. Jain AK. Measuring the effect of fertility decline on the maternal mortality ratio. Stud Fam Plan. 2011;18:247–60.
87. Ross JA, Blanc AK. Why aren't there more maternal deaths? A decomposition analysis. Matern Child Health J. 2012;16:456–63.
88. Ahmed S, Li Q, Liu L, Tsui AO. Maternal deaths averted by contraceptive use: an analysis of 172 countries. Lancet. 2012;380(9837):111–25.

89. Hubacher D, Mavranezouli I, McGinn E. Unintended pregnancy in sub-Saharan Africa: magnitude of the problem and potential role of contraceptive implants to alleviate it. Contraception. 2008;78:73–8.
90. Eke AC, Alabi-Isama L. Long-acting reversible contraception (LARC) use among adolescent females in secondary institutions in Nnewi, Nigeria. J Obstet Gynaecol. 2011;31:164–8.
91. Curry DW, Rattan J, Huang S, Noznesky E. Delivering high-quality family planning services in crisis-affected settings II: results. Glob Health Sci Pract. 2015;3:25–33.
92. United Nations. Department for Economic and Social Information and Policy Analysis. Population Division. The health rationale for family planning: timing of births and child survival. New York: United Nations, Department for Economic and Social Information and Policy Analysis, Population Division; 1994.
93. Conde-Agudelo A, Rosas-Bermúdez A, Kafury-Goeta AC. Birth spacing and risk of adverse perinatal outcomes: a meta-analysis. JAMA. 2006;295:1809–23.
94. Perin J, Walker N. Potential confounding in the association between short birth intervals and increased neonatal, infant, and child mortality. Glob Health Action. 2015;8:29724.
95. Merali S. The relationship between contraceptive use and maternal and infant health outcomes in Tajikistan. Contraception. 2016;93:216–21.
96. Maïga A, Hounton S, Amouzou A, Akinyemi A, Shiferaw S, Baya B, Bahan D, Barros AJ, Walker N, Friedman H. Trends and patterns of modern contraceptive use and relationships with high-risk births and child mortality in Burkina Faso. Glob Health Action. 2015;8:29736.
97. Shiferaw S, Abdullah M, Mekonnen Y, Maïga A, Akinyemi A, Amouzou A, Friedman H, Barros AJ, Hounton S. Trends in contraceptive use and distribution of births with demographic risk factors in Ethiopia: a sub-national analysis. Glob Health Action. 2015;8:29720.
98. Asiki G, Newton R, Marions L, Seeley J, Kamali A, Smedman L. The impact of maternal factors on mortality rates among children under the age of five years in a rural Ugandan population between 2002 and 2012. Acta Paediatr. 2016;105:191–9.
99. Habimana-Kabano I, Broekhuis A, Hooimeijer P. The effect of pregnancy spacing on fetal survival and neonatal mortality in Rwanda: a Heckman selection analysis. J Biosoc Sci. 2016;48:358–73.
100. Brown W, Ahmed S, Roche N, Sonneveldt E, Darmstadt GL. Impact of family planning programs in reducing high-risk births due to younger and older maternal age, short birth intervals, and high parity. Semin Perinatol. 2015;39:338–44.

Mifepristone for Postcoital Contraception

12

Archil Khomasuridze

When in early 1980s of the last century, D. Etienne-Emile Baulieu synthesized the first antiprogesterone-RU 486 or Mifepristone, everybody considered it to be an "Abortion Pill" only. Beyond any doubt, it was a great achievement too, but the life has showed later that the new compound has not only this possibility but also the potential for solution of all problems, when progesterone is not needed.

Today mifepristone is used or can be used for interruption of pregnancy of any duration, treatment of leiomyomas, emergency and ordinary hormonal contraception and cervical ripening and as a painkiller during the physiological labour. This area is widening (Table 12.1).

The big medical possibilities of mifepristone, unfortunately, are not used even in the developed countries. It seems, that the reputation of the preparation is spoiled by its' primary nickname-"Abortion Pill". Needless to say, that all types of abortion are bad, but it remains as reality for millions of women, and it is our obligation to improve its technologies alternatively. One of such alternatives is mifepristone.

What's mifepristone's use for contraception is concerned, even the essence of mifepristone indicates on its' possibililities for all types of hormonal contraception. We're absolutely confident in it on the basis of our experience, as well as mechanisms of action of the compound. But the world's reality is quite different. Mifepristone for contraception is used only in China, Vietnam, Russia and Armenia, when the very developed country, like the USA, permits its usage for abortion only and not for contraception. Meantime the clinical analogue of mifepristone—ulipristal acetate—is registered for contraception. The reason of such situation is mifepristone's simultaneous possibility to interrupt pregnancy, but it is clear as well that it's the excellent contraceptive, the clinical mode of action of which is quite similar to

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Table 12.1 Indications of mifepristone

1. Termination of I, II, and III trimester pregnancies
2. Treatment of leiomyomas
3. Mini-pills
4. Postcoital contraception
5. Mono- and multiphasic low-dose contraception
6. Physiological labour

Table 12.2 Modes of contraceptive actions of mifepristone

1. Receptors of progesterone and progestogens
2. Content of cervical mucosa
3. Endometrium biochemistry
4. Delay and stop of ovulation

any hormonal contraceptive. The result always depends on the method and terms of its usage. It can be used as contraceptive, but also it terminates the pregnancy in case of contraception failure. What's wrong about it?!

By the way, let me acquaint you with my and my colleague's point of view on definition of the embryo's life during pregnancy.

We agree with the opinion that it starts from the moment of gamete fertilization and its interruption can be called as killing, but simultaneously we consider the life in the uterus is *qualitatively different* from the postpartum life and it belongs to the mother, who decides the embryo's fate.

The opinion above can be a moot and we do not intend to dispute it. Let me draw your attention to mifepristone's potential for postcoital contraception or contragestion. First of all, it's necessary to stop on its mode of action. It's beyond any doubt mifepristone's ability to stop pregnancy is due to its antiprogestogenic influence. The components' contraceptive effect is quite clear, because of existing considerable evidence, that it can change cervical and endometrial biochemistry as well, as delay or stop ovulation (Table 12.2).

All the mechanisms mentioned are quite enough to guarantee mifepristone's contraceptive effect for its emergency variation. Mifepristone's ability to interrupt pregnancy, as mentioned above, increases its contragestional value.

Mifepristone, as contraceptive, is not registered in Georgia. We're in its process only. We use 10 mg tablets.

The situation has not been the obstacle on the way of accumulation of serious experience, since we'd the official permission for many years to carry out the profound investigations in the framework of our cooperation with WHO, which covered 10 years' period, when 10 mg mifepristone was clinically evaluated for postcoital contraception in 586 women. It's difficult to imagine, but I cannot afford not to mention with profound confidence that we'd not a single fiasco of unwanted pregnancy. The data was published in *The Lancet* in 2002 (Table 12.3).

We, in Tbilisi, have continued the study later and paid attention to two slightly different and positive nuances. Here are our results. In principal they do not differ

Table 12.3 Pregnancy rates and prevented fractions

	Rate			Prevented fractions (95% CI)	Relative risks (95% CI)	Relative risks (95% CI)
	N	Pregnancies	Expected pregnancies			
Mifepristone	359	20 (1.55%)	108	81% (69.2–87.8)	1	0.87 (0.49–1.56)
Single-dose levonorgestrel	1356	21 (1.47%)	111	82% (70.9–88.7)	0.95 (0.52–1.75)	0.83 (0.46–1.50)
Two-dose levonorgestrel	1356	24 (1.77%)	106	77% (64.9–85.4)	1.15 (0.64–2.05)	1
All levonorgestrel	2712	44 (1.62%)	216	80% (71.2–85.6)	1.05 (0.63–1.76)	–

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from WHO results but still give us the grounds for more optimism, and I'd like share it with you.

The main point is that we had not a single case of unwanted pregnancy during the whole period of using mifepristone for postcoital contraception. What's the reason? In our opinion, one reason can have ethnic origin or our patients were more accurate from the point of view of realizing the provider's information and instructions.

For your information, I've used to work in contraception since 1966 and always considered all hormonal contraceptives have almost 100% efficacy, if properly used, and if we have the fiasco, we've to look for the reason in the patient behaviour or their insufficient informing.

Approximately the same is concerning mifepristone's side effects during its usage for postcoital contraception. Please pay attention to the following comparison (Table 12.4).

The frequency of menstrual irregularities in the women using hormonal contraceptives like levonorgestrel is not comparable with the same indicator of mifepristone, the more we've patented the method for its prophylaxy for this period of time.

To conclude, let me inform that we've used to work with antiprogestogenes intensively since the early 1980s and we witnessed impressionable results, but many questions were born as well. We're sure that many of them are connected with the non-objective attitude to mifepristone. Let me present the few of them:

1. What's the reason that the medical abortion has not substituted the surgical method completely? (We consider the reason is not proper informing of the clients and providers.)
2. What's the reason of the fact that mifepristone is practically not used for termination of II and III trimester pregnancies under medical indications? (We and some our colleagues have the evidence that mifepristone for these purposes is much more effective and less harmful than the traditional methods.)
3. Why is mifepristone not used for cervical ripening and, correspondingly, as a painkiller during the physiological delivery? (Our experience shows that this

method gives the high opportunity to decrease the cervical traumatization during labour.)

4. What's the reason of not so wide usage of mifepristone for the treatment of progesterone-dependent benign formations?
5. Why are the minimal doses of mifepristone not used as mini-pills?
6. What is the reason of non-inclusion of mifepristone as a part of mono- and multiphasic contraceptives?
7. What's the reason that mifepristone is used for emergency contraceptive in China, Vietnam, Russia and Armenia and not used in the USA? (It's clear that it's better than levonorgestrel and not worse, at least, than ulipristal acetate.)

Table 12.4 Side effects within 7 days of menses delay

WHO study				Georgian study (%)
	Group	Number of cases	*P*	
Nausea	Mifeprostone	196/1364 (14%)	0.86	10
	Single-dose levonorgestrel	189/1359 (14%)		15
	Two-dose levonorgestrel	199/1361 (15%)		17
Vomiting	Mifeprostone	12/1364 (%)	0.31	0.5
	Single-dose levonorgestrel	19/1359 (1%)		1.4
	Two-dose levonorgestrel	19/1361 (1%)		1.5
Diarrhoea	Mifeprostone	61/1364 (5%)	0.24	0
	Single-dose levonorgestrel	53/1359 (4%)		1
	Two-dose levonorgestrel	44/1361 (1%)		1.5
Fatigue	Mifeprostone	208/1364 (15%)	0.30	15
	Single-dose levonorgestrel	184/1359 (14%)		16
	Two-dose levonorgestrel	182/1361 (13%)		17
Dizziness	Mifeprostone	123/1364 (9%)	0.82	5
	Single-dose levonorgestrel	132/1359 (10%)		9
	Two-dose levonorgestrel	126/1361 (9%)		11
Headache	Mifeprostone	140/1364 (10%)	0.71	3
	Single-dose levonorgestrel	142/1359 (10%)		7
	Two-dose levonorgestrel	130/1361 (10%)		8
Breast tenderness	Mifeprostone	114/1364 (8%)	0.99	8.8
	Single-dose levonorgestrel	113/1359 (8%)		9
	Two-dose levonorgestrel	115/1361 (8)		10
Lower abdominal pain	Mifeprostone	191/1364 (14%)	0.72	9
	Single-dose levonorgestrel	183/1359 (14%)		15
	Two-dose levonorgestrel	198/1361 (15%)		17
Bleeding	Mifeprostone	258/1364 (19%)	<0.0001	18
	Single-dose levonorgestrel	426/1359 (31%)		30
	Two-dose levonorgestrel	426/1361 (31%)		31
Delay of menses more than 7 days	Mifeprostone	118/1327 (9%)	<0.0001	8
	Single-dose levonorgestrel	62/1334 (5%)		5
	Two-dose levonorgestrel	63/1332 (5%)		6

References

1. von Hertzen H, et al. Lancet. Low dose mifepristone and two regimens of levonorgestrel for emergency contraception: a WHO multicentre randomized trial. 2002;360(9348):1803–10.
2. Trussell J, Raymond E, Cleland K. Emergency contraception: a last chance to prevent unwanted pregnancy. Princeton: Princeton University; 2016.
3. Wertheimer R. Emergency postcoital contraception. Am FamPhysician. 2000;62(10):2287–92.
4. Sarkar NN. The potential of mifepristone (RU-486) as an emergency contraceptive drug. Acta Obstet Gynecol Scand. 2005;84(4):306–16.
5. Finer LB, Zolna MR. Declines in unintended pregnancy in the United States, 2008-2011. N Engl J Med. 2016;374:843–52.
6. Li HWR, Lo SST, Ng EHY, Ho PC. Efficacy of ulipristal acetate for emergency contraception and its effect on the subsequent bleeding pattern when administered before or after ovulation. Hum Reprod. 2016;31:1200–7.
7. Cheng L, Che Y, Gülmezglu AM. Interventions for emergency contraception. Cochrane Database Sys Rev. 2012;(8):CD001324.
8. Turok DT, Sanders JN, Thompson IS, Royer PA, Eggebroten J, Gawron L. Preference for and efficacy of oral levonorgestrel for emergency contraception with concomitant placement of a levonorgestrel IUD: a prospective cohort study. Contraception. 2016;93:526–32.
9. Kapp N, Abitbol JL, Mathé H, Scherrer B, Guilard H, Gainer E, Ulmann A. Effect of body weight and BMI on the efficacy of levonorgestrel emergency contraception. Contraception. 2015;91(2):97–104.
10. United States Food and Drug Administration. FDA communication on levonorgestrel emergency contraceptive effectiveness and weight. Available at: http://www.fda.gov/Drugs/DrugsSafety/PostmarketDrugSafetyinformationforPatientaandProviders/ucm109775.htm. Accessed 24 May 2016.
11. Berger C, Boggavarapu NR, Menezes J, Lalitkumar PG, Gemzell-Danielson K. Effects of ulipristal acetate on human embryo attachment and endometrial cell gene expression in an in vitro co-culture system. Hum Reprod. 2015;30:800–11.
12. Jasam C, Cochon L, Salvatierra AM, Williams A, Kapp N, Levy-Gompel D, Brache VA. prospective, open-label, multicenter study to assess the pharmacodynamics and safety of repeated use of 30 mg ulipristal acetate. Contraception. 2016;93:310–6.
13. Jatlaoui TV, Riley H, Curtis KM. Safety data for levonorgestrel, ulipristal acetate and Yuzpe regimens for emergency contraception. Contraception. 2016;93:93–212.
14. Cameron ST, Berger C, Michie L, Klipping C, Gemzell-Danielsson K. The effects on ovarian activity of ulipristal acetate when 'quickstarting' a combined oral contraceptive pill: a prospective, randomized, double-blind parallel-arm, placebo-controlled study. Hum Reprod. 2015;30:1566–73.
15. Cintia L, Johansen MS. The effect of plan B on teen abortions: evidence from the 2006 FDA ruling. Contemp Econ Policy. 2015;33:418–33.
16. Bullock H, Steele S, Kurata N, et al. Pharmacy access to ulipristal acetate in Hawaii: is a prescription enough? Contraception. 2015;92:388–9.

13 Considering the Pathogenesis of Atherosclerosis to Explain CIMT- But Not CAC-Proven Estrogen Atheroprevention in the Elite Trial

Jenna Friedenthal and Frederick Naftolin

13.1 Menopause, Estrogen, and Arteriosclerosis

The most common cause of death in women is coronary arteriosclerosis (ASVD). This disease begins as a subclinical accumulation of macrophages (plaque) in the subendothelial tissue of the arteries of the heart. The macrophages transform to fat-laden "foam cells" and begin to undergo apoptosis. The dead cells are a site of accumulation of calcium. This chronology is well-established in many animal and human studies [1].

Menopausal women have an increased risk of ASVD. Women who receive estrogen as a treatment after surgical menopause (ET), or as part of menopause after natural menopause (MHT), have a lower age-corrected incidence of ASVD-related events and death from coronary heart disease [2].This chapter will review the need for markers of the presence and progress of ASVD and its precursor lesions and their present availability. Using the recent proof of the salutary effect of postmenopausal estrogen treatment on menopausal women as an example, we hope to make the case for more and longer studies of long-term estrogen treatment of menopausal woman. Finally, we propose that the temporal difference between the development of subclinical plaque and its calcification may explain the recent finding of improved carotid artery intima-media thickness (CIMT), but not coronary artery calcium (CAC) in women taking long-term MHT.

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13.2 The Importance of Noninvasive Vascular Imaging in Experimental Settings

While noninvasive markers of risk for ASVD and cardiovascular disease (CVD) and clinical tests for acute cardiac events are well-established, until recently the monitoring of vascular health by directly visualizing vessel walls was neither feasible nor safe in non-indicated cases [3]. This restriction is especially true in the case of clinical experimentation, and the "work-arounds" have had important impacts on the field. For example, at the time of planning for the Women's Health Initiative (WHI), the lack of satisfactory noninvasive vascular monitoring techniques required dependence on actual cardiac events as the experimental end point. Because obtaining enough subjects to expect sufficient numbers of cardiac events has the power to discern between controls and treated subjects, the WHI resorted to studying older, postmenopausal women who were more likely to have cardiac events. This resulted in cohorts that at the time of entry to the study averaged ~63 years of age and were started on estrogen and progestin more than a decade after their menopause. This had serious negative repercussions regarding the evaluation of vascular and cardiac risk of hormone treatment [4].

Fortunately, in the interim since the initiation of the WHI, noninvasive methods have appeared that allow accurate, precise, and repetitive noninvasive monitoring of the health of vessels to study the effects of menopausal hormone treatment (MHT) during the entire climacteric. These methods allow the detection and progress of the development of subclinical and clinical CVD and have been used in clinical trials testing for cardioprotection by MHT. Among these methods are two, carotid artery intimal-medial thickening (CIMT) and coronary artery calcium imaging (CAC), that complementarily monitor the development of atherogenesis, followed by atherosclerosis [3]. This chapter compares the interaction of CIMT and CAC measurements during the monitoring of the cardioprotective effects of MHT.

13.3 CIMT and CAC as Measures of Atherogenesis and Atherosclerosis

ASVD and CIMT—The process of atherogenesis is triggered by vascular inflammation that induces molecular signals (cytokines, growth factors, etc.) to begin the capture and internalization of monocytes/macrophages into the subendothelial layer of the injured vessel. With time, the accumulation of macrophages develops into a subendothelial thickening, or plaque. The plaque widens, and the vessel wall thickens; this thickening can safely and repeatedly be monitored by ultrasound imaging of the vessel wall. Since the carotid artery in the neck is particularly accessible and anatomically constant, it has been studied for intimal-medial size (thickness, CIMT) during the development of atheromas. Since the CIMT normally increases with age, it is the differential thickening that is used to determine the presence and size/rate of atherogenesis.

Calcification, a marker of plaque degeneration, is not necessary for the appreciation of changes in the CIMT. Since it has been proven that the rate of differential thickening or even that certain thickness of the carotid artery wall has been shown to closely parallel the development of atheromas and atherosclerosis, the thickening of the carotid artery, repeatedly monitored by high-definition ultrasound (CIMT), has become a standard method for noninvasive monitoring of atherogenesis. Increases in CIMT have been shown to be associated with increased risk for future cardiac events [5]; additional studies have reinforced the reliability of CIMT as a predictor for future cardiac disease [6, 7].

CAC monitors advanced ASVD—During arterial wall thickening, plaque macrophages transform into large, fat-filled "foam cells," some of which die and calcify. Some have theorized that calcium accumulate in atherosclerotic vessels is attributable to activity of arterial osteoblasts and osteoclasts [8]. Clumps of calcified vascular cells are X-ray-dense and can be visualized by their adjacency to contrast media flowing through vessels. If the coronary arteries are studied, these are termed coronary artery calcifications (CAC). It was in the 1960s that, using fluoroscopic guidance, calcification in the coronary arteries was first found to be associated with clinical atherosclerosis [9]. Today, quantification of CAC by CT scan has become an important means of evaluating the establishment and progress of ASVD and for quantifying the overall extent of the atherosclerotic burden [10, 11].

13.4 Estrogen's Role in Cardiovascular Disease in Women

Estrogen has long been considered a cardioprotective agent in women. Estrogen administration has a known positive impact on plasma lipid profiles, antiplatelet effects, and antioxidant effects [12]. In addition, estrogen receptors and aromatase are present in human coronary artery endothelium [13]. Abnormalities of the estrogen receptor alpha (ERα) gene have been linked to cardiovascular disease [14]. Previous studies have shown that postmenopausal women experience greater arterial stiffening, a known marker of vascular aging, than premenopausal women [15]. A study by Moreau suggests that estrogen improves endothelial compliance [16–18]. We studied the effects of estradiol on the early events in atherogenesis, showing that estradiol induces polysialylation of neural cell adhesion molecules (nCAM), which disables nCAM to nCAM tethering and capture of monocytes. We proposed that this could interrupt the above chain of events that leads to development of plaque, etc. [19].

Numerous observational studies support the role of estrogen in preventing cardiovascular disease when used as menopausal hormone treatment (MHT) [20–22]. Although the initial interpretation of the results from the Women's Health Initiative [23] raised concerns about the effects of hormone treatment on cardiovascular events, seen above, extensive subsequent research, including WHI re- and sub-analysis, and a prospective observational study by the WHI support the cardioprotective effects of estrogen treatment, especially when administration is started at or close to the onset of menopause [24–26].

In the studies that have separated the components or the epidemiology of CVD in postmenopausal women, the estrogen component of MHT is the active agent of MHT cardioprotection [2].

Risks vs. benefits of estrogen treatment in normal menopausal women, pulmonary embolism—Obtaining and maintaining cardioprotection requires long-term administration of the treatment. While both benefits and complications have been ascribed to postmenopausal estrogen treatment, studies indicate that ET begun during the first decade of menopause does not increase the rate of pulmonary emboli, regardless of age or time since menopause that the ET was started. On the contrary, E+medroxy progesterone is associated with increased occurrence of pulmonary emboli [27]. A word of caution in applying these findings, since the WHI women excluded women with historic early ASVD findings, clinical application of these findings depends on the normalcy of the patients prior to starting long-term ET.

13.5 Using CIMT/CAC to Develop the Rules of Oral Estradiol ± Progesterone (MHT)-Induced Cardioprotection

Since it is not feasible to use invasive testing or closely timed X-ray-based tests such as CACs to monitor treatments, important questions have remained regarding safe and effective harnessing of the cardioprotective effects of MHT.

In March 2016, the ELITE study published results on 643 healthy postmenopausal women stratified according to the length of time since menopause (<6 or ≥10 years) who were randomly assigned to receive either oral estradiol plus vaginal progesterone MHT or placebo. After a median of 5 years' treatment, there was a protective effect of MHT as measured by a higher rate of CIMT rise in untreated postmenopausal controls compared to estradiol-treated women; however, no protection against the rise in CAC was found [28]. This disparity in CIMT/CAC changes has not been explained.

The delay between the recognition of increased CIMT and the amount of CAC may reflect the sequential nature of plaque formation and foam cell death and calcification.

The above findings and the need to have plaque before calcification of dead foam cells can occur could explain the observation of CIMT changes without observed CAC changes in estrogen-treated women [3]. Such a delay of the accumulation of CAC (death of foam cells and their calcification) despite increased CIMT (accumulation of foam cells) is consistent with available data comparing CIMT and CAC in the context of estradiol administration.

(a) The 4-year KEEPS study showed improvement of all measured cardiovascular risk factors, but no protective changes in CIMT or CAC scores [29].
(b) After 5 years of treatment, the ELITE trial showed a difference in CIMT, but not CAC.

(c) The WHI observational study did not study CIMTs but showed a difference in CACs 7.4 years (mean) after starting estrogen treatment [30].
(d) An earlier study of women after one to three decades of MHT showed both CIMT and CAC to be lower in treated women than untreated case controls [31].

In summary, these data support the cardioprotective effects of timely estradiol treatment, with the CAC changes lagging the CIMT increases.

Hypothesis We propose that the lack decreased CAC in the MHT treated women during the ELITE trial may be due to the delay of calcification of vascular plaque. This hypothesis suggests that longer follow-up would show both CIMT and CAC to indicate estrogen's cardioprotective effect.

13.6 Future Studies

The cardioprotective effects of estrogen in women are established, and the importance of timely initiation of ET has been established. However, much work remains to be accomplished. This includes the following:

1. Confirmation and dose/regimen response in properly timed and long duration studies on the cardioprotective effect of ET, with or without anti-estrogenic agents to control endometrial growth.
2. Long-term trials are needed to test the hypothesis regarding the timing disparity of CIMT/CAC changes and to determine benefits or risks associated with such long-term treatments.
3. Effects of MHT must be determined on the expression and molecular makeup of estrogen and progesterone receptors in the vessels of estrogen-treated women.
4. Long-term studies are needed to confirm persistent estrogen-related cardioprotection of postmenopausal women and the proper timing and method of discontinuation after decades of treatment.

References

1. Libby P, Hansson GK. Inflammation and immunity in diseases of the arterial tree: players and layers. Circ Res. 2015;116(2):307–11.
2. Salpeter SR, Cheng J, Thabane L, Buckley NS, Salpeter EE. Bayesian meta-analysis of hormone therapy and mortality in younger postmenopausal women. Am J Med. 2009;122(11):1016–22.
3. Hodis HN, Mack WJ. Atherosclerosis imaging methods: assessing cardiovascular disease and evaluating the role of estrogen in the prevention of atherosclerosis. Am J Cardiol. 2002;89(12A):19E–27E.
4. Tan O, Harman SM, Naftolin F. What can we learn from design faults in the Women's Health Initiative randomized clinical trial? Bull NYU Hosp Jt Dis. 2009;67(2):226–9.
5. Salonen JT, Salonen R. Ultrasonographically assessed carotid morphology and the risk of coronary heart disease. Arterioscler Thromb. 1991;11:1245–9.

6. Iwakiri T, Yano Y, Sato Y, Hatakeyama K, Marutsuka K, Fujimoto S, Kitamura K, Kario K, Asada Y. Usefulness of carotid intima-media thickness measurement as an indicator of generalized atherosclerosis: findings from autopsy analysis. Atherosclerosis. 2012;225:359–62.
7. Geisel MH, Bauer M, Hennig F, Hoffmann B, Lehmann N, Möhlenkamp S, Kröger K, Kara K, Müller T, Moebus S, Erbel R, Scherag A, Jöckel KH, Mahabadi AA, Investigative Group of the Heinz Nixdorf Recall Study. Comparison of coronary artery calcification, carotid intima-media thickness and ankle-brachial index for predicting 10-year incident cardiovascular events in the general population. Eur Heart J. 2017;38:1815–22.
8. Doherty TM, Asotra K, Fitzpatrick LA, Qiao JH, Wilkin DJ, Detrano RC, Dunstan CR, Shah PK, Rajavashisth TB. Calcification in atherosclerosis: bone biology and chronic inflammation at the arterial crossroads. Proc Natl Acad Sci U S A. 2003;100:11201–6.
9. Eggen DA, Strong JP, HC MG Jr. Coronary calcification. Relationship to clinically significant coronary lesions and race, sex, and topographic distribution. Circulation. 1965;32:948–55.
10. Alluri K, Joshi PH, Henry TS, Blumenthal RS, Nasir K, Blaha MJ. Scoring of coronary artery calcium scans: history, assumptions, current limitations, and future directions. Atherosclerosis. 2015;239:109–17.
11. Sangiorgi G, Rumberger JA, Severson A, Edwards WD, Gregoire J, Fitzpatrick LA, Schwartz RS. Arterial calcification and not lumen stenosis is highly correlated with atherosclerotic plaque burden in humans: a histologic study of 723 coronary artery segments using nondecalcifying methodology. J Am Coll Cardiol. 1998;31:126–33.
12. Guetta V, Cannon RO III. Cardiovascular effects of estrogen and lipid-lowering therapies in postmenopausal women. Circulation. 1996;93(10):1928–37.
13. Diano S, Horvath TL, Mor G, Register T, Adams M, Harada N, Naftolin F. Aromatase and estrogen receptor immunoreactivity in the coronary arteries of monkeys and human subjects. Menopause. 1999;6(1):21–8.
14. Ding J, Xu H, Yin X, Zhang FR, Pan XP, Gu YA, Chen JZ, Guo XG. Estrogen receptor α gene PvuII polymorphism and coronary artery disease: a meta-analysis of 21 studies. J Zhejiang Univ Sci B. 2014;15(3):243–55.
15. Westendorp IC, Bots ML, Grobbee DE, Reneman RS, Hoeks AP, Van Popele NM, Hofman A, Witteman JC. Menopausal status and distensibility of the common carotid artery. Arterioscler Thromb Vasc Biol. 1999;19(3):713–7.
16. Moreau KL, Meditz A, Deane KD, Kohrt WM. Tetrahydrobiopterin improves endothelial function and decreases arterial stiffness in estrogen-deficient postmenopausal women. Am J Physiol Heart Circ Physiol. 2012;302(5):H1211–8.
17. Fahraeus L. The effects of estradiol on blood lipids and lipoproteins in postmenopausal women. Obstet Gynecol. 1998;72(5 Suppl):18S–22S.
18. Miller VM, Lahr BD, Bailey KR, Heit JA, Harman SM, Jayachandran M. Longitudinal effects of menopausal hormone treatments on platelet characteristics and cell-derived microvesicles. Platelets. 2016;27(1):32–42.
19. Naftolin F, Mehr H, Fadiel A. Sex steroids block the initiation of atherosclerosis. Reprod Sci. 2016;23(12):1620–5. https://doi.org/10.7326/M14-0353.
20. Grodstein F, Stampfer MJ, Manson JE, et al. Postmenopausal estrogen and progestin use and risk of cardiovascular disease. N Engl J Med. 1996;335:453–61.
21. Bush TL, Barrett-Connor E, Cowan LD, Criqui MH, Wallace RB, Suchindran CM, Tyroler HA, Rifkind BM. Cardiovascular mortality and noncontraceptive use of estrogen in women: results from the Lipid Research Clinics Program Follow-up Study. Circulation. 1987;75(6):1102–9.
22. Grodstein F, Manson JE, Colditz GA, Willett WC, Speizer FE, Stampfer MJ. A prospective, observational study of postmenopausal hormone therapy and primary prevention of cardiovascular disease. Ann Intern Med. 2000;133(12):933–41.
23. Rossouw JE, Anderson GL, Prentice RL, 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;288:321–33.

24. Choi SD, Steinberg EM, Lee HH, Naftolin F. The Timing Hypothesis remains a valid explanation of differential cardioprotective effects of menopausal hormone treatment. Menopause. 2011;18(2):230–6.
25. Manson JE. The 'timing hypothesis' for estrogen therapy in menopausal symptom management. Women's Health (Lond). 2015;11(4):437–40.
26. Clarkson TB, Melendez GC, Appt SE. Timing hypothesis for postmenopausal hormone therapy: its origin, current status, and future. Menopause. 2013;20(s):342–53.
27. Bassuk SS, Manson JE. Oral contraceptives and menopausal hormone therapy: relative and attributable risks of cardiovascular disease, cancer, and other health outcomes. Ann Epidemiol. 2015;25(3):193–200.
28. Hodis HN, Mack WJ, Henderson VW, et al. Vascular effects of early versus late postmenopausal treatment with estradiol. N Engl J Med. 2016;374(13):1221–31.
29. Harman SM, Black DM, Naftolin F, et al. Arterial imaging outcomes and cardiovascular risk factors in recently menopausal women: a randomized trial. Ann Intern Med. 2014;161(4):249–60.
30. Manson JE, Allison MA, Rossouw JE, et al. Estrogen therapy and coronary artery calcification. N Engl J Med. 2007;256(11):2591–602.
31. Ge QS, Tian QJ, Tseng H, Naftolin F. Development of low-dose reproductive hormone therapies in China. Gynecol Endocrinol. 2006;22(11):636–45.

The Effect of Oestrogens on Mood

14

Martin Birkhaeuser

14.1 Introduction

Women of all ages have a higher incidence of depression than men [1–4]. The lifetime incidence of endogenous depression in women is twice the incidence in males (Table 14.1). For many gynaecologists it will be a surprise that mood change is the most common symptom for which women seek treatment at menopause in psychiatric clinics. Almost half of these women are clinically depressed, and over a third experience their first episode of depression in the menopausal transition [5–7]. Among women attending community or university menopause clinics, two-thirds of those at a London site, and three-quarters of those at a San Diego site met criteria for recurrent major depression when evaluated by a psychiatric interview. Because depression in the elderly is an important public health concern [8], an eventual correlation between menopause and depression is of practical importance.

In the late nineteenth century, Emil Kraepelin (died in 1929) developed a new psychiatric nosology [9]. He described "involutional melancholia" as a distinct clinical entity characterized by late onset and symptoms of fear, despondency, agitation and hypochondriacal delusions. In the 1950s and 1960s of the twentieth century, Manfred Bleuler postulated a causal relationship between the endocrine system and psychiatric diseases [10, 11]. In this context, Bleuler had introduced the notion of "endocrine depression". Subsequent research discounted a syndrome of depression at menopause [12] by claiming that depression and menopause are two different entities occurring independently in the same age group. Hence, involutional melancholia was omitted from DSM with publication of the third edition in 1980. In 1984, Osborn stated [14]: "For the doctor confronted with an unhappy menopausal

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Table 14.1 Incidence of different forms of depression in both sexes [1, 2, 13]

	Relation f:m
Unipolar depression	4:1
One depressive episode bipolar I	2:1
One depressive episode bipolar II	1:1
Global proportion of females to males [2]	2:1

No differences in age at start, psychotic symptoms, tentamen, chronicity

woman, who is weepy, irritable and sleeping badly, it is often difficult to decide whether these symptoms are a natural consequence of her endocrine status or due to social or psycho-logical factors. Certainly, a link between depression and the menopause should not necessarily be assumed". If the older literature is contradictory concerning the correlation between depressive dysphoria and oestrogens, it might be due in part to the fact that the women seen and the diagnostic criteria used are not identical between specialists in gynaecological endocrinology and in psychiatry. Only in 1998, Gath [15] postulated that for the tests to be used, the following points had to be respected by all specialists:

- The psychiatric criteria have to be fulfilled.
- The sensitivity has to be established.
- The "test-retest" reliability has to be established.
- The usefulness of the test has to be established.

These criteria made it possible to reassess the possibility of a link between menopause and mood.

14.2 The "Window of Vulnerability"

It is well known that women suffer in their lifetime twice as much from depression than men (Table 14.1). In women, endocrine unstable life periods such as puberty, pregnancy, postpartum and menopause are "windows of increased vulnerability" for depressive states. Overall, the risk for major depression is approximately 1.5–3 times higher in women than in men. The estimated lifetime prevalence in women is 21.3% [16, 17].

A systematic review of the existing cohort studies published in 2007 looked at the independent influence of the menopausal transition on mood including depression, anxiety and other psychological symptoms and concluded that there is no demonstrated pattern of an adverse independent influence of the menopausal transition on mood symptoms in midlife women. The authors stated that the available studies are methodologically too diverse to be definitive [18]. The attitude changed with the publication of the first longitudinal studies. Preliminary data suggested that events related to the late perimenopause might be associated with an increased susceptibility to develop depression in some women [19, 20]. Another early study reported that hot flushes and other climacteric symptoms might affect the quality of a woman's life [21]. Greater longitudinal studies have been needed to confirm one

Table 14.2 "Window of vulnerability": Increased risk for depression in the menopausal transition (longitudinal studies)

Study	Odds ratio (95% confidence interval)	References
Penn Ovarian Aging Study (2004)	2.89 (1.29–0.45) (all participants)	[22]
Penn Ovarian Aging Study (2006)	(no history of depression at inclusion)	[23]
– High CES-D[a] scores (>16)	4.29 (2.39–7.72)	
– Diagnosis of depressive disorder	2.50 (1.25–5.02)	
Harvard Study of Moods and Cycles (2003)	1.77 (1.22–2.57) (all participants)	[24]
Harvard Study of Moods and Cycles (2006)	1.8 (1.03–3.20) (no history of depression at inclusion)	[26]
Study of Women Across the Nation (2007)	1.71 (1.27–2.30) (late perimenopause)	[27–29]

[a]Epidemiologic Studies Depression Scale (CES-D)

for all that menopausal transition is a risk factor for mood changes and depression (Table 14.2, [22–30]). In the Penn Ovarian Aging Study (POAS), the risk of depressive symptoms was nearly three times higher in women in the menopause transition compared with premenopausal women [22]. Women with no history of depression were still 2½ times more likely to report depressed mood in the menopause transition compared with the premenopausal period [23]. Other cohort studies reported similar findings: the Harvard Study of Moods and Cycles [26], the Study of Women's Health Across the Nation (SWAN) [28–30], the Seattle Midlife Women's Health Study [31] and the Melbourne Women's Midlife Health Project [32]. In the prospective Melbourne Women's Midlife Health Project, a large increase in FSH levels over this period was associated with depressive symptoms (OR, 2.6; 95% CI, 1.0–6.7) [33]. All these longitudinal studies confirmed the hypothesis of the "window of increased vulnerability" in peri- and early postmenopausal women.

A recent follow-up publication of the Penn Ovarian Aging Study (POAS) reported that the prevalence of high scores on the Centre for Epidemiologic Studies Depression Scale decreased from 10 years before to 8 years after the final menstrual period (FMP), with a decrease of approximately 15% of baseline per year (odds ratio, 0.85; 95% CI, 0.81–0.89; $P < 0.001$) [34]. Relative to the final menstrual period (FMP), the risk of depressive symptoms was higher in the years before and lower in the years after the FMP. Among women with a history of depression, the likelihood of depressive symptoms was more than 13 times greater overall and 8 times greater after menopause compared with women with no depression history. Among women with no history of depression who first experienced depressive symptoms approaching menopause, the risk of depressive symptoms declined after the FMP, with a significantly lower risk the second year after menopause. There was a correlation with FSH increase. The risk of depressive symptoms after menopause decreased by 35% for each increase by one unit (SD) in the log rate of change of follicle-stimulating hormone (odds ratio, 0.65; 95% CI, 0.46–0.91; $P = 0.01$) before the FMP. A concordant restoration of ovarian function, characterized by an increase of serum oestradiol and a decrease of serum FSH, and a spontaneous amelioration of mood in perimenopausal depression has been observed in a longitudinal study [35].

It may be concluded that the FMP is pivotal in the overall pattern of decreasing depressive symptoms in midlife women, with higher risk before and lower risk after the FMP. In particularly vulnerable women, the menopausal transition might trigger a depressive disorder [16, 17, 23, 32, 37, 38]. The incidence of depressive mood (feeling down, sad, on the verge of tears, lack of drive, mood swings) and anxiety (inner restlessness, feeling panicky) is significantly increased in women with early and precocious menopause [39]. A history of depression strongly increases the risk both before and after menopause. Women without a history of depression before the menopause transition have a low risk of depressive symptoms 2 or more years after the FMP [34].

14.3 Mental Changes, Mood and Vasomotor Symptoms

Psychological symptoms reach from nervousness, aggressiveness, irritability and agitation to instability, anxiety and depressive mood. Menopausal women may feel controlled by an inner tension. Several well-conducted, large observational studies have established a clear association of medium and severe VMS, mainly hot flushes and sweats at night, and psychological symptoms such as anxiety and depression (Fig. 14.1) [18, 37, 38, 40–42]. In women who report both symptoms, depressive symptoms are more likely to precede hot flushes [36]. The main consequence of vasomotor symptoms is a chronic lack of sleep due to frequent VMS epidodes. As VMS, lack of sleep increases the risk for depressive mood (feeling down, sad, on the verge of tears, lack of drive, mood swings) and anxiety (inner restlessness, feeling panicky) in the peri- and early postmenopause. Chronic lack of sleep due to frequent hot flushes and sweats at night correlates with mental symptoms in symptomatic climacteric women [43]. Such a condition, if severe, may end in a state of physical and mental exhaustion with a general decrease in performance, an impaired memory, a decrease in concentration and an augmenting forgetfulness. If not treated adequately, mental changes may generate serious difficulties at the working place as well as at home (partner, children) and with friends and may end in social isolation.

A recent review concluded that there is a bidirectional association between VMS and depressive symptoms [44], but it is still open if there is a relationship between VMS and major depressive disorder.

In addition to VMS, there are many other risk factors for depression in the menopausal transition to be taken into consideration:

- Negative attitude to menopause towards ageing/menopause
- Psychiatry: history of depressed mood/major depression

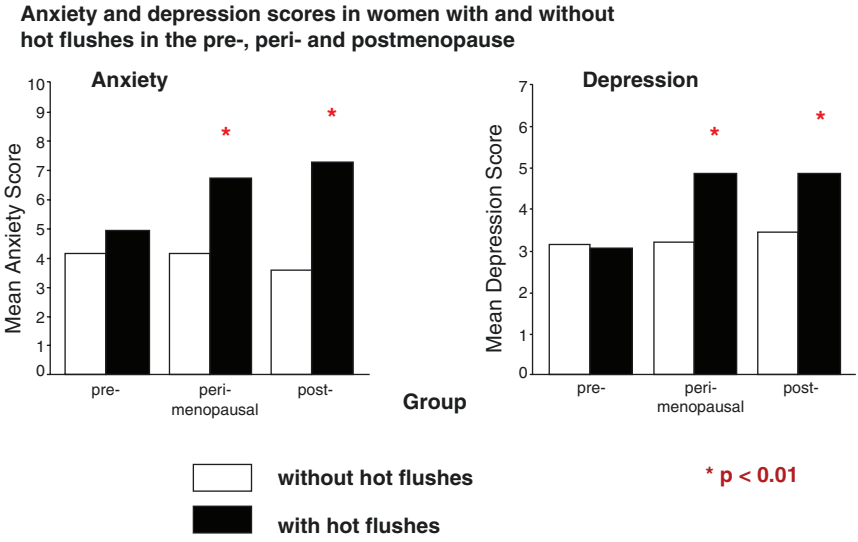

Fig. 14.1 Anxiety and depression scores in women with and without hot flushed in the pre-, peri- and postmenopause. Compared to women without vasomotor symptoms, the risk for symptoms of anxiety and depression is significantly increased in the peri- and the postmenopausal women suffering from hot flushes, but not in premenopausal women [40]

  - Family history
  - Personal history
  - Comorbidity
- Psycho-socio-economic
  - Stressful life events
  - Death in the near family/close friends
  - Unhealthy lifestyle
  - Violation (particularly young age)
  - Marital/partnership concerns
  - Low economic level
  - Unemployment
  - "Empty nest"
- Early menopause
- Premenstrual syndrome
- Education: low educational level
- Ethnicity: white ethnicity

14.4 The Effect of Oestradiol Administration

14.4.1 Animal Studies, Mechanism

Through their brain receptors, oestrogens modulate the metabolism of serotonin and noradrenalin, as do antidepressants, and influence therefore mood, mental function and cognition [16, 17, 45] (Table 14.2). In healthy women, oestrogen affects the dopaminergic, serotonergic and cholinergic systems and brain regions crucial to higher cognitive function and mood. Acute loss of ovarian hormones increases neuronal membrane breakdown and is associated with reduced activation of brain regions critical to memory. Oestrogen deficiency may lead to mental changes including mood.

In the rat, castration provokes depression- and anxiety-like symptoms [46–49]. Rat studies confirmed the notion that beneficial effects of E2 in cognitive tasks may be most evident when E2 is initiated at E2 decline and or not more than 5 months later. In the present study, rats that had improved performance above what is typically considered chance (50%) in the task demanded were those with continuous E2 from 14 to 20 months of age [46]. Rats that administered chronic E2 immediately after OVX have a better performance than rats substituted only 5 months later (see Fig. 14.2). In rats not substituted at all, depression- and anxiety-like symptoms are strongly expressed.

It has been shown in females primates that oestradiol acts at midbrain on neuroregulation [50, 51]. In female macaques whose age corresponded to the human peri- and early postmenopause, the neuropsychological behaviour was better compared to placebo in monkeys substituted by oestradiol. These animals showed less depression-like symptoms, and they had a better memory. In the substituted primates, there was a higher density of newly formed dendritic branching in the brain regions responsible for these functions.

14.4.2 Oestrogen Administration in Peri- and Postmenopausal Women

Twenty years ago, a meta-analysis came to the conclusion that oestrogens improved depressive mood significantly (mean $d = 0.69$; $p = 0.0001$) and that androgens plus oestrogens combined had been even more effective than oestrogens alone (mean $d = 1.37$; $p = 0.003$). The effect size (d-value) used on this meta-analysis is a scale-free measure of the strength of research findings and a measure of the differences between two means. It is expressed in standard deviation units (d-value of 0.00 = no treatment effect). Newer data are consistent in demonstrating that oestrogen therapy decreases aggressiveness and improves affective disorders, mood, depressive symptoms and anxiety in climacteric women with VMS in the peri- and early postmenopause [52–65]. The KEEPS trial, a large recent 4-year RCT, reported that CEE (0.45 mg/day, with cyclic progesterone) but not transdermal oestradiol (0.05 mg/day, with cyclic progesterone) improved depressive symptoms and anxiety compared to placebo [66]. However, observational studies and other RCTs (including

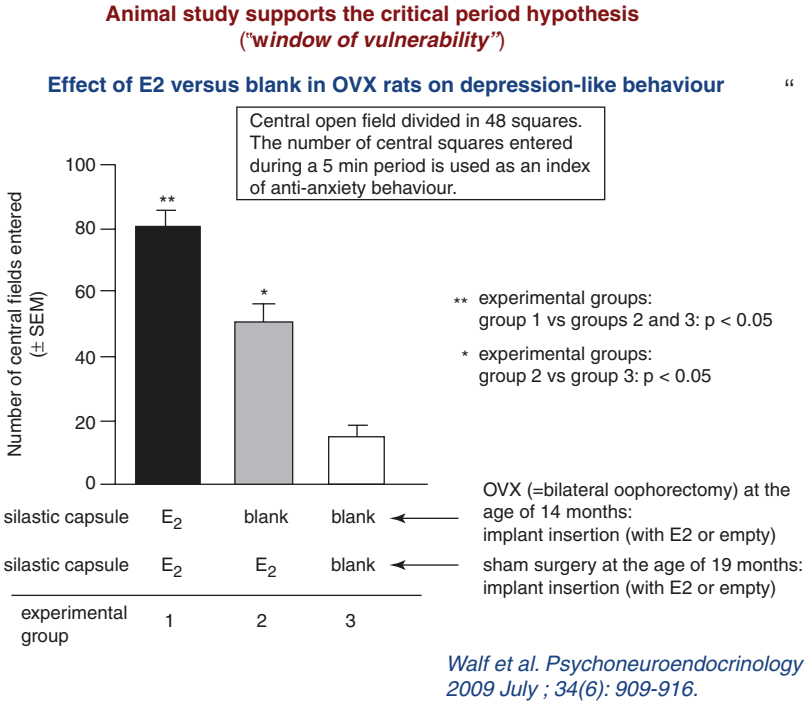

Fig. 14.2 Depression- and anxiety-like behaviour in castrated rats corrected by oestrogens. In the rat, castration provokes depression- and anxiety-like symptoms. As a consequence, castrated rats avoid in the experiment done in this study exposed bright fields in the centre of the test cages and remain within the dark outer fields. The more the animals explore the bright inner fields, the less active and therefore less "anxious" and "depressive" they are. The most active animals are those having been continuously substituted by oestradiol implants after castration leading to stable physiological high-normal serum levels (black column). The most passive and therefore "depressive" and "anxious" are the castrated animals never substituted (white column). This shows that oestrogens override the inhibition induced by castration [46]

our own data, Fig. 14.3; [16, 67]) had shown that efficacy of transdermal oestradiol was at least equal to the one of peroral oestrogens [16, 53, 56, 59, 61, 64, 67] (Fig. 14.3). Inversely, current use of oestrogens decreases the risk for the occurrence of depressive symptoms significantly compared to non-users (OR 0.7; CI 0.5–0.9; $p = 0.01$) [68].

In contrast to these positive results, a 4-month pilot study, a RCT, found no effect on mood for MHT (CEE 0.625 mg/day + continuous medroxyprogesteronacetate = MPA) in women aged 45–55 years [69]. These data are congruent with an earlier observation showing in a placebo-controlled trial that MPA neutralizes the positive effect of CEE alone on depressive symptoms in postmenopausal women [70]. Already in the 1990s, it has been pointed out that some progestins such as MPA block the favourable mental-tonic effect of oestrogens on mood [52, 71]. No such negative impact is known for micronized progesterone, dydrogesterone or NETA.

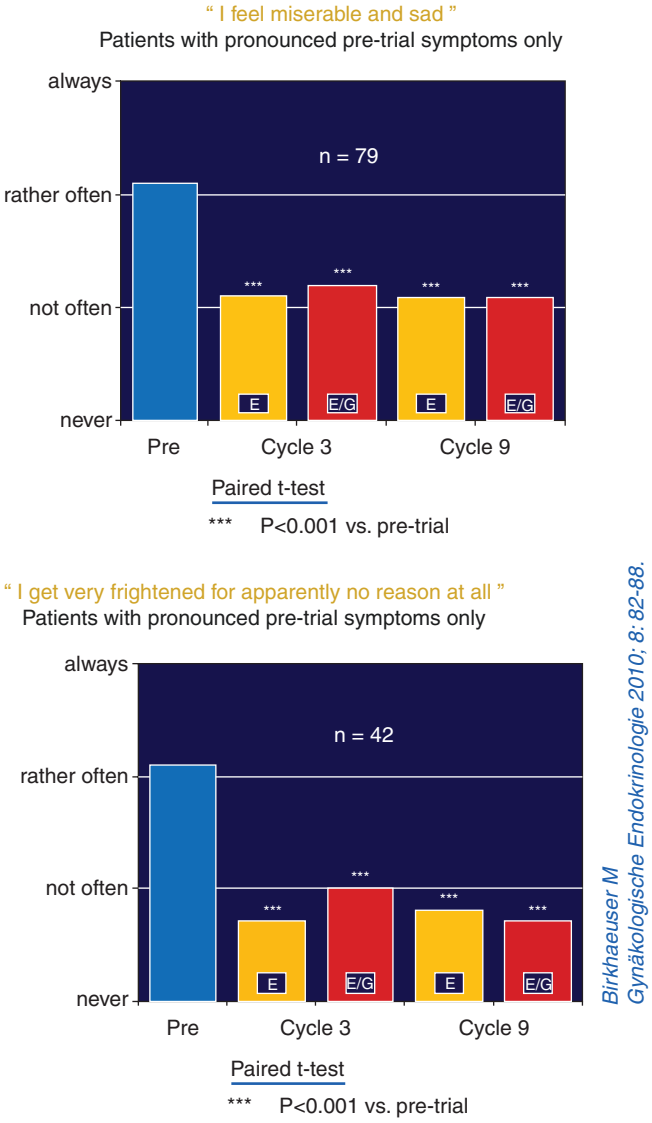

Fig. 14.3 Efficacy of transdermal MHT for the treatment of depressive symptoms. A prospective study over 12 months in 367 symptomatic postmenopausal women has demonstrated that transdermal MHT is also effective in treating depression in the peri- and early postmenopause [16, 67]. Transdermal MHT consisted in the sequential administration of a E2-alone patch for 2 weeks (50 µg/day), followed by a combined E2/NETA-patch (E2 50 µg/day + NETA 250 µg/day) for the next 2 weeks over 12 months. The two questions presented on this figure have been "I feel miserable and sad" and "I get very frightened for apparently no reason at all". The symptoms of depression and anxiety decreased significantly at month 3 and stayed significantly lower until the end of the treatment compared to baseline (paired *t*-test; $p < 0.001$ vs. pre-trial) [67]

Table 14.3 Effects of oestrogens on serotonergic and noradrenergic neurons

Effects on serotonergic system	Effects on noradrenergic system
Modulates serotonin neuronal firing	Increases available norepinephrine
Increases serotonin synthesis	Increases norepinephrine synthesis
Decreases serotonin breakdown	Reduces norepinephrine turnover rate
Affects serotonin receptor subtypes	Alters adrenergic receptor gene expression
Desensitizes serotonin autoreceptors	

The combination of oestrogens with androgens is increasing the effect on mood of oestrogens alone [52, 54]. The combination of oestrogens and androgens may be helpful in women suffering from depressive symptoms together with a loss of libido.

No positive effect on depressive mood has been observed in trials where mostly asymptomatic women in their late postmenopause had been included [58, 72, 73].

Oestrogens may potentiate the effect of antidepressants [74–76]. Oestrogens modulate the metabolism of noradrenalin and serotonin in a similar way to many antidepressants, resulting in an increase of the adrenergic and serotonergic activity (Table 14.3). In a double-blind RCT in 358 depressed postmenopausal women, 72 received fluoxetine (20 mg/day) plus CEE, and 286 received CEE alone, fluoxetine alone or placebo. Patients on ERT plus fluoxetine had a substantially greater mean Ham-D percentage improvement than patients on ERT plus placebo (40.1% vs. 17.0%, respectively; $p = 0.015$); fluoxetine-treated patients not on ERT did not show benefit significantly greater than placebo-treated patients not on ERT [75].

Oestrogens may improve cognition [54, 69, 77], but the data are still controversial although a correlation has been shown between low endogenous oestradiol levels and the decrease in cognitive ability [78].

Conclusion

Women of all ages have a higher incidence of depression than men. The lifetime incidence of endogenous depression in women is twice the incidence in males. Through their brain receptors, oestrogens modulate the metabolism of serotonin and noradrenalin, as do antidepressants, and influence therefore mood, mental function and cognition. In women having a particular vulnerability, the menopausal transition might trigger a depressive disorder. Similarly, the dramatic fall of sexual steroids after delivery including oestrogens is followed in some women by a postpartal depression.

Endocrine instable life phases such as puberty, pregnancy, postpartum and menopausal transition correspond to “windows of increased vulnerability”. As for cardiovascular diseases, these conditions might be a “window of opportunity” for oestrogen use. In the menopausal transition, there is suggestive evidence from clinical trials that oestrogen therapy improves mood, anxiety and depression, particularly in women suffering from vasomotor symptoms. Therefore, in peri- and early postmenopausal women with climacteric vasomotor symptoms, oestrogens might be considered as a first-line treatment for depressive symptoms. There is week evidence that depressed elderly postmenopausal women may profit from a combined administration of oestrogens and fluoxetine. However, SSRIs or

antidepressants remain the first-line treatment of depressive mood in asymptomatic women in their late postmenopause: oestrogens do not improve depression in the late postmenopause. If oestrogen therapy started early might improve cognition, remains today a controversial issue.

References

1. Benazzi F. Female depression before and after menopause. Psychother Psychosom. 2000;69:280–3.
2. Benazzi F. Gender differences in bipolar II and unipolar depressed outpatients: a 557-case study. Ann Clin Psychiatry. 1999;11:55–9.
3. Garcia-Alvarez R. Epidemiology of depression in Latin America. Psychopathology. 1986;19(Suppl 2):22–5.
4. Weissman MM, Bland R, Joyce RP, Newman S, Wells JE, Wittchen H-U. Sex differences in rates of depression: cross-national perspectives. J Affect Disord. 1993;29:77–84.
5. Ayubi-Moak I, Parry BL: Psychiatric aspects of menopause, in Kornstein SG, Clayton AH Women's mental health: a comprehensive textbook. New York, Guilford, 2002, pp 132–143
6. Tam LW, Stucky V, Hanson RE, Parry BL. Prevalence of depression in menopause: a pilot study. Arch Womens Ment Health. 1999;2:175–81.
7. Parry BL. Perimenopausal depression. Am J Psychiatry. 2008;165:23–7.
8. Lebowitz BD, Pearson JL, Schneider LS, et al. Diagnosis and treatment of depression in late life. Consensus state-ment update. J Am Med Assoc. 1997;278:1186–90.
9. Kraepelin E. Psychiatrie. Ein Lehrbuch für Studierende und Ärzte. 6. Auflage. Leipzig: Barth; 1899.
10. Bleuler M. Psychiatrie und Endokrinologie. Geschichte ihre Beziehung in den letzten 30 Jahren. Acta Psychiatr Scand. 1965;41(3):411–8.
11. Bleuler M. Endokrinologie und Psychiatrie. Schweiz Arch Neurol Psychiatr. 1953;71(1–2):360–71.
12. Weissman MM. The myth of involutional melancholia. JAMA. 1979;242:742–4.
13. Perugi G, Musetti L, Simonini E, Piagentini F, Cassano GB, Akiskal HS. Gender-mediated clinical features of depressive illness. The importance of temperamental difference. Br J Psychiatry. 1990;157:835–41.
14. Osborn M. Depression at the menopause. Br J Hosp Med. 1984;32:128–9.
15. Gath D. The assessment of depression in peri-menopausal women. Maturitas. 1998;20:33–9.
16. Birkhäuser M. Depression und Östrogene. Besteht eine kausale Beziehung? Gynäkologische. Endokrinologie. 2010;8:82–8. https://doi.org/10.1007/s10304-009-0317-6.
17. Clayton AH, Ninan PT. Depression or Menopause? Presentation and management of major depressive disorder in perimenopausal and postmenopausal women. Prim Care Companion. J Clin Psychiatry. 2010;12(1):PCC.08r00747. https://doi.org/10.4088/PCC.08r00747blu.
18. Vesco KK, Haney EM, Humphrey L, et al. lnfluence of menopause on mood: a systematic review of cohort studies. Climacteric. 2007;10(6):448–65.
19. Avis NE, Brambilla D, McKinlay SM, Vass K. A longitudinal analysis of the association between menopause and depression: results from the Massachusetts Women's Health Study. Ann Epidemiol. 1994;4:214–20.
20. Schmidt PJ, Haq N, Rubinow DR. A longitudinal evaluation of the relationship between reproductive status and mood in per-imenopausal women. Am J Psychiatry. 2004;161:2238–44.
21. Daly E, Gray A, Barlow D, McPherson K, Roche M, Vessey M. Measuring the impact of menopausal symptoms on quality of life. Br Med J. 1993;307:836–40.
22. Freeman EW, Sammel MD, Liu L, Gracia CR, Nelson DB, Hollander L. Hormones and menopausal status as predictors of depression in women in transition to menopause. Arch Gen Psychiatry. 2004;61:62–70.

23. Freeman EW, Sammel MD, Lin H, Nelson DB. Associations of hormones and menopausal status with depressed mood in women with no history of depression. Arch Gen Psychiatry. 2006;63:375–82.
24. Harlow BL, Wise LA, Otto MW, Soares CN, Cohen LS. Depression and its influence on reproductive endocrine and menstrual cycle markers associated with perimenopause: the Harvard Study of Moods and Cycles. Arch Gen Psychiatry. 2003;60:29–36.
25. Harlow BL, Cohen LS, Otto MW, Spiegelman D, Cramer DW. Prevalence and pre-dictors of depressive symptoms in older premenopausal women: the Harvard Study of Moods and Cycles. Arch Gen Psychiatry. 1999;56:418–24.
26. Cohen LS, Soares CN, Vitonis AF, Otto MW, Harlow BL. Risk for new onset of depression during the menopausal transition: the Harvard study of moods and cycles. Arch Gen Psychiatry. 2006;63:385–90.
27. Bromberger JT, Matthews KA, Schott LL, et al. Depressive symptoms during the menopausal transition: the Study of Women's Health Across the Nation (SWAN). J Affect Disord. 2007;103(1–3):267–72.
28. Bromberger JT, Kravitz HM. Mood and menopause: findings from the Study of Women's Health Across the Nation (SWAN) over ten years. Obstet Gynecol Clin North Am. 2011;38(3):609–25.
29. Bromberger JT, Schott LL, Kravitz HM, et al. Longitudinal change in reproductive hormones and depressive symptoms across the menopausal transition: results from the Study of Women's Health Across the Nation (SWAN). Arch Gen Psychiatry. 2010;67:598–607.
30. Bromberger JT, Kravitz HM, Chang YF, Cyranowski JM, Brown C, Matthews KA. Major depression during and after the menopausal transition: Study of Women's Health Across the Nation (SWAN). Psychol Med. 2011;41(9):1879–88.
31. Woods NF, Smith-DiJulio K, Percival DB, Tao EY, Mariella A, Mitchell S. Depressed mood during the menopausal transition and early postmenopause: observations from the Seattle Midlife Women's Health Study. Menopause. 2008;15(2):223–32.
32. Dennerstein L, Guthrie JR, Clark M, Lehert P, Henderson VW. A population-based study of depressed mood in middle-aged, Australian-born women. Menopause. 2004;11(5):563–8.
33. Ryan J, Burger H, Szoeke C. A prospective study of the association between endogenous hormones and depressive symptoms in postmenopausal women. Menopause. 2009;16(3):509–17. https://doi.org/10.1097/gme.0b013e31818d635f.
34. Freeman EW, Sammel MD, Boorman DW, Zhang R. Longitudinal pattern of depressive symptoms around natural menopause. JAMA Psychiat. 2014;71(1):36–43.
35. Daly RC, Danaceau MA, Rubinow DR, Schmidt PJ. Concordant restoration of ovarian function and mood in perimenopausal depression. Am J Psychiatry. 2003;160:1842–6.
36. Freeman EW, Sammel MD, Hui Lin H. Temporal associations of hot flashes and depression in the transition to menopause. Menopause. 2009;16:728–34. https://doi.org/10.1097/gme.0b013e3181967e16.
37. Weber MT, Maki PM, McDermott MP. Cognition and mood in perimenopause: a systematic review and meta-analysis. J Steroid Biochem Mol Biol. 2014;142:90–8.
38. Deecher D, Andree TH, Sloan D, Schechter LE. From menarche to menopause: exploring the underlying biology of depression in women experiencing hormonal changes. Psychoneuroendocrinology. 2008;33:3–17.
39. Rocca WR, Grossardt BR, Geda YE. Long-term risk of depressive and anxiety symptoms after early bilateral oophorectomy. Menopause. 2008;15:1050–9.
40. Juang KD, Wang SJ, Lu SR, Lee SJ, Fuh JL. Hot flashes are associated with psychological symptoms of anxiety and depression in peri- and post- but not premenopausal women. Maturitas. 2005;52:119–26.
41. Reed SD, Ludman EJ, Newon KM, et al. Depressive symptoms and menopausal burden in the midlife. Maturitas. 2009;62(3):306–16.
42. Terauchi M, Hiramitsu S, Akiyoshi M, et al. Associations among depression, anxiety and somatic symptoms in peri- and postmenopausal women. J Obstet Gynaecol Res. 2013;39:1007–13.
43. Brown JP, Gallicchio L, Flaws JA, Tracy JC. Relations among menopausal symptoms, sleep disturbance and depressive symptoms in midlife. Maturitas. 2009;62:184–9.

44. Worsley R, Bell R, Kulkarni J, Davis SR. The association between vasomotor symptoms and depression during perimenopause: a systematic review. Maturitas. 2014;77:111–7.
45. Craig MC, Murphy DG. Estrogen: effects on normal brain function and neuropsychiatric disorders. Climacteric. 2007;10(Suppl 2):97–104.
46. Walf AA, Paris JJ, Frye CA. Chronic estradiol replacement to aged female rats reduces anxiety-like and depression-like behavior and enhances cognitive performance. Psychoneuroendocrinology. 2009;34:909–16.
47. Walf AA, Frye CA. Effects of two estradiol regimens on anxiety and depressive behaviors and trophic effects in peripheral tissues in a rodent model. Gend Med. 2009;6(1):300–11.
48. Romano-Torres M, Fernández-Guasti A. Estradiol valerate elicits antidepressant-like effects in middle-aged female rats under chronic mild stress. Behav Pharmacol. 2010;21:104–11.
49. Récamier-Carballo S, Estrada-Camarena E, Reyes R, Fernández-Guasti A. Synergistic effect of estradiol and fluoxetine in young adult and middle-aged female rats in two models of experimental depression. Behav Brain Res. 2012;233:351–8.
50. Morrison JH, Brinton RD, Schmidt PJ, et al. Symposium: estrogen, menopause, and the aging brain: how basic neuroscience can inform hormone therapy in women. J Neurosci. 2006;26(41):10332–48.
51. Sanchez RL, Reddy AP, Bethea CL. Ovarian steroid regulation of the midbrain CRF and UCN systems in Macaques. Neuroscience. 2010;171:893–909.
52. Zweifel JE, O'Brien WH. A meta-analysis of the effect of hormone replacement therapy upon depressed mood (published erratum appears in Psychoneuroendocrinology 1997;22:655). Psychoneuroendocrinology. 1997;22:189–212.
53. Schmidt PJ, Rubinow DR. Sex hormones and mood in the perimenopause. Ann N Y Acad Sci. 2009;1179:70–85.
54. Sherwin BB. Affective changes with estrogen and androgen replacement therapy in surgically menopausal women. J Affect Disord. 1988;14:177–87.
55. Soares CN, Prouty J, Born L, Steiner M. Treatment of menopause-related mood disturbances. CNS Spectr. 2005;10:489–97.
56. de Lignières B, Vincens M. Differential effects of exogenous oestradiol and progesterone on mood in post-menopausal women: individual dose/effect relationship. Maturitas. 1982;4:67–72.
57. Wisniewski AB, Migeon CJ, Meyer-Bahlburg HFL, et al. Complete androgen and insensitivity syndrom: long-term medical, surgical, and psychosexual outcome. J Clin Endocrinol Metab. 2000;85:2664–9.
58. Hlatky MA, Boothroyd D, Vittinghoff E, et al. Quality-of-life and depressive symptoms in postmenopausal women after receiving hormone therapy: results from the Heart and Estrogen/Progestin Replacement Study (HERS) trial. JAMA. 2002;287:591–7.
59. Cohen LS, Soares CN, Poitras JR, Prouty J, Alexander AB, Shifren JL. Short-term use of estradiol for depression in perimenopausal and postmenopausal women: a preliminary report. Am J Psychiatry. 2003;160:1519–22.
60. Pearlstein T, Rosen K, Stone AB. Mood disorders and menopause. Endocrinol Metab Clin North Am. 1997;26:279–94.
61. Soares CN, Poitras JR, Prouty J. Effect of reproductive hormones and selective estrogen receptor modulators on mood during menopause. Drugs Aging. 2003;20:85–100.
62. Stahl SM. Why drugs and hormones may interact in psychiatric disorders. J Clin Psychiatry. 2001;62:225–6.
63. Studd J. Depression and the menopause. Oestrogens improve symptoms in some middle aged women. BMJ. 1997;314:977–8.
64. Soares CN, Almeida OP, Joffe H, Cohen LS. Efficacy of estradiol for the treatment of depressive disorders in perimenopausal women: a double-blind, randomized, placebocontrolled trial. Arch Gen Psychiatry. 2001;58:529–34.
65. Schmidt PJ, Nieman L, Danaceau MA, et al. Estrogen replacement in perimenopause-related depression: a preliminary report. Am J Obstet Gynecol. 2000;183:414–20.

66. Gleason CE, Dowling NM, Wharton W, et al. Effects of hormone therapy on cognition and mood in recently postmenopausal women: findings from the randomized, controlled KEEPS-Cognitive and Affective Study. PLoS Med. 2015;12:e1001833206.
67. Birkhäuser M. Long-term substitution with combined transdermal hormone therapy: efficacy in the control of postmenopausal somatic and psychological symptoms. In: Abstract Volume Sixth International Congress on the Menopause, Bangkok, Vol 29; 1990. p. 10–2.
68. Whooley MA, Deborah Grady D, Caule JA. Postmenopausal estrogen therapy and depressive symptoms in older women. J Gen Intern Med. 2000;15:535–41.
69. Maki PM, Gast MJ, Vieweg A, Burriss SW, Yaffe K. Hormone therapy in menopausal women with cognitive complaints: a randomized, double-blind trial. Neurology. 2007;69:1322–30.
70. Sherwin BB. The impact of different doses of estrogen and progestin on mood and sexual behavior in postmenopausal women. J Clin Endocrinol Metab. 1991;72:336–43.
71. Hunter MS. Emotional well-being, sexual behaviour and hormone replacement therapy. Maturitas. 1990;12:299–314.
72. Hays J, Ockene JK, Brunner RL, et al. Effects of estrogen plus progestin on health-related quality of life. N Engl J Med. 2003;348:1839–54.
73. Morrison MF, Kallan MJ, Ten Have T, et al. Lack of efficacy of estradiol for depression in postmenopausal women: a randomized, controlled trial. Biol Psychiatry. 2004;55:406–12.
74. Morgan ML, Cook IA, Rapkin AJ, Leuchter AF. Estrogen augmentation of antidepressants in perimenopausal depression: a pilot study. J Clin Psychiatry. 2005;66:774–80.
75. Schneider LS, Small GW, Hamilton SH, Bystritsky A, Nemeroff CB, Meyers BS. Estrogen replacement and response to fluoxetine in a multicenter geriatric depression trial. Fluoxetine Collaborative Study Group. Am J Geriatr Psychiatry. 1997;5:97–106.
76. Amsterdam J, Garcia-Espana F, Fawcett J, et al. Fluoxetine efficacy in menopausal women with and without estrogen replacement. J Affect Disord. 1999;55:11–7.
77. Henderson VW. Gonadal hormones and cognitive aging: a midlife perspective. Womens Health. 2011;7:81–93.
78. Yaffe K, Lui L-Y, Grady D, et al. Cognitive decline in women in relation to non-protein-bound estradiol concentrations. Lancet. 2000;356:708–12.

Menopause and Ageing

15

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15.1 Menopause and Ageing

Menopause generally occurs around 51 years of age, with an age range varying between 40 and 60 years [1]. The World Health Organization (WHO) defines menopause as the permanent cessation of menses due to the loss of ovarian follicular activity. The final menstrual period is retrospectively assigned after 12 months of amenorrhoea, in the absence of other pathological or physiological causes [2]. Women complain about new and unexpected annoying symptoms and their bodies are at the mercy of intense hormonal and physical changes. The clinical signs associated with this period are vasomotor symptoms, sleep disruption, mood alteration, urogenital complaints and sexual dysfunction. Among these, the most frequent are vasomotor symptoms that afflict 75% of women and usually disappear within 1–5 years.

Within reproductive life, oestrogen production by the ovaries is tightly controlled by negative feedback mechanisms with limited influence exerted by extra-gonadal synthesis. However, approximately 90% of circulating oestrogen is lost at the time of ovarian failure, during the fourth and fifth decade of life, prompting extra-glandular formation of oestrogens by aromatase expression within adipose and skin to

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become the predominant source of sex steroid generation, with additional contribution from the adrenals.

Loss of sex hormones during ageing contributes to changes in body mass, musculoskeletal integrity, sexual dysfunction and long-term risks of health and disease. Interestingly, decreased libido in postmenopausal women may be attributed to reductions in testosterone [3]. The literature concerning the role of sex hormone changes in a raft of additional cognitive changes within ageing men and postmenopausal women is more disparate, with inconsistent studies within both males and females. Persistently elevated FSH within postmenopausal women has, however, been linked to Alzheimer's disease [4].

Moreover, the years around the menopause are associated with weight gain, increased central adiposity and waist circumference. The annual rate of weight gain estimated about 0.5 kg or more is independent of menopausal status, while it is consistently related to chronological ageing. In contrast, adverse changes in either body fat distribution, from a more gynoid pattern to a more android pattern, or body composition with increased fat mass and decreased lean mass may be due to hormonal changes occurring during the menopausal transition. The deleterious change in body fat distribution consists in an increase in abdominal adiposity due to the selective accumulation of fat in the intra-abdominal compartment independently of age and total body fat mass. Specifically, increased abdominal adiposity is known to increase the risk of developing cardiovascular disease.

Data from the Women's Healthy Lifestyle Project provide clear evidence that weight gain and increased waist circumference, along with elevations in lipid levels and other CHD risk factors, are preventable through use of lifestyle intervention in healthy menopausal-aged women. In fact although these changes are inevitable with age and menopause, physical activity may attenuate the impact of both events. Thus, weight gain prevention should be recognized as an important health goal for women before they approach menopause, and women should make regular physical activity.

Perhaps the most recognized change resulting from the menopause is that of osteoporosis. This appears to be driven by reductions in metabolically active trabecular bone occurring due to uncoupling of the bone remodelling cycle secondary to oestrogen loss and leads to a marked increase in fracture risk among elderly women [5].

The significant increase in the risk of heart disease in women undergoing the menopause, coupled with the relatively reduced burden of heart disease among premenopausal women when compared to age-matched males, indicates a cardioprotective role for oestrogen which is lost in later age. Despite these many changes, HRT has met with considerable controversy in females. Its use for the primary or secondary prevention of cardiovascular disease has, for instance, been warranted both ineffective and harmful, at least in its combined continuous form [6].

The second endocrine system whose function declines and that demonstrates an age-related change is the adrenal gland. After reaching the intra-individual maximum levels during the third decade of life, dehydroepiandrosterone sulphate (DHEAS) steadily decline down to 10–20% of its maximum level by around the

age of 70 years [7]. This decline has been termed adrenopause, in spite of the fact that cortisol secretion does not significantly change with age. There is a clear sex difference in DHEAS levels with lower concentrations in women compared with men [8]. Adrenopause is independent of menopause and occurs in both sexes as a gradual process at similar age. The reduced DHEAS/cortisol ratio associated with ageing might contribute to the prevailing catabolic action of cortisol, although the available evidences do not justify dehydroepiandrosterone supplementation in all elderly subjects [9].

15.2 Physical Activity, Diet and Lifestyle

All women at midlife should be encouraged to maintain or achieve a normal body weight, be physically active, adopt a healthy diet, limit alcohol consumption and not smoke. Some women find that avoidance of spicy food, hot drinks and alcohol lessens their vasomotor symptoms. Obesity is associated with a greater likelihood of vasomotor symptoms, although women who are overweight (BMI from 25 to <30 kg m^2), as opposed to obese (BMI $\geq$ 30 kg m^2), are more likely to have severe symptoms [10]. For obese women, weight loss may lessen vasomotor symptoms, as well as reduce the risks of cardiovascular disease; diabetes; urinary incontinence; breast, pancreatic and endometrial cancer; and dementia [11]. Increasing physical activity has been recommended to alleviate vasomotor symptoms; however, this may exacerbate symptoms in women with a low level of fitness. Furthermore, a recent prospective study has shown no benefit of exercise in reducing these symptoms. Regular physical activity is to be encouraged to maintain muscle mass and balance, thereby reducing the risk of fall and fracture. A fairly high and consistent degree of impact exercise seems to be required to improve bone density.

15.3 Hormone Therapy

Hormone replacement therapy (HRT) was exposed to a dramatic impact by the publications of the Women's Health Initiative (WHI) trial in 2002 and the Million Women Study (MWS) in 2003, affecting women's attitudes and physicians' prescribing practice throughout the world. This caused a general decrease of hormone therapy (HT) use. Publications that raised a word of caution about discontinuing use of HT were disregarded [12]. Now, over 15 years after the first publication, there appears to be a revival of HT, which seems to involve publications in leading medical journals [13] and outstanding worldwide organizations such as the International Menopause Society and the Endocrine Society.

Oestrogen deficiency is the principal pathophysiological mechanism that underlies menopausal symptoms, and various oestrogen formulations are prescribed as menopausal hormone therapy, which remains the most effective therapeutic option available (Table 15.1). The addition of progesterone aims to protect against the consequences of systemic therapy with oestrogen only in women with intact uteri [14]:

Table 15.1 Prescription therapy options for management of menopausal symptoms [23]

Treatment option	Benefits	Risks	Patient-specific considerations
Hormone therapy			
Systemic oestrogen alone*	• Symptom relief • Fracture risk reduction • Osteoporosis prevention • Improved QoL	• VTE • Stroke • CVD • Breast cancer (not seen in large clinical trials) • Gallstones	• Oestrogen-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 oestrogen and progestogen*	• Symptom relief • Fracture risk reduction • Osteoporosis prevention • Improved QoL • Colon cancer risk reduction	• VTE • Stroke • CVD • Breast cancer • Gallstones	• Oestrogen-related risk of stroke, VTE and CVD are 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 oestrogen 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 oestrogen 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 oestrogen • 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 oestrogen	Effective against genitourinary syndrome of menopause	Negligible	Significant systemic absorption can transiently occur with excessive use of vaginal oestrogen 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 oestrogen	Approved for management of moderate-to-severe dyspareunia due to vulvovaginal atrophy in the United States and Europe
TSEC (combination of conjugated equine oestrogen and BZA (SERM))*	Symptom relief	Similar risk profile to oestrogen 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 oestrogens and BZA is effective for fracture risk reduction
Tibolone (synthetic steroid with oestrogenic, 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

*Caution advised when considering use in an older postmenopausal woman with existing comorbidities who is remote from onset of menopause

namely, endometrial pathologies, including hyperplasia and cancer. 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.

In the systemic circulation, oestradiol and oestrone are partly bound to sex hormone-binding globulin (SHBG), as well as to albumin, as is testosterone. Increasing or decreasing SHBG levels will affect the amount of unbound oestrogen and testosterone in the circulation [15]. Oral oestrogen therapy increases SHBG synthesis in the liver through the first-pass effect; by contrast, standard-dose transdermal oestrogen does not increase SHBG synthesis. In some women, oral oestrogen therapy results in very high SHBG levels, with a reduction in unbound hormone. This potentially leads to loss of efficacy of the administered oestrogen and/or iatrogenic testosterone deficiency.

For those who require pharmacological therapies, average dose hormone therapy (HT) is the most effective treatment for vasomotor symptoms (VMS) with reductions in both frequency and severity in the order of 75% [16], and HT may improve quality of life in symptomatic women [17]. 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 oestrogens. Transdermal oestradiol does not seem to increase the risk of venous thromboembolic events. Evidence show that transdermal oestrogen ($\leq$50 μg) is associated with a lower risk of deep vein thrombosis, stroke and myocardial infarction compared to oral therapy [18] 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 oestrogen, transdermal oestradiol does not increase the risk of gallbladder disease [19, 20].

Genitourinary syndrome is a relatively new terminology describing vulvovaginal changes at menopause, as well as urinary symptoms of frequency, urgency, nocturia, 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 oestrogen therapy [21]. Oestrogen therapy restores normal vaginal flora, lowers the pH and thickens and revascularizes the vaginal lining. The number of superficial epithelial cells is increased, and symptoms of atrophy are alleviated. Importantly, low-dose vaginal oestrogen improves vaginal atrophy without causing proliferation of the endometrium. Given the documented efficacy and proven safety, vaginal oestrogen is the first-line approach to treat symptoms of vaginal atrophy in the majority of women: vaginal oestrogen is effective, and while systemic absorption does occur, it does not induce endometrial hyperplasia. Concerns about systemic absorption mean that vaginal oestrogens may be avoided in breast cancer patients taking aromatase inhibitors. 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 [22].

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. Of clinical importance is that stopping HT commonly leads to recurrent VMS and may also trigger new onset VMS.

Initiation of hormone therapy 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. Transdermal oestrogen 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 nonhormonal therapies. In general, commencement of hormone therapy is not recommended for women who are aged >60 years [23].

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 oestrogen with a progestogen is needed. Progesterone, which is naturally produced in women 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 antioestrogenic, action is common. The antiandrogenic effect is relevant for dialogist (DNG) and DRSP and minor for nomegestrol acetate (NOMAC). None of them have a glucocorticoid effect. DRSP is different due to its strong antimineralocorticoid action and has a favourable effect on blood pressure. In addition, these progestogens do not interfere with the positive effect of oestrogens on lipid and carbohydrate metabolism, do not augment haemostasis 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 [24]. The inclusion of progesterone appears to increase breast cancer risk, but progestogens are still indicated to prevent endometrial hyperplasia and cancer risk [22].

15.3.1 Tibolone

Tibolone is a synthetic steroid that is rapidly converted to two metabolites with oestrogenic activity and to a third metabolite characterized by a mixed progestogenic/androgenic activity. Tibolone controls hot flushes, sweating and 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 [25] (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 oestrogen-metabolizing breast enzymes [26]. 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.

15.4 Choosing the Emerging Therapy

In the past 2 years, two new pharmaceutical preparations were approved in the United States and Europe for the treatment of menopausal symptoms. An oral selective oestrogen receptor modulator (SERM), ospemifene, has been approved for the treatment of moderate-to-severe pain during intercourse associated with vulvovaginal atrophy [27], and a tissue-specific SERM-oestrogen complex (a combination of oral conjugated equine oestrogen and bazedoxifene (a SERM)) has been approved for the management of moderate-to-severe vasomotor symptoms in women with an intact uterus [28]. Tissue selectivity is achieved through the concurrent use of oestrogen and a SERM, which replaces a progestogen and selectively blocks the undesirable actions of oestrogen. In the case of conjugated equine oestrogen-bazedoxifene, the proliferative effects of oestrogen are blocked in the uterus and possibly also the breast, whereas the bone-sparing actions of oestrogen are preserved. 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 are unclear. This strategy may benefit a certain subset of women, such as those with surgically induced menopause, who have persistent sexual symptoms despite optimization of menopausal hormone therapies (Fig. 15.1).

15.4.1 TSEC (Combination of Conjugated Equine Oestrogen and BZA (SERM))

The rationale for combining oestrogens with a SERM is to retain beneficial effects of oestrogens on VMS, VVA and bone while incorporating the antioestrogenic effects of the SERM on the breast and endometrium to improve the overall safety profile. It was recently demonstrated that CE 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

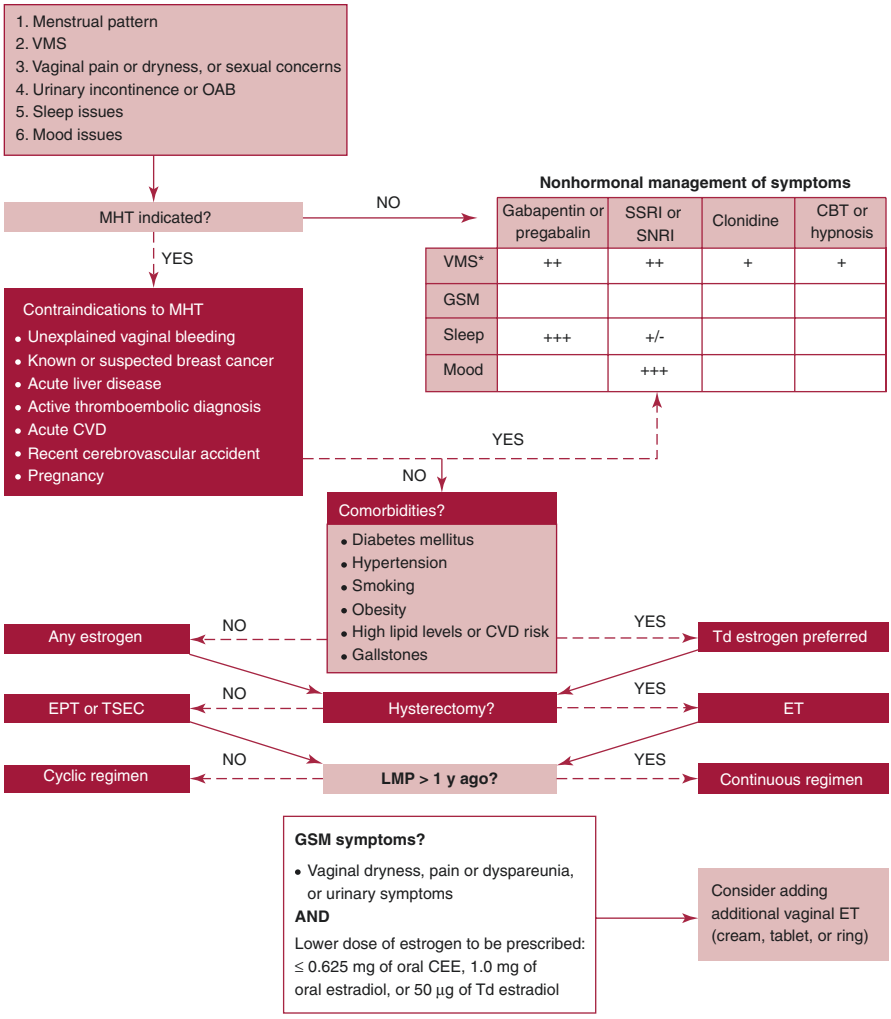

Fig. 15.1 Evidence-based algorithm for management of menopausal symptoms. *CBT* cognitive-behavioural therapy, *CEE* conjugated equine oestrogen, *CVD* cardiovascular disease, *EPT* oestrogen-progestogen therapy, *ET* oestrogen therapy, *GSM* genitourinary syndrome of menopause, *LMP* last menstrual period, *MHT* menopausal hormone therapy, *MQ6* Menopause Quick 6, *OAB* overactive bladder, *SNRI* serotonin-norepinephrine reuptake inhibitor, *SSRI* selective serotonin reuptake inhibitor, *Td* transdermal, *TSEC* tissue-selective oestrogen complex, *VMS* vasomotor symptoms. Modified from [38]

more like the pure antioestrogen fulvestrant than like other SERMs in these tissues. BZA's antioestrogenic effects in endometrial tissue eliminate the need to include a progestin when combined with oestrogens in women with a uterus [29].

Five Phase III, randomized, double-blind Selective estrogens, Menopause, And Response to Therapy (SMART) trials established the efficacy and safety of CE/

BZA use for up to 2 years. CE/BZA is generally well tolerated. Among women treated with CE/BZA in the SMART studies, rates of ischemic stroke, cardiovascular events and VTE were low and similar to placebo.

The tissue-selective oestrogen complex (combination of 0.45 mg of oral conjugated equine oestrogen and 20 mg bazedoxifene (a SERM)) has been approved for the management of moderate-to-severe VMS in the United States and Europe [30].

15.4.2 Ospemifene (SERM)

The recent introduction of ospemifene, an orally available selective oestrogen receptor modulator with the indication of prevention and treatment of VVA, may represent a new option for those patients who are intolerant to long-term use of vaginal oestrogens [31]. It has minimal endometrial stimulation, though it does cause flushes in some women, but there is insufficient evidence to guide the choice of nonhormonal agents for vulvovaginal symptoms.

15.4.3 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" [32]. DHEA serves as a precursor for oestrogens and androgens from foetal 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, such as gamma aminobutyric acid type A, *N*-methyl-D-aspartate and sigma-1 receptors. In addition, DHEA or DHEA sulphate (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. Binding of DHEA to the cell membrane is coupled to recruitment of G proteins such as G{alpha}i2 and G{alpha}i3 that mediate the rapid activation of intracellular signalling cascades.

So, DHEAS represent the most abundant sex steroid in plasma in humans (more than 1000 times higher than oestradiol 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 [33].

Higher endogenous DHEAS levels are independently and favourably associated with executive function, concentration and working memory [34].

Several studies had previously demonstrated that 1-year treatment [35, 36], using administration of 10 mg DHEA daily in symptomatic postmenopausal women with lower (fifth 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 ageing as well as their relation with climacteric symptoms [37].

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 oestrogen 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.

A Phase III multicentre Canadian trial of DHEA/acolbifene in postmenopausal women with moderate-to-severe hot flushes has been completed, but data have not yet been reported. The primary end points of this study were change from baseline to week 12 in frequency and severity of moderate-to-severe hot flushes. Secondary end points consisted of change from baseline to week 12 in VVA (i.e. superficial/parabasal cell counts, pH, atrophy symptoms), as well as sexual function and quality of life (based on questionnaires). Safety/tolerability is also a secondary end point.

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 [30].

Conclusion

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. Based on the WHI, the greatest risk appears to be associated with combined oestrogen-progestin therapy; therefore, recent strategies have focused on eliminating the need for progestins either through use of topical oestrogens without a progestin for VVA or by combining oestrogen(s) or DHEA with potentially safer options (e.g. micronized progesterone, SERMs) to reduce endometrial stimulation.

Menopausal hormone therapy remains the most effective treatment of VMS and is also indicated for GSM (previously called *vulvovaginal atrophy*) and bone protection. With no fixed duration of treatment, the guidelines now state

that MHT should be individualized to account for each patient's unique risk-benefit profile.

The ultimate goal is to get closer to the profile of the ideal menopausal therapy—that is, to relieve bothersome menopausal symptoms and reduce the risk of osteoporosis and cardiovascular disease, without increasing the risk of endometrial or breast cancer.

References

1. Treloar AE. Menstrual cyclicity and the pre-menopause. Maturitas. 1981;3(3–4):249–64.
2. World Health Organization. Research on the menopause in the 1990s. In: Report of a WHO Scientific Group, World Health Organization Technical Report Series; 1996.
3. El-Sakka AI, Hassoba HM. Age related testosterone depletion in patients with erectile dysfunction. J Urol. 2006;176:2589–93.
4. Janicki SC, Schupf N. Hormonal influences on cognition and risk for Alzheimer's disease. Curr Neurol Neurosci Rep. 2010;10:359–66.
5. Wells G, Tugwell P, Shea B, Guyatt G, Peterson J, Zytaruk N, Robinson V, Henry D, O'Connell D, Cranny A, Osteoporosis Methodology Group and The Osteoporosis Research Advisory Group. Meta-analyses of therapies for postmenopausal osteoporosis. Meta-analysis of the efficacy of hormone replacement therapy in treating and preventing osteoporosis in postmenopausal. Endocr Rev. 2002;23:529–39.
6. Marioribanks J, Farquhar C, Roberts H, Lethaby A. Long term hormone therapy for perimenopausal and postmenopausal women. Cochrane Database Syst Rev. 2012;(7):CD004143.
7. Orentreich N, Brind JL, Vogelman JH, Andres R, Baldwin H. Long-term longitudinal measurements of plasma dehydroepiandrosterone sulfate in normal men. J Clin Endocrinol Metab. 1992;75(4):1002–4.
8. Orentreich N, Brind JL, Rizer RL, Vogelman JH. Age changes and sex differences in serum dehydroepiandrosterone sulfate concentrations throughout adulthood. J Clin Endocrinol Metab. 1984;59(3):551–5.
9. Arlt W. Dehydroepiandrosterone and ageing. Best Pract Res Clin Endocrinol Metab. 2004;18:363–80.
10. Gartoulla P, Worseley R, Bell RJ, Davis SR. Moderate-severe vasomotor and sexual symptoms remain problematic for 60-65 years old women. Menopause. 2015;22(7):694–701.
11. Davis SR, et al. Understanding weight gain at menopause. Climacteric. 2012;15:419–29.
12. 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.
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 Intern Med. 2012;157:104–10.
14. 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.
15. Dunn JF, Nisula BC, Rodboard D. Transport of steroid hormones. Binding of 21 endogenous steroids to both testosterone-binding globulin and corticosteroid-steroid binding globulin in human plasma. J ClinEndocrinol Metab. 1981;53:58–68.
16. MacLennan AH, Broadbent JL, Lester S, Moore V. Oestrogen and combined oestrogen/progestogen therapy versus placebo for hot flushes. Cochrane Database Syst Rev. 2004;(4):CD002978.
17. Welton AJ, Vickers MR, Kim J, Ford D, Lawton B, MacLennan AH, WISDOMteam, et al. Health related quality of life after combined hormone replacement therapy: randomised controlled trial. BMJ. 2008;337:a1190.

18. The North American Menopause Society. The 2012 Hormone Therapy Position Statement of the North American Menopause Society. Menopause. 2012;19:257–71.
19. Olie V, et al. Hormone therapy and recurrence of venous thromboembolism among postmenopausal women. Menopause. 2011;18:488–93.
20. Hoibraaten E, et al. 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.
21. Santen RJ. Vaginal administration of estradiol: effects of dose, preparation and timing on plasma estradiol levels. Climacteric. 2014;17:1–14.
22. Roberts H, Hickey M, Lethaby A. Hormone therapy in postmenopausal women and risk of endometrial hyperplasia Cochrane review summary. Maturitas. 2014;77:4–6.
23. Davis SR, Lambrinoudaki I, Lumsden M, Mishra GD, Pal L, Rees M, Santoro N, Simoncini T. Menopause. Nat Rev Dis Primers. 2015;1:15004.
24. Schindler AE. The "newer" progestogens and postmenopausal hormone therapy (HRT). J Steroid Biochem Mol Biol. 2014;142:48–51.
25. 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.
26. 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.
27. 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, placebo-controlled trial. Climacteric. 2015;18:226–32.
28. Pinkerton JV, et al. 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;23:18–28.
29. 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.
30. Genazzani AR, Komm BS, Pickar JH. Emerging hormonal treatments for menopausal symptoms. Expert Opin Emerg Drugs. 2015;20(1):31–46.
31. Palacios S, Castelo-Branco C, Currie H, et al. Update on management of genitourinary syndrome of menopause: a practical guide. Maturitas. 2015;82:308–13.
32. 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.
33. 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.
34. 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;93:801–8.
35. Genazzani AR, Stomati M, Valentino V, et al. Effect of 1-year, low-dose DHEA therapy on climacteric symptoms and female sexuality. Climacteric. 2011;14:661–8.
36. Pluchino N, Ninni F, Stomati M, et al. 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.
37. Pluchino AR, Genazzani N. DHEA replacement for postmenopausal women: have we been looking in the right direction? Climacteric. 2015;18:1–3.
38. Goldstein S. An efficient tool for the primary care management of menopause. Can Fam Physician. 2017;63:295–8.

Reproductive Hormones and Dementia

16

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Dementia is an entity that has been thought of as a disease but is now understood as the outcome of a number of diseases:

> Dementia is a broad category of brain diseases that cause a long term and often gradual decrease in the ability to think and remember that is great enough to affect a person's daily functioning…A dementia diagnosis requires a change from a person's usual mental functioning and a greater decline than one would expect (to be) due to aging….The most common type of dementia is Alzheimer's disease (AD), which makes up 50–70% of cases. Other common types include vascular dementia (VD) (25%), Lewy body dementia (15%), and frontotemporal dementia…. A small proportion of cases run in families. In the DSM 5, dementia was reclassified as a neurocognitive disorder, with various degrees of severity. Diagnosis is usually based on history of the illness and cognitive testing with medical imaging and blood work used to rule out other possible causes. The mini mental state examination is one commonly used cognitive test. (Wikipedia, 2017)

Dementia is increasingly common as the population ages. In the past, the two most common causes of dementia, AD and VD, were treated as occurring independently; however, it has become increasingly clear that inflammatory changes associated with AD and vascular changes associated with VD are commonly found in the same individual [1]. Often, both are found in aging individuals who show signs of diminished cognitive function, emotional disruption, failure of memory, and dysautonomia, which make evaluation of cause and effect difficult. These signs and symptoms may accompany dementia, and it may not be possible clinically to clearly

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Fig. 16.1 Dementia has many causes; it is a final common clinical pathway that, itself, may not reveal the underlying cause

separate AD from VD. Therefore, without specific testing [2], it may not be possible to separate the effects of various agents on the genesis, prevention, or rate of AD- vs. VD-related dementia. Since they are caused by different processes and can have unrelated pathological and clinical progress, research to distinguish the genesis, prevention, progress, and treatment of multi-causal dementia has been slow.

Despite their availability, until recently the use of the imaging techniques that could reveal the onset and course of their underlying processes was either not applied or only performed to confirm a diagnosis rather than to reveal contributing factors to the clinical diagnosis, dementia; see Fig. 16.1. This has seriously hindered the understanding of dementia and its contributing factors and attempts at unraveling its antecedents. For example, the diagnosis and treatment of patients may be delayed by failure to separate organic from functional causes of dementia. Recently, the role of inflammation has been established in the development of organic dementias, such as AD and VD [5, 6]. The lack of determination of the underlying cause of dementia is a particular problem for interpreting published studies that are retrospective and depend solely upon clinical diagnoses made to facilitate medical care.

Fortunately, contemporary techniques of imaging and biomarker accumulation that are specific to lesions that lead to dementia have become available [3]. AD markers usually are concentrated in the limbic brain of the temporal lobe and diencephalon, while imaging studies identifying VD show that it can occur throughout the entire brain [3]. Widespread use of these techniques will allow specific diagnoses in clinical care and research situations.

Most cases of dementia are noticed because they involve cognitive dysfunction, dementia being most often associated with cortical disease, especially the temporal lobe (memory, planning, and emotion) and the prefrontal lobe (judgment, planning, higher functions). Brain shrinkage is a common accompaniment to both conditions; but, since nine out of ten brain cells are not neurons [4], the clinical outcomes of shrinkage do not have a linear relationship with the features of dementia (Fig. 16.2).

Degenerative dementia is often presaged by minimal cognitive impairment (MCI); but, both atherogenesis and neurodegeneration may be silent during their development, only being shown by diagnostic testing. With progression of dementia, connections eventually involve vegetative function (diencephalon) that contributes to the patient's demise [7].

The underlying lesions are well defined. Vascular insufficiency/VD is due to atherosclerosis and narrowing of cerebral small vessels, some of which actually may be due to accumulation of amyloid plaque. AD includes the extracellular accumulation of inflammatory insoluble amyloid (ironically, the result of precipitation of the soluble anti-inflammatory protein) and the intracellular

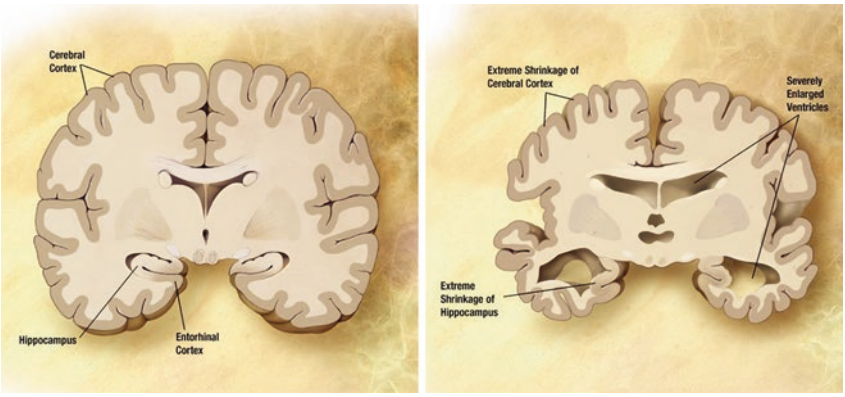

Fig. 16.2 Comparing a mid-frontal section of the normal brain (left) with one of the brain of a subject with AD (right). Imagine the meninges wrapped around the brain on the left, and loosely covering the brain on the right, brain shrinkage. Note that the cortex (gray matter) is formed about smaller gyri, implying that the chief shrinkage is in the non-neuronal white matter, lighter color. In AD, pictured, there is widespread shrinkage, but the most obvious is in the areas of the temporal lobe (lower structures on each image). Shrinkage associated with VD is more uniformly distributed, explaining why significant generalized shrinkage after ischemia and small infarcts may not result in dementia, while a single, strategically placed infarct (stroke) may result in dementia. As discussed in the text, AD and VD can coexist in cases of dementia (Image from Wikipedia, 2005)

accumulation of hyperphosphorylated tau (an activated microtubule assembly protein) [8]. While AD and atherosclerosis may manifest themselves early, and as the result of familial/genetic propensity [6], these are the minority of cases. They may be solitary or combined in the same individual. There is no literature on combined early VD and AD.

There are many practical reasons for healthcare professionals to develop a single descriptor for "dementia." But, the tendency to declare a single diagnosis of the type of dementia rather than "mixed dementia" becomes problematic when one considers the common coexistence of the two underlying diseases in the aging brain. It takes on added significance when considering possibly different effects of estrogen/MHT on the underlying diseases in dementia. For example, treatment of aging women with estrogen/MHT could provoke dementia because of intravascular clotting, as may have been the case in the WHIMS study that could obscure effects on AD [9].

Because of the difficulty in separating the two forms of dementia, especially in the absence of objective signs such as those seen on imaging or on biopsies, unless there are the requisite imaging studies, we address clinical research reports on "dementia" rather than attempting to separate effects of/on AD vs. VD. This practical approach is dictated by the difficulty in separating the underlying mechanisms of "AD" and/or "VD." Since the available literature is mainly comprised of retrospective studies based on clinical impressions rather than specific techniques, the possibility of mixed dementia remains a major obstacle.

Finally, although this chapter focuses on reproductive hormones and dementia, there are many other influences on brain function that may contribute to acute or

chronic dementia; for example, heavy smoking increases the risk of both AD and VD [10]. Other factors, such as reproductive history, metabolic syndrome, medications, trauma, associated neurodegenerative processes, and nonreproductive hormones such as thyroxin are associated with dementia (Fig. 16.1) [11–13]. While other risk factors will not be further addressed, they must be considered as possible co-variables in the development of AD/VD (Fig. 16.2).

16.1 Hormones and Dementia

16.1.1 Sex Steroids/Receptors and Dementia

Many, if not all, "steroid hormone actions" are due to liganding (binding) of a molecule by receptors that then dimerize. The paired, liganded receptors then address a second set of molecular binding sites or DNA configurations that affect either transcription or posttranscriptional modification of the product. In the case of estrogen receptor ligands, there is competition for binding between the putative ligands and other receptor binders. Once the receptor is liganded, receptor dimerization occurs, and the dimer is presented to the estrogen response element (ERE) of genes that are "estrogen responsive." Most human genes contain EREs or portions of EREs and are therefore estrogen responsive. We have elsewhere discussed these actions in detail [14]. Although there are exceptions, for the sake of brevity, we will use the terms "estrogen" and "estradiol" as being equivalent to activation of the above chain of events that lead to estrogen receptor-mediated gene regulation. The same generalities apply to other sex steroids/receptors and will not be repeated [15].

16.1.2 Estrogen(s)/Estrogen Receptors and Dementia

Estrogen receptors are widely expressed throughout the brain, including areas commonly affected in dementia (Fig. 16.3).

The hippocampus is a feed-forward system that acts to capture, process, and distribute information to the rest of the brain. Estradiol regulates hippocampal neurons [16] (Fig. 16.4).

In conjunction with the role of estrogen in dementia, postmortem brain sex steroid levels in both women and men have been shown to be inversely related to the degree of dementia at the time of death [17].

16.2 Estrogen and Cognition

Estrogen and memory—There is evidence of memory sparing if estrogen is administered immediately after oophorectomy. However, despite numerous attempts to show memory-sparing effects of estrogen/MHT on naturally postmenopausal women, evidence has not been adduced of this action [18].

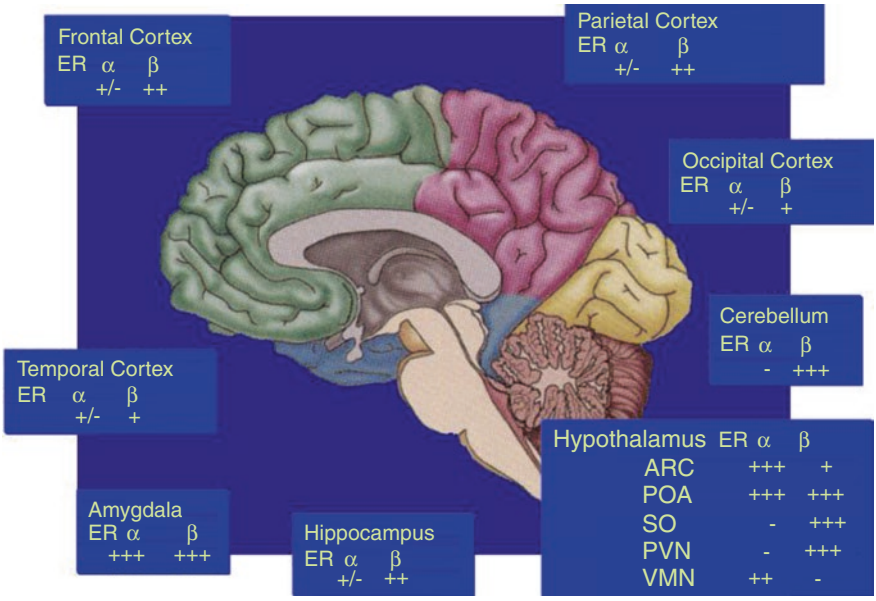

Fig. 16.3 Both estrogen receptor α (ERα) and β (ERβ) are widely represented in the brain. This makes estradiol a target for studies on both AD and VD as causes of dementia

Estrogen and minimal cognitive impairment—Minimal cognitive impairment (MCI) has long been mooted as a forerunner of dementia, particularly AD. However, effects of estrogen on cognition after menopause have not been proven. Recently, a prospective study of estrogen treatment on cognition was reported, again, and definitively shows little or no effect; but, this is not sufficient to assess whether the lack of effect relates to the occurrence of dementia, especially non-AD dementia [19, 20].

16.3 Estrogen and Dementia

Epidemiologic evidence—Although age is the most powerful factor in the occurrence of dementia, there is strong evidence that genetic sex and sex steroids/receptors are somehow associated with dementia [15]. Mainly, this stems from the relationship of the genetic sex to the incidence of age-related dementia, women having more dementia at a given age than men, unless the women took MHT. This has been defined further, to show that the MHT must have started within the first 10 years of menopause, to show the protective effect of MHT [21]. As well, retrospective studies have indicated, but not proven, that hormone treatment of post-reproductive women is associated with age-corrected lower rates of dementia [22, 23].

Drug-use registries—An important recent retrospective analysis of "AD" supports the protective effect of estrogen, although it suffers from the difficulties of being retrospective and depends upon clinical diagnoses [24].

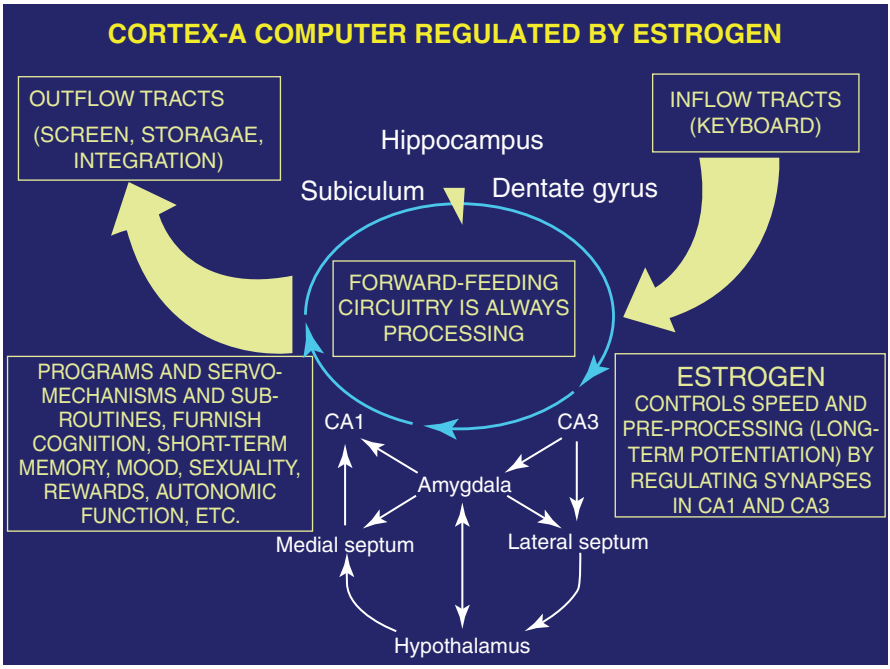

Fig. 16.4 The paired computer chips in the brain. The feed-forward three neuron loop (light blue) in the hippocampus receives information in a manner analogous to a computer chip, and both processes and distributes the information to other areas of the brain that are responsible for mood/emotion (amygdala), memory (cortex), autonomic function (hypothalamus), perception (specialized cortex), etc. Estrogen regulates many functions of the hippocampus, especially the transfer of information from the axons of the inflow tracts to the dendrites of the feed-forward neurons [16]

Prospective studies on estrogen treatment and dementia—During the Women's Health Initiative, a sub-study was undertaken to test for the effects of estrogen (conjugated equine estrogen, CEE) and/or the synthetic progestin medroxyprogesterone acetate (MPA) on the prevalence of dementia. Termed the WHIMS [25], it followed the occurrence of dementia in women who at the age of 65 years began daily CEE + MPA or CEE alone for 6 years. WHIMS' conclusions were that this hormone treatment was the cause of a slight excess of AD in the subjects [26, 27]. Some of the WHIMS survivors were studied with MRI to assess VD vs. AD; however, this remains conjectural [28, 29]. Overall, this study has many problems for interpretation. The primary one is the use of hormones in women who were decades past the menopause, which is fraught with an increase in intravascular clotting, the lack of determination of the status of vascular disease prior to starting MHT, and the use of medroxyprogesterone acetate, an androgen-derived synthetic progestin. There was no testing for minimal cognitive dysfunction at the outset of the study, which might have pointed to whether the dementia was AD, and the diagnoses were by consensus by individuals who had not examined the women. At this time, it is not possible to rule out VD as the underlying lesion [2].

In another prospective cohort study, women who started MHT during the first years of menopause appear to have a lower incidence of dementia than "comparable" subjects who did not use MHT [30]. Unfortunately, there were no pretreatment evaluations, and the contributions of AD and VD to the dementia burden were not determined.

Additional relevant experimental findings—Human arterial vascular endothelium expresses ER and is, therefore, responsive to estrogen [53].

AD—Estrogen has been shown to hinder tau hyperphosphorylation [8] and improve amyloid clearance [5]. Estrogen is anti-inflammatory and antioxidant and promotes apoptosis of immunocytes [23]. Preclinical studies in AD-mutated mice support prevention of AD by estrogen and some progestins [31, 32]. A recent systems biology evaluation concluded that androgen and estrogen receptors are involved in the development of AD [33].

VD—There is considerable evidence of estrogen protection against atherogenesis and arteriosclerosis. This is especially well-demonstrated in the recently published prospective estrogen treatment of menopausal women [34].

Interactions of VD and AD—Considerable attention has been played to the possibility that AD and VD could be mechanistically related. Capture of leucocytes that may penetrate brain parenchyma has been shown [35]. As well, impaired clearance of amyloidβ by the cerebral capillaries has been shown in animal models of AD [36]. The possibility of intravascular pressure changes affecting amyloidβ accumulation in cerebral capillaries has been posited [37]. The accumulation of amyloidβ in the capillaries of the eye is under active study [38].

Studies on capillary and blood-brain barrier aspects of the cerebral vasculature system is of especial interest. Estrogen/ER has been shown to block the binding of monocytes to arterial endothelium [39] to affect the passage of monocytes across the blood-brain barrier [40] and to affect the response to inflammatory signals in the nervous system [41, 42].The possibility that AD and VD are intertwined and that estrogen may be a key factor cannot be dismissed.

Antihormone effects on dementia—While evidence has been accumulating of increased rates of dementia in men taking androgen-blocking treatments, prospective trials are just becoming available [43–45]. On the contrary, and despite tantalizing animal studies indicating an association between antiestrogen treatment and dementia, data indicating a relationship between antiandrogen treatment and dementia has not appeared [46]. However, the apparent increase of cardiovascular complications in long-term aromatase inhibitor treatment may indicate that it will only be with large sample, long-term prospective studies that a role for AIs in dementia will be resolved [47].

Estrogen treatment and dementia—Logical extension of the possible role of estrogen in dementia resulted in a burst of reports that estrogen treatment appeared to improve the dementia of identified subjects; however, prospective clinical trials have not shown either delay or reversal of dementia by sex steroid treatment. [23, 29, 48]. The same result of no effect on dementia was found in a prospective trial of the selective estrogen receptor modulator, raloxifene, on established dementia [49].

Summary—There are many reasons that could explain the apparent lack of experimental effectiveness of sex steroid treatments against dementia. Until these have been ruled out as confounders, and definitive clinical trials performed, it remains prudent to consider sex steroids as possible factors in protection against dementia and to keep in mind possible protective effects of estrogen treatment/replacement. Data is accumulating that this could apply in the cases of mixed dementias.

Androgens and dementia—Although long a subject of interest, the relationship of dementia to testosterone remains unclear [6, 50].

Neurosteroids and dementia—In vitro and animal studies have indicated that allopregnanolone is neuroprotective. However, the results of clinical studies on dementia and neurosteroids are not yet available [31].

Gonadotrophins and dementia—It has recently been reported that gonadotropin-releasing hormone (GnRH) may increase the effect of acetylcholinesterase inhibitor (AChEI) in slowing the decline of patients with dementia. This finding is exciting but requires repetition and further exploration [51].

Human chorionic gonadotrophin also has been raised as having a positive role in the management of dementia [52]. Both hCG and its hyperglycosylated counterpart are active on steroidogenic and TGFβ systems; therefore, this is an interesting suggestion, and further results are awaited.

16.4 General Conclusions

Dementia, regardless of its cause, remains the scourge of aging. As the population of developed countries continues to age, the personal tragedy and cost of dementia rises. One of the most important deficits is the lack of reliable data on the cause(s) of dementia in subjects included in population studies or undergoing clinical trials. This also hinders development of treatment plans and management. Part of the problem is that the structure of our reimbursement systems requires a single diagnosis, which works against precise assessment of the roles of the individual causes of dementia. These requirements were imposed before specific methods were available to diagnose AD vs. VD and to deal with mixed dementias. This is changing, and we can look forward to cause-related datasets from which to plan research and treatment of the underlying condition, rather than the outcome, dementia.

Nowhere is the problem of undiagnosed partitioning of causes of dementia more evident than in determining the role of reproductive hormones in dementia. There are many indications that reproductive hormones, especially estrogen/estrogen receptors, may be protective against dementia. Thus far, preclinical experimental evidence supports prevention of both AD and VD. However, clinical studies have not revealed evidence that reproductive hormones, particularly estrogen, prevent or treat AD. On the other hand, there is evidence of prevention of the most common cause of VD, atherogenesis, and arteriosclerosis and data indicating that estrogen could be protective against vascular-related parenchymal (AD) dementia. This raises the possibility that it is VD, either singly or in mixed dementia cases, that is being prevented/treated by estrogen. We have made a case for this hypothesis in this manuscript.

It is expected that wide use of specific, quantitative imaging and other techniques will define the contributions of AD and VD in individual cases, thereby allowing testing of this hypothesis. Clinical research leading to improved, specific diagnosis and appropriate prevention and interventions is of the highest priority.

Acknowledgements Helpful discussions with Dr. Mony de Leon are gratefully acknowledged.

References

1. Wyss-Coray T. Ageing, neurodegeneration and brain rejuvenation. Nature. 2016;539(7628):180–6.
2. Jiang S, Yan C, Qiao Z, Yao H, Jiang S, Qiu X, Yang X, Fang D, Yang Y, Zhang L, Wang L, Zhang L. Mismatch negativity as a potentail neurobiological marker of early-stage Alzheimer disease and vascular dementia. Neurosci Lett. 2017;24:26–31.
3. Quinn TJ, McCleery J. Diagnosis in vascular dementia, applying 'Cochrane diagnosis rules' to 'dementia diagnostic tools'. Clin Sci (Lond). 2017;25(8):729–32.
4. Hilgetag CC, Helen B. Are there ten times more glia than neurons in the brain? Brain Struct Funct. 2009;213(4–5):365–6.
5. Bowen RL. Sex hormones, amyloid protein, and Alzheimer disease. JAMA. 2001;286(7):790–1.
6. Uchoa MF, Moser VA, Pike CJ. Interactions between inflammation, sex steroids, and Alzheimer's disease risk factors. Front Neuroendocrinol. 2016;43:60–82.
7. Bowen RL, Perry G, Xiong C, Smith MA, Atwood CS. A clinical study of lupron depot in the treatment of women with Alzheimer's disease: preservation of cognitive function in patients taking an acetylcholinesterase inhibitor and treated with high dose lupron over 48 weeks. J Alzheimers Dis. 2015;44(2):549–60.
8. Alvarez-de-la-Rosa M, Silva I, Nilsen J, Pérez MM, García-Segura LM, Avila J, Naftolin F. Estradiol prevents neural tau hyperphosphorylation characteristic of Alzheimer's disease. Ann N Y Acad Sci. 2005;1052:210–24.
9. Zhang T, Casanova R, Resnick SM, Manson JE, Baker LD, Padual CB, Kuller LH, Bryan RN, Espeland MA, Davatzikos C. Effects of hormone therapy on brain volumes changes of post-menopausal women revealed by optimally-discriminative voxel-based morphometry. PLoS One. 2016;14(3):e0150834.
10. Rusanen M, Kivipelto M, Quesenberry CP. Heavy smoking in midlife and long-term risk of Alzheimer disease and vascular dementia. Arch Intern Med. 2011;171:333–9.
11. Munro CA. Sex differences in Alzheimer's disease risk: are we looking at the wrong hormones? Int Psychogeriatr. 2014;26(10):1579–84.
12. Karim R, Dang H, Henderson VW, Hodis HN, St John J, Brinton RD, Mack WJ. Effect of reproductive history and exogenous hormone use on cognitive function in mid- and late life. J Am Geriatr Soc. 2016;64(12):2448–56.
13. Adelborg K, Horváth-Puhó E, Ording A, Pedersen L, Toft Sørensen H, Henderson VW. Heart failure and risk of dementia: a Danish nationwide population-based cohort study. Eur J Heart Fail. 2017;19(2):253–60.
14. Fadiel A, Song J, Tivon D, Hamza A, Cardozo T, Naftolin F. Phenytoin is an estrogen receptor α-selective modulator that interacts with helix 12. Reprod Sci. 2015;22(2):146–55.
15. Vest RS, Pike CJ. Gender, sex steroid hormones, and Alzheimer's disease. Horm Behav. 2013;63(2):301–7.
16. Oberlander JG, Woolley CS. 17β-Estradiol acutely potentiates glutamatergic synaptic transmission in the hippocampus through distinct mechanisms in males and females. J Neurosci. 2016;36(9):2677–90.
17. Rosario ER, Chang L, Head EH, Stanczyk FZ, Pike CJ. Brain levels of sex steroid hormones in men and women during normal aging and in Alzheimer's disease. Neurobiol Aging. 2009;32(4):604–13.

18. Sherwin BB. Estrogen and cognitive functioning in women: lessons we have learned. Behav Neurosci. 2011;126(1):123–7.
19. Henderson VW, St John JÁ, Hodis HN, McClearly CA, Stanczyk FZ, Shoupe D, Kono N, Dustin L, Allayee H, Mack WJ. Cognitive effects of estradiol after menopause: a randomized trial of the timing hypothesis. Neurology. 2016;87(7):699–708.
20. Gleason CE, Dowling NM, Wharton W, Manson JE, Miller VM, Atwood CS, Brinton EA, Cedars MI, Lobo RA, Merriam GR, Neal-Perry G, Santoro NF, Taylor HS, Black DM, Budoff MJ, Hodis HN, Naftolin F, Harman SM, Asthana S. Effects of hormone therapy on cognition and mood in recently postmenopausal women: findings from the randomized, controlled KEEPS-Cognitive and Affective Study. PLoS Med. 2015;12(6):e1001833.
21. Rocca WA. Time, sex, gender, history, and dementia. Alzheimer Dis Assoc Disord. 2017;31(1):76–9.
22. Tschanz JT, Norton MC, Zandi PP, Lyketsos CG. The Cache County Study on Memory in Aging: factors affecting risk of Alzheimer's disease and its progression after onset. Int Rev Psychiatry. 2013;25(6):673–85.
23. Silva I, Mor G, Naftolin F. Estrogen and the aging brain. Maturitas. 2001;38(1):95–100.
24. Mikkola TS, Savolainen-Peltonen H, Tuomikoski P, Hoti F, Vattulainen P, Gissler M, Ylikorkala O. Lower death risk for vascular dementia than for Alzheimer's disease with postmenopausal hormone therapy users. J Clin Endocrinol Metabol. 2016;102(3):870–7.
25. Craig MC, Maki PM, Murphy DG. The Women's Health Initiative Memory Study: findings and implications for treatment. Lancet Neurol. 2005;4(3):190–4.
26. Rapp SR, Espeland MA, Manson JE, Resnick SM, Bryan NR, Smoller S, Coker LH, Phillips LS, Stefanick ML, Sarto GE. Educational attainment, MRI changes, and cognitive function in older postmenopausal women from the Women's Health Initiative Memory Study. Int J Psychiatry Med. 2013;46(2):121–43.
27. Goveas JS, Rapp SR, Hogan PE, Driscoll I, Tindle HA, Smith JC, Kesler SR, Zaslavsky O, Rossom RC, Ockene JK, Yaffe K, Manson JE, Resnick SM, Espeland MA. Predictors of optimal cognitive aging in 80+ women: The Women's Health Initiative Memory Study. J Gerontol. 2016;71(1):S62–71.
28. Coker LH, Hogan PE, Bryan NR, Kuller LH, Margolis KL, Bettermann K, Wallace RB, Lao Z, Freeman R, Stefanick ML, Shumaker SA. Postmenopausal hormone therapy and subclinical cerebrovascular disease: the WHIMS-MRI Study. Neurology. 2009;72(2):125–34.
29. Naftolin F, Harman SM. Preventing Alzheimer disease and cognitive decline. Ann Intern Med. 2011;154(3):211–2; author reply 212–3.
30. Imtiaz B, Tuppurainen M, Rikkonen T, Kivipelto M, Soininen H, Kröger H, Tolppanen AM. Postmenopausal hormone therapy and Alzheimer disease: a prospective cohort study. Neurology. 2017;88(11):1062–8.
31. Irwin RW, Brinton RD. Allopregnanolone as regenerative therapeutic for Alzheimer's disease: translational development and clinical promise. Prog Neurobiol. 2014;113:40–55.
32. Bengtsson SK, Johansson M, Backstrom T, Nitsch RM, Wang M. Brief but chronic increase in allopregnanolone cause accelerated AD pathology differently in two mouse models. Curr Alzheimer Res. 2013;10(1):38–47.
33. Winkler JM, Fox HS. Transcriptome meta-analysis reveals a central role for sex steroids in the degeneration of hippocampal neurons in Alzheimer's disease. BMC Syst Biol. 2013;7:51.
34. Hodis HN, Mack WJ, Henderson VW, Shoupe D, Budoff MJ, Hwang-Levine J, Li Y, Feng M, Dustin L, Kono N, Stanczyk FZ, Selzer RH, Azen SP, ELITE Research Group. Vascular effects of early versus late postmenopausal treatment with estradiol. N Engl J Med. 2016;374(13):1221–31.
35. Pietronigro EC, Della Bianca V, Zenaro E, Constantin G. NETosis in Alzheimer's disease. Front Immunol. 2017;8:211.
36. Morris AW, Carare RO, Schreiber S, Hawkes CA. The cerebrovascular basement membrane: role in the clearance of B-amyloid and cerebral amyloid angiopathy. Front Aging Neurosci. 2014;6:251.

37. Attems J, Jellinger K, Thal DR, Van Nostrand W. Review: sporadic cerebral amyloid angiopathy. Neuropathol Appl Neurobiol. 2011;37(1):75–93.
38. Jentsch S, Schweitzer D, Schmidtke KU, Peters S, Dawczynski J, Bar KJ, Hammer M. Retinal fluorescence lifetime imaging ophthalmoscopy measures depend on the severity of Alzheimer's disease. Acta Ophthalmol. 2015;93(4):e241–7.
39. Curatola AM, Huang K, Naftolin F. Dehydroepiandrosterone (DHEA) inhibition of monocyte binding by vascular endothelium is associated with sialylation of neural cell adhesion molecule. Reprod Sci. 2012;19(1):86–91.
40. Bechmann I, Goldmann J, Kovac AD, Kwidzinski E, Simburger E, Naftolin F, Dirnagl U, Nitsch R, Priller J. Circulating monocytic cells infiltrate layers of anterograde axonal degeneration where they transform into microglia. FASEB J. 2005;19(6):647–9.
41. Bechmann I, Mor G, Nilsen J, Eliza M, Nitsch R, Naftolin F. FasL (CD95L, Apo1L) is expressed in the normal rat and human brain: evidence for the existence of an immunological brain barrier. Glia. 1999;27(1):62–74.
42. Mor G, Nilsen J, Horvath T, Bechmann I, Brown S, Garcia-Segura LM, Naftolin F. Estrogen and microglia: a regulatory system that affects the brain. J Neurobiol. 1999;40(4):484–96.
43. Nead KT, Gaskin G, Chester C, Swisher-McClure S, Leeper NJ, Shah NH. Association between androgen deprivation therapy and risk of dementia. JAMA Oncol. 2017;3(1):49–55.
44. Hershman DL, Unger JM, Wright JD, Ramsey S, Till C, Tangen CM, Barlow WE, Blanke C, Thompson IM, Hussain M. Adverse health events following intermittent and continuous androgen deprivation in patients with metastatic prostate cancer. JAMA Oncol. 2016;2(4):453–61.
45. Nead KT, Gaskin G, Chester C, Swisher-McClure S, Dudley JT, Leeper NJ, Shah NH. Androgen deprivation therapy and future Alzheimer's disease risk. J Clin Oncol. 2016;34(6):566–71.
46. Overk CR, Lu PY, Wang YT, Choi J, Shaw JW, Thatcher GR, Mufson EJ. Effects of aromatase inhibition versus gonadectomy on hippocampal complex amyloid pathology in triple transgenic mice. Neurobiol Dis. 2012;45(1):479–87.
47. Seruga B, Zadnik V, Kuhar CG, Marinko T, Cufer T, Zakotnik B, Zorman D, Ocana A, Amir E. Association of aromatase inhibitors with coronary heart disease in women with early breast câncer. Cancer Invest. 2014;32(4):99–104.
48. Li R, Singh M. Sex differences in cognitive impairment and Alzheimer's disease. Front Neuroendocrinol. 2014;35(3):385–403.
49. Henderson VW, Ala T, Sainani KL, Bernstein AL, Stephenson BS, Rosen AC, Farlow MR. Raloxifene for women with Alzheimer disease: a randomized controlled pilot trial. Neurology. 2015;85(22):1937–44.
50. Seidl JN, Massman PJ. Relationships between testosterone levels and cognition in patients with Alzheimer disease and nondemented elderly men. J Geriatr Psychiatry Neurol. 2015;28(1):27–39.
51. Meguro K. Cholinesterase inhibitors are compatible with psychosocial intervention for Alzheimer disease patients suggested by neuroimaging findings. Psychiatry Res. 2017;259:29–33.
52. Rao CV. Involvement of luteinizing hormone in Alzheimer disease development in elderly women. Reprod Sci. 2017;24(3):355–68.
53. Diano S, Horvath TL, Mor G, Register T, Adams M, Harada N, Naftolin F. Aromatase and estrogen receptor immunoreactivity in the coronary arteries of monkeys and human subjects. Menopause. 1999;6(1):21–8.

Vaginal Aplasia Creatsas Vaginoplasty

17

George Creatsas

Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome is a rare congenital abnormality of the female genital tract presented with aplasia of the uterus and the upper two-thirds of the vagina in an otherwise normal 46XX individual. The incidence is approximately one case in 4000 women. The syndrome is frequently associated with other nongynecological defects, such as urinary tract anomalies, vertebral deformities, and to a lesser extent auditory and cardiac lesions. Furthermore the absence of the vagina and the uterus has a profound psychological impact on the young woman's sense of femininity, so that the demand for a sexual life makes the creation of a neovagina strongly advisable [1].

Diagnosis is based on the history, the clinical and gynecological examination, and the ultrasonography, including the urinary tract examination to exclude relative anomalies, as well as laparoscopy and hysteroscopy to diagnose and classify the relative uterovaginal anomaly.

Several techniques of vaginal reconstruction, surgical or nonsurgical, have been reported as the Creatsas vaginoplasty, the Frank's procedure, the Williams vaginoplasty, the McIndoe operation, the Vecchietti's technique, and others [2–4].

The Creatsas vaginoplasty is a modification of the Williams procedure. It is a simple, safe, and quick operative method resulting in a functioning vagina, similar to normal. We developed our technique in 1981 and until now we have performed 254 cases.

The operation starts with three incisions (using electrocautery), at the third, sixth, and ninth o'clock positions of the hymen. This opening prevents postcoital bleeding during the first sexual intercourse. The vulval tissues are put under tension by four Allis clamps (Fig. 17.1a). A U-shaped incision is followed on the labia (Fig. 17.1b). The upper edge of the incision ends 4 cm laterally to the external

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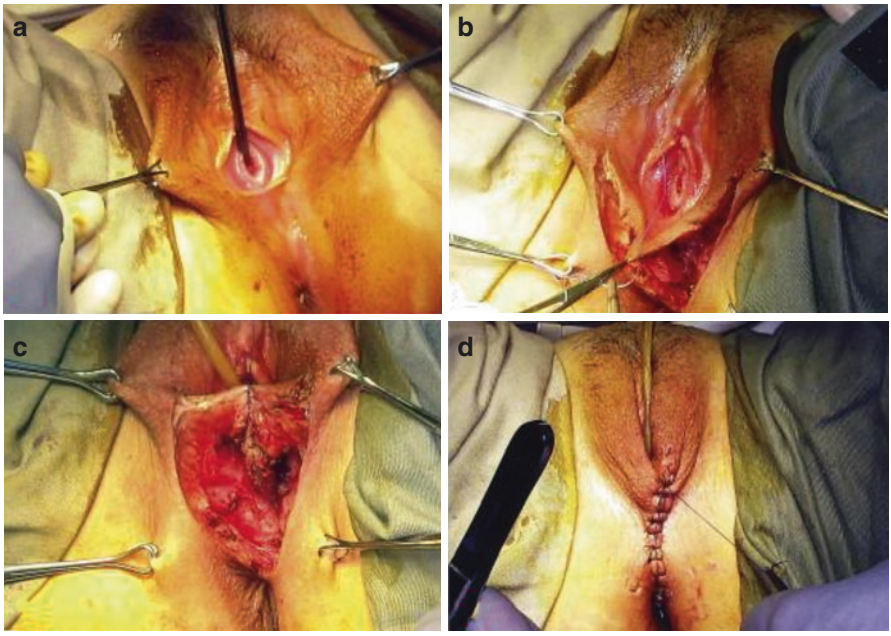


Fig. 17.1 (**a**) Placement of the Allis clamps and catheterization of the urethral meatus. (**b**) A U-shaped incision in the vulva, mobilization of the tissues, and placement of the first suture. (**c**) Completion of the first layer of sutures between the inner skin margins. (**d**) Closing of the second layer and completion of the operation [4, 5]

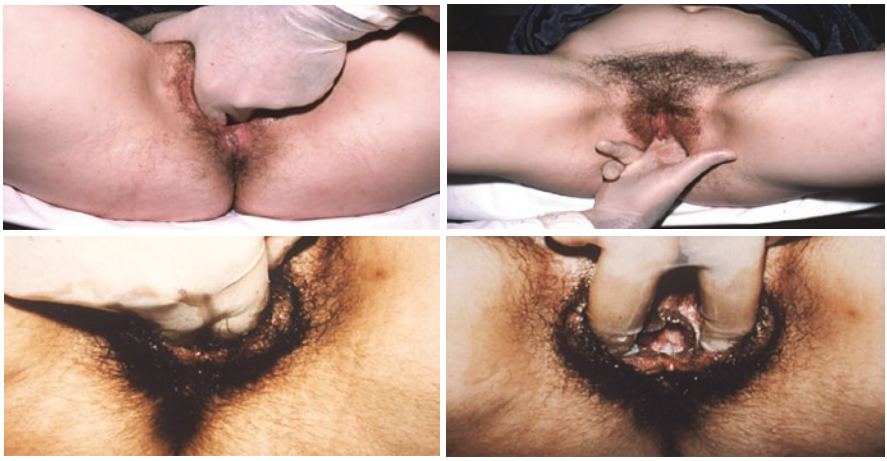
urethral meatus. After mobilizing the tissues, a meticulous hemostasis is required to avoid postoperative hematomas and tissue necrosis [4, 6].

Close of the inner skin margins is followed. The knots are placed inside the created neovagina, to avoid early decomposition, which could lead to wound opening.

A layer of sutures is followed to approximate the subcutaneous tissue and the perineal muscles. Finally, the external skin is closed (Fig. 17.1d). For the closing of both the skin layers (Fig. 17.1b, c) interrupted absorbable 2-0 sutures are used, starting posteriorly and proceeding anteriorly.

The criterion for the success of the operation is the creation of a neovagina up to 10–12 cm in depth and 4–5 cm in width. The functional dimensions of the neovagina are measured using sonovaginography [7]. A clinical reexamination at 4 weeks and 6 months after the operation and then on a yearly basis is recommended. Following our procedure, no significant postoperative complications were reported, and all patients have a satisfactory sexual intercourse. A mean hospital stay up to 6 days is required to prevent postoperative complications such as dehiscence during bathing at home and to maximize patient compliance.

There is no need for postoperative vaginal dilatations, which impairs the psychological impact of the patient [1, 5, 8, 9].



The McIndoe's vaginoplasty was a commonly used vaginoplasty among other available operative techniques. However several complications were reported, such as the injuries of the neighboring organs. Also graft shrinkage, due to the development of granulomatous tissue, caused neovaginal stenosis. The aesthetic outcome should be taken into consideration.

The Vecchietti's operation and its laparoscopic version are frequently performed in several European centers over the last years, with a low perioperative morbidity and a short recovery period. Potential important complications may occur, as passing the cutting needle from the abdominal wall to the retrohymenal fossa. Frequent follow-up evaluations to adjust the device's tension and the use of dilators after the removal of the apparatus are also required [10].

The sigmoidal colpoplasty is an intraperitoneal operation that carries intraoperative risks and complications. Satisfactory anatomical and functional results have been reported by the use of pelvic peritoneum from the pouch of Douglas [2].

The Frank's technique requires daily use of manually operated vaginal dilators for a long period of time. Despite the good results of the method and the absence of surgical and anesthetic risks, young patients often cannot maintain the required compliance.

In contrast to other methods, Creatsas vaginoplasty provides elasticity of the tissues, formatting the lower part and the introitus. The neovagina permits pleasant and uncomplicated sexual intercourse (Tables 17.1 and 17.2). The latter may be attempted shortly after the operation to alleviate the patient's stress. Almost all of our patients described their sexual life as satisfactory or adequate, which shows that sexual satisfaction is usually achieved.

In conclusion, the aim of all methods is the creation of a vaginal channel of adequate functional depth and width, with axial deviation similar to normal. Our experience shows that the Creatsas vaginoplasty is a simple, quick, and effective vulvo-perineoplasty that satisfies all the requirements.

Table 17.1 Creatsas vaginoplasty

Total MRKH cases	*200*
Mean age at operation	17.2 (r:13–26)
Depth of vaginal dimple	
3–20 mm	157 (78.5%)
20–30 mm	43 (21.5%)
Remnants of uterine horns	167 (83.5%)
Accessary ovary	1
Urinary tract anomalies	*89 (44.5%)*
Unilateral kidney	62
Solitary pelvic kidney	10
Horseshoe kidney	9
Double renal pelvis/ureter	8
Skeletal malformations	18 (9%)
Scoliosis	11
Humpback	4
Klippel-Feil syndrome	3
Hearing loss	9 (4.5%)

Creatsas et al. Fertil Steril 2010
Operations until the year 2010

Table 17.2 Postoperative results of Creatsas vaginoplasty

Creatsas vaginoplasty—results	
Attempted sexual activity	49 (74.5%)
>4 sexual partners	77 (38.5%)
Genital wart infections	46 (23%)
Mean surgical time (min)	28 (r: 23–43)
Neovagina	
10–12 cm depth/5 cm width	191 (95.5%)
7–9 cm depth/2–3 cm width	9 (4.5%)
Postoperative complications	
Wound opening	8 (4%)
Coital bleeding	0
Mean hospital stay (days)	6 (r: 3–11)
Quality of sexual life	
Satisfactory	189 (94.5%)
Adequate	10 (5%)
Unsatisfactory	1 (0.5%)
Dyspareunia	0
Pregnancies	2

Creatsas et al. Fertil Steril 2010

References

1. Laggari V, Diareme S, Christogiorgos S, Deligeoroglou E, Christopoulos P, Tsiantis J, et al. Anxiety and depression in adolescents with polycystic ovary syndrome and Mayer-Rokitansky-Küster-Hauser syndrome. J Psychosom Obstet Gynaecol. 2009;30(2):83–8.
2. Cai B, Zhang JR, Xi XW, Yan Q, Wan XP. Laparoscopically assisted sigmoid colon vaginoplasty in women with Mayer-Rokitansky-Kuster-Hauser syndrome: feasibility and short-term results. Br J Obstet Gynecol. 2007;114:1486–92.
3. Capraro VJ, Gallego MB. Vaginal agenesis. Am J Obstet Gynecol. 1976;124:98–107.
4. Creatsas G, Deligeorolgou E, Christopoulos P. Creation of a neovagina after Creatsas modification of Williams vaginoplasty for the treatment of 200 patients with Mayer- Rokitansky-Kuster- Hauser syndrome. Fertil Steril. 2010;94(5):1848–52.
5. Creatsas G, Deligeoroglou E, Makrakis E, Kontoravdis A, Papadimitriou L. Creation of a neovagina following Williams vaginoplasty and the Creatsas modification in 111 patients with Mayer-Rokitansky-Küster-Hauser syndrome. Fertil Steril. 2001;76:1036–40.
6. Deligeoroglou E, Christopoulos P, Creatsas G. A unique case of descending salpingitis and functioning endometrium in a Mullerian remnant in a woman with Mayer-Rokitansky-Kuster-Hauser syndrome. Fertil Steril. 2005;83:1547–8.
7. Botsis D, Deligeoroglou E, Christopoulos P, Aravantinos L, Papagianni V, Creatsas G. Ultrasound imaging to evaluate Creatsas vaginoplasty. Int J Gynaecol Obstet. 2005;89:31–4.
8. Deligeoroglou E, Kontoravdis A, Makrakis E, Christopoulos P, Kountouris A, Creatsas G. Development of leiomyomas on the uterine remnant of two women with Mayer-Rokitansky-Kuster-Hauser syndrome. Fertil Steril. 2004;81:1385–7.
9. Fedele L, Bianchi S, Frontino G, Fontana E, Restelli E, Bruni V. The laparoscopic Vecchietti's modified technique in Rokitansky syndrome: anatomic, functional, and sexual long-term results. Am J Obstet Gynecol. 2008;198(377):1–6.
10. Marques Hde S, dos Santos FL, Lopes-Costa PV, dos Santos AR, da Silva BB. Creation of a neovagina in patients with Rokitansky syndrome using peritoneum from the pouch of Douglas: an analysis of 48 cases. Fertil Steril. 2008;90:827–32.

18 Is Reproductive Microsurgery Dead or Has Its Demise Been Greatly Exaggerated?

Victor Gomel

The indications for reproductive surgery in the era of assisted reproductive technology (ART) are twofold: (1) primary treatment of tubo-ovarian disease, adhesions, and other pelvic pathologies to give the patient the opportunity to achieve spontaneous conception and (2) treatment of uterine, adnexal, and other conditions that would adversely affect the outcome of assisted reproduction.

Tubo-ovarian disease, adhesions, and other pelvic pathologies are the principal cause of infertility in approximately one-third of cases. Surgery was the only option for tubo-peritoneal infertility until the early 1990s when the take-home baby rate with IVF reached 12.3% in the USA. During the 1960s, 1970s, and 1980s, nearly all of the abdominal and pelvic operations were performed through a laparotomy incision. Large sponges were used to retract the bowel; the peritoneal cavity was exposed to the room atmosphere and to the heat of the lights illuminating the operative site. Intraoperative irrigation was rarely performed, and when it was, saline solution was used. The outcomes were modest and failures largely due to postoperative adhesions.

18.1 Microsurgery

The initial goal of developing microsurgery and microsurgical tenets was to prevent or at least reduce postoperative adhesions and to improve surgical outcomes. In addition to reducing trauma at the site of surgery, we had to develop a system that decreased injury to the mesothelial cells that line the peritoneum and inflammation in the peritoneal cavity. The microsurgical principles were developed over time with the use of nonhuman animal experiments and clinical observations in the human,

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which included systematic second-look laparoscopies, performed 8–12 weeks after the initial surgery.

The microsurgical principles include:

1. Delicate handling of tissues and judicious use of electrical and laser energy
2. Frequent intraoperative irrigation of exposed tissues with heparinized lactated Ringer's solution at room temperature to prevent desiccation of the peritoneum and decrease clotting of blood in the peritoneal cavity
3. Prevention of foreign body contamination of the peritoneal cavity and use of lint-free surgical pads soaked in the heparinized Ringer's solution before use
4. Meticulous pinpoint hemostasis that minimizes adjacent tissue damage using a microelectrode or a very fine bipolar forceps
5. Identification and use of proper cleavage planes
6. Complete excision of abnormal tissues
7. Excision and removal of broad adhesions (shallow adhesions are simply divided mechanically)
8. Precise alignment and approximation of tissue planes
9. Performing a thorough lavage with the use of heparinized Ringer's solution at the end of the procedure to remove any blood clots, foreign body, or debris that may be present in the peritoneal cavity
10. Leaving 300–500 mL Ringer's solution, to which 500–1000 mg hydrocortisone succinate is added, in the peritoneal cavity before total peritoneal closure
11. Use of magnification, as necessary: permitting prompt identification of abnormal morphologic changes, recognition and avoidance of surgical injury, and application of the preceding principles with the use of appropriate fine instruments and suture materials
12. Administration of one or two doses of dexamethasone postoperatively

With open cases, at the end of the procedure, an ilioinguinal nerve block is performed. This enables the patient to move more easily in the immediate postoperative period and to require less analgesic medication.

Thus, microsurgery is a surgical philosophy as much as a technique: a delicate surgical approach designed to minimize peritoneal trauma and tissue disruption and to prevent postoperative adhesions while increasing the accuracy of the procedure and improving the outcome. Indeed this surgical approach significantly improved outcomes, intrauterine pregnancies, and birth rates.

The microsurgical approach and use of magnification with an operative microscope also permitted to treat certain conditions with a more effective, less invasive technique and obtain better results. A good example is that it permitted to perform a tubo-cornual anastomosis instead of a tubo-uterine implantation, which required a fundal incision from one cornu to the other into the uterine cavity.

The microsurgical technique was initially used in open procedures of reconstructive tubal surgery and soon after in other types of reproductive procedures as myomectomy, ovarian cystectomy, endometriosis, etc. Recognizing that laparoscopy provided a surgical access route, we commenced initially to treat patients with distal

tubal disease by laparoscopy, applying the same microsurgical principles. Included were cases of salpingo-ovariolysis, fimbrioplasty, salpingostomy, and tubal pregnancy. Evidently, at the time, these procedures were performed by monocular vision, viewing the operative field directly through the ocular of the laparoscope. Experience with operative laparoscopy resulted in the modification of open interventions for more complex cases, and the formal laparotomy approach was replaced by a small mini laparotomy incision, permitting such procedures to be performed on an ambulatory basis.

18.2 In Vitro Fertilization and Assisted Procreation

There was globally significant interest in learning and applying these techniques from the mid-1970s until the mid-1990s when in vitro fertilization results improved significantly and clinics offering this service became more readily available. The birth rates per initiated cycle doubled from 1990 to 1999 from 12.3 to 25.4% in the USA. This rate appears to have stabilized in the USA at around 26–30% during the last several years (2002–2015). In the USA in 2015, of the 231,936 initiated cycles 91,090 were fresh nondonor eggs or embryo cycles; these yielded a live birth rate of 21.8%. The 69,882 transfer cycles of frozen nondonor embryos yielded a live birth rate of 44.3%.

Part of this success is due to the transfer of multiple embryos. This in turn results in a high proportion of multiple fetal pregnancies and eventually in preterm and low birth weight infants. In Europe where the transfer of the number of embryos is regulated by several states, the pregnancy and birth rates are much lower; so are the rates of multiple pregnancies. Despite a trend in reducing the numbers of embryos transferred, in the USA in 2014, a single embryo was transferred only in 23.6% of cases. Two embryos were transferred in 54.1%, three in 12.4%, four in 3.2%, five in 1.1%, and six or more in 0.2%.

Such practice is associated with a tremendous increase in preterm and low birth weight infants and the associated important sequels of perinatal mortality and morbidity, including cerebral palsy. The rates of preterm births for single infants from single fetus, singletons from multiple fetuses, twins, and triplets and greater order of multiples in the USA in 2014 were 10.9%, 16.8%, 56.8%, and 98.7%, respectively. The rates of low birth weight infants were very close to those of the premature infants. The large proportion of multiple births, with the associated increased obstetrical complications, neonatal complications, and deaths, causes great societal costs and significant financial burden and emotional costs for the parents.

The significant increase in live birth rates with ART was accompanied with the commercialization of the technology and its services, all over the world. In parallel fashion, there was a significant decline in the practice and teaching of reproductive surgery. IVF now is frequently offered as a primary treatment option, in most cases of tubal factor infertility. These changes have occurred despite the major progress that gynecologic surgery has experienced: introduction of microsurgical techniques and the predominant use of laparoscopic surgical access that readily permits the application of these techniques in reproductive surgery. However, based on the

current US outcomes of a birth rate of 28% per initiated cycle, the cumulative probability of live birth after three cycles of treatment would be around 52%. Furthermore, several studies have shown conclusively that a large percentage of couples do not wish to complete three cycles of IVF, even when these are paid by the state or covered by insurance. Reports from France, where IVF is covered by the state, corroborate with these findings. Others refuse to have IVF for religious or ethical reasons, and many would find the cost of IVF prohibitive, since the procedure is not covered in many states and countries, while other treatment modalities are, as is the case in British Columbia in Canada.

For the infertile woman with tubo-ovarian and pelvic pathology, there are only two realistic options to achieve a pregnancy: reconstructive surgery or IVF. The presence of a credible alternative, in IVF, permits the reproductive surgeon to operate on patients with a better prognosis, which was not the case until the early 1990s when the take-home baby rate per initiated cycles reached 12%. We have known for a long time that one of the important factors influencing surgical outcome was the degree of tubal damage and extent of pelvic disease and adhesions. Operating on patients with better prognosis translates in superior outcomes, which has been well demonstrated.

18.3 Fertility-Promoting Reconstructive Microsurgery

The goal of fertility-promoting surgery is to restore the anatomic and functional integrity of the reproductive organs. With appropriate investigation, preparation, and appropriate surgical skills, many reconstructive procedures can be performed during the initial diagnostic laparoscopy using microsurgical techniques: salpingo-ovariolysis, salpingostomy, and even tubo-tubal anastomosis for reversal of sterilization. This is also applicable to other pelvic pathologies, such as endometriosis, uterine myomas, etc. Intrauterine conditions may be treated by performing a concomitant hysteroscopy.

In the absence of any other infertility factors, laparoscopic salpingo-ovariolysis yields intrauterine pregnancy rates of 50% to over 60%. Laparoscopic fimbrioplasty for tubal phimosis, which frequently also necessitates a preliminary salpingo-ovariolysis, yields intrauterine pregnancy rates of 40–50%. In a series of 40 such cases, reported by this author, 19 women (48%) had live births. Periadnexal adhesions are also often present with hydrosalpinges; a preliminary salpingo-ovariolysis would be followed by salpingostomy (salpingoneostomy). Liberal use of laparoscopic salpingostomy will yield live birth rates of 20–30%. With favorable (mild) cases, these rates are significantly higher. In a series of 90 women whose surgery was performed by this author, the overall live birth rate was 32.4%. Based on the American Fertility Society classification, 73 of these cases had severe tubal damage, and only 17.8% of these had live births, whereas in those classified as having mild damage, 58.8% had one or more births.

The deleterious effect of large hydrosalpinges (visible at sonography) on IVF results has been well established. Preliminary laparoscopic salpingectomy or proximal tubal occlusion by application of clips improves the IVF outcomes. It has

been demonstrated that prior salpingostomy, instead, provides an equally beneficial effect with IVF, and it offers the woman the potential of achieving a pregnancy naturally. The American Society for Reproductive Medicine's (ASRM) most recent committee opinion published in 2012 fortunately supports what we have been proposing and writing since the mid-1990s. In this particular issue, the report reads as follows: "Although IVF is preferred over salpingostomy for mild hydrosalpinges in older women and for those with male factor or other infertility factors, salpingostomy before IVF may improve the subsequent likelihood of success of IVF while still giving the patient the option to attempt spontaneous conception. Patients with poor-prognosis hydrosalpinges are better served by salpingectomy followed by IVF."

Microsurgery is ideal for tubo-tubal anastomosis and produces excellent results that are principally dependent on the length and status of the reconstructed tube. Most occlusions are caused by a disease process; rarely they are congenital or due to remains of an old tubal pregnancy. In cases of reversal of sterilization, the remaining tubal segments are usually normal. A proper microsurgical anastomosis yields a tube that is normal albeit shortened. Women who are under 35 years of age with normal ovarian function and a fertile male partner can anticipate birth rates of 70–75%, with most pregnancies occurring within the first 12 months. Those over 35 years old can anticipate a cumulative live birth rate of ±50%. The birth rate in those >40 years old is not negligible. In a multicenter survey carried out in the Netherlands in 1990, the birth rate among 78 women aged 40 years and over, after a minimum period of 1 year of follow-up, was 44%. Recent publications support this evidence.

The procedure can be performed through a minilaparotomy incision, with laparoscopic access as well as robotic assistance. There is a great variation in reported results. The principal factors that affect the outcome are the age of the woman partner, which plays a paramount role in fertility regardless of the mode of treatment, the length of the available tubal segments, the degree of excellence of the microsurgical technique, and the selection of patients. When these parameters are met, the live birth rates are high, and the ectopic pregnancy rate is <2%; if they are not met, the birth rates are low, and the tubal pregnancy rates are high.

A relatively recent study analyzed the delivery rates of two groups with similar characteristics: One of the groups underwent IVF and the other surgical reversal of sterilization. The cumulative delivery rates over 72 months in the IVF and reversal groups were 52% and 59.5%, respectively. In those <37 years of age, they were 52.4% and 72.2%, respectively ($P = 0.12$). The average cost per delivery was 11,707€ for IVF and 6,015€ for surgical reversal. Obviously, costs may vary depending on the country and/or jurisdiction.

The ASRM's committee opinion regarding sterilization reversal supports the position held in our center for more than three decades: "There is good evidence to support the recommendation for microsurgical anastomosis for tubal ligation reversal; it can be accomplished by minilaparotomy as an outpatient procedure." Similar results may be obtained by laparoscopy if the procedure is performed "in a fashion identical to open microsurgical tubal anastomosis. Operating times are prolonged."

And "Only surgeons who are very facile with laparoscopic suturing and who have extensive training in conventional tubal microsurgery should attempt this procedure."

Tubo-cornual anastomosis became possible with the introduction of gynecologic microsurgery and replaced tubo-uterine implantation, which was performed previously. Microsurgical tubo-cornual anastomosis (TCA) for pathologic proximal tubal occlusion offers several advantages over tubal implantation: It maintains the integrity of the uterine cornu; it preserves a longer tube; it obviates the need for a cesarean section, except for obstetrical reasons; and it yields better results. Before recommending this procedure, it is imperative to ensure that the cornual region is indeed occluded with a pathologic process and that all other parameters of fertility, both female and male, are normal. In published reports on cases of true pathologic proximal tubal occlusion, live birth rates range from 38 to 56%. In a series of 48 women followed for >1 year, reported by this author, 27 (56.3%) had one or more births. Most of the studies were published before 1997. There has been a paucity of recent publications, which suggests decreased utilization of this procedure. TCA is a relatively difficult procedure, and therefore technical excellence and experience have an important influence on the outcome. The ASRM's committee opinion regarding microsurgical TCA for pathologic cornual occlusion states: "Unless the proximal blockage on HSG is clearly due to salpingitis isthmica nodosa, selective salpingography or tubal cannulation can be attempted… Before performing this procedure, there should be confirmation of normal distal tubal anatomy." And "IVF is preferred to resection and microsurgical anastomosis. Microsurgery may be considered after failed tubal cannulation if IVF is not an option for the patient, but it should be attempted only by those with appropriate training."

Microsurgery also permits reconstruction for complex situations, for example, a tubo-ovarian transposition, with preservation of their vascular pedicles. In such cases, the prognosis that the surgical reconstruction offers is not necessarily proportional to the technical difficulty of the procedure.

Conclusion

Assisted reproductive techniques are being used increasingly as primary treatment for infertility. This is largely the result of the commercialization of the ART technology and its services. The industry is well capitalized and is lucrative. Yet it is important to stress that even if couples were to undergo three successive cycles of IVF, nearly 50% would fail to obtain a baby. Many patients need to have reproductive surgery before IVF for various conditions such as myomas, adnexal tumors, endometriosis, etc. Yet, there has been a significant decline in the practice and teaching of reconstructive surgery and microsurgery in gynecology. Such teaching and practice made the gynecologist a more refined surgeon, attributes that would be regrettable to lose.

As evident from the preceding, reconstructive surgery when well performed, in properly selected cases, offers satisfactory results. Furthermore, it offers the

couple the opportunity to attempt a pregnancy over a long period of time and to conceive more than once out of the same procedure. This and the available data suggest that there should be a real place for reconstructive surgery. The preservation of such skills will require a concerted effort on the part of the teaching institutions.

It is gratifying that the ASRM, with their most recent committee opinion, has clearly recognized the important role of reconstructive microsurgery in the treatment of infertility. It is to be hoped that this will stimulate increase in the training and practice of this discipline.

The development of operative laparoscopy, tubal microsurgery, and IVF in the last 40 years has significantly improved the outlook of couples suffering from tubal infertility. These are complementary approaches that can be used singly or in combination. When both alternatives are equally available to the patient and used, a much greater overall cumulative birth rate can be obtained.

In the preface of the book, *Microsurgery in Female Infertility*, published by Little Brown, in January 1983, we find the following statement: "This manuscript has been completed during a time of rapid change and expansion with the understanding that it represents not an endpoint but merely an accounting at a given point in time. Further developments are also occurring in the area of IVF and embryo transfer (IVF & ET), which will undoubtedly produce improved results. Nonetheless, I do not consider the techniques of microsurgery on the one hand and IVF & ET on the other as competitive; on the contrary, I see them as complementary, enabling us to achieve a greater success rate among those patients presenting with complex fertility problems." This statement is still valid today.

Suggested Reading

1. American Society for Reproductive Medicine. ASRM 2012 committee opinion: role of tubal surgery in the era of assisted reproductive technology. Fertil Steril. 2012;97:539–45.
2. Centers for Disease Control and Prevention. Assisted reproductive technology 2014 national summary report. Available at: www.cdc.gov/art/ART2014.
3. Kupka MS, Ferraretti AP, de Mouzon J, Erb K, d'Hooghe T, Castilla JA, et al. Assisted reproductive technology in Europe, 2010: results generated from European registers by ESHRE. Hum Reprod. 2014;29:2099–113.
4. Gomel V. The place of reconstructive tubal surgery in the era of ART. Reprod Biomed Online. 2015;31:722–31.
5. Gomel V. Reconstructive tubal microsurgery and assisted reproductive technology. Fertil Steril. 2016;105:887–90.
6. Koninckx PR, Gomel V. Role of the peritoneal cavity in the prevention of postoperative adhesions, pain, and fatigue. Fertil Steril. 2016;106:998–1010.
7. Koninckx PR, Gomel V. Introduction: quality of pelvic surgery and postoperative adhesions. Fertil Steril. 2016;106:991–3.
8. Gomel V, Koninckx PR. Microsurgical principles and postoperative adhesions: lessons from the past. Fertil Steril. 2016;106:1025–31.

Technological Breakthroughs inPOP Surgery

19

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19.1 Pelvic Organ Prolapses (POP)

The pelvic floor in women is a complex and highly vulnerable structure. Injuries and functional modifications of this complex due to pregnancy, life events, and aging often lead to pelvic organ prolapse (POP). Following the definition of a joint report by the two leading urogynecological societies [1], POP is defined as "any descent of one or more of the anterior vaginal wall, posterior vaginal wall, the uterus (cervix) or the apex of the vagina (vaginal vault or cuff scar after hysterectomy)." The different types of prolapse include apical vaginal prolapse, i.e., uterus and vaginal vault (after hysterectomy when the vaginal vault prolapses); anterior vaginal wall prolapse, i.e., cystocele (bladder prolapse), urethrocele (urethra prolapse), and paravaginal defect (pelvic fascia defect); and posterior vaginal wall prolapse, i.e., enterocele (small bowel prolapse), rectocele (rectum prolapse), and perineal deficiency. Women may present prolapse of one or more of these anatomical structures. POP may be associated with other pelvic floor dysfunctions such as sexual dysfunction, urinary incontinence (UI), chronic obstructive defecation syndrome (ODS), and constipation. Typical symptoms of POP are vaginal bulging, pelvic pressure, vaginal bleeding, discharge and infection, and low backache. All these symptoms have a profound social, psychological, and sexual impact, and they severely affect quality of life [2].

POP is a common condition affecting 40–60% of parous women. When defined by symptoms, prevalence estimates of pelvic organ prolapse range from 2.9 to 5.7% with an expected 46% increase in the number of women with POP by 2050 [3]. The number of patients needing surgical correction of POP has increased in the recent

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year; therefore, 30% of women aged 50–89 years require a consultation for pelvic floor dysfunction, and the life-time risk of surgical repair is estimated at 11%, with almost one-third of the patients requiring repeat surgery [4].

19.2 Surgical Strategies forAdvanced POP Treatment

Therapies for symptomatic POP include surgical and nonsurgical options. Generally, conservative or mechanical treatments (pelvic floor physical therapy, pessary placement) are considered for women with moderate of prolapse, those who wish to have pregnancy, the frail, or those women who don't want to undergo surgery. In cases of unsuccessful conservative management or for advanced-stage POP, surgical correction is the best treatment for patients. Reconstruction of pelvic floor defects can be obtained through restoration of anatomical supports that should be comparable with the original anatomical structures. Selection of the correct surgical approach in the treatment of advanced-stage pelvic organ prolapse is a complex decision process which involves several factors. Surgery can be adapted to accomplish different targets based on the age and functional status of patients: woman's personal surgical history and objectives, individual risks of surgical complications, prolapse recurrence, and de novo symptoms affect surgical planning and the choice of surgical procedure for advanced POP.

The presence of a loss of apical support is one of the most challenging situations in POP surgery. Apical defect is usually present in women with prolapse that extends beyond the hymen; therefore, adequate support for vaginal apex is an essential component of a long-term surgical repair [5]. There are several good options for surgical repair of apical vaginal defects; thus, searching for a safe, effective, and durable repair is an ongoing process. Historically, repair of vaginal apex prolapse could be performed vaginally or abdominally and more recent by laparoscopic traditional and robotic approach. Goals of surgical repair of apical prolapse include restoration of proper anatomy, maintenance of sexual function, and durability. Surgery objective is the suspension of the upper vagina, which may include the uterus or the vaginal vault in post-hysterectomy women. Several surgical techniques, both with and without synthetic mesh, have been described for transvaginal approach to apical prolapse. The most common procedures include fixation of the apex to sacrospinous ligaments, uterosacral ligament, or iliococcygeus muscles. The main advantages of the vaginal approach include decreased morbidity, shorter hospitalization and convalescence, and low costs. Unfortunately, long-term success rates are lower compared with abdominal approach such as sacrocolpopexy [3]. Recent concern and FDA warnings regarding adverse effects of transvaginal mesh implants in 2008 and 2011 led to the return of abdominal reconstruction surgery [6].

19.3 Abdominal Apical Defect Surgery

The gold standard for the treatment of apical prolapse is the abdominal sacral hystero-cervicopexy (ASC) [7, 8]. During ASC, a bridge of graft material is attached to the prolapsed vagina (or cervix) and secured to the anterior longitudinal ligament of

the sacrum. ASC is associated with a lower risk of awareness of prolapse, recurrent prolapse, repeat surgery for prolapse, postoperative SUI, and dyspareunia than vaginal operations; however, ASC shows a longer operating time than vaginal approaches [9]. As technology has improved, minimally invasive techniques have been developed demonstrating equal effectiveness than open ASC, with decreased morbidity and mortality.

Traditional laparoscopic ASC (L-ASC) was not universally adopted secondary to its requirement of advanced laparoscopic skills not accessible to the majority of gynecologic surgeons and long learning curve. Laparoscopic ASC is a technically challenging procedure particularly because of the need of deep pelvic dissections (vesicovaginal space, rectovaginal space) and the need of the presacral ligament identification. The presacral ligament area is also surrounded by critical structures, such as the right ureter, the middle sacral vessels, the left iliac vein, and the caval bifurcation. Many surgeons choose to perform vaginal surgery in order to avoid increased morbidity associated with abdominal approach.

Development of new strategies to suspend the apex, which avoid these challenges and are technically easier, is therefore key to permit broader dissemination of minimally invasive abdominal procedures for advanced apical POP [10]. Lateral mesh suspension to the abdominal wall (ALS) is a recently developed surgical approach that fulfills the concepts of a newer easy technical strategy to suspend the apex. This new technique was proposed by JB Dubuisson in 1998. The procedure does not require posterior dissection of the rectovaginal space or isolation of the sacral promontory nor the placement of sutures at this level. The technique requires a deep dissection of the vesicovaginal space up to the level of the bladder trigon and the placement of a T-shaped mesh which is sutured to the anterior vaginal wall, uterine cervix, and isthmus. Two long lateral arms are then retracted through retroperitoneal tunnels to reach the lateral abdominal wall. This allows lateral and apical traction of the apex that is not displaced posteriorly as performed in ASC but in the center of the pelvis. Laparoscopic-ALS (L-ALS) is easier than L-ASC due to the reduced dissection and the lower number of sutures required. The large series of L-ALS performed by Dubuisson demonstrated the feasibility and the effectiveness of the laparoscopic lateral colpouterine suspension with mesh reinforcement in the treatment of advanced anterior and apical prolapse, showing a high anatomical and functional success rate [11–13].

19.4 Robotic-Assisted Apical Surgery

In the last 15 years, the use of the da Vinci Surgical System (Intuitive Surgical Inc., Sunnyvale, CA, USA) has modified laparoscopy surgery and has become widely adopted by many pelvic surgeons across fields including gynecology, gynecologic oncology, urogynecology, urology, and colorectal and surgical oncology. The robotic technology is more intuitive than standard laparoscopy as the system mimics the surgeon's maneuvers in the console. The increased magnification with three-dimensional vision, the seven degrees of freedom of the instruments, and the physiologic tremor filtering are the main features of robotic technology. These

features provide pelvic surgeons with an enhanced ergonomic setting, simplifying complex laparoscopic tasks such as suturing or performing deep pelvic dissection with safety and efficiency as primary factors. The addition of the fourth robotic arm further improves surgeon efficiency with very little reliance on the side surgeon to perform various steps during a typical POP repair. Audio communication between the console and side surgeon and nursing staff provides an additional advantage, as does the use of a teaching console in terms of surgical education [14].

For these reasons, robotic-assisted pelvic floor surgery has become an important component of the pelvic surgeon's armamentarium in the treatment of symptomatic POP. The choice of conventional or robotic-assisted laparoscopic approach for a minimally invasive procedure is influenced by laparoscopic skill, surgical preference, and comfort level of the surgeon. Even if robotic surgery may confer many benefits over conventional laparoscopy, these advantages should continue to be weighed against the cost of the technology (the robotic system itself, the instrumentation, and the maintenance). The robotic instrumentation is limited to ten uses before requiring replacement, thereby increasing the costs associated with robotic-assisted surgery. Furthermore, initialization of a robotic surgery program requires prolonged operating room time that also adds to the cost of this surgical approach [14].

Because of high technically challenging difficulties, ASC seems to be one of the best procedures to be performed by robotic platform, and several studies agree and emphasize the improved outcomes (in terms of blood loss and hospital stay) following robotic-assisted sacrocolpopexy (R-ASC) compared with open surgery [15, 16] and laparoscopy [17]. The ideal candidates for robotic-ASC (R-ASC) are patients with severe (grade III or IV) apical and/or multi-compartment prolapse. R-ASC is ideal for women who desire definitive reconstruction with concomitant intra-abdominal procedures such as supracervical hysterectomy or women who have relapse of vaginal vault suspension. R-ASC is also ideal for young women who desire definitive reconstruction with the lowest overall failure rate, lowest risk of mesh complications, and risk of dyspareunia.

The ideal candidates for robotic-assisted lateral suspension (R-ALS) are patients with symptomatic anterior and apical prolapse. In this procedure, robotic assistance allows effective and accurate dissection of the vesicovaginal space up to the retrotrigonal area. This step is essential to obtain placement of the mesh in a position that allows full correction of advanced anterior prolapse. A key element for the efficacy of the procedure stays in a solid fixation of the mesh to the anterior vaginal wall and apex, and robotic assistance allows for a rapid and extremely reproducible placement of sutures. Furthermore R-ALS allows conservation of the uterus. Many patients today express a desire to preserve their uterus due to a perceived benefit with sexual function, body image, or simply cultural beliefs. From an anatomical and functional standpoint, a preserved uterus will continue to divide the pelvis into two compartments and to participate to natural straining for evacuation and urination; from a surgical standpoint, the complications related to total or supracervical hysterectomy are avoided. R-ALS may be an alternative to sacral colpo-cervicopexy for high-morbidity patients and may represent a useful alternative to

management of presacral area such as in pelvic abnormalities cases that make mesh stitching to the sacrum difficult (low presacral left iliac vein, presacral varicocele) or in difficult promontory dissection cases such as when a fatty presacral space is present. Finally, lateral suspension may be a salvage strategy to manage women with apical prolapse relapse that were previously treated with ASC. The most published studies on robotic-ALS have reported only small series of cases [10, 18] and short follow-up, and no systematic reviews exist.

Conclusion

POP repairs continue to evolve over time in search of better and more durable repairs that will potentially improve patient satisfaction and reduce the need for reoperation. Robotic technology allows the pelvic surgeon fine instrumentation for deep pelvic dissection with safety and efficiency as primary factors. Whether robotic POP surgery potential advantages will turn into more solid and durable reconstruction is not proved, and it is currently a matter of debate. An area that also needs to be explored is whether robotic assistance may reduce the risk of mesh-related complications because of more precise development of the deep pelvic spaces and of the highly reproducible placement of sutures that optimizes the mesh tension and minimizes wrinkling. The efficiency of robotic POP procedures can be improved with dedicated operating room staff, a knowledgeable side surgeon, minimal equipment changes, and adherence to specific steps for robotic procedures. Well-trained and experienced side surgeons and assistants can significantly improve operating room efficiency and can actively anticipate the console surgeon's needs. With improved efficiency, the costs of robotic POP surgery can be reduced substantially. Robotic-assisted sacrocolpopexy and robotic-assisted lateral suspension are well tolerated and feasible techniques that do not require advanced laparoscopic training and can be accomplished by a skilled open or vaginal pelvic surgeon. Future prospective, randomized studies are needed to determine long-term efficacy, morbidity, and patient satisfaction of R-ASC and R-ALS procedures.

References

1. Haylen BT, de Ridder D, Freeman RM, Swift SE, Berghmans B, Lee J, et al. An International Urogynecological Association (IUGA)/International Continence Society (ICS) joint report on the terminology for female pelvic floor dysfunction. Neurourol Urodyn. 2010;29(1):4–20.
2. Jelovsek JE, Barber MD. Women seeking treatment for advanced pelvic organ prolapse have decreased body image and quality of life. Am J Obstet Gynecol. 2006;194(5):1455–61.
3. Maher C, Feiner B, Baessler K, Schmid C. Surgical management of pelvic organ prolapse in women. Cochrane Database Syst Rev. 2013;(4):Cd004014.
4. Olsen AL, Smith VJ, Bergstrom JO, Colling JC, Clark AL. Epidemiology of surgically managed pelvic organ prolapse and urinary incontinence. Obstet Gynecol. 1997;89(4):501–6.
5. Brubaker L. Sacrocolpopexy and the anterior compartment: support and function. Am J Obstet Gynecol. 1995;173(6):1690–5; discussion 1695–6.
6. Murphy M, Holzberg A, van Raalte H, Kohli N, Goldman HB, Lucente V. Time to rethink: an evidence-based response from pelvic surgeons to the FDA Safety Communication: "UPDATE

on Serious Complications Associated with Transvaginal Placement of Surgical Mesh for Pelvic Organ Prolapse". Int Urogynecol J. 2012;23(1):5–9.
7. Maher CF, Qatawneh AM, Dwyer PL, Carey MP, Cornish A, Schluter PJ. Abdominal sacral colpopexy or vaginal sacrospinous colpopexy for vaginal vault prolapse: a prospective randomized study. Am J Obstet Gynecol. 2004;190(1):20–6.
8. Paraiso MF, Jelovsek JE, Frick A, Chen CC, Barber MD. Laparoscopic compared with robotic sacrocolpopexy for vaginal prolapse: a randomized controlled trial. Obstet Gynecol. 2011;118(5):1005–13.
9. Maher C, Feiner B, Baessler K, Christmann-Schmid C, Haya N, Brown J. Surgery for women with apical vaginal prolapse. Cochrane Database Syst Rev. 2016;(10):Cd012376.
10. Simoncini T, Russo E, Mannella P, Giannini A. Robotic-assisted apical lateral suspension for advanced pelvic organ prolapse: surgical technique and perioperative outcomes. Surg Endosc. 2016;30(12):5647–55.
11. Dubuisson JB, Eperon I, Jacob S, Dubuisson J, Wenger JM, Dallenbach P, et al. Laparoscopic repair of pelvic organ prolapse by lateral suspension with mesh: a continuous series of 218 patients. Gynecol Obstet Fertil. 2011;39(3):127–31.
12. Dubuisson JB, Chapron C. Laparoscopic iliac colpo-uterine suspension for the treatment of genital prolapse using two meshes: a new operative laparoscopic approach. J Gynecol Surg. 1998;14(4):8.
13. Veit-Rubin N, Dubuisson JB, Gayet-Ageron A, Lange S, Eperon I, Dubuisson J. Patient satisfaction after laparoscopic lateral suspension with mesh for pelvic organ prolapse: outcome report of a continuous series of 417 patients. Int Urogynecol J. 2017;28(11):1685–93.
14. Rosenblum N. Robotic approaches to prolapse surgery. Curr Opin Urol. 2012;22(4):292–6.
15. Elliott CS, Hsieh MH, Sokol ER, Comiter CV, Payne CK, Chen B. Robot-assisted versus open sacrocolpopexy: a cost-minimization analysis. J Urol. 2012;187(2):638–43.
16. Lee RK, Mottrie A, Payne CK, Waltregny D. A review of the current status of laparoscopic and robot-assisted sacrocolpopexy for pelvic organ prolapse. Eur Urol. 2014;65(6):1128–37.
17. Seror J, Yates DR, Seringe E, Vaessen C, Bitker MO, Chartier-Kastler E, et al. Prospective comparison of short-term functional outcomes obtained after pure laparoscopic and robot-assisted laparoscopic sacrocolpopexy. World J Urol. 2012;30(3):393–8.
18. Dallenbach P, Veit N. Robotically assisted laparoscopic repair of anterior vaginal wall and uterine prolapse by lateral suspension with mesh: initial experience and video. Int Urogynecol J. 2014;25(8):1137–9.

Preterm Birth as a Syndrome

20

Gian Carlo Di Renzo, Irene Giardina, Eleonora Brillo, and Valentina Tosto

20.1 Definition of Prematurity

Accumulating observations now indicate that preterm labor, preterm premature rupture of membranes (P-PROM), pre-eclampsia, small for gestational age (SGA), large for gestational age (LGA), recurrent miscarriage, and many other conditions that we deal with in clinical practice are not discrete entities, but are syndromes with more than one cause the so-called great obstetrical syndromes [1].

Accumulating evidence suggests that preterm birth is indeed a syndrome attributable to multiple pathologic processes: many causes and many phenotypes!

Preterm birth is defined as a preterm delivery that occurs before 37 weeks of gestation. In approximately 50% cases, labor occurs spontaneously with contractions, cervical changes, and intact membranes, in 30% of cases after a premature rupture of membranes (P-PROM), in 20% of cases it is induced when the continuation of pregnancy involves an unacceptable risk to both the mother and/or the child (a maternal disease, such as pre-eclampsia, abruptio placentae, placenta previa, and/or fetal conditions, such as severe growth restriction and intrauterine fetal death).

Prematurity in relation to different complications and prognostic implications is divided into:

- Late Preterm: 34–36.6 weeks
- Moderate Preterm: 32–33.6 weeks
- Low Preterm: 28–31.6 weeks
- Very-Low Preterm: <28 weeks

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The lower limit of very-low preterm distinguishes the fetus who will be born preterm from abortion and it varies in different nations in relation to the legislation.

A joint group of the Society for Maternal-Fetal Medicine (SMFM), NICHD, ACOG, and American Academy of Pediatricians (AAP) in 2014 suggested to indicate how "periviable period" the gestational age between 22 and 25 weeks, where survival varies per week from 6%, 26%, 55%, and 72%, respectively [2].

About 85% of premature births belong to late or moderate preterm class, 10% to low preterm class, and only 5% to very-low preterm class.

20.2 Epidemiology

In Europe, preterm delivery incidence varies between 5 and 18%, while only 0.3–1.5% occurs before 28 weeks, with an obviously much poorer outcome.

In high-income countries, between two-thirds and three quarters of neonatal deaths occur in 6–11% children born alive before 37 weeks (from EUROPERISTAT 2008). Babies born before 32nd week of gestation are at particularly high risk of adverse outcomes, with rates of infant mortality around 10–15% and cerebral palsy around 5–10% [3, 4]. However, also infants between 32 and 36 weeks of gestation show worse outcomes compared to newborns at term [5–7].

The preterm infant also has an increased risk of early mortality and to develop chronic diseases [8, 9]. Many countries have reported increased rates of preterm birth in the last two decades, and this trend was recently confirmed by a global WHO survey [10].

There are many explanations which may justify the increased preterm birth rate. One is the rise of twin pregnancies associated with infertility treatments and increased age mother in the search for pregnancy. The preterm birth rate in twin pregnancies is 40–60%, compared to 5–10% of singletons [11, 12]. Secondly, survival of the highly premature infants has been greatly improved in recent decades because of advances in perinatal medicine, with the use of prenatal corticosteroids and postnatal surfactant. This improved prognosis has changed the operators perception of the risk associated with prematurity. This policy has led therefore to the increase in the absolute number of preterm births [13].

20.3 Identification of Preterm Labor

Before undertaking any therapeutic strategy, careful identification of the pregnant patient at risk is needed, so as to detect manageable conditions and fetal and/or maternal contraindications.

The criteria for diagnosing preterm labor are not precise because the etiology and the sequence of events that precede preterm labor are not yet fully understood. Symptoms reported by patients with suspected preterm labor are: pelvic pain, back pain, menstrual-like cramps, and vaginal discharge.

Prediction of preterm labor is based on the detection of risk factors and specific markers.

20.3.1 Risk Factors

There are numerous medical history risk factors involved in promoting the onset of preterm birth. The identification of risk factors it is an integral part in the diagnosis of preterm birth risks.

As the cause of labor still remains elusive, the exact cause of preterm birth is also unsolved. In fact, the cause of about 50% of preterm births has never been determined. Labor is a complex process involving many factors. Different pathways have been identified that can result in preterm birth and have considerable evidence:

1. Precocious maternal-fetal endocrine-paracrine activation
2. Uterine overdistension (hydramnios, placental abruption, multiple pregnancies)
3. Decidual bleeding
4. Intrauterine inflammation/infection [14].

Identifying women at high risk of giving birth early would enable the health services to provide specialized care for these women to delay the birth or make sure they will be born in the appropriate place (e.g., a hospital with a special care baby unit, the so-called NICU) [15]. Risk scoring systems have been suggested as a possible way of identifying these women [15]. However, there is no definitive agreement in this area, so it is unclear whether the risk scoring systems would help to prolong pregnancy reducing the number of preterm births. The risk factors that most often related to preterm birth are associated to maternal background and/or factors during current pregnancy, such as infections.

Some relevant considerations on the main risk factors reported in the literature in single pregnancies are summarized below:

- Maternal age: women <18 years and >40 years have a higher risk of preterm birth (OR 1.5–2)
- Ethnicity: black population has a consistently higher risk than the white-Caucasian. The causes of these differences are not entirely clear; genetic factors are possibly intertwined with environmental ones)
- Socio-economic hardship: socio-economic disparities are associated with many other factors, including education, nutritional status, maternal smoking, drugs abuse, infections, stress). Within the industrialized countries, the preterm birth is considerably more common among socially disadvantaged women. The social unrest, if defined by the type of employment, income, or education level, is associated with an increased risk of preterm delivery; the reasons for this trend are unclear.
- Marital status: pregnancy in unmarried women is associated with an increased risk of prematurity between all ethnic groups and at different age groups. The reasons for this effect are not known, but they are commonly attributed to the relative loss of social support or resources.

- Low BMI and obesity: BMI <18 is associated with an increased risk of preterm birth. There is also agreement on the fact that obesity increases the risk.
- Previous obstetric history: a previous spontaneous preterm delivery is the most important risk factor for a subsequent preterm birth. The risk of recurrence varies from 14 to 22% for one previous preterm birth, from 28 to 42% for two previous preterm deliveries, and even more than 67% after three previous preterm birth. Others risk factors for preterm birth are represented by induced abortions in the second quarter, previous cerclage for cervical incontinence, and previous interventions on the cervix (such as conization, deep diathermocoagulation, and extended loop). Women who had a preterm birth with medical indication in the first pregnancy have a greater risk of spontaneous or medically indicated preterm birth in the second as well. Mothers who were born themselves preterm or have a sister who gave birth to a baby preterm are at increased risk to deliver preterm.
- Actual obstetric history: uterine overdistension from hydramnios, presence of large myoma (>5 cm diameter), uterine malformations, recurring metrorrhagia of the first, second, and third trimesters are all factors associated with preterm birth. Hypertensive disorders of pregnancy are commonly related to preterm birth, especially for incidental complications such as placental abruption.
- Infertility treatments: pregnancies from assisted reproduction, especially high level of technology, have a high risk of PTL, even if singleton.
- Interpregnancy time: an interval inferior to 6 months between two pregnancies involves a greater risk of preterm birth.
- Infections: early bacterial vaginosis is associated with increased risk of preterm birth. Urinary tract infections increase the risk of pyelonephritis which increases preterm delivery incidence.
- Smoke: smoking increases the likelihood of preterm birth through unclear mechanisms. Cigarette smoking appears to explain some of the socio-economic disparities of the preterm birth, given its higher prevalence between groups of socio-economically disadvantaged women. Stop smoking is directly related to a significant decrease in the rate of preterm birth.
- Stress: there is a significant association between chronic stress/catastrophic events, major maternal anxiety, and preterm delivery. The mechanisms that link stress/emotions to the preterm birth are not yet clarified although the levels of CRH may play a role.

20.3.2 Markers

To improve the accuracy of diagnosis of threatened preterm birth delivery two methods have been mostly substantiated in the last decade:

– Transvaginal ultrasound cervical length (abiophysical test)
– Research of fetal fibronectin (fFN), PAMG1, or IGF-BP1 in cervical-vaginal secretions (biochemical test)

Cervical length is a good predictor of preterm birth in all populations.

Cervicometry can be applied as screening test, but the literature presents controversial data about the universal screening application of cervicometry. FIGO proposes it (FIGO 2015); NICE states that there are not yet enough evidences to propose it [16].

It is recommended to perform ultrasound cervicometry measurement in correspondence of the anatomy scan of the second trimester (20–22 weeks of gestation). The finding of a cervical length <2.5 cm is associated with an increasing risk of preterm labor/birth, with a sensitivity between 30 and 60% [17, 18]. The recommended scan time is at least 5 min, and the shortest and the best CL has to be reported for clinical management.

fFN is a glycoprotein produced by the chorion and it functions as a "glue" between placenta, amino-chorion membranes, and decidua. It is normally found in cervical-vaginal secretions up to 16–18 week of gestation, and it reappears around term or at the time of preterm labor. It is believed that fFN is a marker of chorio-decidual interface alteration due to infection or inflammation, placental abruptio, or mechanical factors. This test is mainly used to exclude a preterm delivery rather than to identify it. In fact, its negative predictive power (90–97%) has been shown to be significantly greater than its positive predictive power (<40%) for preterm delivery within 7–14 next days.

Phosphorylated insulin-like growth factor binding protein (phIGFBP-1) is produced by the placenta decidual cells, and it is thought to be released into the cervical-vaginal fluid after tissue damage to the chorio-decidual interface [19]. A qualitative test from vaginal swab between 22 and 34 weeks of gestation can be used to identify women at risk for preterm birth: a positive cervical phIGFBP-1 test increases the probability of delivery within 7 days from 17.8 to 54.5%; a negative test decreases the risk to 6.0% [19].

The contextual use of biophysical and biochemical tests reaches a high negative predictive value (100%), making it a very useful method to identify patients truly at risk and so candidates to tocolytic therapy and steroid prophylaxis.

More recently, a new test (PartoSure test) based on the research of placental alpha-1 microglobulin (PAMG-1) in cervical-vaginal fluid has been evaluated; it has been shown that the positive test in patients symptomatic with intact membranes and cervical dilatation ≤3 cm indicates the possibility of a spontaneous preterm delivery within 7 days with a high degree of accuracy (around 80%). A negative result indicates that spontaneous preterm delivery within 14 days is highly unlikely (negative predictive value around 95–98%). The PartoSure test seems to be the single most accurate test for prediction of imminent preterm delivery, with excellent positive and negative predictive values in symptomatic patients.

20.3.2.1 Management of Preterm Birth

Tocolysis and administration of corticosteroids (CS) to induce lung fetal maturation are the first therapeutic tools for the management of the so-called threatened preterm labor. Also bed rest and adequate hydration are usually recommended in these patients.

20.4 Corticosteroids Administration

Antenatal maternal corticosteroids (CS) administration consists of betamethasone or dexamethasone 12 mg intramuscularly injected in two doses, 24 h apart (standard regimen). The full effect of therapy occurs after 48 h the first dose and within the first 7 days [20–23].

CS administration is associated to a reduction of 60% in the incidence of respiratory distress syndrome (RDS), 30–40% reduction in the risk of major intraventricular hemorrhage (IVH), and necrotizing enterocholitis (NEC) in the preterm newborns.

The strength of the evidence on the effectiveness of CS depends on the gestational age: scarce before the 26 weeks and after the 34th week.

The rescue course (repetition of a single CS dose) is supported in women who received a previous full treatment >2 weeks sooner if the gestational age is still <32 6/7 weeks, and only if it is likely that the patient will give birth within the next day [24].

The antenatal CS therapy is recommended for women with pregestational and gestational diabetes at risk of preterm delivery although this should be accompanied by interventions to prevent maternal hyperglycemic alterations [24].

20.5 Tocolysis

The use of tocolytics is associated with a delay of childbirth of 24–48 h up to a maximum of 7 days, but it apparently does not reduce the incidence of preterm birth nor the worse neonatal outcomes. Its use is functional to allow effective administration of CS, and/or control the uterine contractile activity during a possible transfer to an appropriate center equipped with NICU. The tocolytics have not been shown to reduce perinatal morbidity and mortality related to prematurity.

Therefore, the main objectives of tocolysis are to gain time for antenatal CS to become effective and to gain time to allow in utero transfer to a hospital with intensive care unit [25].

Actually, the main tocolytic agents are:

- *Inhibitors of prostaglandin synthesis* (Indomethacin, Naproxen, Ketoprofen, Diclofenac). The possible dosage used is 50 or 100 mg by suppository; alternatively 50 mg per os could be used, followed by 25–50 mg per os every 6 h. Tocolysis by antiprostaglandin drugs must be limited to 32 week, and the duration of administration should not exceed 48 h for the risk of fetal negative effects, such as intrauterine premature closure of the ductus arteriosus, insufficiency of the tricuspid valve, renal dysfunction or failure, necrotizing enterocholitis [26].
- *Calcium antagonists*. Nifedipine, the main utilized calcium antagonist, acts by inhibiting calcium passage through the plasmatic membrane, interfering reversibly with voltage-dependent ion channels.

The first dose is usually 10 mg per os, to be repeated after 20 min if the contractions persist. Oral therapy can be carried out for 10–20 mg every 4–6 h [27].

Avoid sublingual administration for the risk of maternal hypotension.

- *Beta-sympathomimetics* (Ritodrine, Salbutamol, Terbutaline, Ixosuprine, Exoprenaline). Substances with beta2-adrenergic effect at the uterine level and partial beta1-adrenergic activity [28].
- *Oxytocin antagonists* (Atosiban). In analog to oxytocin, it is able to block myometrium and decidual receptors of oxytocin competing specifically with the substance. It is characterized by fast uterus-specific action and dose-dependent effect.

There are three steps of administration: initial bolus dose of 6.75 mg minute, followed by an infusion of 18 mg/h for 3 h and then 6 mg/h for up to 45 h. The rare maternal side effects are nausea, headache, dizziness, tachycardia, hypertension, hyperglycemia, and allergic reaction [29]. The relative safety of Atosiban for the mother and the fetus suggests its use as a first-line drug.

- *Magnesium sulfate.* Many investigations have confirmed the poor tocolytic effect of this substance. Moreover, many studies have now confirmed its better use for neuroprophylaxis and prevention of cerebral palsy in the preterm newborn (4 g in 20–30 min, followed 1 g/h for 24 h) within 32 week of gestation [30, 31]. The adjunctive administration of aminophylline (480 mg/die min 48 h) can also significantly reduce the rate of intraventricular hemorrhage in neonate born at less than 30 week of gestation [32].

There is no evidence about advantages of using tocolytic drugs' combinations.

20.6 Preventive Tools

20.6.1 Progesterone and Progestogens

Progesterone has been suggested to promote myometrial relaxation by regulating and maintaining high levels of cyclic adenosine monophosphate and nitric oxide synthetase, lowering levels of oxytocin and by inhibiting formation of myometrial gap junctions. It can be administered by many different routes: oral, vaginal, and intramuscular. The rate of absorption is dependent on which pharmaceutical form is used, the blood flow at the site of administration, and the solubility in the tissues. The modulation of maternal immune response, the suppression of inflammatory cascade, reduction of uterine contractility, the improvement of the utero-placental circulation explain the growing interest in the progesterone and its therapeutic application during pregnancy [33]. A growing body of evidence suggests that progesterone plays a role in preventing preterm delivery.

As for prevention, vaginal progesterone administered to asymptomatic women with a short cervix (defined as less than 25 mm) in the midtrimester reduces the rate of preterm birth <33 weeks by 45% and decreases the rate of neonatal complications, including neonatal respiratory distress syndrome [34, 35]. In women with a previous spontaneous preterm birth, the administration of 17-alpha hydroxyprogesterone caproate (17 OHP-C) as well the use of vaginal micronized progesterone reduces the rate of preterm birth <37 weeks by 34–45% and decreases the need for neonatal oxygen supplementation [35, 36].

According to guidelines published in recent years by various societies (RCOG, ACOG, EAPM, SOGC) concerning the management of preterm labor, the use of progesterone can be considered as follows:

- Early prophylaxis (from 12 to 36 weeks of gestation) with either micronized progesterone (100 vaginal daily) or 17 OHP-C (injection intramuscularly of 250 mg weekly) to prevent a recurrence in case of prior history of PTB.
- Vaginal micronized progesterone in singleton and twin pregnancy (200 mg vaginal daily), with or without prior history of PTB and a short cervical length (<25 mm) at 18–23 weeks since it has been found to reduce the rate of PTB and perinatal morbidity and mortality.
- Vaginal micronized progesterone after one episode of threatened preterm labor (200 mg vaginal daily).

20.7 Cervical Cerclage and Cervical Pessary

Other nonpharmacological approaches, such as cervical cerclage or cervical pessary in patients at high risk for PTL, can be considered only on the basis of the specific obstetric risk factors.

The literature shows evidence that cerclage provides clear and proven benefits only in circumstances diagnosed with cervical incompetence [37]. Results of randomized trials have not generally supported this practice, essentially based only on a poor obstetric history. Recently, it has been demonstrated that cervical cerclage is efficacious only in cases at high risk for PTB combining previous history of three or more late abortions or three or more preterm delivery, history of preterm labor, and an objective decrease in cervical length (patients with cervical shortening, evaluated by transvaginal ultrasound) or increase cervix dilatation in asymptomatic patient [38]. The emergency cerclage associated with the administration of tocolytic agents has shown controversial affects [38].

The efficacy of different types of cerclage has not been tested rigorously. Each technique has its advantages and pitfalls. Transvaginal techniques (cerclage of McDonald or Shirodkar) differ in the anatomical level of the cerclage suture. Based on current evidence, cervical cerclage should not be used in twin gestations. It is always advised to discuss the benefits/risk ratio and take the patient preference into consideration.

In recent years, it has been also considered the preventive effect of placing a cervical pessary (a model introduced by the German gynecologist Arabin more than 50 years ago) in a population of appropriately selected high risk women screened for cervical

length assessment at the midtrimester scan (non-symptomatic patients, singleton pregnancy, short cervix <25 mm, at 20–24 weeks gestation as risk marker), without prior cervical incompetence. Various studies show significant reduction of preterm birth without increasing the rate of vaginal infections, but not all authors confirmed these data. The evidence about the use of cervical pessary is still in progress [39–41].

20.8 P-PROM (Preterm Premature Rupture of the Membranes)

P-PROM is associated with about 30% of preterm deliveries. It has multifactorial etiopathogenesis, with a clear prevalence for the infectious cause.

In a woman reporting symptoms suggestive of P-PROM, perform a speculum examination to look for pooling of amniotic fluid and:

- If pooling of amniotic fluid is observed, do not perform any further diagnostic test.
- If pooling of amniotic fluid is not observed, consider performing a biochemical test such as insulin-like growth factor binding protein-1 test or placental alpha-microglobulin-1 with vaginal fluid. The PAMG1-based test has been found to be more accurate and it is the only one which has been compared with the evaluation of the effective rupture of membranes by coloring amniotic fluid with intra-amniotic injection of a dye and looking at the discharge of the colored amniotic fluid from the vagina [37].

Antibiotic prophylaxis is generally not recommended in women at risk for preterm birth in absence of signs of infection. Instead, antibiotic prophylaxis is recommended in presence of documented P-PROM or signs of infection (use a combination of clinical assessment and tests for diagnosis of infection: serum C-reactive protein, white blood cell count, and measurement of fetal heart rate using cardiotocography). Pregnant women symptomatic for bacterial vaginosis or other infections should be treated.

In women at risk for preterm birth, it is preferable to administer broad spectrum antibiotics versus Gram+, Gram- and anaerobics, with evidence of improved perinatal morbidity and mortality [16, 42].

Pregnant women with P-PROM should be better treated with oral clarithromycin 250 mg twice a day for a maximum of 10 days or until the woman is in established labor. For women with P-PROM who cannot tolerate aminoglycosides, it should be considered oral penicillin for a maximum of 10 days or until the woman is in established labor.

Do not offer amoxicillin plus clavulanic acid as prophylaxis for intrauterine infection or P-PROM because of a documented risk of neonatal necrotising enterocolitis.

There is increasing evidence to support the use of clindamycin over metronidazole in presence of vaginal dysbiosis [37].

20.9 Mode of Delivery

The mode of delivery of preterm infants is controversial. Neonatal outcome depends on many factors including perinatal management, gestational age, corticosteroids administration, signs of inflammation (chorioamnionitis), singleton pregnancy versus multiple pregnancy, specific maternal e/o fetal pathologies [37].

The best practice consists in consider benefits/risk ratio of the vaginal versus the cesarean delivery on the basis of the specific case.

Preterm gestational age alone is not a valid and sufficient indication for cesarean section, unless there are specific obstetrical (maternal e/o fetal) indications. Instrumental delivery by vacuum should be avoided in very preterm gestational age. Guidelines of the RCOG and EAPM don't recommend the use of vacuum extractor below 34 weeks of gestation and consider that the safety has not clearly established between 34+0 and 36+0 weeks [37, 43].

20.10 Delayed Cord Clamping

Delayed cord clamping seems to improve neonatal outcome, particularly in neonates born preterm. In fact, it is associated with less need for red blood cell transfusion, increasing the hemoglobin and hematocrit levels and decreasing the risk of intraventricular hemorrhage and necrotizing enterocolitis. Data on long-term outcome of the procedure are lacking. The optimal time to delay cord clamping is not well established (but there is an agreement that at least a minute should be waited) and few more studies are in progress. The procedure is simple, and it should therefore be applied if possible, unless there are strong contraindications [37].

Conclusion

Preterm birth is a great obstetric syndrome, which must be handled with clear plans and protocols involving a medical team. The management needs to be customized possibly case by case, but always trying to comply with approved and shared guidelines.

Accurate identification of women truly in preterm labor allows appropriate application of interventions that can improve neonatal outcome: antenatal corticosteroid therapy, group B streptococcal infection prophylaxis, magnesium sulfate for neuroprotection, and transfer to a center with an appropriate level of nursery.

Management of preterm labor should be directed towards establishing the cause, ensuring delivery under optimal conditions, and consideration of the pros and cons of delaying delivery to increase gestational age. In practice, this means that women admitted in threatened preterm labor should be appropriately assessed to determine the optimal time for delivery. The presence of fetal compromise or intrauterine infection can hinder prolonging the pregnancy, whereas early gestational age and uncomplicated preterm labor with intact membranes can mitigate a delay in delivery. The decision should be based on a risk–benefit analysis.

References

1. Di Renzo GC. The great obstetrical syndromes. J Matern Fetal Neonatal Med. 2009;22(8):633–5.
2. Raju TNK, et al. Periviable period. Executive summary of a Joint Workshop by the Eunice Kennedy Shriver National Institute of Child Health and Human Development, Society for Maternal-Fetal Medicine, American Academy of Pediatrics, and American College of Obstetricians and Gynecologists. Obstet Gynecol. 2014;123:1083–96.
3. Larroque B, et al. Neurodevelopmental disabilities and special care of 5-year-old children born before 33 week of gestation (the EPIPAGE study): a longitudinal cohort study. Lancet. 2008;371:813–20.
4. Zeitlin J, et al. Differences in rates and short-term outcome of live births before 32 weeks of gestation in Europe in 2003: results from the MOSAIC cohort. Pediatrics. 2008;121:e936–44.
5. Kramer MS, et al. The contribution of mild and moderate preterm birth to infant mortality. Fetal and Infant Health Study Group of the Canadian Perinatal Surveillance System. JAMA. 2000;284:843–9.
6. Gouyon JB, et al. Neonatal outcome associated with singleton birth at 34–41 weeks of gestation. Int J Epidemiol. 2010;39:769–76.
7. Boyle EM, et al. Effects of gestational age at birth on health outcomes at 3and 5 years of age: population based cohort study. BMJ. 2012;344:e896.
8. Crump C, et al. Gestational age at birth and mortality inyoung adulthood. JAMA. 2011;306:1233–40.
9. Crump C, et al. Early-term birth (37–38 weeks) and mortality in young adulthood. Epidemiology. 2013;24:270–6.
10. Blencowe H, 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.
11. De Mouzon J, et al. Assisted reproductive technology in Europe, 2007: results generated from European registers by ESHRE. Hum Reprod. 2012;27:954–66.
12. Blondel B, et al. Preterm birth and multiple pregnancy in European countries participating in the PERISTAT project. BJOG. 2006;113:528–35.
13. Goldenberg RL, et al. The preterm birth syndrome: issues to consider in creating a classification system. Am J Obstet Gynecol. 2012;206:113–8.
14. Simhan HN, Caritis SN. Prevention of preterm delivery. N Engl J Med. 2007;357(5):477–87.
15. Davey MA, Watson L, Rayner JA, Rowlands S. Risk-scoring systems for predicting preterm birth with the aim of reducing associated adverse outcomes. Cochrane Database Syst Rev. 2015;(10).
16. NICE. Preterm labour and birth: methods, evidence and recommendations. Guidelines N 25 Nov 2015
17. Tsoi E. Sonographic measurement of cervical length in threatened preterm labor in singleton pregnancies with intact membranes. Ultrasound Obstet Gynecol. 2005;25:353–6.
18. Sotiriadis A. Transvaginal cervical length measurement for prediction of preterm birth in women with threatened preterm labor: a meta-analysis. Ultrasound Obstet Gynecol. 2010;35:54–64.
19. Conde-Agudelo A, Romero R. Cervical phosphorylated insulin-like growth factor binding protein 1 test for the prediction of preterm birth: a systematic review and metanalysis. Am J Obstet Gynecol. 2016;214(1):57–73.
20. RCOG. Green Top Guideline No 7: Antenatal corticosteroids to reduce neonatal morbidity and mortality. London: Royal College of Obstetricians; 2010.
21. Gyamfi-Bannerman C, Thom EA, Blackwell SC, et al. Antenatal betamethasone for women at risk for late preterm delivery. N Engl J Med. 2016;374(14):1311–20.
22. Society for Maternal-Fetal Medicine (SMFM). Implementation of the use of antenatal corticosteroids in the late preterm birth period in women at risk for preterm delivery. Publications Committee. www.smfm.org.

23. Elimian A, et al. Antenatal corticosteroids: are incomplete courses beneficial? Obstet Gynecol. 2003;102:352–5.
24. ACOG Committee Opinion. Antenatal corticosteroid therapy for fetal maturation. Obstet Gynecol. 2011;117:422–4.
25. Royal College of Obstetricians and Gynaecologists (RCOG). Tocolysis for women in preterm labor. London: RCOG. p. 13.
26. King J, et al. Cyclo-oxygenase (COX) inhibitors for treating preterm labour. Cochrane Database Syst Rev. 2005;(3):CD001992.
27. Flenady V, Wojcieszek AM, et al. Calcium channel blockers for inhibiting preterm labour and birth. Cochrane Database Syst Rev. 2014;(6):CD002255.
28. Neilson JP, et al. Betamimetics for inhibiting preterm labour. Cochrane Database Syst Rev. 2014;(2):CD004352.
29. Flenady V, Reinebrant HE, et al. Oxytocin receptor antagonists for inhibiting preterm labour. Cochrane Database Syst Rev. 2014;(6):CD004452.
30. ACOG Committee on ObstetricPractice Society for Maternal-Fetal Medicine. Committee opinion n°573: Magnesium sulphate use in obstetric. Obstet Gynecol. 2013;122:727–8.
31. Chang E. Preterm birth and the role of neuroprotection. BMJ. 2015;350:g3661.
32. Di Renzo GC, Mignosa M, Gerli S, Burnelli L, Luzi G, Clerici G, et al. The combined maternal administration of magnesium sulfate and aminophylline reduces intraventricular hemorrhage in very preterm neonates. Am J Obstet Gynecol. 2005;192(2):433–8.
33. Di Renzo GC, Giardina I, Clerici G, Mattei A, Alajmi AH, Gerli S. The role of progesterone in maternal and fetal medicine. Gynecol Endocrinol. 2012;28(11):925–32.
34. Romero R, Nicolaides K, Conde-Aqudelo A, Tabor A, et al. Vaginal progesterone in women with an asymptomatic sonographic short cervix in the midtrimester decreases preterm delivery and neonatal morbidity: a systematic review and metaanalysis of individual patient data. Am J Obstet Gynecol. 2012;206(2):124.e1–19.
35. Romero R, Dey SK, Fisher SJ. Preterm labor: one syndrome, many causes. Science. 2014;345(6198):760–5.
36. 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.
37. Di Renzo GC, Cabero Roura L, Facchinetti F, Helmer H, Hubinont C et al. Preterm labor and birth management: reccomendations from the European Association of Perinatal Medicine. 2017. http://www.tanfonline.com/loi/ijmf. Accessed 20 May 2017.
38. Berghella V, Rafael TJ, et al. Cerclage for short cervix on ultrasonography in women with singleton gestations and previous preterm birth: a meta anlysis. Obstet Gynecol. 2011;117:663–71.
39. Arabin B, Alfirevic Z. Cervical pessaries for prevention of spontaneous preterm birth: past, present and future. Ultrasound Obstet Gynecol. 2013;42:390–9.
40. Abdel-Aleem H, et al. Cervical pessary for preventing preterm birth. Cochrane Database Syst Rev. 2013;(5):CD007873
41. Nicolaides K, et al. Randomised trial of a cervical pessary to prevent preterm singleton birth. N Engl J Med. 2016;374(11):1044–52.
42. ACOG. Prevention of early onset group B streptococcal disease in newborns. Committee Opinion N485. Obstet Gynecol. 2011;117:1019–27.
43. Operative Vaginal Delivery. Green-Top Guideline n 26, 2001. Royal College of Obestrics and Gynecology.