Howard J.A. Carp *Editor*

Progestogens in Obstetrics and Gynecology

Second Edition

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ISBN 978-3-030-52507-1 ISBN 978-3-030-52508-8 (eBook)
https://doi.org/10.1007/978-3-030-52508-8

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

Foreword

In 1930, WM Allen and GW Corner used the name progestin (later renamed progesterone in 1935) to describe a hormone which was responsible for implantation, end embryo survival. They could not have imagined the profound relevance of the hormone and implications for the development of a class of drugs, which are in widespread use and affect our daily lives. For many years, progesterone was thought of as a hormone only affecting pregnancy, and the wider implications of triggering the progesterone receptor were relatively ignored. In the 1960s, the contraceptive pill became available and caused a revolution in the way that women could plan their pregnancies. Progestogens were introduced to modulate the estrogen used to inhibit ovulation in the contraceptive pill.

Since then a whole host of synthetic drugs known as progestogens have come into clinical use. The main use of progestogens is in pregnancy. Progestogens are used in luteal support, to prevent miscarriage, and to prevent preterm labor. Progestogens are used outside of pregnancy, for abnormal uterine bleeding, cycle control, hormone replacement therapy, and even in the prevention and treatment of endometrial cancer. Today, we know that progesterone is found in nonmammalian vertebrates. Progesterone had a physiological role as an anti-inflammatory agent and neurosteroid long before mammalian pregnancy had evolved. Therefore, it is hardly surprising that progestogens are being used as possible anti-inflammatory agents in endometriosis, and even in male in traumatic brain injury, and in multiple sclerosis.

Just as the early investigators in the 1930s could not realize the implications of their discovery, it is difficult to prophesy the future. A new field of development is receptor modulators. Mifepristone is a progesterone receptor modulator. It was introduced as an abortifacient. However, today new uses are being developed for receptor modulators. Experimental work with uterine fibroids may entirely change the management of fibroids and affect the whole approach to surgery for gynecological conditions.

This book brings together all the aspects of progestogens in gynecological (and non-gynecological) practice. There are chapters governing basic scientific topics such as physiology and pharmacology. The major applications of progestogen

therapy in luteal support, miscarriage, preterm labor, contraception, abnormal uterine bleeding, etc. have been described in depth. However, in clinical practice, there are always controversies, leaving the clinician puzzled as to how to help the patient. The different progestogens with their overlapping effects on estrogen, androgen, glucocorticoid, and mineralocorticoid receptors are described in order to allow the clinician to make the most appropriate choice of progestogen. It is hoped that this book will be read by gynecologists, endocrinologists, general practitioners, and associated disciplines, who wish to keep up to date and gain a comprehensive view of developments.

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Preface to the Second Edition

Since the first edition of this book, much new knowledge has accumulated regarding the progestogens. In the field of infertility, the Lotus trials have added new opportunities for supporting the luteal phase. Similarly, in miscarriage, new research has clarified the role of progestogens, but alas, has brought up as many questions and controversies as have been clarified. Hence, Chaps. 4 and 5 have been updated considerably to show the current trends and new controversies. Chapter 14 on "Progestogens in Non-gynecological Indications" was a novel concept in the first edition. The subject has been broadened. Today progestogens are used in a wide variety of neurological conditions, which has necessitated rewriting the entire chapter. In addition, the use of progestogens has become modified in both endometrial and breast tumors requiring updates of these two important subjects.

With all the changes mentioned above, progestogens are still probably the most widely used class of drugs in medical practice. Millions of women use progestogens in the contraceptive pill daily for many years. Progestogens are widely used to protect the endometrium in postmenopausal replacement therapy, cycle regulation, abnormal uterine bleeding, and endometriosis. However, the clinician is often in a quandary, as to which progestogen is most appropriate in any clinical situation. The

actions of progestogens overlap with other steroids. Progestogens have estrogenic or antiestrogenic actions, androgenic or antiandrogenic actions, and glucocorticoid or mineralocorticoid actions. Each may have advantages or disadvantages depending on the clinical situation. Additionally, much evidence has accumulated regarding the pro-thrombotic effects of certain progestogens. Hence, definite choices are necessary for prescribing endocrine contraception, where thrombosis may be a risk in healthy women. Progestogens also have other side effects including stimulatory effects on the breast, possibly predisposing to breast carcinoma, breakthrough bleeding, acne mood changes, loss of libido, and dryness of the vagina.

All of the above actions of progestogens have been incorporated into this book, which discusses the actions and uses of progestogens in depth. The book is planned for general gynecologists and specialists working in the field. Each contributing author is an authority on a specific area of progestogen use. I would like to thank each author for the time and effort taken in preparing the manuscript to make the publication of this second edition possible.

Tel Aviv, Israel Howard J. A. Carp

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Chapter 1
Physiology of Progesterone

Edi Vaisbuch, Offer Erez, and Roberto Romero

1 Introduction

The corpus luteum was first discovered in 1672 by Reinier de Graaf and named in 1689 by Marcelo Malpighi. Malpighi proposed that the corpus luteum produces the ovarian follicles and that the yellow substance, like egg yolk, serves to nourish the ovum. In 1903, Fraenkel demonstrated that excission of the corpora lutea of rabbits, before implantation, prevented implantation. Moreover, lutectomy in early pregnancy (<14 days) resulted in pregnancy loss. In 1929, Corner and Allen reported that injecting extracts of the corpus luteum into castrated adult female rabbits induces a characteristic alteration of the endometrium identical to progestational proliferation, previously shown to be due to the presence of corpora lutea in the ovaries. Allen and Corner subsequently demonstrated that in ovariectomized rabbits (at the 18th hour of pregnancy), the presence of progestational proliferation induced

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H. J. A. Carp (ed.), *Progestogens in Obstetrics and Gynecology*,
https://doi.org/10.1007/978-3-030-52508-8_1

by corpus luteum extracts may sustain normal implantation as well as embryo survival and growth; whereas in the absence of progestational proliferation, the embryos never survived beyond the fourth day. Therefore, the extracts of corpus luteum were essential for both implantation and early pregnancy maintenance.

In 1930, Allen proposed the name "progestin" to refer to the hormone responsible for these biological effects. In 1934 four different groups reported purification and characterization of "progestin". Each group suggested a different name to refer to the main corpus luteum hormone, and the name "progesterone" came by consensus after a meeting of the League of Nation's Health Organization in 1935.

The major target organ of progesterone is the reproductive tract; however, progesterone has a systemic effect and influences other organs including, but not limited to, the mammary glands, the nervous system and brain, the heart, the bones, and the endocrine and immune systems (Fig. 1.1) [1, 2]. In the reproductive system, progesterone, in association with estrogen, is involved in the development and sexual maturation of the reproductive organs and orchestrates the menstrual cycle [3–5]. This chapter describes the specific effects of progesterone on the uterus (myometrium and endometrium) and the cervix during the normal menstrual cycle and pregnancy. Detailed discussion on the effect of progesterone on organs outside the female reproductive tract is described in other chapters of this book.

2 The Mechanisms of the Cellular Action of Progesterone

Progesterone can evoke genomic or non-genomic responses upon its interaction with target cells. The term "*genomic actions*" refers to the cellular (nuclear) response involved in the activation of the genetic machinery, resulting in modulation of DNA expression. The genomic actions of progesterone (*the Classical pathway*) are largely, but not only, mediated by the progesterone receptor (PR) [6]. The term "*non-genomic actions*" (*the Non-Classical pathways*) indicates the cellular responses to progesterone that involve alternative pathways, such as the activation of signal-transduction cascades, the generation of intracellular second messengers, and the modulation of protein kinases and ion fluxes (Fig. 1.2) [7, 8].

2.1 Genomic Actions of Progesterone and the Cytosolic Progesterone Receptor

The classical cytosolic PR, [6] a member of the steroid/nuclear receptor superfamily of ligand-activated nuclear transcription factors is a mediator of the genomic actions of progesterone. In resting conditions, this receptor is localized in the cytosol, within a large complex of proteins, including heat shock proteins and FK506-

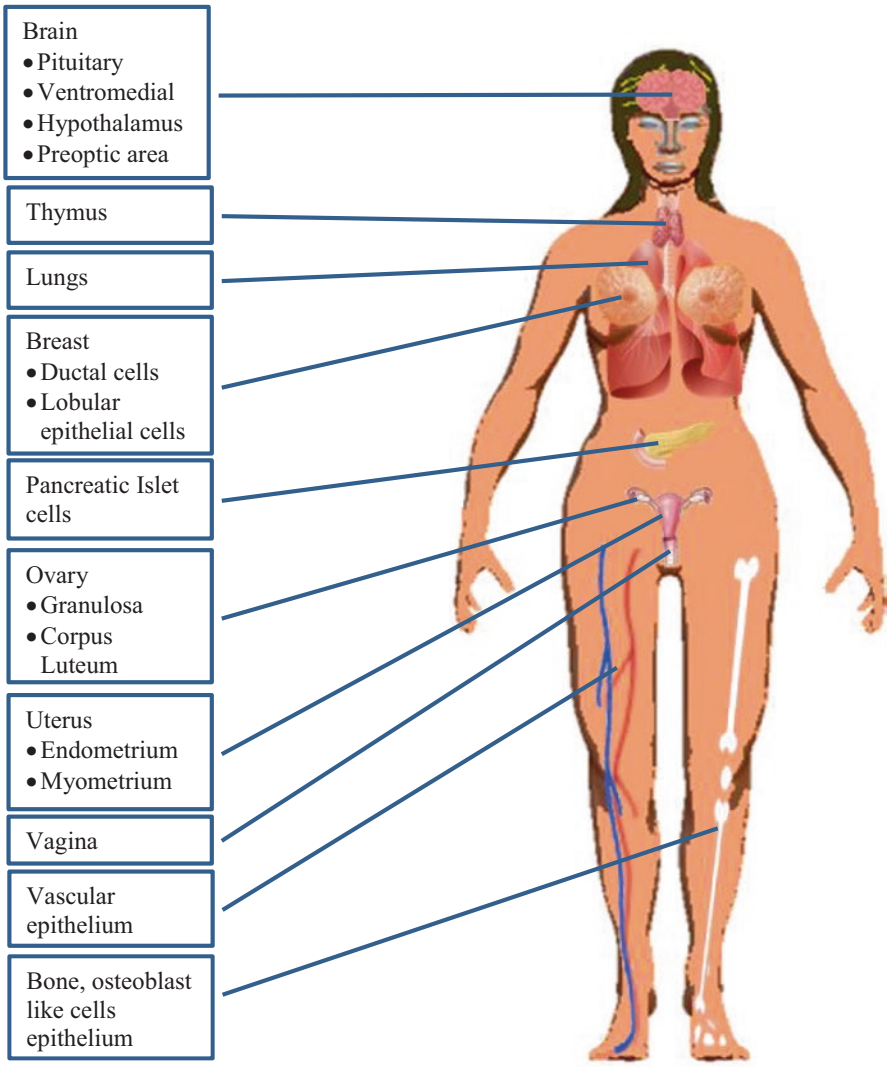

Fig. 1.1 Tissue and cell types expressing progesterone receptors. (Modified from Graham JD, Clarke CL [185])

binding proteins, contributing to maintaining it in a transcriptionally inactive state (Fig. 1.3) [9].

The cytosolic receptor can be activated by ligand-dependent [9] and ligand-independent mechanisms [10]. In the ligand-dependent pathway, progesterone gains access into the cell through passive diffusion or facilitated transport, and binds to the receptor, which changes its conformation (including dimerization and shedding of the heat shock proteins) [11]. This process allows the dissociation of the PR from

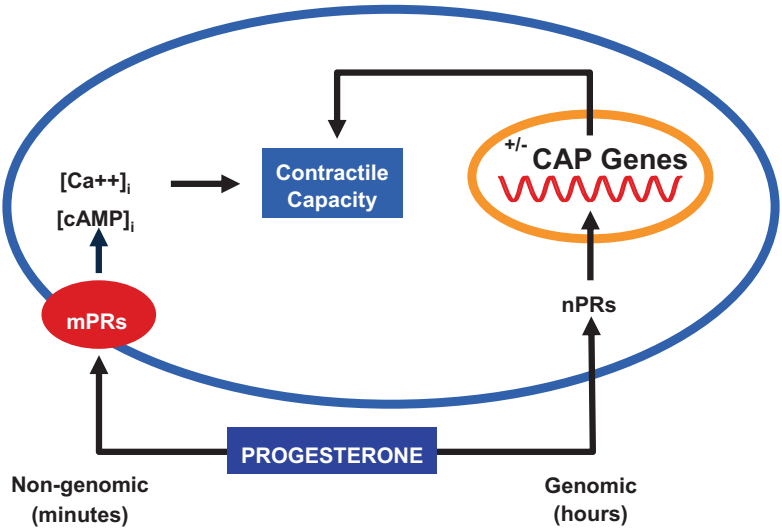

Fig. 1.2 Central Paradigm for genomic and non-genomic progesterone actions on myometrial cells. Reproduced with permission from Thieme Publishers: Mesiano S. *Myometrial progesterone responsiveness* [186]

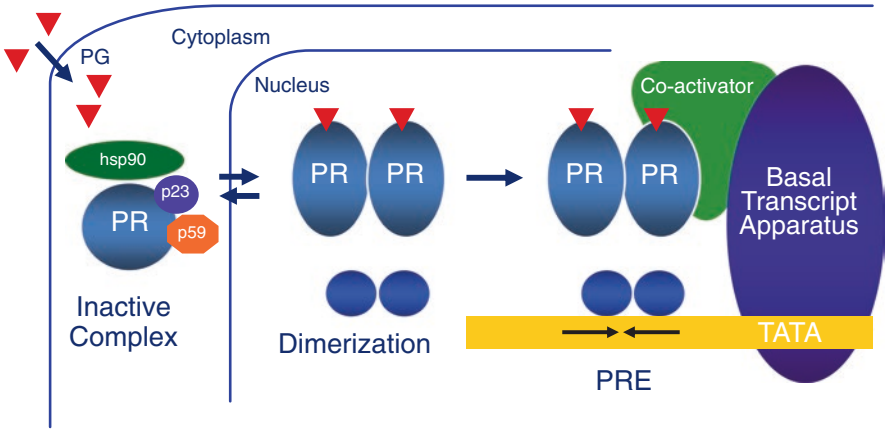

Fig. 1.3 Progesterone activation of the cytosolic progesterone receptor. Reproduced with permission from Elsevier: Leonhardt SA, Boonyaratanakornkit V, Edwards DP. *Progesterone*

the chaperone complex, its translocation into the nucleus, and finally its interaction with the DNA, where binding in a homodimeric form to cis-acting DNA progesterone response elements (PREs) modulates the transcription of target genes [9]. The ligand-independent activation of the PR, instead, is the result of cross-talk between membrane receptors and intra-cellular kinases, including a cAMP-dependent kinase, the cyclin A/cyclin-dependent kinase-2 (Cdk2), the mitogen-activating pro-

tein kinase (MAPK), the stress-activated p38 MAPK, and the protein kinases A and C [12].

The progesterone receptor gene (PGR) encoding the human nuclear PR is located on chromosome 11q22.1 and has eight exons. Alternative splicing allows for the synthesis of different isoforms of the receptor [13]. The two major isoforms of the PR are progesterone receptor-A (PR-A) and B (PR-B). These isoforms, although characterized by a different length, do not differ in their amino acid sequence: PR-B is 933 amino acids in length, while PR-A lacks 165 amino acids at the N-terminal [14]. *In vitro*, PR-B is a stronger trans-activator than PR-A, whereas PR-A acts as a trans-repressor of PR-B and of other steroid receptors [15, 16]. Structurally, both isoforms consist of an amino-terminal region, a centrally located DNA binding domain, and a carboxy-terminal hinge region containing nuclear localization signals as well as the ligand-binding domain. Three transcription activation function (AF) domains have been identified within the PR amino acid chain. AF-1 is located upstream of the DNA-binding domain, while AF-2 is located in the ligand-binding domain [17]. AF-3 is unique to the PR-B isoform and is located within the N-terminal region [15, 18]. In addition, an inhibitory function region, located between the AF-1 and AF-3 domains, has been proposed to be responsible for the auto-inhibition and trans-repression of the PR [19]. Interestingly, most of the evolutionary changes in the human PR took place in this region [20]. (For more information on the structure of the Human Progesterone Receptor gene, see http://www.ncbi.nlm.nih.gov/gene/5241).

A third isoform of the PR, the PR-C, was also described [21, 22]. The PR-C is a 60kD N-terminally truncated isoform, lacking the DNA-binding domain, but containing the hormone-binding region with the sequence for dimerization and nuclear localization [21, 23]. The cytoplasmatic PR-C has been suggested to inhibit PR-B activity by sequestrating the locally available progesterone [23]. The nuclear PR-C can form heterodimers with PR-B, therefore interfering with its binding to the response elements in the DNA [23]. In contrast, PR-C can enhance the progestin-induced transcriptional activity of the PR-A and PR-B isoforms, either by sequestrating the co-repressors and/or by increasing the capacity of the heterodimers of PR-A or PR-B with PR-C to recruit co-activators [21]. In this manner, PR-C could be involved in the modulation of the transcriptional activity of PR-A and PR-B, contributing to the pleiotropic effects of progestins [21]. Additional isoforms of the PR, such as PR-S [24] and PR-M [25] have also been identified and partially characterized. It has been proposed that the tissue responses to progesterone may be affected by changes in the expression ratio of the different isoforms [22].

Importantly, the validity of the immunoassay used in the identification of some of the PR isoforms, such as PR-C and PR-M, has been questioned, [26, 27] as the nuclear PR antibodies used may cross-react with cytoskeletal proteins (α-actinin, desmin and vimentin). Hence, these antibodies are not specific for these PR isoforms [27].

2.2 The Role of Co-Regulators in Progesterone Signaling

The activity of the nuclear PR is regulated not only by the hormone itself but also by co-regulators (co-activators and co-repressors) as well as by chromatin modifiers [28]. Co-regulators can enhance or inhibit gene transcription by creating a functional link between the ligand-activated receptors, the DNA and the transcription factors [29]. The existence of "intermediary factors" in the PR nuclear signaling was described more than four decades ago by the group of B.W. O'Malley [30]. Since then, the interest in co-regulators has increased, given their possible involvement in the "transcriptional interference" in the tissue-specific responses, evoked by nuclear receptor ligands and selective receptor modulators (i.e., Tamoxifen and Raloxifene), and their roles in the pathogenesis/progression of neoplastic disease [31]. Thus, the possible involvement of progesterone co-regulators in the modulation of myometrial progesterone action should be taken into account [32, 33].

Progesterone co-activators include members of the "Steroid Receptor Co-activator" (SRC/p160) family, [34] such as SRC-1, SRC-2, and SRC-3, which share a strong sequence homology [34, 35]. The involvement of these progesterone co-activators in normal growth, puberty, and female reproductive function, as well as in mammary gland development, is supported by studies of genetically modified animals. SRC-1 [36, 37] is an important co-activator in the uterus, whereas SRC-3 is in the mammary gland, [37, 38] and SRC-2 is in both organs [32, 33]. Of note, SRC-1 and SRC-2 knockout mice manifest a deficient uterine response to progesterone stimulation. However, SRC-1 knockout mice preserved their fertility, [36] whereas SRC-2 knockout mice had an early block of embryo implantation [32]. Progesterone receptor co-activators share an NRbox (also called the LXXLL motif) necessary for binding to the "co-activator binding groove" in the receptor [35, 39].

Co-repressors of the progesterone receptors inhibit transcription factor recruitment and down-regulate the receptor-dependent gene expression. This is accomplished preferentially by recruiting histone deacetylases, [40] which enhance tight nucleosome-DNA interactions and increase chromatin compaction [35]. However, the molecular basis of the interactions between steroid receptors and co-repressors is not well-defined [35].

2.3 Non-genomic Actions of Progesterone

The identification of steroid receptors on cells lacking a functional nucleus, (i.e., spermatozoa, erythrocytes and platelets) suggests non-genomic steroid actions. This is a fast-track rapid response system, in contrast to the long response time (i.e., hours/days) of the "genomic" pathways [7, 8]. The first evidence in support of the existence of non-genomic progesterone actions came from the study of progesterone responses in germ cells (oocytes and spermatocytes). Some of the non-genomic actions exerted by progesterone on these germ cells include changes in intracellular

calcium concentrations, [41, 42] promotion of Na^+ [43] and Cl^-[44] fluxes, inhibition of adenylate cyclase activity with a consequent decrease in intracellular cAMP levels, [45] and the involvement in G proteins-phospholipase C- inositol trisphosphate, and diacylglycerol signaling [46, 47]. Fig. 1.3.

"Membrane-initiated steroid signaling" defines the non-genomic activities of progesterone that are secondary to the activation of membrane-bound progesterone receptors (mPRs) [7, 8]. Evidence supporting the idea that at least some of the non-genomic actions of progesterone are mediated by mPRs includes: (1) progesterone application outside the cell is more effective in decreasing intracellular cAMP concentrations than upon its cytoplasmic microinjection; [48] (2) progesterone activity is sustained after conjugation with synthetic polymers [49, 50] or its covalent binding to large molecules, such as albumin, [46, 51] which prevent progesterone access into the cytosol; (3) progesterone effects are reduced in the presence of antibodies directed toward progesterone membrane-binding proteins; [42] and (4) the non-genomic activities of progesterone, such as Ca^{2+} influx, are not affected by inhibitors of genomic progesterone responses, including RU38486 and RU486 [41, 52] Fig. 1.4.

Progesterone high-affinity binding proteins and receptors have been identified on the cellular membranes of a variety of cells such as spermatozoa, [41, 42, 52]

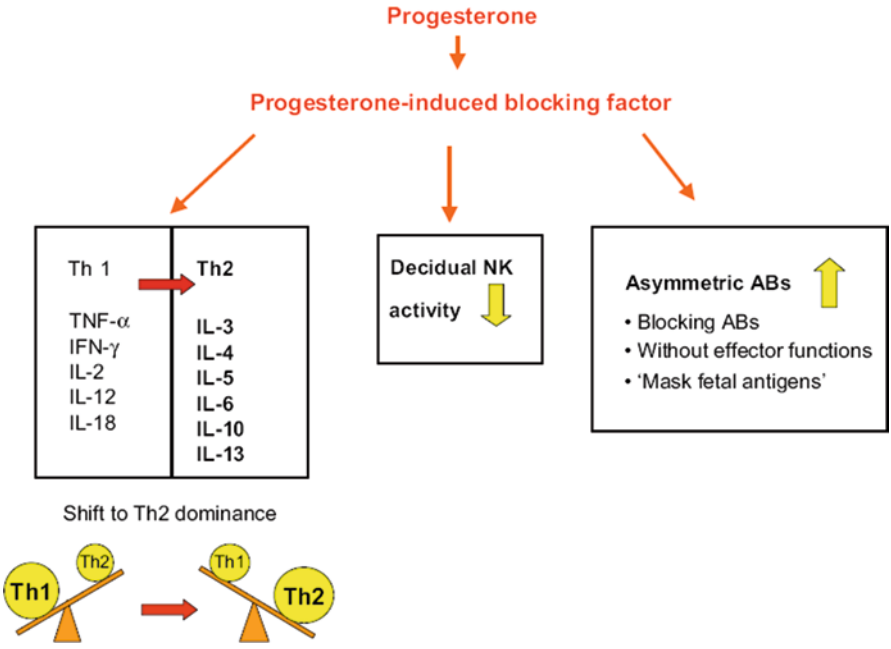

Fig. 1.4 The effect of progesterone and progesterone induced blocking factor (PIBF) on maternal immune system during pregnancy. Reproduced with permission from Elsevier: Walch KT, Huber JC, *Progesterone for recurrent miscarriage: truth and deceptions.* Best Pract Res Clin Obstet Gynaecol, 2008. 22:375–89 [59]

porcine liver microsomes, [53, 78] and porcine vascular muscle cells [54] as well as in the brains of female mice knocked out for the classical PR [55]. Some researchers have previously argued that the existence of a progesterone-binding site does not necessarily indicate that the receptor is functionally active in terms of cellular signaling, and that a characterization of non-classical receptors is still required [7].

Non-genomic progesterone receptors display different affinities, binding capacities, and dose response/competition curves for progesterone and other molecules sharing progestin structure. For example, the recombinant human mPRγ, produced in an *E. coli* expression system, has a high-affinity, saturable, single binding site for progesterone and several of its hydroxylated derivatives; however, recombinant human mPRγ does not bind and has no affinity for synthetic progestins and anti-progestins [56]. Similarly, there is evidence indicating the presence of at least two distinct membrane-surface progesterone receptors in capacitated human spermatozoa: a high-affinity site specific for progesterone and a low-affinity site that binds with equal affinity to 11β-hydroxyprogesterone and 17α-hydroxyprogesterone [41].

3 The Physiologic Effects of Progesterone

3.1 The Effect of Progesterone on the Immune System

The immune system in the female reproductive tract faces two opposing challenges: the consistent exposure to infectious pathogens and, in contrast, the need to be tolerant to both the allogenic spermatozoa and the semi-allogenic fetus. To overcome these challenges, the female sex steroids (i.e., estrogen and progesterone) control the function of the innate and adaptive immune systems in the reproductive tract according to the changes occurring through the menstrual cycle and during pregnancy [57, 58]. Indeed, in the rat uterus, major histocompatibility complex (MHC) class II positive cells, macrophages, granulocytes, and dendritic cells were more abundant in the endometrial stroma and around the uterine glandular epithelium in the estrus stages of the menstrual cycle relative to the diestrus stages in which progesterone is the dominant hormone [59]. Moreover, ovariectomy in mice results in a decrease in the number of uterine macrophages that can be restored by hormonal treatment [2].

The uterine/decidual natural killer (uNK) cells, which are different from the peripheral NK cell population, [1] are also affected by progesterone. uNK cells have a role in promoting blastocyst implantation and maintenance of pregnancy [60, 61]. During the mid-late luteal phase, the numbers of this unique population of NK cells is elevated [62, 63] as a result of the increased decidual concentrations of interleukin (IL)-15, and IL-15 mRNA [64]. Their number further increases during the early stages of pregnancy and decreases from mid-gestation to term [62]. The immunologic recognition of pregnancy also leads to a higher expression of PR on the

membrane of uterine NK cells [65] and decreased cytotoxic activity in comparison to the non-pregnant state [66].

During normal pregnancy, progesterone diverts the T-cell response toward Th-2 rather than Th-1, leading to higher secretion of IL-6 and IL-10, as well as supporting B-cell antibody production [67–69]. During pregnancy, there is also a change in the antibody population and a shift toward asymmetric "blocking" antibodies (i.e., those glycosylated by mannose-rich oligosaccharide on only one of the Fab regions; although asymmetric antibodies can combine with antigens, they poorly activate phagocytosis, complement fixation, and cytotoxicity). The prevalence of asymmetric antibodies increases from 9% in the non-pregnant state to 29% in pregnant women [70]. Yet, they can compete with symmetric and competent antibodies having the same specificity and thus block the actions of symmetric antibodies. Asymetric antibodies may be a mechanism to reduce the antibodies' mediated response against the invading trophoblast during pregnancy and to control the equilibrium of maternal anti-fetal immune responses [71].

It has been reported that the effects of progesterone on the T-cell response, B-cell activity, generation of asymmetric antibodies, and NK cytotoxicity are mediated by a progesterone-induced blocking factor (PIBF) (Fig. 1.4), a 34 kDa immunoregulatory protein synthesized by PR-positive lymphocytes and CD56+ decidual cells [72]. The actions of PIBF include: (1) enhancement of the production of asymmetric antibodies; [73] (2) diverting the T-helper response toward Th-2 activity, resulting in increased concentrations of IL-3, IL-4, and IL-10 as well as decreased IL-12 production; [74] and (3) the latter, combined with the inhibition of perforin secretion by PIBF in a dose-dependent manner, reduces the cytotoxic activity of NK cells [74, 75]. In summary, progesterone affects all arms of the immune system and propagates maternal tolerance to the semi-allogenic fetus.

3.2 The Role of Progesterone in Non-pregnant Women

3.2.1 Progesterone and the Menstrual Cycle

Progesterone participates in the control of ovulation, the preparation and stabilization of the endometrium before implantation, the regulation of the implantation process, and the maintenance of pregnancy [76]. During the follicular phase of the menstrual cycle, estrogen predominates and has a major role in the proliferation of the endometrium while progesterone concentration is relatively low. Progesterone predominates during the secretory phase (maximal concentrations occur in the mid-luteal phase), inhibits the endometrial proliferation induced by estrogen, and changes the endometrial morphology to the secretory type [77]. However, the glandular and vascular elements continue to grow, resulting in progressive tortuosity [77]. Progesterone stimulates glycogen vacuole formation within glandular cells, resulting in the active secretion of glycoproteins and peptides by the glands into the endometrial cavity as well as in edema of the endometrial stromal tissue [78]. In the

mid-luteal phase, progesterone is responsible for the transformation of stromal cells into decidual cells, which is critical for the establishment of pregnancy. In the absence of conception, the degeneration of the corpus luteum exerts a physiological progesterone withdrawal resulting in menstruation [76].

Previous exposure to estrogen is essential to stimulate synthesis of PR in endometrial cells. Progesterone can then exert its anti-estrogenic effect on the endometrium [79] through several proposed potential mechanisms, such as the down regulation of estrogen receptor expression, [80] the conversion of estradiol to a less active form (estrone sulphate) via the stimulation of 17-hydroxysteroid dehydrogenase and sulfotransferase, and the suppression of estrogen-mediated synthesis/secretion of specific proteins (e.g., transcription of the proto-oncogene c-fos mRNA) [81].

In addition, progesterone increases the expression of tissue factor (TF) and plasminogen activator inhibitor-1 during decidualization [82]. It has been suggested that an increase in decidual TF concentration is needed to secure rapid hemostasis during blastocyst implantation and placentation as well as the control of postpartum hemorrhage [82]. The association between the decidual expression of TF and progesterone was established by the differences in TF expression in confluent stromal cell cultures derived from proliferative phase endometrium. Stromal cell cultures treated with mifepristone did not increase their TF expression; moreover, administration of mifepristone to cell cultures previously exposed to estradiol+MPA (medroxyprogesterone acetate) or estradiol+progesterone decreased their TF content and TF mRNA expression [83]. Therefore, a low progesterone concentration could contribute to less-effective decidual hemostasis, which may lead to increased decidual bleeding, and a subsequent spontaneous miscarriage or preterm delivery.

3.2.2 Progesterone and the Myometrium in the Non-pregnant Uterus

Uterine contractile activity throughout the menstrual cycle is partially regulated by estrogen and progesterone [84, 85]. This process has been proposed to be mediated by cyclic changes in estrogen and progesterone receptor expression in the endometrium and sub-endometrium [86]. The decrease in the progesterone concentration in the transition from the luteal phase of one menstrual cycle to the follicular phase of the subsequent cycle is followed by increased uterine contractility, which aids in clearing menstrual contents [85]. The rise in estrogen concentration during the late follicular phase further increases uterine contractility, preparing the uterus to facilitate sperm motility toward the Fallopian tube [85]. During the luteal phase, following an increase in progesterone concentration, the uterus is relatively quiescent [84, 85].

Of note, studies in non-pregnant women demonstrated that plasma progesterone concentrations do not reflect the actual progesterone concentrations in the myometrium. Akerlud et al. [87] measured the estrogen and progesterone concentrations in the non-pregnant uterus of women with normal menstrual cycles, demonstrating that there is no correlation between plasma and tissue progesterone concentrations

in the same individual, although the progesterone concentrations in the plasma and myometrial tissue change during the menstrual cycle. The authors reported that in peri- and postmenopausal women, the myometrial concentration of progesterone remains comparable to those in menstruating women, despite a substantial decline in the plasma concentration of progesterone, suggesting that the myometrial uptake of ovarian hormones may be saturated even if plasma concentrations are relatively low. Moreover, the myometrial/plasma ratio of progesterone decreases significantly during the luteal phase [87]. This decrease may be due to down-regulation of the myometrial progesterone receptors following accumulation of progesterone in the myometrium [88].

3.2.3 The Effect of Progesterone on the Uterine Cervix during the Menstrual Cycle

The uterine cervix is a primary end organ that is responsive to pubertal hormonal action, cyclical changes in sex hormones during the menstrual cycle, pregnancy, labor, and menopause [89–91]. The expression of PR changes significantly in the glandular epithelium of the cervix, reaching its peak in the early secretory phase and declining sharply afterward [92]. Progesterone has a dramatic effect on the constituents of the cervical mucus and, hence, on the function of cervical secretions [93, 94]. The cervical mucus becomes thick and sticky under the influence of progesterone. Indeed, one of the suggested mechanisms by which progestins exert their contraceptive effect is through changes in the chemical properties of mucus [95, 96].

The available data suggest that progesterone has an effect on the cervix in the non-pregnant state; however, how and to what extent this effect is important in physiologic and pathologic conditions have yet to be determined.

3.3 Progesterone and Pregnancy

3.3.1 The Role of Progesterone in the Maintenance of Normal Pregnancy and Parturition

Estrogen and progesterone play a central role in pregnancy [97]. The corpus luteum is the main source of progesterone until the seventh week of gestation; then the placenta takes over as the main source of progesterone between 7 and 9 weeks of gestation, a transition termed the “luteal-placental shift” [98, 99] (Fig. 1.5). Indeed, ovariectomy before 8 weeks of gestation results in abortion, but the procedure has no effect on the pregnancy if performed after 9 weeks of gestation. Maternal plasma progesterone concentrations rise during pregnancy from 40 ng/mL in the first trimester to 160 ng/mL in the third trimester [99]. At term, the placenta produces approximately 250 mg of progesterone per day, of which 90% is secreted to the maternal circulation and only 10% into the fetal circulation. However, the fetal

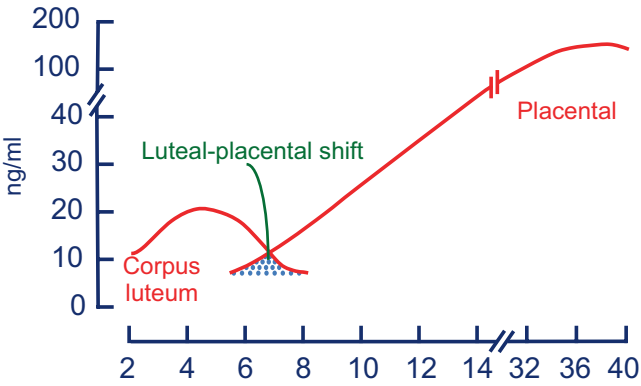

Fig. 1.5 The luteal-placental shift in progesterone production during pregnancy. Reproduced with permission from Elsevier: Yen SSC. *Endocrine-metabolic adaptation in pregnancy*, in *Reproductive endocrinology*, Yen SCC Jaffe RB, Editors. 1991, W.B. Saunders: Philadelphia. p. 936–971 [188]

plasma progesterone concentration is seven-fold higher than the maternal concentration, probably due to the differences in their volume of distribution [100]. However, neither parturition at term [101] nor preterm [102] are associated with significant changes in amniotic fluid progesterone concentrations.

During pregnancy, progesterone is thought to maintain myometrial quiescence and inhibit cervical ripening, while estrogens have been implicated in increasing myometrial contractility and excitability as well as in the induction of cervical ripening prior to the onset of labor [103, 104]. However, before spontaneous parturition, the changes in sex-steroid serum concentrations differ between different species. In many species, a fall in maternal serum progesterone concentration occurs prior to the onset of parturition.

Luteolysis is a crucial component in the mechanism of parturition in the rat, mouse and rabbit [105, 106]. An increase in local progesterone metabolism in both the uterus [107] and cervix [108] was associated with the onset of labor in mice. In sheep and goats, an increase in fetal plasma cortisol induces the placental production of P450 C17 enzymes (17α-hydroxylase and C17–20 lyases), which catalyze the conversion of progesterone to androstenedione, which is transformed into estrogen by aromatases [109]. However, in primates (including humans) and guinea pigs, there is no apparent change in the circulating maternal progesterone concentration before parturition. The human placenta, lacks P450 C17 enzymes and, therefore, cannot synthesize estrogen and androstenedione from C21-progestins; thus, progesterone is the final product of the human placenta.

A serum "progesterone withdrawal" has not been demonstrated in humans or guinea pigs; yet, progesterone is considered important in pregnancy maintenance because inhibition of its action could result in parturition in both species. Administration of anti-progestins [i.e., mifepristone or onapristone] to pregnant women, [110] primates [111] or guinea pigs [103] can induce abortion and/or labor. [97, 127] Alternative mechanisms for the suspension of progesterone action without

a serum progesterone withdrawal have been proposed, including: (1) binding of progesterone to a high-affinity protein that reduces the functional active form; [112] (2) an increase in cortisol concentration during late pregnancy, which may compete with progesterone binding to the glucocorticoid receptors, resulting in a functional progesterone withdrawal; [113] and (3) the conversion of progesterone to an inactive form within the target cell before interacting with its receptor. Indeed, the human amnion and chorion can convert progesterone to the inactive 20α-dihydroxyprogesterone, and this metabolite increases with gestational age and around the time of parturition [114, 115]. However, none of these hypotheses have been proven; [116] therefore, the focus of investigation has shifted to the abundance and modulation of estrogen-progesterone receptor expression and to progesterone's binding capability to its nuclear response element.

3.3.2 The Nuclear Progesterone Receptor in the Myometrium during Pregnancy and Parturition

Conflicting results have been reported regarding the role of the PR in the myometrium during human pregnancy and parturition [116, 117]. The conflicting results may be due to the existence of multiple receptor isoforms, whose myometrial expression is spatially and temporally regulated throughout gestation [118]. Thus, it is likely that the results of the studies may be affected by the sampling site and the specificity of the assay. Of note, initial studies on PR expression in the human myometrium did not distinguish between the different isoforms and were performed on biopsies isolated from the lower uterine segment. In contrast, more recent studies tested the expression of the different receptor isoforms and focused preferentially on the fundal myometrium. The latter is more likely to reflect the molecular changes that mediate uterine contractility than the lower uterine segment, which reacts in favor of dilatation [22]. Finally, the non-genomic progesterone actions have broadened the research on the mechanism of labor toward identification of membrane progesterone receptors that may participate in the suspension of progesterone action.

The key mechanisms explaining the functional progesterone withdrawal include either a reduction of the total number of progesterone receptors within the target tissue or a relative increase of inhibitory PR isoforms. Rezapour et al. [119] investigated the expression of progesterone receptors in the myometrium of women at term not in labor and in the active phase of spontaneous labor, and found significant changes in the distribution of receptors after the onset of labor. The active normal labor group had a higher receptor concentration in the upper uterine segment as well as a higher upper-to-lower uterine segment receptor ratio than the not-in-labor group. Of interest, myometrial PR concentrations were lower in oxytocin-resistant labor than in normal labor. Although progesterone is involved in labor-associated changes in the myometrium through receptor-mediated processes, Rezapour et al. [119] suggested that progesterone is not an inhibitor of myometrial contractility and thus, not consistent with the progesterone withdrawal theory. However, there are *in vitro* reports indicating that progesterone stimulates myometrial tonus and

frequency of contractions [120] and that it has an anti-tachyphylactic effect on oxytocin-induced myometrial contractions [121].

Pieber et al. [116] analyzed the labor-associated changes in the expression of PR-A and PR-B in myometrial samples obtained during term cesarean deliveries from women not in labor and in labor. While PR-A expression was detected only in the presence of effective labor, PR-B was equally expressed in labor and not-in-labor samples. Transient transfection of myometrial cells with PR-A and PR-B confirmed that the over-expression of PR-A has a dominant repressive effect on the transcription of progesterone sensitive genes within human term myometrial cells. The authors interpreted that the expression of PR-B occurs throughout gestation and is required for pregnancy maintenance, whereas a higher expression of PR-A in the presence of effective labor at term may contribute to "functional progesterone withdrawal" [116]. The increase in the expression of the inhibitory PR-A, [116, 122] and in the PR-A/PR-B ratio in the human myometrium, was interpreted as the possible underlying mechanism of the "functional progesterone withdrawal".

The change in the PR-A/PR-B ratio occurs at the mRNA level [123]. The abundance of mRNAs encoding for PR-A and PR-B and estrogen receptors (ERα and β) were compared in the lower uterine segment in women at term in labor and not in labor. The mRNA levels of ERα and of the homeobox gene *HOXA10* were used as markers of progesterone responsiveness. In the laboring myometrium, the mean relative abundance of mRNAs encoding for PR-A, PR-B, and ERα was significantly increased compared to non-laboring tissue, whereas ERβ was low and did not differ between the groups. There was a significant two- to three-fold increase in the PR-A/PR-B ratio in laboring compared to non-laboring specimens. Of interest, in non-laboring myometria, the PR-A mRNA levels and the PR-A/PR-B mRNA ratio positively correlated with mRNA of ERα and *HOXA10* in the laboring myometrium. These positive correlations were interpreted as an indicator that progesterone responsiveness is inversely related to the PR-A/PR-B gene expression ratio and decreases at the onset of labor. Moreover, ERα could be an early gene, whereas *HOXA10* may possibly be a late gene responding respectively to changes in the PR-A/PR-B expression ratio. The positive correlation detected in non-laboring myometria between ERα mRNA levels and those of contraction-associated genes, such as cyclooxygenase-2 (COX-2), and the oxytocin receptor, suggests that the process of human parturition is initiated within myometrial cells well before the onset of active labor [123].

3.3.3 The Membrane Progesterone Receptor during Pregnancy and Parturition

The first report on the existence of high-affinity membrane-associated progesterone binding sites within the uterine tissue dates back to the 1984 study of Haukkamma et al. [124]. It was noted that uterine membrane-associated receptors differ from their soluble cytosolic counterparts, previously identified in the human uterus, in terms of the specificity of their ligands.

Labor and sex-steroids differentially modulate the mPRs. Karteris et al. [125] reported the expression of two different functional mPRs (mPRα and mPRβ) in the myometrial cells of pregnant humans that are directly coupled to G-inhibitory proteins. This expression results in the inhibition of adenyl cyclase, a subsequent decline in cAMP concentrations and increased phosphorylation of the myosin light chain, which facilitates myometrial contractions. The authors proposed that, during labor, progesterone acts preferentially on its membrane receptors, a modus operandi that promotes the shift from quiescence to a contractile state. This change results from the altered PR-B/PR-A ratio, the changes in sex-steroids, and the existence of complex cross-talk between the nuclear and membrane progesterone receptors [125].

Fernandes et al. [126] combined bioinformatic analyses with the expression profile of mPRs to define their role in cycling human endometrium and gestational tissues. Sequence analysis suggested that these receptors belong to the "progestin and adiponectin receptors" family. The onset of parturition was associated with a marked reduction in myometrial mPRα and mPRβ transcripts. Of interest, the levels of mPRα expression were high in the placenta, and inversely correlated with that of the nuclear PR, indicating that mPRα may have an important functional role, particularly in reproductive tissues expressing low levels of nuclear PR [126].

3.3.4 Progesterone Oxytocin Responsiveness and Ca^{2+} Fluxes

Progesterone reduces the myometrial responsiveness to oxytocin through genomic [127] and non-genomic [128, 129] pathways. However, the exact mechanisms by which progesterone blunt uterine responsiveness to oxytocin is unclear. Three potential mechanisms have been proposed: (1) progesterone represses oxytocin receptor synthesis through its genomic action; [127] (2) direct interaction between progesterone and its metabolites with the oxytocin receptor; [130] or (3) the continuous presence of intracellular high progesterone concentrations may alter the responsiveness of the oxytocin receptor through non-genomic effects [131].

The oxytocin receptor needs a cholesterol-rich microenvironment to become stable in its high-affinity state [132]. The intracellular binding of progesterone to the multi-drug-resistant P-glycoprotein interferes with cholesterol transport to and from the plasma membrane, and higher intracellular concentrations of progesterone inhibit cholesterol esterification, [133] which reduces cholesterol concentrations in the plasma membrane [134]. Additionally, progesterone also increases the activity of 3-hydroxy-3-methylglutaryl (HMG-CoA) reductase, increasing the synthesis and membrane concentrations of the cholesterol precursors that are less active in their support of the high-affinity oxytocin receptor [135]. The depletion of active membranous cholesterol forms leads to a low-affinity mode of the oxytocin receptor, which may reduce its intracellular activity [136]. A decrease in the intracellular progesterone concentrations restores the cholesterol transport, leading to an increase in the active cholesterol concentration in the plasma membrane that supports the

activity of the high-affinity oxytocin receptor, thus regaining its uterotonic effect [137].

Some of the activities of progesterone on the myometrium may be mediated by its effects on the activity and metabolism of cAMP [138] and the inhibition of transmembrane Ca^{2+} entry [139]. Treatment of human myometrial smooth muscle cells with MPA resulted in a significant reduction in the oxytocin-mediated increase in intracellular Ca^{2+} concentration [139].

3.3.5 The Interplay Between NFκB and Progesterone in Pregnancy Maintenance and in the Onset of Labor

Nuclear factor kappa B (NFκB) is a transcription factor family classically associated with inflammation. Data indicate that myometrial NFκB activity changes with labor and its activation is regulated in a spatio-temporal fashion. It has been proposed that NFκB is an upstream regulator of multiple labor-associated processes, including the formation of contraction-associated proteins, inflammatory mediators (e.g., cytokines), uterotonic phospholipid metabolites (e.g., prostaglandins), and the induction of extracellular matrix remodeling [140, 141]. The stimuli and mechanisms responsible for NFκB activation in spontaneous labor have not yet been elucidated. Increasing local concentrations of surfactant protein A, [142] accumulation of advance glycation end-products, [143] the amnion cells' mechanical stretch, [144, 184] and the paracrine or autocrine pro-inflammatory effects of the corticotrophin-releasing hormone [145] have been proposed as potential candidates.

NFκB activation favors the myometrial expression of inhibitory isoforms of the PR. Evidence in support of this role includes: (1) a spatial correlation is suggested by the enhanced expression of PR-B and PR-C along with NFκB activation during labor, and these changes are selective to the fundal human myometrium; [22] (2) a temporal correlation has been proposed given the correlation of PR isoform expression and local NFκB activation in the pregnant mouse uterus and in the human fundal myometrium; [22, 142] (3) intra-amniotic injection of surfactant protein A to pregnant mice promoted uterine NFκB activation and preterm labor as well as a rapid increase in uterine levels of PR-B and PR-C; [22, 142] (4) intra-amniotic injection of the NFκB inhibitor (SN50) caused a decrease in the uterine levels of PR-B and PR-C; [22, 142] and (5) *in vitro* models demonstrated that the activation of the NFκB pathway in response to IL-1β treatment is associated with an increased expression of all three PR isoforms (PR-A, PR-B and PR-C) in myometrial cells [22].

The PR-mediated activation of target genes that modulate uterine contractility is antagonized by NFκB. Kalkhoven et al. [146] reported the existence of a mutual trans-repression between the PR and the RelA(p65) subunit of NFκB in different cell lines. This repression was independent from the PR isoforms and the cell type. The authors suggested that the most likely mechanism involved is a direct interaction between the two proteins that would result in an inactive heterodimeric complex on the DNA, which prevents co-factors and members of the basal

transcriptional machinery from initiating transcription [146]. Other possible explanations for the mutual repression of RelA(p65) and PR include binding of these transcription factors to their respective cognate DNA elements, or competition for the same co-activators or transcription intermediary factors (transcriptional interference or squelching) [146]. A similar mutual negative interaction between NFκB and PR activity was reported in human amnion cells [147]. Stimulation of these cells with IL-1β resulted in NFκB activation, followed by a repression of progesterone-dependent transcription, even in the presence of excess PR [147]. This may be the case during spontaneous labor in humans: indeed, the constitutive activity of NFκB reported in human amnion cells in the presence of labor may contribute to the loss of myometrial quiescence, both by repressing the PR activity and increasing the expression of COX-2. The authors proposed that the increase in NFκB activity, near to, or at the time of labor, may represent a "watershed point at which labor becomes inevitable" [147].

The anti-inflammatory activity of progesterone may contribute to the prolongation of pregnancy by direct or indirect attenuation of the NFκB-mediated inflammatory cascade. Several observations support this view: (1) over-expression of the PR in amnion cells was associated with significant repression of NFκB reporter expression; [147] (2) the IL-1β induced up-regulation of COX-2 mRNA in immortalized human fundal myometrial cells was suppressed by exogenous administration of progesterone and associated with a rapid induction of the NFκB transactivation inhibitor, kBα; [148] (3) progesterone down-regulates cytokine production by human leukemia cell lines, mediated, at least in part, by suppression of NFκB activity; [149] and (4) physiological concentrations of progesterone suppress both the spontaneous and the IL-1(α and β)-mediated production of IL-8 by the uterine cervical fibroblasts in pregnant rabbits [150].

In contrast, Vidaeff et al. [151] demonstrated that pre-treatment of HeLa cells with progesterone before exposure to IL-1β resulted in a significant decrease in NFκB protein subunit p65 in the cytoplasm. However, pre-treatment of HeLa cells did not reduce the amount of nuclear p65 or affect the nuclear translocation of p65. The authors suggested that any possible role played by progesterone in preterm labor prevention is not exerted through anti-inflammatory mechanisms of NFκB down-regulation [151].

3.3.6 Changes in Myometrial Progesterone Co-Regulators during Pregnancy

The possibility that changes in the activity of co-regulators can contribute to the functional progesterone withdrawal is currently an object of investigation. Condon et al. [152] proposed that a decline in the levels of PR co-activators in the pregnant uterus at term may antagonize PR function and contribute to the initiation of labor. Analysis of the mRNA and protein expression of PR co-activators in the fundal myometrium of 12 women in labor and 12 women not in labor revealed that the laboring myometrium was associated with a lower mRNA and protein expression

of SRC-2, SRC-3, and CBP (the cAMP-response element-binding protein) than non-laboring myometrial samples, while SRC-1 expression was relatively unchanged before and after the onset of labor.

Term gestation was associated with a decrease in the levels of histone H3 acetylation in the human and mouse uteri. Treatment of pregnant mice with trichostatin, a histone deacetylase inhibitor, delayed the onset of labor by 24 to 48 hours. Altogether, these results suggested that reduced uterine expression of progesterone co-activators at term would lead to a reduction in histone acetylation, thus resulting in an impaired PR responsiveness and a functional progesterone withdrawal [152].

In 2005, a novel progesterone co-repressor, polypyrimidine tract-binding protein-associated splicing factor (PSF), was identified in the rat myometrium: [153] its mRNA expression increased as term approached and was up-regulated prior the onset of labor. PSF interferes with PR binding to its DNA response element and enhances PR degradation. Within the human myometrium, PSF expression was significantly up-regulated as pregnancy progressed, particularly within the upper uterine region, and levels remained elevated in labor. Co-immunoprecipitations and DNA-binding assays showed that PSF directly interacts with the nuclear PR and its glucocorticoid receptor and specific co-regulatory proteins within the human myometrium [154]. These findings are suggestive of a role for myometrial PSF as a nuclear co-regulator and a potential contributor to functional progesterone withdrawal [153–155].

3.3.7 Progesterone Receptor in Fetal Membranes, Decidua, Placenta

There is evidence that all three isoforms, PR-A, PR-B and PR-C, are expressed in the decidua and fetal membranes, yet there is still controversy concerning the predominant isoform [156–159]. A Western Blot analysis conducted by Goldman et al. [156] revealed that the major isoform in the human decidua is PR-B, whereas in the human amnion, it is PR-C. In contrast, in a review on this subject, Taylor et al. [159] reported that immunohistochemical, Western blotting and real-time reverse transcription polymerase chain reaction techniques provide evidence that the major PR isoform in the human decidua is PR-A, whereas in the human term fetal membranes and syncytiotrophoblast, it is PR-C [159].

The quantitative and qualitative expression of the PR isoforms in the decidua and fetal membranes may be subject to significant changes during labor [156, 157]. Our group reported that, in fetal membranes obtained from women in labor, there is a PR-A predominance and a higher PR-A/PR-B ratio than in women not in labor, in which PR-B is the predominant isoform [157]. Similarly, Mesiano et al. [123] reported that mRNA encoding for PR-A and the PR-A/PR-B expression ratio increased significantly in the human myometrium at term in association with labor. Although the role of progesterone receptors in the fetal membranes has not yet been elucidated, it has been proposed that a shift in progesterone isoform expression may be part of a “feto-maternal signaling pathway in the initiation of labor” [158].

3.3.8 The Effect of Progesterone on the Cervix During Pregnancy

Progesterone exerts biological effects in the uterine cervix, and a withdrawal (in rats, rabbits and sheep) or decline in progesterone action (guinea pigs and primates) [109, 139] has been proposed as a key control mechanism for cervical ripening [103, 150, 160, 161]. Evidence in support of this view includes the following: (1) administrat ion of anti-progestins to women in the mid-trimester and at term induces cervical ripening [162] but not labor, [103] which may not begin at all or may be delayed by days or weeks after cervical ripening has been accomplished; and (2) the administration of a PR antagonist to pregnant guinea pigs, [163, 164] and old-world monkeys [165]. Cervical responsiveness to anti-progestins increases with advancing gestational age, and the effect of anti-progestins in the cervix is not always accompanied by changes in myometrial activity. Indeed, Stys et al. [166] demonstrated a dissociation between the effects of progesterone in the myometrium and those in the cervix. Contrary to the acute nature of uterine contractions, the process of cervical ripening is gradual in normal pregnancies and may start weeks before labor and delivery [167].

Cervical ripening is a multifactorial process affected by a myriad of factors [104, 168] and characterized by slow changes in the composition of the extra-cellular matrix. The precise mechanisms by which a blockade of progesterone action may induce cervical changes are poorly understood. Both *in vitro* and *in vivo* studies have supported the key role of progesterone in this process. A decline in progesterone action may induce cervical changes through pro-inflammatory mediators, including IL-8, [169] nitric oxide, [161] prostaglandins [169] and matrix-degrading enzymes [170]. Cervical remodeling and ripening may be influenced by NFκB, which can oppose progesterone action, [22, 107, 146–148, 151] thus providing a link between inflammation, a decline in progesterone action and cervical ripening. The effects of progesterone on cellular and extra-cellular components of the cervix are discussed below.

3.3.8.1 Collagen Remodeling

The mechanical properties of the cervix are largely determined by collagen and proteoglycans that comprise the extra-cellular matrix of the connective tissue. Shortly before labor, the cervix's collagen fibers become less densely packed and the collagen concentration decreases [168]. These changes are mediated, in part, by increased collagenase activity [171]. In pregnant women, there is a decrease of 30% to 50% in the collagen concentration of the uterine cervix in comparison to the non-pregnant state [172].

Winn et al. [173] have investigated the individual and combined effects of relaxin, estrogen and progesterone on growth, softening and histological characteristics of the cervix of ovariectomized non-pregnant gilts. When administered alone, progesterone had no effect on cervical growth and only a modest effect on cervical softening; when progesterone was administered with relaxin, there was an increased

extensibility of the cervix in comparison to progesterone alone or to its combination with estrogen. In addition, the combination of progesterone and relaxin maximally decreased the collagen/amorphous ground substance [173]. The administration of mifepristone to pregnant rats at mid-gestation was associated with marked cervical changes, including decreased tensile strength, reduced collagen organization, and increased matrix metalloproteinase-2 (MMP)-2 mRNA expression [174]. Additionally, the collagen fibrils in the cervix had a shorter mean length and smaller mean diameter after mifepristone treatment. Collectively, this evidence suggests that progesterone suppresses cervical collagenolysis, one of the major processes of cervical ripening before labor [174].

3.3.8.2 Changes in Glycosaminoglycans

Glycosaminoglycans (GAGs) are an important component of connective tissues and the extracellular matrix. Softening of the cervix is associated with changes in GAG, [175] specifically, an increase in total GAGs, hyaleronic acid and water content, while sulphated GAGs decrease [176, 177]. Carbonne et al. [167] determined the effects of progesterone on PGE_2-induced changes in GAG synthesis in human cervical cell cultures. Progesterone did not prevent changes in GAG production (usually considered to reflect cervical ripening); moreover, a high concentration of this hormone even favored these changes. The authors hypothesized that this paradoxical finding may account for the early changes in the consistency of the cervix and for the alteration in GAG content that can be observed as early as the first trimester [177]. Another explanation for this counterintuitive finding is that progesterone has a different effect on the cervix and on the body of the uterus. Increasing concentrations of progesterone during pregnancy may play a key role in the gradual ripening of the cervix and promote the myometrial quiescence and down-regulation of gap junctions in the uterus.

3.3.8.3 Suppression of Metalloproteinases

The degradation of collagen in the cervix is mediated primarily by MMPs, and their effects can be repressed by their endogenous inhibitors (TIMPs) [178]. In the cervical fibroblast of rabbits, progesterone decreases the levels of proMMP-1, proMMP-3, and the steady-state levels of the respective mRNAs in the culture media, and increases the concentrations of TIMPs more effectively than that of estradiol-17 beta [179]. Similarly, Imada et al. [178] reported that physiological concentrations of progesterone suppressed IL-1-mediated production of proMMP-9 and its mRNA in a dose-dependent manner. The authors concluded that, in the rabbit uterine cervix, progesterone is a physiological suppressor of the proMMP-9 production at the transcriptional level [178]. However, the nature of the effect of progesterone may vary according to its concentration.

The effect of anti-progestins on cervical MMP expression has also been studied. Onapristone augmented the expression of MMP-3 mRNA in rabbits [170]. In contrast, mifepristone increases the expression of MMP-2 mRNA, but not of MMP-9 or MMP-3, [174] suggesting that the anti-progestins differ not only in their specificity to progesterone but they may also differ in the mechanism by which their effect is exerted.

3.3.8.4 Modulation of the Inflammatory Response in the Uterine Cervix

Macrophages, neutrophils and eosinophils are thought to play a central role in the remodeling of the cervical connective tissue by production of cytokines and proteolytic enzymes in response to inflammatory stimuli, and they have a regulatory role in cervical ripening [180, 181]. Ramos et al. [182] investigated the mechanism through which eosinophilic invasion is modulated during the second half of pregnancy in rats. Exposure to 17β-estradiol together with progesterone resulted in very poor eosinophilic infiltration, but the progesterone inhibition of eosinophilic infiltration was reversed by co-administration of mifepristone. The authors suggested that the progesterone effect is mediated through the progestin receptor [182].

Human cervical cells release IL-8, [183] a neutrophilic chemotactic and activating agent, [184] which is thought to initiate cervical ripening by promoting neutrophils chemotaxis to the cervix and their activation within the cervical stroma [183, 184]. Denison et al. [169] demonstrated that the release of IL-8 by cervical explants was significantly stimulated by PGE_2 and inhibited by progesterone. The release of the secretory leukocyte protease inhibitor (an inhibitor of neutrophil function) by cervical explants was significantly stimulated by progesterone and inhibited by PGE_2 [169].

4 Conclusions

Progesterone is the key hormone in pregnancy maintenance; it is involved in all processes during pregnancy, from the preparation of the uterine decidua, myometrium and cervix during the menstrual cycle through blastocyst implantation, sustaining myometrial quiescence, cervical competence and modulation of the maternal immune system. There is accumulating evidence that progesterone withdrawal during parturition in humans is probably functional and involves a shift in the balance between progesterone and cortisol as well as in the changes in the genomic and non-genomic effects of progesterone at the cellular level.

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Chapter 2
Pharmacology of Progestogens

Adolf E. Schindler

1 Introduction

Progesterone is the only natural progestogen, synthesized, produced and released by the corpus luteum of the ovary during the luteal phase. Its unique features include an increase in the basal body temperature, secretory changes in the endometrium in preparation for fertilisation and ovum implantation. In addition to these basic biological effects, the progestogens may, to differing degrees, suppress the hypothalamic pituitary axis, an effect which mainly accounts for the contraceptive effect of some progestogens. Progestogens may also affect abnormal endometrial tissue such as hyperplastic endometrium and endometriosis which have been chronically and excessively stimulated by endogenous or exogenous estrogens.

In 1934 progesterone was isolated from animal corpora lutea and structurally identified [1]. The structure of progesterone is shown in Fig. 2.1. In the 1940s the manufacture of progesterone was made possible, by synthesis from the plant sterol "diosgenin" [1]. However, it was soon realized that progesterone could not be properly absorbed by the gut. Progress in pharmacological development led to the development of "micronization"of progesterone, which improves oral as well as parenteral absorbtion [2]. However, bioavailability is low (approximately 5%). Other progestogens, have different absorbative properties and different bioavalabilities. Dydrogesterone, developed in 1961 by Philips-Duphar, in 1961, is a retroprogesterone, derived from progesterone by ultra violet light exposure. This isomeric change permits oral absorption and has been calculated and an increased 28% bioavailability.

The broad variety of progestogens available with different progestogenic potencies, as well as a whole array of partial effects, warrants a detailed presentation and description of the progestogens.

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H. J. A. Carp (ed.), *Progestogens in Obstetrics and Gynecology*,
https://doi.org/10.1007/978-3-030-52508-8_2

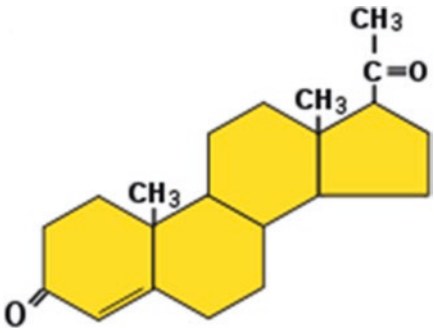

Fig. 2.1 Structure of progesterone

Table 2.1 Progestogens as prodrugs

Prodrug	Clinically relevant compound
Norethinodrel	Norethisterone
Trimegestone	Promegestone
Tibolone	3αOH-tibolone
	3βOH-tibolone
	Δ4-isomer
Desogestrel	3-keto-desogestrel
Norgestimate	Levonorgestrel

2 Classification of Progestogens

Progestogens are all steroid compounds which agonise the progesterone receptor. However, different progestogens have different intensity regarding progestogenic action per molecule. Some of the compounds used therapeutically act as prodrugs, which need to be metabolized before the respective function is obtained (see Table 2.1). Progestogens not only have different structures, but each progestogen expresses a different pattern of partial effects. This partial effect pattern is responsible for the different clinical effects and side effects seen for each progestogen.

Progesterone and the synthetic products agonising the progesterone receptor are known as progestogens. If progesterone is excluded the term "progestin" is used. An overview of the various types of progestogens is shown in Table 2.2 [3, 4]. The development of the classification of the progestogens started in the 1950s. Progestogens have been classified into "generations" dependent on when first ptroduced. Removal of the C19-Methyl-group increased progestogenic activity and oral resorption but decreased androgenic action. The introduction of a 17α-Ethyl-group produced ethisterone, which had a much higher binding affinity to the progesterone receptor. Both processes together produced the progestogen norethisterone (norethindrone in the United States), (a second generation progestogen), which is highly active, well tolerated and has been clinically available since 1957 [5]. 1951 Norethisterone acetate (NETA) was synthesized by Schering and Norethinodrel by Searle. In the 1960's, the prodrugs norethisterone (NET), lynestrenol, ethinylethinodiol acetate, norethinodrel

Table 2.2 Classification of progestogens, and partial effect pattern modified from [3, 4]

Progestogen	Anti-gonadotrophic	Estrogenic		Androgenic		Gluco corticoid	Anti mineralo corticoid
		Pro	Anti	Pro	Anti		
Progesterone	+	−	+	−	+	±	±
Pregnane derivatives: non acetylated							
Dydrogestrone	−	−	+	−	±	−	±
Medrogesterone	+	−	+	−	±		
Pregnane derivatives: acetylated							
Medroxyprogesterone acetate	+	−	+	±	−	+	−
Megestrol acetate	+	−	+	±	+	+	−
Chlormadinone acetate	+	−	+	−	+	+	−
Cyproterone acetate	+	−	+	−	++	+	−
19-Norpregnane derivatives: non acetylated							
Demegestone	+	−	+	−	−	−	−
Promegestone	+	−	+	−	−	−	−
Trimegestone	+	−	+	−	±	−	±
19-Norpregnane derivatives: acetylated							
Nomegestrol acetate	+	−	+	+	±	−	−
Nesterone	+	−	+	−	−	−	−
19-Nortestosterone derivatives: estranes							
Norethisterone (Norethindrone)	+	+	+	+	−	−	−
Norethisterone acetate	+	+	+	+	−	−	−
Norethynodrel	+	+	±	±	−	−	−
Lynestrenol	+	+	+	+	−	−	−
Tibolone (metabolites)	+	+	−	+	−	−	−
Dienogest	+	±	+	−	+	−	−
19-Nortestosterone derivatives: gonanes							
Levonorgestrel	+	−	+	+	−	−	−
Norgestimate	+	−	+	+	−	−	−
Desogestrel (etogestrel)	+	−	+	+	−	−	−
Gestodene	+	−	+	+	−	+	+
Spironolactone derivative							
Drospirenone	+	−	+	−	+	−	+

++ Strongly positive
+ Positive
± Weakly positive
− Negative

and DL-norgestrel appeared on the market. The first progesterone derivative 17-acetoxyprogesterone was developed by Schering in 1954 followed by medroxyprogesterone acetate in 1957. This was followed by medrogestone acetate and chlormadinone acetate in 1959. The retroprogesterone dydrogesterone was formed from progesterone by UV light exposure [6]. In 1961 the third generation orogestogens,became

availabl, cyproterone acetate, followed by desogestrel in 1972 (Organon). Thereafter gestodene, dienogest and the spirolactone derivative drospirenone followed (fourth generation) [7].

3 Pharmacokinetics and Pharmacology of Progestogens

Pharmacokinetics such as absorption, distribution and excretion determine how much of the progestogen is available to the tissues, by measuring the blood levels and the amount that enters the cell is regulated by the extent to which the progestogen is bound to carrier proteins. Carrier proteins cannot cross the cell membranes. The pattern of distribution of the progestogens is mainly regulated by binding to transport proteins and steroid receptors in the tissues.

Generally, all progestogens are bound in the blood with low affinity and high capacity to albumin. However, some of the progestogens derived from 19-Nortestosterone, such as norethisterone (norethindrone) are also bound with high affinity but low capacity to sex hormone binding globulin (SHBG), while others, such as progesterone itself can be bound to the corticosteroid-binding globulin (CBG). The binding of progestogens to transport proteins is reversible, so that a change in binding protein concentration may contribute to the variation or variability of a progestogen. The non-protein-bound (unbound or free fraction) of a steroid is available for metabolism in steroid metabolising cells or binds to a receptor in target cells.

Progestogens given orally reach a maximum concentration within one to 3 h. Information on bioavailability and half-life has been derived from frequent blood sampling during the first 24 h after oral administration. Bioavailabilty represents the amount of the progestogen that is found in the circulation (area under the curve). The half-life is the time in hours in which the progestogen has been absorbed to one half of its highest level. The longest half life is found with drospirenone (31–32 h), whereas norethisterone has the shortest half life (8 h). Details are summarized in Fig. 2.2.

Among progestogens there are great differences in bioavailability. Progesterone itself has a bioavailability of less than 5%, dydrogestrone has a bioavailabilty of 28% and nomegestrol of 60% [4]. The bioavailability of progestogens derived from 19-nortestosterone can reach more than 90%. The distribution of some progestogens bound to SHBG, CBG, albumin and free fraction is shown in Table 2.3.

The clinical effects of the progestogens is not only dose dependent but also influenced by the different partial effect pattern of each progestogen, as summarized in Table 2.2. Each progestogen has a different partial effect pattern, which can modify the final biological effect of each progestogen. Acquaintance with the partial effect pattern will enable the clinician to choose the optimal progestogen.

Progestogens also differ according to the affinity for various steroid receptors such as the progesterone receptor (PR), estrogen receptor (ER), androgen receptor (AR), mineralocorticoid receptor (MR) or glucocorticoid receptor (GR). Affinity

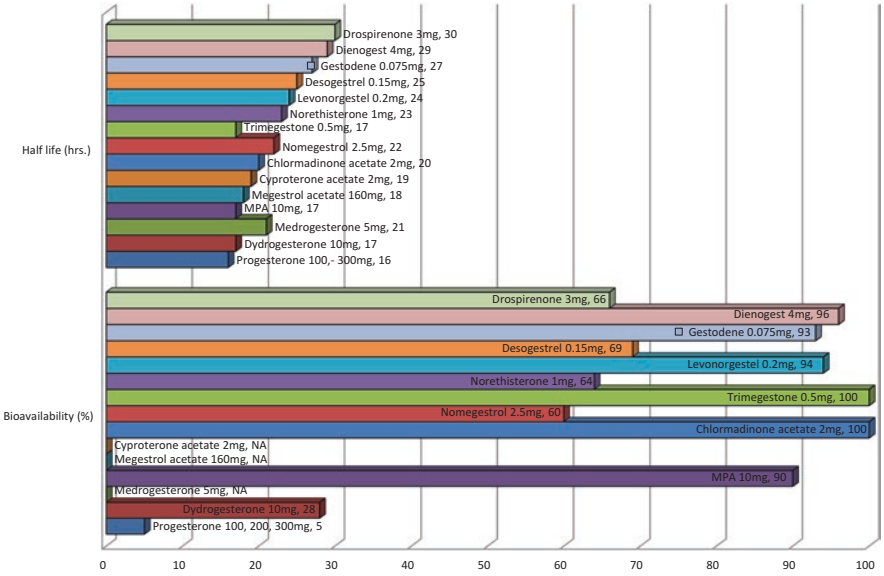

Fig. 2.2 Dose, bioavailability and half-life of progestogens modified from [3, 4]. *Dydrogesterone 17 h with metabolites

for the different receptors is summarised in Table 2.3. Affinity for different receptors is influenced by specific receptor binding proteins (Table 2.3). The clinical consequences of receptor binding are shown in Table 2.4.

A most recent area of interest and controversies reflected the influences of the various progestogens on thromboembolic risk [8].

4 Thrombotic Risk of Progestogens

Thrombosis can develop in the venous or in the arterial part of the vascular system. Venous thrombosis is more common. The basis for the thromboembolic effect is: vascular epithelial trauma, venous stasis, and hypercoagulability or the development of a post thrombotic condition. In addition, there are various risk factors for developing thrombosis such as; increasing age, increased body weight, pregnancy or postpartum, family and personal history of thrombosis, immobility as seen after surgery or an accident, long-distant travel, and smoking, the presence of a hereditary thrombophilia, of antiphospholipid syndrome.

Thrombosis has been seen mainly with the use of estrogen/progestogen combinations, as with the contraceptive pill. In estrogen/progestogen combinations, the main risk factor is the estrogen rather than the progesterone. The estrogen factors associated with risk are mainly the estrogen dose. Hence, since the introduction of the combined oral contraceptive pill in the 1960s the trend has been to reduce the dose of estrogen. Early contraceptive pills contained 100mcg of ethinyl estradiol.

Table 2.3 Relative binding affinities of progestogens to steroid receptors and serum binding proteins. Modified from [3, 7]

Progestogen	PR	AR	ER	GR	MR	SHBG	CBG	Albumin bound	Free
Progesterone	50	0	0	10	100	0	36	79.3	2.4
Dydrogesterone	75	0	–	–	–	–	–		
Chlormadinone acetate	67	5	0	8	0	0	0		
Cyproterone acetate	90	6	0	6	8	0	0		
Medroxyprogesterone acetate	115	5	0	29	160	0	0		
Megestrol acetate	65	5	0	30	0	0	0		
Nomegestrol	125	6	0	6	0	0	0		
Promegestone (R5020)	100	0	0	5	53	0	0		
Drospirenone	35	65	0	6	230	0	0		
Norethisterone	75	15	0	0	0	16	0	60.8	3.7
Levonorgestrel	150	45	0	1	75	50	0	50	2.5
Norgestimate	15	0	0	1	0	0	0		
Desogestrel (Etonogestrel)	150	20	0	14	0	15	0	65.5	2.5
Gestodene	90	85	0	27	290	40	0	24.1	0.6
Dienogest	5	10	0	1	0	0	0		

PR: progesterone receptor (promegestone =100%)
AR: androgen receptor (metribolone = 100%)
ER: estrogen receptor (estradiol-17β = 100%)
GR: glucorticoid receptor(dexamethason = 100%)
MR: mineralocorticoid receptor(aldosterone = 100%)
SHBG: sex hormone binding globulin (dihydrotestosterone = 100%)
CBG: corticosteroid-binding globulin (cortisol = 100%)
ND = not determined

Table 2.4 Comparison of partial effects and metabolic effects of dydrogesterone, medroxyprogesterone acetate and norethisterone

Progestogen	Dydrogesterone	MPA	Norethisterone (Norethindrone)
Androgenic	No	Mildly	Yes
Estrogenic	No	No	Metabolites
Glucocorticoid	No	Yes	No
HDL cholesterol	No effect	↓ (reduces E effect)	↓↓ (androgen effect)
Glucose metabolism	No effect	↓glucose tolerance	↓glucose tolerance

To-day's pills contain only 20 or even 15 mcg of ethinyl estradiol with no decrease in efficacy. Additionally, different estrogens have been introduced such as estradiol and estradiol valerate. As estradiol valerate is metabolized to estrone sulfate and estriol, the circulating estradiol levels are low, explaining the low liver metabolism and therefore the lower risk of thrombosis [9, 10].

In the nineties, different progestogens seemed to be associated with thromboembolic risk. Third generation progestogens were thought to be more thrombogenic than the second generation progestogens. However, biochemical parameters pointed towards an inherited or acquired predisposition to thrombosis. These are listed in Table 2.5.

Table 2.5 Predisposing risk factors for thrombosis

1. Activated protein C resistance, which is most commonly due to the factor V Leiden mutation
2. Protein C (G20201A) mutation
3. Lack of Antithrombin III
4. Lack of protein C
5. Lack of protein S
6. Antiphosphorlipid antibodies including Anticardiolipin antibodies, β_2 glycoprotein 1 antibodies, lupus anticoagulant

Table 2.6 Prothrombotic effect of various progestogens compared to levonorgestrel

Levonorgestrel	1.00
Norethisterone	0.98
Norgestimate	1.19
Desorgestrel	1.82
Gestodene	1.86
Drosperinone	1.68
Cyproterone acetate	1.88

(Adapted from References 11, 13–15)

The absolute risk of venous thrombosis is 3.1/100.000 women who do not use the contraceptive pill, but the risk rises to 6.29/100,000 women who use the combined oral contraceptive pill. Most of the thromboses occur in the first year of use. The incidence of thrombosis is 4.17/100,000 in the first year, falling to 2.98/100000 women after 1–4 years of use, and 2.76 in women using combined oral contraceptives after 4 years.

The thromboembolic risk is modified depending on the used progestogen, and its metabolism particular in the liver. The effect on liver metabolism can lead to an increase or inhibition of protein synthesis, leading to different levels of circulating Sex hormone binding globulin (SHBG), Corticosteroid binding globulin (CBG) and Thyroid binding globulin (TBG). The progestogens are different in their biological partial effect pattern [3]. Progestogens by their different partial effects (androgenic, antiandrogenic, estrogenic and antiestrogenic) modify the effect of estrogens on hemostasis. Progestogens with glucocorticoid partial effect such as medroxyprogesterone acetate and cyproterone acetate increase the activity of the thrombin receptor, hence stimulating procoagulatory activity of the vessel wall. Additionally oral hormonal contraceptives containing desogestrel or gestodene increase the risk of venous thrombotic embolism by 70% compared with levonorgestrel containing contraceptives [11, 12]. The thrombotic effect of different progestogens on venous thromboembolism is shown in Table 2.6. (based on [13–15]).

Progestogen-only contraceptives such as the levonorgestrel IUD do not increased the risk of venous thromboembolism [16], and can therefore be used in women with thrombophilias, or on women with past thromboses on anticoagulation,

5 Conclusions

Progestogens are different: in structure and in action profile. Besides the common progestogenic effect each progestogen has a particular partial effect pattern, which has utmost relevance when clinically used. Effects and possible side effects can be influenced or determined by this.

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Chapter 3
Progestogens in Infertility Practice

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1 Introduction

The physiological role of progesterone is to prepare the endometrium for implantation and to support pregnancy. The name progesterone is derived from the Latin word 'Gestare' meaning to bear or carry. It is also believed that the name progesterone is derived from progestational steroidal ketone [1]. Progesterone is secreted primarily from the corpus luteum of the ovary during the second half of the menstrual cycle and from the placenta during pregnancy. After ovulation, progesterone, secreted by the corpus luteum induces transformation of the proliferative endometrium into the secretory type which is essential for implantation. The endometrium then undergoes specific morphological changes, which are termed 'decidualization'. Decidualization involves slowing of endometrial proliferation, decreasing lining thickness, developing complex secretory endometrial glands, and providing more surface area within the spiral arteries. Progesterone also thickens the cervical mucus making it non-elastic. Luteal 17-beta-estradiol and progesterone, then support pregnancy. All cellular types and structures localized in the functional layer of the endometrium are targets for progesterone action, namely, stromal cells, epithelial

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H. J. A. Carp (ed.), *Progestogens in Obstetrics and Gynecology*,
https://doi.org/10.1007/978-3-030-52508-8_3

glands, and spiral arteries. However, in addition to the endocrine effects, progesterone has numerous immuno-modulatory effects.

In 1976 Georgeanna Seegar Jones first described luteal deficiency (also known as luteal phase defect or luteal insufficiency, as a condition in which the corpus luteum produces inadequate amounts of progesterone for implantation, or placentation, or a lack of an adequate endometrial response, due to a suboptimal number of receptors. Luteal deficiency has been claimed to be responsible for:- subfertility, implantation failure, and recurrent pregnancy loss. However, the whole concept of luteal deficiency has been controversial since its conception, with many doubting the existence of the condition. Similarly progesterone supplementation has also been controversial, with numerous workers doubting any beneficial effects. This chapter will examine the role of luteal deficiency in clinical infertility practice in general, and assisted reproduction in particular.

2 Role of Progesterone in Endometrial Ripening

In the follicular phase of the cycle, estrogen induces endometrial proliferation. In the luteal phase, progesterone induces changes in endometrial morphology converting the proliferative endometrium to a secretory endometrium. In the secretory phase, the endometrial glands and blood vessels become more tortuous. Glycogen accumulates in vacuoles within the glandular cells, leading to secretion of glycoproteins and peptides into the endometrial cavity, and the stroma becomes edematous. Under the influence of progesterone, stromal cells are transformed into decidual cells, with accompanying infiltration of natural killer (NK) cells, T cells, and macrophages. Pinopode formation coincides with increased progesterone levels and down-regulation of progesterone receptors during the window of implantation Pinopodes may extract fluid from uterus, facilitating closer contact between blastocyst & endometrium. Progesterone increases osteopontin (OPN, a ligand for integrin $\alpha_v\beta_3$ secretion) a bridging molecule between the embryo and endometrium. In the mid luteal phase, Leukemia inhibitory factor, (LIF, a cytokine which is essential for implantation in muridae) is upregulated. In fact, antiprogestin treatment results in reduced LIF expression. HOXA-10 & 11 genes are up-regulated by estrogen and progesterone. HOXA-10 mediates integrin involvement in early embryo–endometrial interactions. HOXA-10 expression is required for pinopode formation in the mouse.

3 Role of Progesterone in Implantation

Normal luteal function is essential for initiating pregnancy. After adequate estrogen priming, progesterone induces secretory transformation of the endometrium which improves endometrial receptivity [2]. In the “window of implantation” the

endometrial epithelium acquires a functional and transient ovarian steroid-dependent status, which allows blastocyst adhesion [3]. Progesterone induces the proliferation and differentiation of stromal cells [4]. Progesterone receptor synthesis is controlled by estrogens through estrogen receptors during the proliferative phase. If the synthesis of the estrogen receptors is inhibited then progesterone leads to a fall of both estrogen and progesterone receptors. Various experimental studies have reported down regulation of progesterone receptor epithelial cell expression during the luteal phase of the menstrual cycle [5].

Local vasodilatation and uterine musculature quiescence is also promoted by progesterone by inducing nitric oxide synthesis in the decidua [6]. Francin et al., [7] investigated the consequences of uterine contractions at the time of embryo transfer. The authors reported that on the day of embryo transfer, a high frequency of uterine contractions hindered the transfer outcome, possibly by expelling the embryos from the uterine cavity. Additionally, there was a negative correlation between the frequency of contractions and progesterone concentrations [2].

Decreased endometrial receptivity is the main factor responsible for the low implantation rates in IVF [8].

3.1 Cytokines Acting in Implantation

Numerous cytokines are active in implantation in order to modulate the inflammatory response, remodel tissues and to induce endocrine effects. The entire picture is far from complete, but some of the cytokine effects are listed below. Cytokines such as Interleukin (IL)-3, Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) and Epidermal Growth Factor (EGF) stimulate placental cell proliferation [9] in vitro, and may enable the trophoblast to secrete hCG and hPL [10]. Interferon **γ** (IFNγ) leads to remodelling of the spiral arteries to utero-placental arteries [11]. Interleukin −1 (IL-1) has many effects, both pro-inflammatory and anti-inflammatory. IL-1 stimulates IL-6, IL-8, LIF, TNFα, PGE2, PGF production. IL-1 induce COX-2 gene expression which mediates prostaglandin synthesis, induces MMP-1 productionand increases the activity of MMP-9, which are involved in implantation. IL-1 modulates hCG& CRH synthesis and attenuates progesterone production by granulosa cells. Additionally in animal models, blocking IL-l reduced the number of implanted blastocysts. Interleukin-6, (IL-6) is associated with hCG release, and increases MMP9.

Leukemia inhibitory factor (LIF) is essential for implantation. LIF mRNA is induced by estrogen. Female mice with no LIF gene, have normal blastocysts which fail to implant. Injecting LIF−/− mice with LIF causes viable pregnancies. In humans, LIF causes cytotrophoblast differentiation to an anchoring phenotype due to increased fibronectin synthesis. Infertile patients with multiple implantation failure have been reported to have dysfunctional LIF production compared to fertile women [12]. Interleukin-15 (IL-15) increases trophoblast invasion, modulates MMP-1 and maintains uterine natural killer (NK) cells.

3.2 Action of Progesterone on Cytokines

Progesterone has been shown to induce changes in the functions of a number of immune-competent cells by different molecular and cellular mechanisms. Progesterone stimulates the activity of some specific enzyme matrix metalloproteinases [13] and adhesion molecules [14], inhibits antibody production and suppresses T-cell activation and cytotoxicity [15] and directly or indirectly modifies the activity of NK cells, which are the most numerous lymphoid cells locally [16].

Progesterone is associated with decreased IFNγ & increased IL-10 in endocervical fluid [17]. Progesterone up regulates LIF mRNA expression in vitro [18]. Progesterone inhibits NK activity at the feto-maternal interface, inhibits the release of arachidonic acid, and favours the production of asymmetric, pregnancy-protecting antibodies. The cytokine effects of the progestogen dydrogesterone have been investigated more than progesterone itself. Dydrogesterone, inhibits IFNγ and TNFα production [19]. Dydrogesterone increases the levels of IL-4 and IL-6 [19]. Dydrogesterone Inhibits NK activity at the feto-maternal interface, and in preterm labour, dydrogesterone is associated with significantly higher serum levels of IL-10, and lower concentrations of IFNγ than controls [20].

4 Luteal Phase Insufficiency

4.1 Formation of the Corpus Luteum (CL)

The CL contains a heterogenous population of cells including steroidogenic cells, fibroblasts, immune cells, and endothelial cells. The steroidogenic cells are the large and the small luteal cells that are derived from the granulosa and theca cells of the ruptured follicle, respectively [21]. The granulosa cells of the pre-ovulatory follicle are not vascularized, the blood supply stops at the basement membrane. Following ovulation, basement membrane integrity is lost, tissue re-modeling takes place and vessels, originating from the thecal vasculature, invade the granulosa-luteal cells [22]. Over the next few days, intensive angiogenesis takes place and a capillary network extends throughout the fully differentiated CL. In humans, both vascular density and the endothelial area of each vessel increase markedly from the luteinized granulosa cells of the early CL to the mid-luteal stage [23]. Neoangiogenesis is important for CL function and is controlled by various angiogenic factors, such as vascular endothelial growth (VEGF), fibroblast growth factor, angiopoietins, and insulin-like growth factors.

The process of neovascularization is regulated by pituitary LH [24]. Luteinizing hormone activates matrix metalloproteinases that degrade extracelluar matrix associated with the blood vessels. The CL produces progesterone, estrogens, and non-steroidal substances, such as inhibit A. Apart from the pituitary gonadotropins,

different local substances may also regulate the CL life span and function. Such substances include growth factors, peptides, steroids, and prostaglandins [24].

Normal luteal function requires optimal pre-ovulatory follicular development, luteinization of the granulosa cells to produce progesterone, continued tonic luteinizing hormone (LH) support, vascularization of the corpus luteum (CL), and estrogen to induce progesterone (P4) receptors in the endometrium [25]. The normal corpus luteum life span is 14 days in natural ovulatory cycles unless the life span is prolonged by human chorionic gonadotropin (hCG). hCG is secreted by the developing blastocyst after implantation [26]. By inducing secretory changes, following adequate estrogen priming, progesterone induces normal endometrial receptivity.

4.2 Evaluation of the Luteal Phase

Luteal phase insufficiency generally stems from an insufficiency of estrogen and progesterone production after ovulation. At mid-cycle, the gonadotropin surge has important physiological roles, including induction of luteinization of the granulosa cells, resumption of oocyte meiosis, rupture of the pre-ovulatory follicle, and formation of the CL. Among other events, post-LH surge changes include a shift in steroidogenesis within the follicle with a marked decrease in estradiol (E2) concentrations and a gradual increase in serum progesterone concentrations [27, 28]. The mid cycle FSH surge also plays an important role by promoting nuclear maturation, i.e. the resumption of meiosis, as well as LH receptor formation on the luteinizing granulosa cells, securing the function of the corpus luteum during the following luteal phase. Additional alterations involve uncoupling of gap junctions between granulosa cells and the plasma membrane of the oocyte, a process that seems to be important for the resumption of meiosis [29]. For the transfer of cholesterol from the outer to the inner surface of the mitochondrial membrane steroidogenic acute regulatory protein (StAR) is important [30]. The StAR protein is absent from the granulosa cells before the onset of the LH surge which explains the inability of the granulosa cells to produce progesterone [31].

Various investigations have been suggested methods to assess luteal insufficiency. The original method was by endometrial biopsy taken 2 days prior to menstruation and histologically dated according to Noyes et al's [32] criteria. Luteal deficiency was assumed if the dating lags more than 2 days behind the chronological age. However, biopsy is taken in a non pregnancy cycle and assumed to reflect the situation in a pregnancy cycle. Although many authors have tried to use plasma progesterone levels as a test of luteal function, plasma levels may be unreliable due to the pulsatile secretion of progesterone [33]. Blood may be drawn at a pulse peak or nadir. There may also be normal hormone levels in the presence of abnormal histology may also be due to a deficiency of progesterone receptors rather than a deficiency in progesterone itself. Usadi et al., [34] have reported a lack of correlation between hormone progestogerone levels and endometrial histology. More recent studies have involved assessing daily urinary hormone excretion of luteinizing

hormone, estrone glucuronide, and pregnanediol glucuronide during three or more cycles [35], scanning electron microscopy [36], immunohistochemical analysis of estrogen and progesterone receptors [37] and the endometrial receptor (ERA) assay [38].

4.3 Luteal Insufficiency in Stimulated Cycles

4.3.1 Non ART Cycles

Ovulation may be stimulated by clomiphene citrate, letrazole or gonadotrophins, with or without ART. Luteal insufficiency may be due to the inhibition of LH in the early luteal phase by steroids secreted in supra-physiologic levels in stimulated cycles [39] which exert a negative feedback on the gonadotrophins at the level of the pituitary via the hypothalamo-pituitary-ovarian axis. The disordered negative feedback is mainly due to an altered hormonal milieu in stimulated cycles with controlled ovarian hyperstimulation leading to multifollicular growth and development. If luteal phase hormonal support is not present in assisted reproduction technique (ART) cycles, the serum estrogen and progesterone levels drop, thus leading to a decrease in the implantation rates and pregnancy rates due to luteal phase insufficiency [40]. There may be two types of luteal phase defect: one is associated with the presence of immature follicles, and the other where the follicles are mature. In both types, supplemental therapy with progesterone is effective and mandatory in creating a healthy uterine environment [41] for better implantation rates.

In women undergoing ovulation induction, multiple follicles of different size might ovulate at different times, thus expanding the fertilization window. It can be expected that sex steroid concentrations, both estradiol (E2) and progesterone, after multiple ovulation will be significantly higher [42]. These high concentrations may not only influence the receptivity of the endometrium, but may also cause luteal insufficiency [43] as high concentrations of steroids through negative feedback on the pituitary-hypothalamic axis, inhibit the production of luteal LH, which is mandatory for luteal progesterone production. This is seen to a lesser degree in clomiphene citrate induced cycles. Clomiphene citrate acts as an estrogen receptor modulator blocking the estrogen receptors at the level of the hypothalamus, which in turn increases release of gonadotrophins from the pituitary. Gonadotrophin release supports the corpus luteum in the luteal phase of the cycle. Letrozole is an aromatase inhibitor recently employed as ovulation inducer. However, it does not seem to exert the antagonist effect on endometrial estrogen receptors as clomiphene does. Luteal support does not seem beneficial in clomiphene stimulated cycles [44, 45], nor letrazole cycles.

In non-IVF cycles with gonadotropins, two meta-analyses [45, 46] have demonstrated the benefit in improving reproductive outcomes with vaginal progesterone use as luteal phase support, both in terms of clinical pregnancy rates and in the likelihood of live births per cycle.

4.3.2 ART Cycles

Progestogen luteal phase supplementation has its main effect when GnRH analogues are used. GnRH agonists used for pituitary downregulation inhibit the release of gonadotrophins from the pituitary leading to a luteal phase defect by premature luteolysis. Similarly GnRH antagonist cycles cause direct suppression of the pituitary causing luteolysis and foreshortening of the luteal phase [47]. A significant negative correlation has been reported between both pre-ovulatory estradiol concentrations and day 16 progesterone levels and the concentration of cytosolic progesterone receptor (cPR) [48], while advanced endometrial maturity tends to be associated with low concentrations of cPR. Furthermore, natural cycles have been characterized by low cytosolic E-2 receptors (cER) and high cPR, whereas the concentration of both receptors was greatly reduced in stimulated cycles. Due to receptor abnormalities, the endometrium can be progesterone deficient even if plasma progesterone levels are normal [49].

GnRH agonists are being increasingly used to trigger final oocyte maturation in GnRH-antagonist in vitro fertilization (IVF) cycles. The agonist trigger is used to significantly reduce the risk of ovarian hyperstimulation syndrome (OHSS). The decreased risk of OHSS is due to rapid luteolysis in the early luteal phase. When agonist triggering is used, the mean LH concentration was decreased significantly [50]. Therefore progesterone supplementation is essential in GnRH triggered cycles.

5 Types of Hormone Supplementation in Infertility

In normal ovulatory sub-fertile women, with primary or secondary infertility, 92% of cycles show normal luteal function. Therefore, luteal support seems to be unnecessary [51]. However, iatrogenic LPD is seen with the use of controlled ovarian stimulation, and gonadotropin-releasing hormone (GnRH) analogues for in vitro fertilization (IVF). Iatrogenic LPD has provided an opportunity to study the endocrine and endometrial abnormalities during the luteal phase and the impact of pharmacological intervention. Various regimens of hormone supplementation have been used. These are discussed below.

5.1 Human Chorionic Gonadotropin (hCG)

HCG is used as a trigger in doses varying from 5000 to 10,000 U. hCG mimics the LH surge seen in natural ovulatory cycles and facilitates the final oocyte maturation prior to ovulation. The half-life of hCG has been estimated to be 2.3 days and serum hCG levels are known to be detectable by immunoenzymetric methods for up to 14 days after the injection. The long half life of hCG produces a prolonged luteotrophic effect, securing good reproductive outcomes. However this very factor, the

longer half life of hCG, contributes to the increased risk of developing the ovarian hyperstimulation syndrome (OHSS). Of the various mechanisms by which hCG may rescue the corpus luteum, an increase in both E_2 and progesterone levels appears to be the most likely [52]. The usual dose for luteal phase support is 1500–2500 IU twice per week from the day of embryo transfer (ET) continued until the day of the pregnancy test or until 8–10 weeks of gestation. This is especially important when GnRH agonist is used as a triggering agent. Meta-analyses comparing hCG with progesterone have shown it to be associated with either better or at least similar pregnancy rates to that seen with progesterone [53, 54]. Van den Linden et al. [55], published the results of a metaanalysis to assess luteal phase support (progestogen, hCG or GnRH agonists) in the Cochrane database. The review comprised 94 studies of 26,198 women. The results are summarised in Table 3.1. hCG administration did not improve the clinical pregnancy rate (OR 1.30 95% CI 0.90–1.88) but significantly increased the ongoing pregnancy and live birth rates when compared to placebo or no treatment (OR = 1.76 95% CI 1.08–2.86) and showed similar results to progesterone supplementation [55]. However, the risk of ovarian hyper-stimulation syndrome (OHSS) associated with hCG in stimulated IVF cycles limits its use as a luteal support. As stated above, luteal phase dynamics differ after GnRHa triggering in GnRH antagonist-treated cycles. Whether hCG offers a safe and effective luteal support in this group of women without the risk of OHSS is yet to be fully elucidated [56], but has been described in a small series of 6 patients [57].

Table 3.1 Progestogens in Luteal Phase Support. (Adapted from Van der Linden et al., (51a))

	OR	Side Effects	Studies (Women)
hCG vs placebo or no Rx Clinical pregnancy rate	1.30 (0.09–1.88)	OHSS	5 (746)
hCG vs placebo or no Rx Ongoing pregnancies or live births	1.76 (1.08–2.86)	OHSS	3 (627)
Progestin / placebo, no Rx Clinical pregnancy rate	1.89 (1.30–2.75)		7 (841)
Progestin / placebo, no Rx Clinical pregnancy rate	1.77 (1.09–2.86)		5 (642)
Progestin vs hCG Clinical pregnancy rate	1.20 (0.94–1.43)	OHSS	11 (13787)
Progestin vs hCG Ongoing pregnancies or live births	0.92 (0.54–1.57)	OHSS	4 (434)
Intramuscular vs vaginal progesterone Clinical pregnancy rate	1.14 (0.97–1.33)		13 (2932)
Intramuscular vs vaginal progesterone Ongoing pregnancies or live births	1.24 (1.03–1.50)		7 (2039)
Progestin / progestin, GnRHa Clinical pregnancy rate	0.62 (0.48–0.81)		9 (2435)
Progestin / progestin, GnRHa Ongoing pregnancies or live births	0.62 (0.48–0.81)		10 (2861)

5.2 Micronized Progesterone

Micronized progesterone is today the most widely used form of luteal support. Micronized progesterone can be administered orally, rectally or vaginally. However, the bioavailability of micronized progesterone following oral administration is variable as progesterone is metabolised in the liver to pregnanelone and pregnanediol and thereby inactivated. Hence, endometrial changes are inconsistent [58] due to first pass metabolism. In addition, side effects such as nausea, abdominal bloating, drowsiness, sedation are common with oral administration. The vaginal route of administration is widely used, due to ease of administration and high bioavailability as hepatic degradation is avoided. Intra-vaginal administration results in a high uterine concentration of progesterone with relatively low levels in the peripheral circulation. Vaginal micronised progesterone is available in both capsule and gel forms. The daily dose is 600–800 mg/day in 2–3 divided doses, although no dosage finding study has been performed; and 90 mg of gel (8%) once or twice daily. Pregnancy rates are similar with both forms of vaginal preparations [59, 60]. The disadvantages of vaginal micronized progesterone include local irritation in some women, discharge from the gel or capsule, and staining of the clothes. The divided dose may also be inconvenient, as daily routines need to be interrupted for insertion. Additionally, vaginal administration is inconvenient to some patients.

When all progestogens were compared to placebo or no treatment in Van der Linden et al's [55] metaanalysis, progestogens were found to give a significantly better pregnancy rate than placebo or no treatment. (OR = 1.89 95%CI 1.30–2.75)

5.3 Intramuscular Progesterone

Progesterone in oil, 50–100 mg daily as an intramuscular injection is another form of luteal support. With the availability of vaginal progesterone, the intramuscular route is less often used than previously. Pain, rash and abscess at the injection site and the need for daily visits for intramuscular injection by trained staff, are important factors precluding routine use. Occasional occurrence of acute eosinophilic pneumonia has been reported in otherwise healthy women. However, if the vaginal route of administration is unacceptable or if there is severe local irritation, the intramuscular route of administration may be used. Intramuscular progesterone is more popular in the U.S. Again as with other reimens of treatment, there is a difference whether clinical pregnancy rates or ongoing pregnancy rates and live births are considered as the end points. The results quoted in Van Der Linden et al's (55 51a) metaanalysis are OR 1.14 (95% CI 0,97–1.33) (Table 3.1) for the clinical pregnancy rate but OR 1.24 (95% CI 1.03–1.50) for ongoing pregnancies or live births. However, a prospective trial [61] showed micronized vaginal progesterone gel to produce significantly higher pregnancy rates than intramuscular progesterone in the younger patient. However, both appeared to be equally efficacious in the older patient.

5.4 Estradiol Plus Progesterone

Luteal estradiol supplementation has been used in addition to progesterone support in an attempt to improve IVF outcomes, both in women with low luteal estrogen levels or electively in all treatment cycles. Transdermal estradiol patches delivering a dose of 100 ug/day or oral or vaginal estradiol 4–6 mg/day together with progesterone have all been used with variable results. The current evidence of benefit is limited to a higher implantation rate seen in one single study [62]. The addition of estradiol to progesterone support has not been shown to improve the pregnancy rate [55, 63].

5.5 Progesterone with Gonadotrophin-Releasing Hormone

Small bolus doses of GnRH have been used in an attempt to improve pregnancy rates in antagonist-treated cycles where GnRHa is used to trigger ovulation. Triptorelin 0.1 mg has been administered on the day of oocyte pick-up, embryo transfer and 3 days afterwards, or in multiple doses. The original reports suggested an improvement in both pregnancy and live birth rates [64–66]. However, the updated Van der Linden (51a) does not confirm the earlier optimism (Table 3.1) and the clinical pregnancy rate and ongoing pregnancy pates are worse than with progestogen alone.

5.6 Synthetic Progestogens

Synthetic progestogens derived from 19-nor testosterone have stimulatory effects on the androgen receptors. Therefore, although effective, these preparations are not recommended in infertility practice for the fear of inducing androgenic side effects on a female fetus. Androgenization of a female embryo has been seen in laboratory rats, but has never been reported in humans.

Dydrogesterone is a stereoisomer of progesterone, manufactured by conversion of progesterone with ultra violet light. It has been extensively used in over 90 million women, in 90 countries over 40 years and has been found safe for use in pregnancy [67]. Dydrogesterone has a 50% higher affinity for the progesterone receptor than progesterone itself [68], and has no stimulatory or inhibitory effect on the androgen receptor. Dydrogesterone also has other advantages. It is available as an oral preparation. Although metabolised in the liver, the metabolite dihydrodydrogesterone, is active on the progesterone receptor, unlike the metabolites of progesterone itself.

Chakravarty et al. [69], have compared oral dydrogesterone to micronised vaginal progesterone and found them to be equally effective. Two studies have reported

dydrogesterone to have a superior effect to micronized progesterone itself. Iwase et al. [70], has found dydrogesterone to be associated with a significantly higher live birth rate. Patki and Pawar [71] have found a statiscically significant increase in pregnancy rates with use of 30 mg dydrogesterone as compared to vaginal micronised progesterone in ART cycles.

A double-blind, randomized, multicenter non-inferiority study (LOTUS I) was conducted in which women undergoing fresh cycle IVF treatment were randomized to oral dydrogesterone (DYD) 10 mg TID or micronized vaginal progesterone (MVP) 200 mg TID [72]. The LOTUS I trial was an international Phase III randomized control trial, performed across 38 sites, from August 2013 to March 2016. The results (Table 3.2) showed that dydrogesterone was not inferior to micronized progesterone. However, the trial did not attempt to show superiority. When the odds ratios were compared there was a slightly increased although non significant trend in favour of dydrogesterone. Additionally, oral dydrogesterone was well tolerated as well as had a similar safety profile to MVP [72].

The LOTUS II study was a randomized open label multicenter, phase III, non inferiority study in 37 IVF centers in ten countries from 2015–2017 [73] (Table 3.3), comparing dydrogesterone to micronized progesterone gel. 1034 women age 18 to 42 years were included in the study. In this study the non-inferiority of oral dydrogesterone was again demonstrated. Again, dydrogesterone was well tolerated and had a similar safety profile to MVP gel [73]. Again there was a similar slightly increased, but not significant benefit to dydrogesterone. Even when the figures for both LOTUS trials are combined, the benefit of dydrogesterone was not significant. It would be beneficial to increase the power of both trials to see if superiority could be demonstrated. If so, dydrogesterone may replace micronized progesterone in the light of its oral rather than vaginal administration. In a recent metaanalysis of 7 trials of 3508 patients comparing dydrogesterone to micromised progesterone, a statistically significant benefit was seen after dydrogesterone (OR 1.7 95%CI (1.02–1.35) [74].

Table 3.2 -LOTUS 1 study results

	Dydrogesterone (n = 497)	Micronised vaginal progesterone (n = 477)	Difference
Pregnancy rate at 12 weeks	37.6%	33.1%	4.7%
Live birth rate	34.6%	29.8%	4.9%

Table 3.3 LOTUS II study results

	Dydrogesterone (n = 520)	Micronised vaginal progesterone (n = 514)	Difference 95%CI
Pregnancy rate at 12 weeks	38.7%	35.0%	4.7%
Live birth rate	34.4%	32.5%	4.9%

6 Conclusions

To summarize, the body of scientific evidence confirms equal efficacy of intramuscular and vaginal progesterone preparations in therapeutic doses for luteal phase support.

An appropriate dose of any medication should be the dose which is optimally effective, most convenient to administer, as well as cost-effective.

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Chapter 4
Progestogens in Threatened Miscarriage

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1 Introduction

Threatened miscarriage is defined by the National Library of Medicine, Medical Subject Headings (MeSH 2012), as bleeding during the first 20 weeks of pregnancy while the cervix is closed. It is the most common complication in pregnancy, occurring in 20% of all pregnancies. Based on the presence of bleeding alone, without ultrasound determined viability, it was said that the condition may progress to miscarriage in approximately one half of cases [1, 2], or may resolve. These figures are probably still true before viability can be determined by ultrasound. However, threatened miscarriage may include anything from spots of blood to potentially fatal shock. The treating physician is faced with the question whether any treatment can effectively prevent the pregnancy from being miscarried. Progestational agents have been prescribed since the nineteen fifties in order to prevent miscarriages. There is much theoretical data to support the use of progestogens. Progestogens enhance implantation, affect the cytokine balance, inhibit natural killer cell activity at the feto-maternal interface, inhibit the release of arachidonic acid, prevent myometrial contractility and prevent cervical dilatation. Indeed lutectomy prior to seven weeks causes miscarriage [3]. Mifepristone blocks the progesterone receptor, leading to fetal death and placental separation. Therefore, progestogen supplementation may be indicated, and if so, which progestogen. In addition, the results of treatment need to be compared to the natural history of the condition. There are a number of trials in the literature comparing progestogens to no treatment or placebo, stretching back

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H. J. A. Carp (ed.), *Progestogens in Obstetrics and Gynecology*,
https://doi.org/10.1007/978-3-030-52508-8_4

over 50 years. This chapter will summarise the results of treatment, pitfalls and confounding factors.

2 Natural History

There are problems in determining the natural history of threatened miscarriage. In the older literature, there was no ultrasound performed in order to detect the fetal heartbeat. In many cases, bleeding may have occurred after fetal death. Table 4.1 shows the subsequent history after detection of a fetal heartbeat in a number of observational studies. As can be seen 8.7% of threatened miscarriages subsequently terminated in miscarriage. 92.3% continued developing.

Weiss et al. [8] enrolled patients into a database on presenting with a viable embryo at 10–14 weeks. If the patient reached 10–14 weeks, the chance of miscarrying prior to 24 weeks was 1–2%. Another source of ascertainment of the miscarriage rate after threatened miscarriage is from the control group of randomized trials of progestogens in threatened miscarriage. These can be seen in Figs. 4.2 and 4.3. In these nine studies the miscarriage rate was higher. 129 pregnancies of 519 resulted in miscarriage (24.9%). However, these figures are not corrected for the presence of a fetal heartbeat prior to enrollment in the various trials.

In recurrent miscarriage, vaginal bleeding is a common complication occurring in 50 of 162 women in Reginald's series [11] and 50 of 102 patients in the author's series [12]. The reason for this bleeding remains unclear. 75% of recurrent miscarriages are blighted ova [12]. However, even in the presence of a subsequent live embryo, bleeding still occurs in 40–50% of patients. The likelihood of a pregnancy loss after the detection of a fetal heartbeat was 69/359 (14.2%) in Li et al.'s series [13] and 22.7% of 185 study patients with multiple spontaneous miscarriages in Laufer et al.'s [14] series.

3 Diagnosis of Luteal Deficiency

There is no clear definition of luteal phase deficiency, and no reliable tests to diagnose the condition. Some of the possible tests are described below.

Table 4.1 Prognosis after detection of fetal heartbeat (observational studies)

Series	Miscarriages	Total	Proportion miscarrying (%)
Tongsong et al. [4]	14	255	5.6
Tannirandorn et al. [5]	3	87	3.4
Falco et al. [6]	23	149	15.4
Bennet et al. [7] Subchorionic hematoma	48	516	9.3
Total	88	1007	8.7

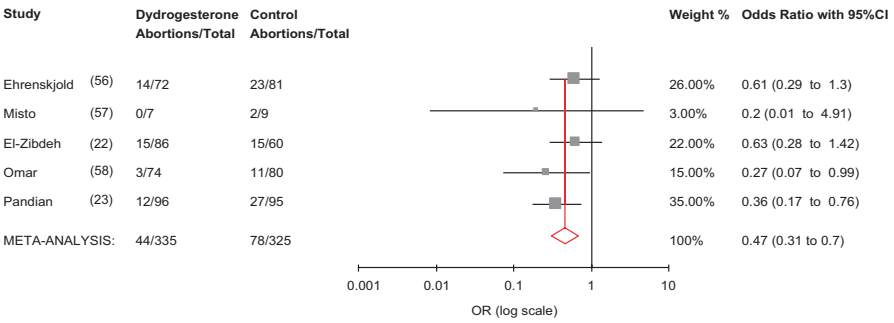

Fig. 4.2 Metaanalysis of dydrogesterne in threatened miscarriage [9]. Reproduced by permission from Informa Healthcare

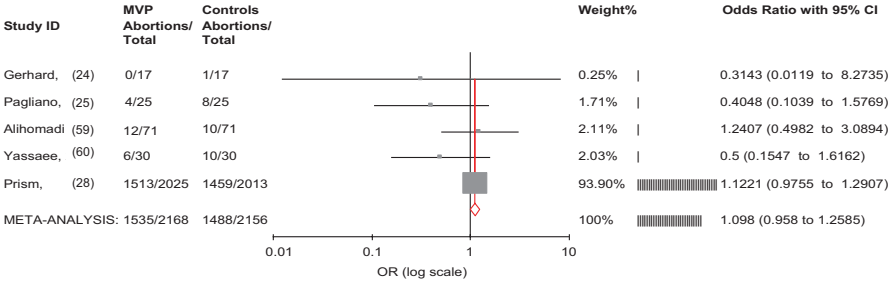

Fig. 4.3 Metaanalysis of vaginal micronized progesterone in threatened miscarriage. Figures adapted from Lee et al. [9] and PRISM [10]

3.1 Progesterone Levels

Serum progesterone levels have been used to make prognoses about the continued development of pregnancy and even to diagnose pregnancy loss. In the 1990's there were various small series trying to predict pregnancy outcome by serum progesterone levels. These have been superceeded by a metaanalysis of seven prospective cohort studies, evaluated the diagnostic accuracy of a single serum progesterone measurement to predict the possibility of a viable pregnancy, miscarriage, or ectopic pregnancy [15]. The study included 26 cohort studies (9436 pregnant women). The thresholds of progesterone used ranged from 3.2 to 11 ng/mL (10 to 35 nmol/L). After meta-analysis of five studies (1998 participants) with similar cut-off values (3.2–6 ng/mL), a single progesterone measurement predicted a non-viable pregnancy with pooled sensitivity of 74.6%. If progesterone was lower than the cut-off value (3.2–6 ng/mL), the probability of a non-viable pregnancy was 99.2% compared with 44.8% if progesterone was higher. There was no information on high levels of progesterone predicting a good outcome. However, it must be borne in mind that progesterone is secreted in a pulsatile fashion. Serum progesterone levels can fluctuate eight-fold in a 90-min period during the midluteal phase and range

from 2.3 to 40.1 pg/mL during a 24-h period in the same healthy subject [16]. Blood may be drawn at a pulse peak or pulse nadir. Progesterone levels should be assessed together with hCG levels and ultrasound findings in order to reach a valid conclusion regarding embryonic viability.

3.2 Other Markers of Luteal Phase Insufficiency

The endometrial molecular marker, nuclear cyclin E, changes in intensity and subcellular localization during the menstrual cycle, and has been reported to be a possible marker of. endometrial development [17]. Cyclin E has even been used as an indicator to determine if the endometrium responds to progesterone supplementation prior to pregnancy [18].

Pillai et al. [19] have published a systematic review of prospective studies that investigated various biochemical markers to determine outcomes in women with threatened miscarriage. 15 studies (1263 women) were eligible for the meta-analysis. The review highlighted the role of hCG, pregnancy associated plasma protein A (PAPP-A), estradiol and cancer antigen 125 (CA 125) as predictors of outcome in threatened miscarriage. CA 125 appeared to be the most promising marker. CA 125 showed a sensitivity of 90% (CI 83–94%), specificity of 88% (CI 79–93%). The chorio-decidual plate produces large amounts of CA 125 in early pregnancy and with tropho-decidual detachment at the time of miscarriage, CA 125 is released into the bloodstream.

Serum hCG is the most common marker used at the beginning of pregnancy. However, it reaches a peak at approximately 9 weeks of pregnancy, and physiologically decreases thereafter. Hence in Pillai et al.'s metaanalysis it was not useful once fetal viability had been established.

Pillai et al. [19] concluded that biochemical markers can be used to predict the outcome of threatened miscarriage, particularly serum CA 125. However, in order to reliably interpret the biochemical markers in early pregnancy, specific cutoff values need to be establishes, and require interpretation together with ultrasound.

3.3 Cause of Luteal Phase Insufficiency

Luteal phase deficiency may be secondary to abnormal follicle formation, associated with poor oocyte quality, or a decreased response to progesterone by the endometrium [20]. Hormone levels may be normal, but histology abnormal due to deficiency of progesterone receptors or endometrial ripening. As with other presumptive causes of miscarriage, low hormone levels may be a result of miscarriage rather than its cause. In the blighted ovum or after embryonic death, there is no villous circulation. Trophoblastic failure after villous circulatory failure results in low hCG levels. If hCG does not stimulate the corpus luteum, progesterone levels

will fall explaining the mechanism of expulsion, but not necessarily that of embryonic death, or the cause of miscarriage. Therefore in threatened miscarriage, diagnosis and treatment cannot be based on progesterone levels.

4 Confounding Factors

The results of progestogen treatment may be confounded as threatened miscarriage, may be due to separation of the placenta in a normal embryo, or a defence mechanism to prevent the continued development of an abnormal embryo, including abnormalities which are incompatible with life. The most important confounding factors are embryonic structural malformations, or chromosomal aberrations. In missed abortions 200 of 233 embryos have been reported to be structurally abnormal on embryoscopy [21]. These defects included: anencephaly, encephalocele, spina bifida, syndactyly, pseudo-syndactyly, polydactyly, cleft hand and cleft lip. Without embryoscopy these embryos would not have been diagnosed, and the patient might have been treated empirically with progesterone. If included in a trial, the results would be skewed in favour of a negative effect. However, embryoscopy is advanced technique, which is not usually available. Additionally, 70% of miscarriages are blighted ova. Therefore, it is impossible to tell if a rudimentary embryo may have been structurally abnormal. At present, ultrasound is not sufficiently sensitive to diagnose these very early anomalies.

Up to 60% of sporadic miscarriages [22–24] may be caused by chromosomal aberrations in the embryo. The most common aberrations which are incompatible with life include 16 Trisomy and polyploidy. Additionally, with the introduction of comparative genomic hybridization (CGH) a further 15% of so called normal embryos on conventional karyotyping, have been diagnosed with genetic aberrations [25, 26], and the numbers may increase if whole exome screening is used.

Progesterone cannot correct chromosomal aberrations, or severe anomalies which are incompatible with life. Unfortunately, the abortus is not usually tested genetically. Hence, it is unclear whether miscarriage after supplemental progesterone is due to failure of treatment or confounding of the results by embryonic genetic aberrations.

Before a trial of progesterone can be said to be conclusive, other predictive factors should be taken into account.

5 Effect of Progestogen Supplementation

Progestogens have been used since the 1950s to prevent threatened miscarriage terminating as miscarriage. It has been difficult to prove whether progestogens are effective, due to the generally good natural history, and the effect of confounding factors. Recently, two systematic reviews have been performed. Wahibi et al. [27]

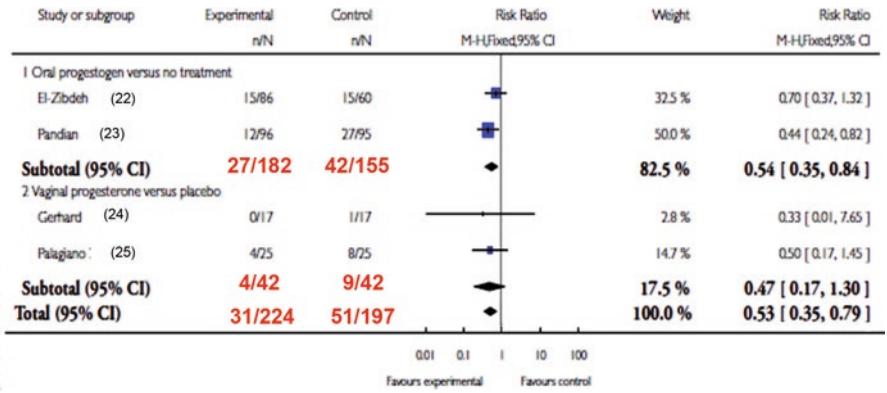

Fig. 4.1 Metaanalysis of RCT's on progestogen supplementation. Modified from Wahabi et al. [27]. Original reproduced by permission of John Wiley and Sons

carried out an analysis of two trials of oral dydrogesterone compared to placebo [28, 29] and two trials of vaginal progesterone [30, 31] (see Fig. 4.1). The overall figures showed a statistically significant benefit, OR = 0.53 (CI 0.35–0.79) in favour of progestogen supplementation. However, the analysis consisted of only four studies. It is interesting to note that in the women who were treated with vaginal progesterone the treatment was not statistically effective in reducing miscarriage when compared to placebo (RR = 0.47 95% CI, 0.17–1.30) whereas oral progestogen (dydrogesterone) was effective (RR = 0.54 CI 0.35–0.84). The author subsequently published a trial of dydrogesterone in threatened miscarriage. The original intention was to assess all progestogens. However, no relevant trials were found for micronized or intramuscular progesterone, in addition to those assessed in Wahibi et al.'s [27] metaanalysis. Carp's [32] metaanalysis included five randomized studies. 660 patients were eligible for analyses of pregnancy maintenance. The results (Fig. 4.2) showed that the effect of dydrogesterone on the risk of miscarriage in women with threatened miscarriage appeared to be substantial. There was a statistically significant reduction in the odds ratio for miscarriage after dydrogesterone compared to standard care of 0.47 (CI 0.31–0.7). The 24% miscarriage rate in control women (78/325) was reduced to 13% (44/335) after dydrogesterone administration (11% absolute reduction in the miscarriage rate).

Lee et al. [33] have since publishes a metaanalysis of progestogens in threatened miscarriage. There is a subgroup metaanalysis of four trials (286 patients) of vaginal progesterone. Not one had a statistically significant effect, and the metaanalysis, although showing a trend to a lower miscarriage rate, did not reach statistical significance, (OR = 0.72, CI 0.39–1.34). In 2019, the results of the PRISM study were published [34] A total of 4153 women, were randomly assigned to receive progesterone (2079 women) or placebo (2074 women). The incidence of live births after at least 34 weeks of gestation was 75% (1513 of 2025 women) in the progesterone group and 72% (1459 of 2013 women) in the placebo group (RR, 1.03; 95% CI,

1.00 to 1.07). This 3% difference was not statistically significant. Coomarasamy et al. [35] have subsequently argued, that in the subgroup of women with 1 or more miscarriage(s) and current pregnancy bleeding, the live birth rate was 75% with progesterone vs 70% with placebo (RR 1.09, 95% CI 1.03–1.15). In women with 3 or more previous miscarriages and current pregnancy bleeding; live birth rate was 72% (98/137) with progesterone vs 57% (85/148) with placebo (RR 1.28, 95% CI 1.08–1.51; $p = 0.004$). The authors suggested offering micronized progesterone to women with vaginal bleeding and a history of one or more previous miscarriage(s). This course of action was estimated to result in an additional 8450 live births per year in the United Kingdom. However, even if the figures in Lee et al.'s [33] meta-analysis are added to the PRISM [34] study, the results still do not reach statistical significance, as shown in Fig. 4.3.

Shearman and Garrett [36] found 17 hydroxyprogesterone caproate to have no beneficial effect in threatened miscarriage. Additionally, when myometrial tissues were suspended in organ chambers and exposed to varying concentrations of progesterone or 17 hydroxyprogesterone caproate [9], 17 hydroxyprogesterone caproate actually stimulated contractility unlike progesterone itself which significantly inhibited spontaneous contractility. 17 hydroxyprogesterone caproate is therefore not recommended for threatened miscarriage.

Unfortunately, no studies control for embryonic genetic aberrations. In recurrent miscarriage, there are two trials of micronized vaginal progesterone [10, 18]. Although both are very different in design, The PROMISE trial [10] did not account for embryonic genetic aberrations, and found progesterone to have no beneficial effect, whereas Stephenson et al. [18] only included patients losing euploid embryos. The result of progesterone supplementation was significantly beneficial. The inclusion of inappropriate patients with aneuploid embryos may have confounded the results in the PROMISE [10] trial reducing them to insignificance.

6 Subgroups of Threatened Miscarriage

6.1 Subchorionic Hematoma

Subchorionic hematoma is a common occurrence in threatened miscarriage, being found in approximately 18% of all cases of bleeding during the first trimester [37, 38]. A metaanalysis by Tuuli et al. [39] which assessed trials in which the presence of a fetal heart was not identified, included 1735 women with a subchorionic hematoma. 17.6% of pregnancies progressed to miscarriage. There is one observational study on the natural history of subchorionic hematoma in threatened miscarriage after detection of the fetal heart [7]. The incidence of miscarriage was 8.9%, similar to other cases of threatened miscarriage. The authors concluded that, "For women with a subchorionic hematoma that is sonographically identified, fetal outcome is dependent on size of the hematoma, maternal age, and gestational age".

A variety of grading systems have been used for characterizing the size of the hematoma, including subjective grading as small, moderate, or large, the volume of the hematoma from its ultrasound measurement and estimation of hematoma size as a fraction of gestational sac size, and estimated fraction of the gestational sac surrounded by hematoma. Heller et al. [37] compared these four grading systems in order to predict fetal outcome. The earlier in pregnancy that the hematoma was seen, the worse the outcome ($p < 0.00001$, logistic regression). The rates of demise were 19.6% at 7 weeks or earlier, 14.6% for 7–8 weeks, and 3.6% later than 8 weeks. The best grading system was the hematoma size as the estimated fraction of the sac size when compared to the subjective hematoma size and fraction of the sac surrounded by hematoma.

Figure 4.4 shows a patient with a subchorionic hematoma from the author's series.

There are two trials of progestogens in subchorionic hematoma. Both are open labelled observational studies. In the first study, Pelinescu-Onciul et al. [40] treated 125 women with micronized progesterone 600 mg/d. 18.7% of pregnancies terminated in miscarriage. In the second study [41], 100 women, with threatened miscarriage and a viable embryo received dydrogesterone. There were 93 live births and 7 miscarriages. The difference in results was significantly better in the

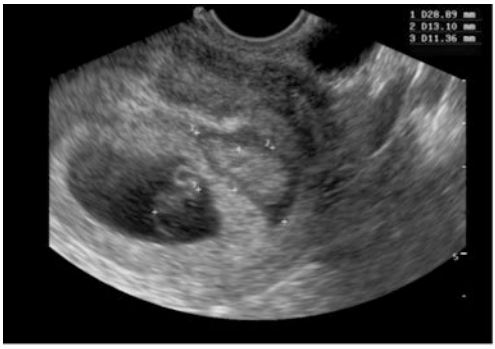

Fig. 4.4 Subchorionic hematoma at 7 and 9 weeks

dydrogesterone group. (RR, 2.04 CI, 1.05–3.97). However, these results should be treated with caution due to the methodological flaws of comparing two separate cohorts of patients who were not randomized.

6.2 Threatened Miscarriage After Recurrent Miscarriage

As stated above, vaginal bleeding occurs in 40–50% of recurrently miscarrying women, even when the pregnancy is viable. Progestogen supplementation has been used in recurrent miscarriage with varying degrees of success, as described in the next chapter. As in threatened miscarriage, treatment can only affect a live embryo, or an embryo at a stage prior to 5.5 weeks (usually the earliest that a fetal heart can be detected). Only one study has assessed treatment of threatened miscarriage after recurrent miscarriage [34]. As mentioned above, there was no beneficial effect of miscronised vaginal progesterone when administered to patients with threatened miscarriage, subgroup analysis showed a statistically significant benefit when administered to patients with two or more or three or more previous miscarriage and subsequent threatened miscarriage. The live birth rate was 72% with progesterone vs 57% with placebo (RR, 1.28, CI 1.08–1.51; $p = .004$). .

7 Safety and Side Effects

Safety and side effects are always a major worry when drugs are used in pregnancy. The major concerns about safety have centered on the effect of progestogens on the androgen receptor. The original progestogens were derivatives of 19 Nor testosterone. These compounds led to varying degrees of masculinization of female fetuses in rats. The masculinization included clitoral enlargement, and varying degrees of labial fusion. However, these side effects are not seen with other progestins. On the contrary, progesterone itself has anti-androgenic effects and has been reported to lead to hypospadias. In a case–control study, a relationship was reported between the use of progesterone and isolated hypospadias in two uncontrolled observational studies [42, 43]. Carmichael et al. [44], studied the risk of hypospadias and periconceptional progestin intake. Progestin-related hypospadias was reported by 42 (8.4%) case mothers and 31 (2.4%) control mothers, for intakes from 4 weeks before conception to 14 weeks after. The crude odds ratio for progestin use at any time was 3.7 (95% CI 2.3–6.0). Additionally, Rock et al. [45] reported one case of undescended testis and one case of meningomyelocele in 93 women treated with progesterone in the first trimester. Check et al. [46] found two cardiovascular malformations,

omphalocele, hydrocephalus and club foot with cleft palate in 382 women exposed to either progesterone or 17-α hydroxy-progesterone. These studies had no control group.

Progesterone itself, has been reported as safe, with no increase in the frequency of congenital anomalies [47, 48]. In 1999, the US FDA classified micronized progesterone, as a category B drug. It appears that progesterone treatment does not increase the risk of nongenital birth defects.

However, there are side effects with micronized progesterone. If given orally, the hormone is degraded in liver. In miscarriage, there is little data on efficacy data, and there is extreme variability in plasma concentrations [49]. Side-effects include nausea, headache and sleepiness. If micronized progesterone is administered vaginally, hepatic metabolism is avoided. The suppositories are not painful, and side effects are few. However, there are problems with patient compliance, as vaginal tablets are not acceptable in some cultures. In addition, vaginal administration is uncomfortable if there is bleeding or discharge and the suppositories may be washed out if bleeding is severe. There are also side effects concerning patient comfort, as the patient has to leave her daily activities, find a clean room, lie down to insert the progesterone and rest for 20 min to allow absorption. She has to repeat this inconvenience 2–3 times per day. The internet contains numerous reports and images of side effects such as excessive and irritant vaginal discharge, vulval edema, irritation etc. These side effects are not usually mentioned in the professional literature.

17 hydroxyprogesterone by intramuscular injection has numerous side effects including: extreme pain, swelling, itching and other local reactions at the injection site, abscesses formation, hypersensitivity reactions, cough, dyspnea, tiredness, dizziness, genital itching, and increased risk of gestational diabetes, mood swings, headaches, bloating, abdominal pain, perineal pain, constipation, diarrhea, nausea, vomiting, joint pain, depression, decreased sex drive, nervousness, sleepiness, breast enlargement, breast pain, dysuria, polyuria, UTI, vaginal discharge, fever, flu-like symptoms, back pain, leg pain, sleep disorder, upper respiratory infection, asthma, acne and pruritus. There have been concerns regarding the vehicle, castor oil, which may induce labor by stimulating release of prostaglandins [50, 51]. Three clinical studies in singleton pregnancies have all shown increased risk of miscarriage compared to placebo [52–54]. 17 hydroxy progesterone acetate or caproate are therefore not recommended for threatened miscarriage.

In order to assess the safety of dydrogesterone, all twenty two studies originally considered for Carp's [32] metaanalysis on dydrogesterone were reviewed. The follow up data on 1380 patients suggests that the side effects including birth defects are minimal. Additionally, a review of birth defects associated with dydrogesterone use during pregnancy [55] concluded that clinical experience with dydrogesterone provided no evidence of a causal link between maternal use during pregnancy and birth defects. It is estimated that between 1977 and 2005 approx. 38 million women treated with dydrogesterone, and more than 10 million fetuses exposed. There also seem to be no major side effects in the mother.

8 Conclusions

In the light of observational studies showing that 90% of threatened miscarriages continue developing after detection of a fetal heartbeat, it is difficult to give recommendations. If the embryo is aneuploid, it will be miscarried whatever treatment is advised. However, progestogen administration has been shown to reduce the number of threatened miscarriages developing to miscarriage. 17 hydroxy progesterone caproate seems to increase the likelihood of miscarriage, and is therefore not recommended. Dydrogesterone however, has been assessed on 660 patients in a systematic review, and the effect seems to be substantial, reducing the odds ratio for miscarriage by 47%. The effect of micronized progesterone requires further clarification, and there is conflicting evidence regarding efficacy, and the patient needs to be aware of the inconvenience.

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Chapter 5
Progestogens and Recurrent Miscarriage

Narmada Katakam and Luciano G. Nardo

1 Introduction

Progesterone has been implicated as being essential for successful embryo implantation, and for the prevention of miscarriage. In fact, progesterone was one of the first reported as treatment for the prevention of RM as early as in 1950 [1]. In this chapter, we discuss the available evidence and review the rationale for the use of progestational agents in cases of recurrent miscarriage.

RM has been defined as either three or more consecutive spontaneous pregnancy losses [2] or two or more consecutive losses [3]. The fact that RM can be defined as two or more losses, complicates the interpretation of epidemiological studies and makes the available research on this subject rather heterogeneous. Miscarriage is defined in North America as pregnancy loss prior to twenty weeks. In Europe, the term miscarriage includes all pregnancy losses from the time of implantation until 24 weeks of gestation, although advances in neonatal care have resulted in babies surviving before this gestation.

The difference between RM and sporadic miscarriage is that statistically as the demonstrated effect is being repeatedly observed the chances are that the causality is due to a systemic and recurring factor. Women with recurrent miscarriages tend to lose genetically normal pregnancies compared to women with sporadic miscarriages [4]. The incidence of recurrent miscarriages is approximately 1% of couples

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H. J. A. Carp (ed.), *Progestogens in Obstetrics and Gynecology*,
https://doi.org/10.1007/978-3-030-52508-8_5

trying to conceive [2], which is higher than the expected incidence of (0.34%) if RM were to occur by chance alone [5]. Hence, RM is probably a separate clinical entity to sporadic pregnancy loss. The 0.34% chance was calculated assuming a sporadic miscarriage rate of 15%, which is probably an underestimation.

The aetiology of RM has been extensively researched. The underlying causes could be either maternal or fetal in origin. Maternal causes may include uterine factors such as endometrial pathology, endometrial receptivity and/or congenital uterine anomalies, hormonal imbalance and insufficiency, infections, defective immunoregulation, hereditary or acquired thrombophilia such as antiphospholipid syndrome, chromosomal abnormalities such as Robertsonian translocations. Fetal causes include chromosomal abnormalities and structural malformations. Nevertheless, in up to 50% of cases no cause can be identified [6].

The prognosis of RM has been reported to be better in secondary than in primary recurrent miscarriage [7]. A descriptive cohort study of 987 women who presented with RM in a tertiary centre showed that approximately two thirds of women with RM succeeded in having a live birth within five years after the first consultation, but a full third did not. There was a significantly decreased chance of at least one subsequent live birth with increasing maternal age and increasing number of miscarriages at first consultation [8].

Recurrent miscarriage is a devastating experience for the patient and a dilemma for the clinician. The emotional and psychological implications for the patient are very significant. There may be feelings of desperation, frustration, guilt, depression, low self-esteem and distrust which may overwhelm the patient. As a result, the physicians have never stopped seeking ways of treating RM, especially as these patients are willing to try anything to have a live birth.

2 The Role of Progesterone

Progesterone's role in the successful implantation of an embryo led to progesterone being called "the hormone of pregnancy." It was shown over 40 years ago that surgical removal of the corpus luteum before the eighth week of pregnancy lead to spontaneous miscarriage. All progestogens are placentotrophic and their use was thought to improve trophoblastic proliferation into the spiral arteries. Progestogens are generally thought to be safe and have become standard treatment for luteal support in assisted reproduction. Worldwide the demand from women for any treatment that provides hope drives clinicians to prescribe progestogens or progesterone. Since the first use of progestogen preparations in the 1950s the supposed therapeutic benefit has been controversial and disputed. The debate regarding the efficacy of this treatment is ongoing.

2.1 Potential Mechanisms of Action of Progestogens in Preventing RM

Implantation has been described as a three-stage process. The first stage comprises the apposition of the blastocyst to the endometrium. The second stage involves the adhesion of the embryo to the endometrial epithelium. In this stage the blastocyst can no longer be removed by just being flushed out. Adhesion is due to cell surface glycoproteins, the specific mechanisms of which, are still being studied. The two first stages are mainly mediated by integrins, mucins, trophinins and tastins. The third stage is the invasion and embedding of the trophoblast. The invasive stages consist of two phases: early and deep invasion. Early invasion is an interplay between matrix metalloproteases (MMP) secreted by the embryo and tissue inhibitors of these proteases (TIMMP) secreted by the endometrium. During deep invasion there is an interaction between T-helper (Th) 1 cytokines preventing implantation and Th2 cytokines enhancing implantation [9].

Implantation failure has been thought to be the cause of many cases of recurrent miscarriage, as well as other reproductive sequelae such as recurrent assisted conception failure and pre-eclamptic toxaemia of pregnancy [10]. There are various ways in which progesterone influences and regulates implantation, irrespective of the mode of administration, whether oral, rectal, vaginal or intramuscular.

2.1.1 Disturbed Metabolism of Cholesterol and Progesterone in Recurrent Miscarriages

In order to establish pregnancy, the maternal decidua is invaded by extravillous trophoblasts (EVTs) and a blood supply is established for the growing fetus. There is increased expression of the HDL-receptor, scavenger receptor class B type I, elevated levels of hydroxy-delta-5-steroid dehydrogenase 3 beta- and steroid delta-isomerase 1 (HSD3B1). HSD3B1 is a rate-limiting enzyme in progesterone synthesis. Decreased HSD3B1 expression has been reported in EVTs in women with recurrent miscarriage [11].

2.2 Effects of Progesterone on the Uterus and the Endometrial Environment

2.2.1 Endometrial Development and Luteal Phase Deficiency

Estrogens and progesterone secreted cyclically by the ovaries control morphological and functional changes in the endometrium, leading to an optimal endometrial environment for implantation (commonly referred to as the ‘implantation or nidation window’). The nidation window is approximately 6 days after the LH surge

[12] (approximately day 20 in a 28-day cycle). However, there is no consensus as to the duration of such a window [13]. Work by Navot in human embryos suggested that the implantation window lasts 4–5 days synchronous with peak progesterone concentrations [14, 15].

The term Luteal phase deficiency or luteal phase defect (LPD) is a term that has been used to describe a decrease in the amount or the duration of progesterone secretion from the corpus luteum or lack of an adequate endometrial response to ovarian steroids [16, 17]. The gold standard for the diagnosis of LPD has traditionally been the morphological examination of a precisely timed luteal phase endometrial sample according to the Noyes' criteria [18]. Progesterone supplementation aids the creation of a more receptive endometrial environment. Over the years, there have been several reports of significant variability in the histological evaluation of human endometrium dating [19, 20]. The need and usefulness of histological dating of the endometrium itself has been questioned [21], and although it has served its cause, it is nowadays considered outdated.

In recent years scientists have been seeking novel approaches to characterise the endometrium. They have utilised the ability to describe morphological changes using scanning electron microscopy (SEM), and the development of techniques that focus on molecular aspects of endometrial development. Using SEM of the uterine epithelium, some researchers [22, 23] have shown the existence of specialised cell surface structures called pinopodes (or uterodomes). The development of pinopodes has been associated with the adhesion of blastocysts to the luminal epithelium [24] and have thus been considered as markers of receptivity. Progesterone stimulates the appearance of pinopodes, whereas oestrogens cause their regression. Supraphysiological levels of oestradiol such as those achieved during controlled ovarian stimulation have been associated with impairment of uterine receptivity [25]. However, other studies have failed to demonstrate a reliable pattern of pinopodes expression [26, 27], and their significance as markers of endometrial receptivity remains a matter of debate.

Despite the above controversies, the role of progesterone in successful implantation is not disputed. The failure to synchronise the complex mechanisms involved in the crosstalk between the endometrium and the embryo results in failure of implantation. Progesterone deficiency could contribute to the pathophysiology of recurrent pregnancy loss by delaying endometrial development. Low progesterone levels have been found in recurrent miscarriage with delayed endometrial ripening [28–30].

An increase in the secretion of estradiol precedes ovulation and promotes the proliferation and differentiation of uterine epithelial cells. It is then followed by the secretion of progesterone, which induces stromal cell development [31]. Progesterone acts on the endometrium via specific progesterone receptors (PR) or by changing the isoforms ratio and possibly their expression level. Receptor synthesis is controlled by estrogens through estrogen receptors during the proliferative phase. By down-regulating estrogen receptors, progesterone leads to a fall of both estrogen and progesterone receptors [32].

Polymorphisms of progesterone receptors (PROGINS) have been reported to act as a risk-modulating factor in women with RM. Receptor polymorphisms may cause an alteration in the biological function of the PR and can be associated with an individual susceptibility to pregnancy loss, though this concept has not been confirmed in a recent meta-analysis [33]. It appears that inappropriate endometrial development can occur even with sufficient progesterone levels [34], possibly due to genetic variation of progesterone receptors. The concept of absolute or relative progesterone deficiency in the pathophysiology of recurrent miscarriage, could explain why progesterone treatment may benefit some but not all women with unexplained RM.

2.2.2 Induction of Uterine Quiescence

In animal models, progesterone has been recognised as one of the major causes of inhibition of myometrial contractility. Withdrawal of progesterone is responsible for the initiation of labour. In humans, there is no detectable progesterone withdrawal, but biochemical events suggest 'functional progesterone withdrawal'. Potential mechanisms include changes in receptor isoforms and decreased myometrial sensitivity to progesterone. Nitric oxide (NO) generated in the pregnant uterus has been shown to maintain uterine relaxation [35]. Several studies have shown that progesterone enhances NO production in the endometrium [36–39]. Pharmacological withdrawal of progesterone by administration of 3-beta-hydroxysteroid dehydrogenase inhibitors or mifepristone (RU486) is associated with the onset of labour, and has been widely used to terminate early pregnancy by competitively blocking the PR [40].

2.3 Immunological Role of Progestogens

Maternal recognition of fetal antigens does not appear to compromise pregnancy. On the contrary it induces functional modifications that allow the conceptus to survive and develop. Maternal immune tolerance is established in the decidua, probably at the feto-maternal interface. There is a significant body evidence that progesterone facilitates an immune environment conducive to the early development of pregnancy.

2.3.1 Involvement of Progesterone in Maternal Cytokine Production

Progesterone-induced blocking factor (PIBF) is a protein synthesised by lymphocytes of pregnant women in the presence of progesterone. Progesterone receptors on lymphocytes are moderated by the immunological recognition of pregnancy [41]. PIBF is associated with both the immunomodulatory [42] and anti-abortive [43–45]

properties of progesterone. Lymphocytes from women with normally developing pregnancies produce significantly more PIBF than those of women with failing pregnancies.

In pregnancy there is a physiological shift in the decidual cytokine pattern from a Th1 response to a Th2 response. The cytokine shift may be modulated by PIBF [46]. Th1-type pro-inflammatory cytokines (TFN-α, IFN-γ, IL-2) support allograft rejection and are thought to be detrimental to pregnancy. TNF-α activates natural killer (NK) cells, promotes apoptosis of the trophoblast and initiates coagulation, at least in mice [47]. Interferon-γ can induce expression of major histocompatibility antigens on the trophoblast, where they are not normally expressed. Th2-cytokines (TGF-β2, IL-3, IL-4, IL-5, IL-10) inhibit pro-inflammatory Th1 responses, and seem to benefit pregnancy maintenance [48]. TGF-β2 induces trophoblast proliferation, IL-4 and IL-10 inhibit prothrombinase.

The activation of peripheral blood mononuclear cells (PBMC) by trophoblast antigens confirmed that women with RM have a predominately Th1 cytokine profile [49]. Increased production by PBMC of Th1 cytokines and decreased levels of Th2 cytokines have been demonstrated in non-pregnant women with recurrent early pregnancy losses [50]. Bates and colleagues failed to demonstrate the proposed defect in the shift from Th1 to Th2 cytokines in women with RM. Instead, increased production of IL-4 and IL-10 was shown in such women, along with reduced IFN-γ in pregnant women [47]. Nevertheless, the case for a possible association between maternal Th1 dominance and recurrent miscarriage is strong. Researchers are therefore faced with the challenge of determining the optimal Th1/Th2 cytokine balance and trying to manipulate it towards an immune favourable environment.

Progesterone has been reported to be associated with a decrease in IFN-γ (Th-1) and increase in IL-10 (Th-2) in endocervical fluid [51]. In addition, progesterone up-regulates Leukemia inhibitory factor (LIF) mRNA expression in vitro [52]. LIF is essential for implantation in muridae.

Dydrogesterone is the most commonly used progestogen to support early pregnancy. Dydrogesterone can be administered orally and has high affinity with the PR, resembling endogenous progesterone in its pharmacology and biochemistry. Raghupathy and colleagues investigated the effects of dydrogesterone therapy on Th1 and Th2 cytokines production in RM. Downregulation of Th1 cytokines (TNF-α, and IFN-γ) and stimulation of Th2 cytokines (IL-4 and & IL-6), and induction of PIBF production was reported [53]. Other researchers have suggested that the induction of PIBF production in humans could be the indirect mechanism by which dydrogesterone improves pregnancy outcome [54].

2.3.2 Involvement of Progesterone in Maternal Natural Killer (NK) Cells

The number of peripheral large granular lymphocytes or natural killer cells (pNK) cells have been associated with RM [55, 56]. However, the number or killing activity of pNK cells may not reflect the condition in the endometrium where implantation occurs, Hence, the potential role of pNK cells in the pathophysiology of

miscarriage remains uncertain. Elevated uNK cells have been found in luteal-phase endometrial biopsies of women with recurrent miscarriage compared to the pre-implantation endometrium in normal pregnancies [57]. Interestingly a recent meta-analysis that evaluated uNK cells expressed as percentage of the stromal cells failed to demonstrate a significant difference between these two groups [58]. However, the studies included in the meta-analytical pooling appeared to suffer from significant heterogeneity.

Natural Killer cells, which are present in the endometrium in the luteal phase of the menstrual cycle become decidual NK cells in pregnancy. They increase in number in the first trimester [59] However, uNK dNK cells display limited cytotoxicity [60]. They are thought to control trophoblastic invasion through the production of immunoregulatory cytokines and angiogenic factors, and are involved in remodelling of the decidual blood vessels [61–63] If uNK cells are associated with RM, the mechanism is unclear. Although uNK cells do not express progesterone receptors, both the number and function of NK cells are influenced indirectly by progesterone [64]. Szekeres-Bartho and colleagues have demonstrated that a low proportion of PIBF-positive lymphocytes are inversely related to NK cell activity and pregnancy loss [65]. PIBF is thought to contribute to the suppression of decidual NK cells cytolytic activity [66].

The role of progesterone on maternal NK cells is still being evaluated. When interpreting studies on NK cells, one should take account of the compartment (peripheral blood, endometrial, decidua etc.) in which the cells or other prognostic markers are investigated.

2.3.3 Involvement of Progesterone in Anti-trophoblast Antibodies

The presence of various molecules has been implicated in the increased incidence of recurrent miscarriage, including anti-trophoblast antibodies (ATAB) [67]. Antibodies that cross react with HLA-negative syncytiotrophoblasts. ATAB were expressed in 17% of women with two or more miscarriages and 34% of women with three or more miscarriages. In-vitro studies using ATAB positive and ATAB negative sera from women with recurrent miscarriage were performed and their effect on hCG and progesterone secretion by JEG-3 cells were analysed. hCG and progesterone production were found to be inhibited and thus ATAB's interfere with early pregnancy [67].

3 The Evidence for Progesterone Use in Recurrent Miscarriage

There have been several meta-analyses evaluating the use of progestogens for the prevention of subsequent miscarriage after RM. A meta-analysis in the Cochrane database concluded that there was probably a slight benefit for women receiving

progestogen in terms of live births (RR 1.07, 95% CI 1.00 to 1.13), in six trials [68]. However, the papers included in the meta-analyses, did not account for the confounding factor of embryonic aneuploidy, nor did they stratify for the number of miscarriages, the particular progestogen used, maternal age or primary or secondary aborter status, all of which impact on the outcome. One study of dydrogesterone, by El Zibdeh et al. [69] was excluded from the earlier meta-analyses due to quasi-randomisation rather than true randomisation. The effect of confounding factors can easily be seen in the case of vaginal micronized progesterone, where two studies have produced conflicting results. Stephenson and colleagues [70] studied the role of vaginal progesterone in women with two or more unexplained miscarriages under 10 weeks and miscarriages with chromosome errors excluded. Pregnancy was only allowed after vaginal micronised progesterone increased Cyclin E (a marker of endometrial maturation [71]) levels. After correction of Cyclin E levels, vaginal micronized progesterone 100–200 mg was administered every 12 hours. The ongoing pregnancy rate increased from 6% (16/255) to 69% (OR, 2.1 CI 1.0–4.4) with progesterone [70]. The 'Progesterone in recurrent miscarriage (PROMISE) [72] study', however, (a randomised, double blind, placebo controlled international multicentre trial of micronized vaginal progesterone) took no account of embryonic aneuploidy or state of the endometrium. Using these non-selective criteria, there was no evidence of progesterone leading to a significant difference between the groups for any of the outcomes.

Another systemic review and meta-analysis [73] of 10 trials which included 1586 women used micronized progesterone in two trials and medroxyprogesterone, cyclopentylenol ether of progesterone, dydrogesterone, or 17-hydroxyprogesterone caproate in eight studies. This metaanalysis investigated first trimester progesterone supplementation in women with unexplained recurrent miscarriage. The miscarriage risk was lower (RR 0.72, 95% CI 0.53–0.97) and live birth rate was higher (RR 1.07, 95% CI 1.02–1.15) after progesterone supplementation when all the progestogens were analysed as a whole.

The editor of this book has published a metaanalysis of three trials of dydrogesterone [74]. Two of the trials were randomized, one was quasi randomized. There was a 10.5% (29/275) miscarriage rate after dydrogesterone administration compared to 23.5% in control women (OR 0.29 CI 0.13–0.65). Chapter 2 describes the difference in bioavailability and receptor binding between progesterone and dydrogesterone.

On summarising the above literature and trials, it seems that there is an advantage to using progestogens in recurrent miscarriage. However, it remains to define a population who can respond, and the appropriate diagnostic tests to determine who can benefit.

4 Safety of Progestogens for Prevention of Recurrent Miscarriage

4.1 Safety of the Mother

Millions of women have used progesterone or progestogens, and 39 million women have been assumed to have been exposed to dydrogesterone alone [75]. Few studies have accounted on the maternal adverse effects of progesterone use to prevent miscarriage [76]. However, few side effects have been reported. Typical maternal side effects of progestogens such as nausea, bloating, dizziness, breast tenderness, mood changes and cephalalgia may occur, but can also be attributed to the physiological changes occurring in early pregnancy.

Theoretically there could be concerns that progesterone may delay spontaneous miscarriage, by promotion of uterine quiescence or even aid retention of chromosomally abnormal embryos. However, it seems that the mechanism in immunologically mediated pregnancy losses may be different to that in those due to aneuploidy. In the latter event implantation fails altogether, whereas in immunologically mediated miscarriages there is adequate implantation and subsequently an immunologically mediated process of vasculitis, inflammation leading to thrombus formation. Progestogens only influence chromosomally normal embryos by regulating immunomodulation [77, 78].

4.2 Safety of the Fetus

There have been reports suggesting an association between intrauterine exposure to progestogens in the first trimester of pregnancy and genital abnormalities in both male and female fetuses. Some progestogens such as ethisterone have been thought to induce mild virilisation of the external genitalia in the female fetus [79, 80]. However, virilisation has only been seen in rat fetuses, the lack of evidence in humans has made it impossible to quantify the risk. Additionally, virilisation was only seen with progestogens derived from 19-nor testosterone, Virilisation has never been seen with progesterone derived progestogens (see Chap. 2). Carmichael and colleagues [81] reported that maternal intake of progestins in early pregnancy is associated with an increased risk of hypospadias in the male fetus due to an anti-androgenic effect (OR 3.7, 95% CI 2.3, 6.0). Other studies do not indicate an increased risk with exposure to progestins. Interestingly dydrogesterone has less of an anti-androgenic effect than progesterone itself. The urogenital groove is fused by 16 weeks of gestation, in order to avoid the anti-androgenic effect, some authors

have recommended that progesterone containing medications should be avoided in the first trimester of pregnancy [82]. However, avoidance in the first trimester precludes use in recurrent pregnancy loss, where the majority of losses occur in the first trimester.

5 Future Research

The lack of robust data has generated the need for well-conducted studies to assess the validity of intervention with progestogen supplementation in RM. In a small unpublished study, it was reported that 90% of obstetricians and gynaecologists called for a definitive placebo controlled randomised trial [83]. The potential pitfalls for such a study are many, and careful design is of paramount importance. Matching age and number of miscarriages is one component. Stratification for primary versus secondary miscarriage is another factor which has to be taken into account. Commencing progesterone in the luteal phase, mode of administration, type of progestogen are other essential features to be considered. Unfortunately, heterogeneity in human populations is unavoidable even in patients with exactly same clinical characteristics. Ideally, all pregnancies should have been conceived with the same partner, which is again almost impossible to elicit. The exclusion of other causes of miscarriage is also difficult to ascertain, as there are subjective variables such as the presence of embryonic aneuploidy.

6 Conclusions

Progesterone is a 'pro-gestational' agent that maintains the pregnant state. The immunomodulatory function of progesterone appears to be decisive in early pregnancy. It is therefore quite possible that there may be a role for its use in women with unexplained RM. The paucity of good quality evidence of effect and inclusion of heterogeneous patients in trials may be responsible for contradictory and ever-changing views amongst clinicians. However, absence of evidence is not evidence of absence of the role of progesterone in RM.

Research into RM is ongoing and focused on several areas, mostly at the biomolecular level. Factors such as circulating pro-coagulant microparticles, glycoproteins, hCG and glycodelin, leptin receptors and TNF-α inhibitors are being further investigated. As our understanding of the pathophysiology of recurrent miscarriage improves new causes are likely to be identified, leading to individualisation of treatment for RM.

Progesterone is safe to use in pregnancy, inexpensive and easy to administer. Until more robust evidence is available, women who are desperate to have a live birth despite multiple pregnancies, can be offered progesterone supplementation, with an explanation of the conflicting results.

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Chapter 6
Progestogens in Preterm Labour Prevention: An Update

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1 Introduction

Preterm births (PTBs) refer to premature deliveries which occur prior to 259 days of gestation or below 37 gw. The condition is prelevant presenting a major public health problem worldwide. 15 million preterm deliveries occurred globally in 2010, accounting for 1 million infant deaths [1]. In the USA, 1/8 of the deliveries in recent years were preterm; 85% of which were associated with severe perinatal morbidity and mortality [2]. Of 3.1 million global neonatal deaths 35% were due to PTB complications [3]. Approximately 20% of preterm deliveries were iatrogenic, for fetal and/or maternal indications (pre-eclampsia, placental abruption, intrauterine growth restriction, placenta praevia, maternal cholestasis, non-reassuring fetal monitoring test and monochorionic-monoamniotic twin pregnancy complications) [4]. Another 20–30% of PTB cases were related to preterm premature rupture of membranes (P-PROM), 20–25% were a result of intra-amniotic inflammation or infection and 25–30% were associated with unexplained (spontaneous) preterm labor (PTL) [5].

Perinatal mortality increases more than three times in women with PTB (51.7/1000 births) which is also a leading cause for severe perinatal morbidity, such as neurodevelopmental deficiency, cerebral palsy, seizures, blindness, deafness, bronchopulmonary dysplasia, retinopathy of prematurity, gastrointestinal complications, cardiovascular and metabolic disorders [6, 7]. Additionally, medical care of preterm newborns is expensive involving large costs to healthcare systems and families. Interventions that reduce the rate of PTB would have a profound impact on the medical, financial and emotional burden for these children, their families and the healthcare systems.

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H. J. A. Carp (ed.), *Progestogens in Obstetrics and Gynecology*,
https://doi.org/10.1007/978-3-030-52508-8_6

Clinical risk identification remains the first and most important approach to women at a risk for preterm delivery and it is based on non-specific socioeconomic factors, previous history of PTB, short cervix measured on transvaginal ultrasound, infections/inflammatory status detection. There are several non-modifiable and modifiable risk factors related to preterm delivery. Once women at risk are identified, clinicians can apply the available strategies to prevent this event.

Progesterone plays a key role in the maintenance of uterine quiescence (during the latter half of pregnancy) due to a decrease in stimulatory prostaglandin production, inhibition of contraction associated protein gene expression, suppression of the cytokine inflammatory response thus preventing ascending infection dissemination, as well as a reduction in gap-junction formation and cervical stroma degradation. Progesterone alters not only estrogen synthesis in fetal membranes and the placenta, but also fetal endocrine-mediated effects. It decreases the number of oxytocin receptors and suppresses the activation of the pro-inflammatory cascade. Moreover, term and preterm labour onsets are both mainly associated with functional withdrawal of progesterone activity at a uterine level [8].

The body of scientific literature is growing with publications on the role of progesterone in obstetrical care, especially in PTL prevention. In this regard, progestogens have been largely studied in clinical trials for the prevention of PTBs in the last decades. Researchers and clinicians have demonstrated great interest in characterising the pharmacokinetic and pharmacodynamic profile of progestogens in order to define and plan the best preventive strategies.

This chapter focuses on the mechanisms whereby progestogens may reduce the PTL risk, the optimal preparation, dosage and route of administration. Progesterone is the most studied prophylactic agent for the prevention of PTBs and the improvement of neonatal outcome, but there is a lack of consistency in the reported beneficial effects. The different results may be due to the multifactorial etiology of PTL activation, the various patient cohorts recruited in clinical trials and the use of different types and routes of administration of progesterone and progestogens.

2 Role of Progesterone in Obstetrics

Progesterone is a hormone which plays a well-recognized essential role in the process of reproduction. It is used in the treatment of different gynecological pathologies (endometrial hyperplasia, dysfunctional uterine bleeding, amenorrhea, luteal phase deficiency, pre-menstrual syndrome, contraceptive use) and obstetrical conditions (assisted reproductive technologies, threatened miscarriage, prevention of RPL, history of previous preterm delivery, threatened PTL).

The role of progesterone in the maintenance of pregnancy comprises the modulation of maternal immune response [9–11], the suppression of the pro-inflammatory cascade [12], the inhibition of uterine contractility [13–15], and its' beneficial effects on utero-placental perfusion [16, 17]. The physiological aspects of progestogens actions have been widely reported, especially for progesterone itself

(P4) and the synthetic molecule of 17-alpha hydroxyprogesterone caproate (17-OHP-C). Progestogens inhibit IFN-gamma and TNF-alpha, but increase IL-4 production [18]. Dydrogesterone has been associated with higher levels of IL-10 and increased progesterone-induced blocking factor (PIBF) [18, 19], thus demonstrating a significant anti-inflammatory role. Vaginal micronized progesterone significantly reduces metalloproteinase expression (both of MMP-9 and MMP-2) and lipopolysaccharide (LPS) action on fetal membranes, suggesting a possible protective mechanism in the prevention of infection-associated PTB [20]. Regarding the effects on the myometrium, progesterone has been shown to have a tocolytic action both *in vitro* and *in vivo* throughout pregnancy, decreasing myometrial oxytocin receptor concentration. Early studies published in the 1960s and 1970s demonstrated a reduction of PTBs in various high risk populations of pregnant patients where 17-OHP-C was administered intramuscularly [9–12]. In the 1990s the interest in the relationship between progesterone/progestogens and PTL grew and the body of scientific literature expanded.

At present, we know that the effects of progesterone/progestogen therapy are dose-dependent and related to the route of administration, although sometimes based on controversial clinical data. Some authors have suggested that 17-OHP-C inhibits myometrial contractility in a dose-dependent manner [21], whereas others have observed no effect of 17-OHP-C in miscarriage prevention, but demonstrated a reduction in the incidence of PTBs and low birth-weight newborns [22].

3 Routes of Administration: Key Differences

Although the pharmacokinetics and pharmacodynamics of progesterone have been well studied since 1935, when progesterone was first synthesized, the optimal variant to use remains controversial because of the various routes of administration, doses and biochemical structure. The rate of absorption is dependent on which pharmaceutical form is being used and the blood flow at the site of administration. Progesterone can be administered by many different routes: orally, vaginally, intramuscularly, per rectum, and by transdermal patches.

Some information derived from animal models has suggested that vaginal progesterone might uniquely alter the inflammatory milieu of both the cervix and the endometrium, with consequent significant potential clinical advantages [23]. Other studies have also supported the vaginal route of administration in comparison to intramuscular injection. There is also a randomised control trial (RCT) comparing both vaginal and intramuscular progesterone administration in pregnant women with a previous history of PTB. Significantly fewer preterm deliveries were observed among women on vaginal progesterone [24].

Geographical differences exist worldwide according to the types, dosage and routes of administration of progesterone. In a large multi-institutional study conducted in the National Institute of Child Health and Human Development (NICHD) Maternal-Fetal Medicine Units Network, Meis et al., demonstrated that 17-OHP-C

given intramuscularly decreased recurrent the PTB rate by 30% (RR 0.66, CI, 0.54–0.81) [25]. Adoption of prophylactic progestin therapy was associated with a decreased OR of reccurent PTB before 35 and 37 weeks of gestation after adoption of program based on early progestin therapy [26]. Hence, current practices in the US is to offer 17-OHP-C, when a pregnant patient presents with a history of a previous spontaneous PTB. Additionally, 17-OHP-C is the only approved progesterone treatment by the Food and Drug Administration (FDA) for the prevention of PTL in the US. However, it may be reasonable to consider the use of vaginal progesterone, which although not FDA approved for this indication, was found by several studies to be effective in PTB prevention [22].

The main pharmacokinetic and pharmacodynamic progesterone features and their relation to the route of administration are reported below.

3.1 Oral Administration

Oral administration is associated with better patient compliance, but has several disadvantages, such as the extreme variability in plasma concentrations due to individual variation in gastric filling and enterobiliary circulation. Moreover, food intake may influence the rate of drug absorption by reducing gastric emptying, decreasing gastrointestinal (GI) motility, increasing GI secretions and splanchnic blood flow. Oral progesterone intake is also influenced by passage through the liver and hepatic metabolism. The metabolites produced by the liver are not progestogenically active as demonstrated by the discrepancy between the measured progesterone levels and endometrial histology [27].

Several synthetic oral progestogens have been developed to overcome progesterone's low oral bioavailability. Their pharmacological effects, however has revealed many side effects, such as androgenic effects, fluid retention tendency, alterations in lipoprotein profile, headache, mood disturbance, nausea, sleepiness, GI discomfort. Micronization of progesterone into particle sizes of <10 microns increases the available surface area and enhances the aqueous dissolution rate thus improving intestinal absorption. Suspension in oil and packaging in gelatin capsules has also further enhanced intestinal absorption [28].

3.2 Intramuscular Administration

Historically, the most common route of administration was intramuscular [28]. IM administraton results in optimal blood progesterone levels. Consequently, the IM route can be used in patients with vaginal bleeding and does not need more than a single daily or weekly dose [29]. However, IM use is associated with local discomfort and pain, and occasional local non-septic abscesses at the site of injection. Moreover, other side effects have also been described, such as tiredness, dizziness,

headache, bloating, abdominal pain, constipation, diarrhea, nausea, vomiting, joint pain, depression, sleepiness, breast tenderness, flu-like symptoms, infection predisposition and hypersensitivity reactions. Recent studies, have also associated the intramuscular application of 17-OHP-C with an increased rate of gestational diabetes (GD) due to its' diabetogenic effects by promoting pancreatic beta-cell hyperplasia and thus increasing insulin production [30]. Rebarber et al. studied GD in patients who received 17-OHP-C injections to prevent recurrent PTB: The GD incidence in the 17-OHP-C treated group was 12.9% compared to 4.9% in the control group ($p < 0.001$) without medication [31]. However, 17-OHP-C is the only agent approved to date by the US FDA for prevention of recurrent PTB [30].

3.3 Vaginal Administration

The vaginal route is the preferred route of administration in clinical practice in numerous centres worldwide. The scientific literature supports vaginal progesterone application as an effective strategy for PTB prevention. The vaginal route of administration results in higher concentrations of progesterone within the uterus due to the so called *first uterine pass effect* [29]. Three different hyphotheses have been reported to explain the first uterine pass effect: (1) high immediate concentration within the vaginal and uterine cells; (2) presence of portal-like lymphatic vessels which link the upper vagina to the uterus; (3) presence of a reverse circulation system, much like the portal system, with vein to artery diffusion between the upper vagina and the uterus. Bulletti et al., demonstrated that a first uterine pass effect occurred when progesterone was administered vaginally, confirming that the vaginal route permits targeted drug delivery to the uterus, maximising the desired effects while minimising the potential adverse systemic side effects related to other routes of administration [32]. After vaginal application the uterine progesterone tissue concentration was found to exceed more than tenfold the levels seen after systemic administration. The time to reach peak concentration was generally slightly less than that after oral administration of micronised progesterone. Plasma concentrations with a plateau-like profile and more constant progesterone concentrations over time are other advantages deriving from the vaginal route of administration. Additionally, the vaginal application does not seem to interfere with glucose metabolism or the induction of GD [33].

3.4 Rectal Administration

Recently, Afridi et al., compared the efficacy of oral dydrogesterone and a micronized progesterone rectal suppository (Cyclogest) in the prevention of PTB among patients at a risk for PTL. Group A was given oral dydrogesterone (10 mg twice daily) while group B was given a cyclogest pessary (400 mg daily) per rectum at

bedtime. The authors observed a good profile with rectal administration of progesterone: prophylactic micronized progesterone per rectum was more effective in decreasing the incidence of PTBs in high risk cases of prematurity compared to dydrogesterone and was further associated with less maternal and neonatal complications [34].

Additionally, Elder et al., observed that rectal progesterone decreased uterine contractions and reduced PTBs, which led to significant perinatal mortality reduction ($p < 0.05$) [35].

Abdali et al., also observed the effect of rectal progesterone on the latent phase and maternal and neonatal outcomes in females with preterm premature rupture of membranes (PPROM). Rectal progesterone (400 mg per night) was administered until delivery or completion of the 34th gestational week in a group of patients with PPROM between 26 and 32 weeks. The trial was placebo controlled. The median latent phase was 8.5 days in the intervention group vs. 5 days in the control group in the 28th–30th weeks of gestation, which was significantly higher ($p = 0.001$). Moreover, in the neonates, the birth-weight was significantly higher in the intervention group ($p = 0.03$) [36]. However, a recent systematic review and metaanalysis on progestogens in PPROM in singleton gestations showed that there was no differences in the latency period for women who received rectal progesterone (one trial assessed rectal progestogen, and one trial had three arms that compared 17-α hydroxyprogesterone caproate, rectal progestogen, and placebo) [37].

The small sample sizes and single source of data and still limit the general acceptance of these studies, although the daily use of rectal progesterone could represent another possible therapeutical option.

Table 6.1 summarises the main different routes of progesterone administration.

Table 6.1 Progesterone routes of administration

Route	Pharmacokinetic and dymamic features
Oral	• Rapid increase and gradual decrease in plasma circulation • First liver pass effect with biological active metabolites • Specific target organs: uterus, brain • Metabolism in the gut (bacteria with 5b-reductase activity), in the intestinal wall (5a-reductase activity) and in the liver (5b-reductase, 3a- and 20a-hydroxylase activities)
Intramuscular	• Supraphysiological plasma concentrations • 17-OHP-C seems to interfere with glucose metabolism (diabetogenic effect?)
Vaginal	• Stable plasma concentrations and consistent tissue levels • First uterine pass effect with targeted delivery into the endometrium • Minimal systemic effects • Metabolism: normal vaginal bacteria and mucosa seem devoid of 5a- and 5b-reductases. After vaginal absorption, only a small increase in 5a-pregnanolone levels were observed and 5b- pregnanolone levels were notaffected • No evidence of effects on glucose metabolism profile
Rectal	• Good profile on clinical efficacy (PTB reduction in higher risk patients,neonatal outcomes) • Lower maternal side effects

4 Progesterone and PTB: "Which, When and How"

Only 50% of all PTBs occur in women with identifiable risk factors, making screening and surveillance difficult. The major factor having the greatest predictive value at present is a previous history of preterm delivery, which is associated with a 1.5- to 2.0-fold risk increased risk [7]. Attempts to treat premature labor once established, have been largely inefficient resulting in only 2–7 days of pregnancy prolongation. The most effective intervention in the prevention of PTL is the use of progestogens, which contribute to the reduction of PTB and the attendant long-life unfavorable consequences [22, 26].

The mechanisms of human parturition include complex biochemical, physiological, anatomical and clinical events that develop both mother and fetus in both term and pre-term pregnancies. This pathway, probably involving a multifactorial basis, comprises: decidual/fetal membrane activation, increased uterine contractility and cervical ripening (dilatation and effacement). The key role of progesterone in PTB prevention is related to the progesterone receptor (PR) isoform expression, its' anti-inflammatory and immunomodulatory function.

Before undertaking any therapeutic strategy, careful identification of high risk patients is mandatory. Thus, previous obstetrical history (previous spontaneous PTB), current clinical signs and symptoms (abdominal pain, uterine contractility, cervical modifications on vaginal examinations) and instrumental/laboratory evaluations by transvaginal ultrasound cervical length (CL) measurement, biochemical detection of PTB risk factors such as fetal fibronectin (fFn), placental alpha microglobulin-1(PAMG-1) test, phosphorylated insulin-like growth factor binding protein (phIGFBP-1) tests are essential in any subsequent decision making process.

Once a threatened PTL diagnosis is made, tocolysis and administration of corticosteroids to induce fetal lung maturation are the first therapeutic tools. Additionally, bed rest and hydration are often recommended although none has proven to be clearly effective. Progesterone, 17-OHP-C as well as other progestogens have been tested in several clinical trials for the prevention of PTB [38]. The most relevant scientific evidence was based on data of progesterone (P4) and 17-OHP-C used as a prophylactically in patients with threatened PTB. The results are controversial. On the whole, progesterone and progestogens appeared to be beneficial, especially in view of cost, availability and biological safety [39, 40].

In women with prior history of PTB, the incidence of recurrent PTL was significantly reduced by weekly intramuscular administration of 17-OHP-C. A recent meta-analysis, however, suggested that daily vaginal progesterone started at about 16 gw is a reasonable alternative to weekly 17-OHP-C for PTB prevention in singleton pregnancies with a previous history of PTB [41].

Recent guidelines recommended offering prophylactic vaginal P4 to women with no history of spontaneous preterm delivery or mid-trimester pregnancy loss in whom transvaginal sonography between 16 and 24 gw revealed a CL <25 mm [41]. However, 17-OHP-C has been found to increase the incidence of GD three fold [30, 31]. In addition, the most recent scientific opinions on 17-OHP-C use, have not

Table 6.2 Types, routes of administration, dose and intervals of progesterone use [34, 42]

Type	Route of administration	Dose (mg)	Interval
17-OHP-C	Intramuscular injection	250	Weekly
Micronized progesterone	Vaginal soft capsule	100, 200, 400	Daily
Micronized progesterone	Vaginal gel	90	Daily
Micronized progesterone	Oral (capsule)	200, 400	Daily
Micronized progesterone	Rectal (pessary)	400	Daily

Adapted from Koun et al.

found 17-OHP-C to be beneficial in preventing preterm birth. The PROLONG study, a large multicenter, international, randomized double-blind trial, found that 17-OHP-C did not decrease recurrent PTB: there were no significant differences in the frequency of PTB < 35 weeks between the 17-OHP-C group and a placebo group (17-OHP-C 11% vs placebo 11.5%) [42].

Vaginal micronized progesterone (in soft capsules of 200 mg or 90 mg gel) administration is usually the preferred route and the optimal biochemical regimen in current clinical practice [41]. Jarde et al. in a recent updated systematic review and network meta-analysis confirmed that vaginal progesterone was the only intervention with consistent evidence of effectiveness for preventing preterm birth in singleton at risk pregnancies overall and in those with a previous PTB [43]. Table 6.2 summarises the types, route of administration, dosage and intervals of progesterone use to prevent PTBs based on the most relevant scientific studies and guidelines [44].

5 Progesterone and Twin-Pregnancy

The available data on progesterone use in twin-pregnancy is controversial. Many studies did not find any beneficial effect of progesterone use in twin pregnancy, while only few recent studies reported a potential advantages in terms of gestational length and neonatal outcomes. In both twin and triplet pregnancies, neither micronized progesterone nor 17-OHP-C had been shown to prevent preterm delivery [38]. More recently, a meta-analysis based on data from 13 RCTs demonstrated that treatment with progestogens, either intramuscular 17-OHP-C or vaginal micronised progesterone, did not prevent PTBs, or improve perinatal outcome in unselected women with uncomplicated twin gestation [45]. A recent Cochrane analysis from 2017 included 17 studies ($n = 4773$) on vaginal progesterone or 17-OHC-P use versus placebo use or no treatment in multiple pregnancies without additional selection criteria at a risk for PTB [46]. There was considerable heterogeneity among studies and predominantly poor study quality. However, no significant differences were observed in terms of the PTB rate neither with 17-OHP-C use at <37 gw (RR 1.05; 95% CI 0.98–1.13) nor use at <28 gw (RR 1.08; 95% CI 075–1.55) compared to the placebo/no treatment group. The same results were observed with vaginal progesterone used

to decrease the PTB rate at <37 gw: RR 0.97; 95% CI 0.89–1.06) and at <28 gw: RR 1.22; 95% CI 0.68–2.21.

Individual patient data meta-analysis by Romero et al., from 2017 on the administration of vaginal progesterone versus placebo or no treatment in 303 asymptomatic twin pregnancies with a CL of ≤25 mm in the second trimester of pregnancy did however, demonstrate a significant reduction in the PTB rate at <33 gw as a primary outcome (31.4 vs. 43.1%; RR 0.69; 95% CI 0.51–0.93) and an improvement in neonatal outcome as a secondary outcome, e.g. reduction in respiratory distress syndrome (RR 0.70; 95% CI 0.56–0.89), neonatal mortality (RR 0.53; 95% CI 0.35–0.81) and a reduction in babies with birth weight of <1500 g (RR 0.53; 95% CI 0.35–0.80) [47].

6 Progesterone as a Tocolytic Agent and/or for Maintenance Therapy?

Progesterone has been assessed as a tocolytic agent, but various studies have demonstrated a weak and slow capacity to inhibit uterine contractions, where 17-OHP-C seemed to have absolutely no effect [38]. Thus, the use of progesterone for acute tocolysis is irrational or may play a role only in conjuction with other tocolytic agents with synergistic effects (atosiban, beta-agonists, indomethacin, nifedipine etc.). Recently, Ashraf investigated the efficacy of combined therapy of using nifedipine with vaginal progesterone in the management of acute threatened PTL. Acute tocolytic therapy with nifedipine was successful in the majority of patients. The additional daily use of vaginal progesterone suppositories resulted in significant prolongation of pregnancy as well as reduction not only in the rate of neonatal low birth weight but also in neonatal intensive care unit (NICU) admissions. Mean pregnancy prolongation was 11.13 ± 5.08 days in group A (only on nifedipine), while it was 29.73 ± 3.10 days in group B (on a combined therapy with nifedipine and vaginal progesterone of 200 mg/daily, $p \leq 0.001$ [48]. However, there was insufficient evidence to recommended progesterone/progestogens use alone for primary tocolysis. There was also no evidence that progesterone or 17-OHP-C combined with other commonly used tocolytics led to effective prolongation of pregnancy or a significant decrease in the rate of PTBs.

Data on the use of progesterone for maintenance treatment was controversial. While RCTs of low quality showed promising results, high quality studies did not reveal any significant differences regarding the rate of PTBs <37 gw, the latency period until delivery and the neonatal outcome between the progesterone/17-OHP-C group of patients and the placebo or no treatment group [49]. Significant differences in the methodology, the inclusion and outcome criteria, the mode of application and the dosage of substances as well as the inadequate statistical power as a result of the low number of cases, make the interpretation and comparability of studies difficult. Therefore, well-designed randomized, placebo-controlled, double-blinded

studies with uniform primary outcome criteria are necessary in order to clarify whether progesterone is of clinical benefit not only for patients with manifested preterm contractions but also as a maintenance treatment after arrest of PTL is achieved. The optimal route of administration and the optimal dosage also require clarification.

7 Future Strategies on PTB Prevention

The most recent clinical data suggest a positive effect of combined treatment based on progesterone use and other strategies to prevent preterm delivery in high risk patients. In particular, recent studies have reported beneficial results derived from the combined use of vaginal progesterone and Arabin's pessary or cerclage. Melcer et al. observed that for women with singleton pregnancies with a short CL, the combined treatment of Arabin's cervical pessary and vaginal progesterone led to a lower rate of preterm delivery <34 gw and prolonged gestation compared to those women who were only on vaginal progesterone [50].

Shor et al. compared the outcome of pregnancy in women with a short CL managed with four different treatment protocols: vaginal progesterone, cervical cerclage and Arabin's cervical pessary (group A), Arabin's cervical pessary and vaginal progesterone (group B), cervical cerclage and vaginal progesterone (group C), or vaginal progesterone alone (group D). These combined approach resulted in promising strategies in pregnant women who had a short CL and a high background risk for preterm delivery [51].

Similar approaches seem to be promising also in twin pregnancies. Zimerman et al., compared twin pregnancies with a short ≤25 mm cervix in the second trimester of pregnancy between 16 and 28 gw on combined treatment for PTB with Arabin's cervical pessary and intravaginal micronised progesterone of 200 mg/daily with a control group on a conservative regimen. The treatment group had a lower incidence rate of PTL before 28 gw. However, further prospective studies are required the efficacy and the use of Arabin's cervical pessary in twin pregnancies [52].

8 Conclusions

Recent years have seen the publication of numerous clinical trials using progestogens for the prevention of several obstetrical complications, including RPL, threatened miscarriage and PTB. As a result of different inclusion criteria and the use of different progestogens and their route of administration, it is difficult to draw a comparison from these studies and to propose an absolute regimen of treatment for daily clinical practice. Taking into account the most recent and relevant RCTs,

geographical differences and differences in management, the following evidence-based recommendations confirm to be reasonable:

- Progesterone and progestogens show an interesting medical profile in all pregnancy phases.
- Early PTB risk assessment is mandatory (prevention, prediction, education).
- Prophylactic progestogen therapy consists of the use of micronized progesterone form (P4) administered daily as a vaginal suppository (sometimes orally) or the use of 17-alpha hydroxyprogesterone caproate (17-OHP-C) in oil suspension administered weekly in the form of an intramuscular injection.
- In asymptomatic women with singleton pregnancies and a short CL on ultrasound of ≤25 mm the daily administration of vaginal progesterone (in capsules of 200 mg or 90 mg gel) until 34 gw leads to a significant reduction in the PTB rate and an improvement in neonatal outcome.
- 17-OHP-C is administered weekly from 16 to 36 gw in the form of an intramuscular injection. However, the latest shows a preference for vaginal micronized progesterone.
- The latest data also suggests a positive effect of progesterone treatment in cases of twin pregnancies with a short CL on ultrasound of ≤25 mm [44].
- Progesterone should not be used as an agent for primary tocolysis. However, it can be used in conjuction with tocolytic agents (in the acute phase) and for maintenance treatment (after the acute phase).
- Promising future pathways for PTB prevention (including twin pregnancies) could be combined treatment comprising progesterone and Arabin's pessary or cerclage [50].

Even if progesterone use shows a general good profile of safety in terms of possible short- and long-term consequences, exposure should be avoided if not indicated. Careful patient selection is crucial for treatment success.

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Chapter 7
Abnormal Uterine Bleeding

Eran Zilberberg and Howard J. A. Carp

1 Introduction

Abnormal uterine bleeding (AUB) is defined as bleeding of abnormal duration or quantity usually defined as above 80 ml/month [1]. AUB is one of the most frequent gynecological complaints, and its prevalence is estimated to occur in 20% or more of women [2].

During the past, descriptive terms have been used to characterize AUB—menorrhagia for heavy uterine bleeding, metrorrhagia for bleeding between periods, polymenorrhea and oligomenorrhea for frequent bleeding or infrequent bleeding (respectively). In 2011, the International Federation of Gynecology and Obstetrics (FIGO) [3] introduced a new nomenclature for AUB for non-gravid reproductive-aged women, known by the acronym PALM-COEIN. This system divides the etiologies for AUB to two groups: (1) Structural causes: PALM (Polyp, Adenomyosis, Leiomyoma, Malignancy and hyperplasia); (2) Nonstructural casues: COEIN (Coagulopathy, Other, Endometrial, Iatrogenic, Not yet classified).

Initial evaluation should assess the source and clinical features of the bleeding and exclude organic causes for the bleeding such as fibroids, polyps, carcinoma of cervix or endometrium, coagulation defects, and systemic disease. As the endometrium is a hormonal sensitive tissue, progestogens are a significant factor in treatment. As outlined in other chapters, the choice of hormonal (progestogen) or

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H. J. A. Carp (ed.), *Progestogens in Obstetrics and Gynecology*,
https://doi.org/10.1007/978-3-030-52508-8_7

surgical therapy varies according to the diagnosis, the patient's needs and fertility desire. This chapter will discuss the different types of progestogen in use for AUB as well as other treatment modalities.

2 Physiology of Menstruation

Lockwood [4] has given a full account of the pathophysiology of menstruation and the changes in AUB. Briefly, following menstruation, repair of the functional layer takes place. Stem cells in the endometrial stratum basalis proliferate, thus producing a new functional layer under the influence of estrogen secreted by the ripening follicle. The endometrial epithelial and stromal cells proliferate. The stromal cells express vascular endothelial growth factor (VEGF) which induces angiogenesis [5] and the endothelial cells express angiopoietin-2 (Ang-2) [6]. In the secretory phase progesterone produced by the corpus luteum induces changes in the endometrium. Around the new blood vessels, progesterone augments expression of Angiopoietin-1 (Ang-1), from the stromal cells. Ang-1 stabilizes the vessels and blocks further angiogenesis by an anti-mitotic action [7]. The anti-mitotic action also prevents further stromal proliferation and is akin to the anti-mitotic action which is used therapeutically in endometrial cancer (see Chap. 11). Progesterone also induces tissue factor (TF) mRNA and protein in the stromal cells [8]. TF is a receptor for coagulation factor VII and its active form, factor VIIa. TF initiates the clotting cascade, The cascade eventually leads to fibrin production. Decidualized stromal cells continue expressing TF throughout pregnancy [9] leading to the increased tendency to thrombosis in pregnancy. Progesterone also induces a second hemostatic protein, plasminogen activator inhibitor-1 (PAI-1). In addition to its anti-fibrinolytic properties PAI-1 restrains trophoblast invasion [10]. Hence, the luteal phase is associated with hemostatic, anti-fibrinolytic and antiproteolytic properties.

In the absence of pregnancy, luteal regression leads to progesterone withdrawal. The falling progesterone level leads to reduction of TF and PAI-1 expression [11]. When the falling level of progesterone reaches a threshold, the spiral arteries in the endometrial stratum basalis tightly coil and constrict. The vasoconstriction leads to ischemia and necrosis in the functional layer.

Progesterone also inhibits expression of metaloproteinases 2, 3 and 9 (MMP-2, 3 and 9) expression. Progesterone withdrawal augments their expression by endometrial stromal cells. Progesterone withdrawal is also associated with up-regulation of the neutrophil and macrophage chemoattractants, interleukin-8 (IL-8) and macrophage chemoattractant protein-1, respectively [12]. Thus, progesterone withdrawal is associated with increased MMP expression and chemokines which promote leukocyte infiltration which add to the proteolytic milieu, promoting menstrual bleeding and tissue sloughing. As the spiral arteries relax, there is bleeding

into the necrotic endometrium which, together with the chemical changes in the endometrium lead to menstruation. After progesterone withdrawal, there is an increase in prostaglandin (PG) synthesis and a decrease in PG metabolism [13]. PG synthesis via COX-2 is particularly relevant in the vascular compartment, since this provides an explanation for the action of non-steroidal anti-inflammatory agents in the treatment of menstrual disorders including heavy and painful periods. Moreover, prostaglandin E (PGE) synergises with IL-8 to increase capillary permeability, which would facilitate the efflux of leucocytes into the surrounding tissues [14].

2.1 Pathophysiology of Anovulatory Bleeding

Anovulatory AUB is usually seen in adolescents and premenopausal women. In both cases there is bleeding from an endometrium which has been stimulated by estrogen, without progesterone modulation. In adolescents, the unopposed estrogen is often due to immaturity of the feedback mechanisms in the hypothalamic-pituitary-ovarian axis. There are various possibilities. If the negative feedback requires only a certain amount of estrogen to inhibit FSH secretion, but the estrogen level never reaches high enough levels to release LH. Falling FSH levels will lead to follicular degeneration and falling estrogen levels. Hence, the endometrial shadow will be thin on ultrasound, and bleeding may be irregular in occurrence with polymenorrhea or acyclic bleeding. However, if the negative feedback requires higher than normal levels of estrogen to inhibit GnRH release, the excess levels of unopposed estrogen may lead to hyperplasia and prolonged cycles (oligomenorrhea) and subsequent prolonged heavy bleeding. Again, there is no positive feedback and LH release.

In the perimenopause, estrogen production is low compared to the reproductive years. Prolonged exposure to unopposed estrogen may also lead to endometrial hyperplasia, and prolonged heavy bleeding.

The mechanism of AUB in anovulation is due to estrogen breakthrough or withdrawal alone. There are none of the stabilising effects of constantly increasing estrogen levels or of post-ovulatory progesterone. While VEGF and Ang-2 are produced there is not enough Ang-1 to stabilise the vessels and block excess angiogenesis. There is no TF or PAI-1, hence local blood clotting is sub optimal as is the anti-fibrinolytic effect of PAI. When estrogen levels stay stable or fall, the endometrial lining cannot be maintained as it is estrogen dependent. In the absence of progesterone, there is no orderly constriction of the spiral arterioles, and no orderly necrosis of the functional endometrium. The bleeding therefore occurs from excess of fragile blood vessels, with suboptimal thrombosis to stop the bleeding, and possibly excessive fibrinolysis.

3 Diagnosis of AUB

The causes of AUB vary by age. In adolescents, anovulatory cycles, coagulopathies, infections and complications of pregnancies are the most common causes. During the reproductive years anovulation is still a common issue, but there may be other causes such as hormone imbalances induced by contraceptives, structural problems as fibroids, adenomyosis and endometrial polyps. In the perimenopausal woman anovulation is again very common, structural issues such as fibroids are still relevant but endometrial hyperplasia and cancer become more prevalent. The common causes in the postmenopausal woman are vaginal, and endometrial atrophy and complications of hormonal replacement therapy and cancer.

The clinical management of AUB is dependent on the diagnosis. History, examination and diagnosis may differ according to the patient's age. In the adolescent, the likelihood of organic disease such as malignancy is low. Clinical abdominal examination usually gives little information as to cause, and if the adolescent is a virgin, vaginal examination is inappropriate. Consequently, if imaging is normal, there is probably little need to rule out organic disease. However, in the perimenopausal patient, the chance of organic disease is higher, and there is a need for clinical examination to assess uterine size, speculum examination of the cervix, and cervical cytology to exclude malignant changes. Additionally, clinical examination is insufficient, imaging is almost mandatory and even more invasive diagnostic techniques such as endometrial biopsy may be indicated. In the reproductive years, pregnancy should be excluded.

In addition, it must be borne in mind that the patient presents for consultation, because the amount of bleeding seems abnormal for her. There may not necessarily be more than 80 ml of bleeding, but it is necessary to accept the patient's subjective distress at an abnormally perceived bleeding pattern. A quantitative estimate of the amount of bleeding can be obtained by a pictorial blood loss assessment chart. The chart requires that the patient uses a points system to quantify the amount that pads or tampons are soaked in blood, and the number of days of bleeding. However, pictorial blood loss assessment chart is often difficult to apply in clinical practice. Recently, smartphone applications have become available, in which the patient can record the amount of bleeding.

3.1 Imaging

Ultrasound, Hysteroscopy, and sonohysterography are used to image the uterus. MRI can also be useful, but is not a primary modality for assessing AUB. These imaging techniques are invaluable for making a diagnosis and directing treatment.

3.1.1 Ultrasound

The first-line modality for pelvic imaging in a woman with AUB is the transvaginal ultrasound (TVUS). In cases where TVUS is inappropriate (virgin patient) or when assessing a large finding (ovarian or uterine) the use of transabdominal US might be beneficial. Ultrasound can detect endometrial thickness, small submucous myomas, adenomyosis, polyp, etc. Figure 7.1 shows a sonogram of an atrophic or hypoplastic endometrium. Figure 7.2 shows a sonogram of endometrial hyperplasia. The ultrasound examination is also used to confirm or refute a diagnosis suspected on the basis of abnormal findings at palpation (e.g. uterine intramural or subserous myomas, or adnexal masses). Ultrasound assessment should also include examination of the adnexa and the urinary bladder, as abnormal bleeding may be explained by a hormone-producing ovarian tumour or a tumour in the urinary bladder. Doppler ultrasonography may provide additional information for characterizing endometrial and myometrial abnormalities, particularly arterio-venous malformations.

If a polyp, adenomyosis or leiomyoma are found, the treatment is surgical or interventional (uterine artery embolization for uterine fibroids or magnetic resonance guided high focus ultrasound for uterine fibroids and adenomyosis) and therefore outside of the scope of this chapter. If no abnormalities are found, endometrial biopsy should be considered.

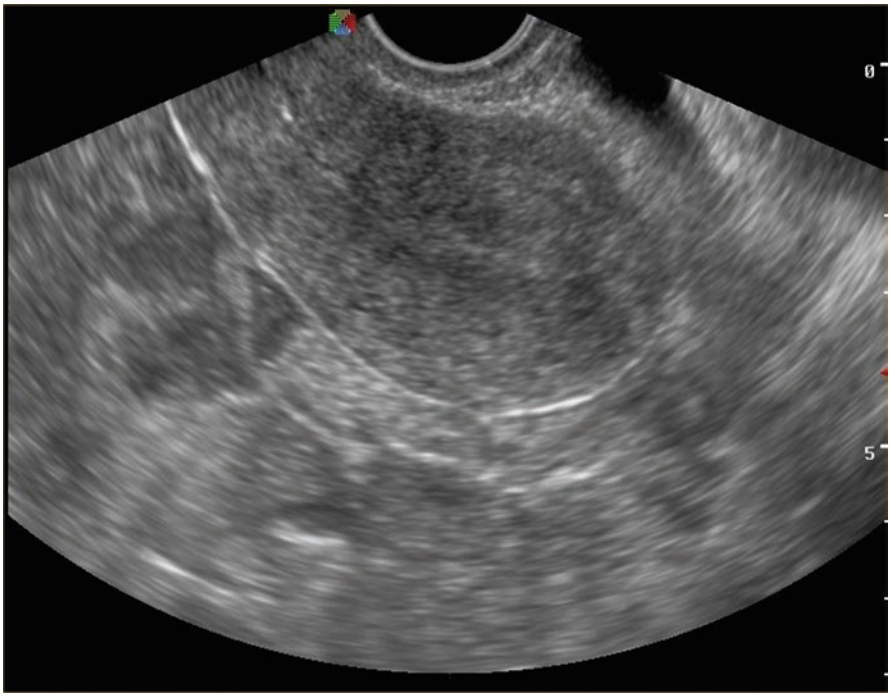

Fig. 7.1 Endometrial atrophy. The endometrial shadow can be seen as a thin feint line

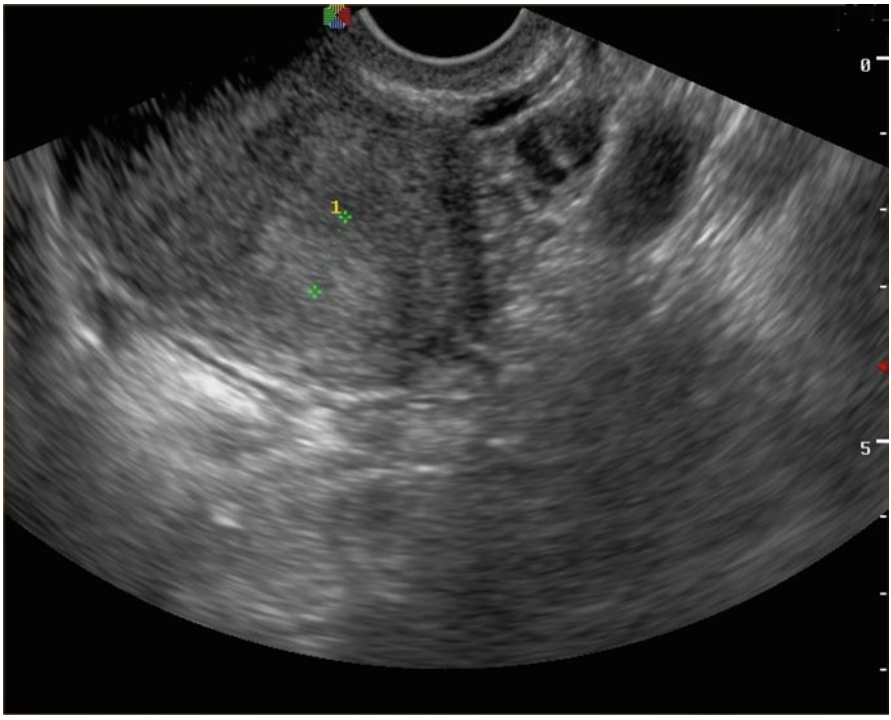

Fig. 7.2 Endometrial hyperplasia. The thickened endometrial shadow can be seen between the two calipers

Doppler flow studies can be added to diagnose arterio-venous malformations and to detect neovascularization, which is of importance in diagnosing malignancy.

3.1.2 Hydrosonography

Hydrosonography is also known as saline-contrast sonohysterography, saline infusion sonography (SIS) or sonohysterography. SIS clarifies the presence of focal lesions protruding into the uterine cavity [15]. If no focal lesions are present in the uterine cavity, the odds of malignancy decrease 20-fold, and the odds of any endometrial pathology decrease 30-fold [16]. A smooth endometrium at SIS is a strong sign of normality. Three dimensional hydrosonography constitutes an improvement in the imaging abilities of SIS, and has been shown to be superior to the older, 2D technique [17].

As most focal lesions cannot be removed, or only be partially removed by blind endometrial sampling, such as pipelle biopsy, or dilatation and curettage, focal lesions should be hysteroscopically resected under direct visual control.

3.1.3 Magnetic Resonance Imaging (MRI)

MRI is not generally recommended as a first-line procedure for investigating AUB. MRI is a good second line procedure if ultrasound reveals a bulky, polymyomatous uterus, or if adenomyosis is suspected. MRI has the advantage of distinguishing between myomas, sarcomas, and adenomyosis. Therefore, MRI can also optimize treatment strategy regarding the use of major surgery, or minimally invasive procedures. MRI can also provide a diagnostic assessment of the endometrium when the uterine cavity is inaccessible [18].

3.1.4 Hysteroscopy

Diagnostic hysteroscopy can diagnose endometrial focal lesions, such as polyp, retained products of conception, caesarean section niche, etc. and atrophy and hyperplasia. Hysteroscopy also has the advantage of allowing a targeted biopsy to be taken, particularly in focal lesions which may be missed by blind endometrial sampling techniques. The likelihood of endometrial cancer diagnosis after a negative hysteroscopy result is 0.4–0.5% [19]. The biggest advantage of hysteroscopy over the other modalities is the possibility to treat at the same procedure, and not merely to diagnose. The European guidelines [20] suggest that hysteroscopy is a second line procedure when ultrasound suggests a focal lesion, when biopsy is not diagnostic, or as an operative procedure if medical treatment fails after 3–6 months.

4 Biopsy

Histological examination is considered the gold standard for making a diagnosis of uterine pathology. Endometrial sampling for the diagnosis or exclusion of mostly hormonally induced endometrial changes (hyperplasia or endometrial cancer) is most often performed with a pipelle. The biopsy also may provide information about the hormonal status of the endometrium. An important limitation of pipelle biopsy is that the pipelle samples an average of only 4% of the endometrium with a reported range of 0–12% [21]. Usually a polyp is an incidental finding during endometrial sampling and is most often not entirely removed by pipelle.

Classically, endometrial sampling was performed by dilatation and curettage (D&C). Pipelle biopsy has replaced D&C, as pipelle biopsy is an office procedure, thus less invasive and less expensive than D&C. In addition, pipelle biopsy does not require the general anesthetic necessary for D&C. Additionally, D&C has been reported to lack the ability to identify uterine focal lesions [22], and blind excision of focal lesions by curettage may be incomplete. Both, D&C and pipelle biopsy show similar success rates for detecting endometrial pathology. The biggest disadvantage of these two techniques is diagnosing focal lesions [23] where hysteroscopy might be indicated.

The question arises as to when sampling is indicated. In the adolescent, there is little place for biopsy, unless absolutely necessary. Goldstein, [24] summarised five large prospective studies in women with postmenopausal bleeding. An endometrial thickness of ≤4 mm on transvaginal ultrasound with bleeding was associated with a risk of malignancy of 1 in 917 (3 cancers in 2752 patients). Goldstein concluded that in postmenopausal bleeding, biopsy is not indicated when endometrial thickness is ≤4 mm. Furthermore, if biopsy is performed in patients with a thin endometrium, it is most likely that no tissue would be obtained for histology. In a study of 97 consecutive patients with post-menopausal bleeding evaluated by endometrial biopsy, only 82% of the patients with an endometrial thickness ≤5 mm ($n = 45$) had a successful Pipelle biopsy completed, and only 27% of them produced a sample which was adequate for diagnosis. The results on postmenopausal women can be extrapolated to premenopausal women. However, in women with endometrial hyperplasia, endometrial sampling is indicated, as there is a high possibility of malignancy.

Endometrial sonographic thickness as an indicator of the need for biopsy is problematic in premenopausal women with AUB, as endometrial thickness changes throughout the menstrual cycle. In our hands, the use of hormonal assessment prior to endometrial sampling has proven to be very clinically useful: determination of blood estradiol, progesterone and beta-hCG levels prior to endometrial sampling can avoid sampling of a pregnant or postovulatory endometrium and allow reassessment of the ultrasound findings and endometrial thickness in view of the hormonal state of the patient.

5 Bleeding Dyscrasias

Bleeding diatheses generally present as heavy menstrual bleeding commencing at menarche and are present in 10.7% of patients with HMB compared to 3.2% of control women. Von Willebrand's disease is the most prevalent defect associated with HMB with a prevalence of 5–20% [25]. Screening includes activated partial thromboplastin time (aPTT) and ristocetin cofactor assay. Treatment consists of combined hormonal contraceptives which presumably induce TF and PAI-1 levels to compensate for the hemostatic defect.

6 Principles of Treatment

Treatment has a number of objectives: to lessen or stop the bleeding, and to provide long term relief. AUB due to a structural problem (polyp, adenomyosis, and leiomyoma) can be treated surgically. Medical management of endometrial cancer has

been described in Chap. 12. The primary goal of medical therapy should be to stabilize and heal the damaged endometrium with estrogen to provide initial haemostasis, followed by combined estrogen/progestogens for endometrial stability and induction of a menstruation-like withdrawal bleeding. The induced bleed may be stronger than normal menstruation, due to a medical "curettage" of a thickened endometrial layer. However, the induced bleed is usually limited in time, especially if hormonal therapy is continued afterwards. This basic plan of action should be modified according to the patient's needs, desire for fertility, anemia, endometrial thickness etc. In addition to hormonal therapy, other medical treatment modalities are available such as NSAIDS, tranexamic acid, and receptor modulators. Some specific modifications are listed below.

6.1 Acute Uterine Bleeding

In acute AUB hemodynamic stability should be assessed and a pregnancy test performed. Uterine curettage is the first line therapy when dealing with profuse bleeding and hemodynamic instability. But in most cases the medical situation allows the physician to initiate treatment with hormonal preparations.

6.1.1 High Dose Intravenous Estrogen

Intravenous conjugated equine estrogen (CEE) is approved by the US Food and Drug Administration (FDA) for the treatment of acute AUB. The mechanism of action of the estrogen in these cases is the rapid growth of endometrium over a denuded epithelial surface [26].

IV conjugated equine estrogen (25 mg in each dose, can be repeated after 3–5 h if necessary) has been reported to stop bleeding in 72% of patients within 8 h of administration compared with 38% of participants treated with a placebo [27]. An antiemetic is often required with this regimen. Little data exist regarding the use of IV estrogen in patients with cardiovascular or thromboembolic risk factors, hence, these patients might not be candidates for high dose estrogen treatment.

If the bleeding stops, the IV treatment should be stopped, and oral maintenance treatment should be started with progesterone treatment or combined oral contraceptives, in order to convert the endometrium to a secretory form. Cycling with progestogen should be maintained for 3 months. If the bleeding does not subside after 8 h, surgical intervention may be required. The simplest form of intervention is insertion of a Foley catheter, and expansion of the balloon to cause tamponade. Tamponade can be followed by dilation and curettage if not previously performed. In very rare cases, when all other treatment fails to stop profuse bleeding, hysterectomy might be indicated.

6.1.2 Hemodynamically Stable Patients

In hemodynamic stable patients, hormonal treatment is the preferred treatment method. High dose oral estrogen may be used to cause rapid endometrial proliferation with conjugated equine estrogen 2.5 mg up to four times a day. The dose can be reduced to two times a day when the bleeding becomes moderate. This regimen is given for up to 21–25 days. After the bleeding subsides treatment with progestogen should be administered e:g Medroxyprogesterone acetate (MPA) 10 mg a day. An alternative form of treatment is high dose combined oral contraceptive. If high dose oral contraceptives are used a dose of three pills per day may be required with the resulting side-effects of large doses of hormones. Munro et al. [28] compared the results of oral contraceptives three times daily for 1 week with MPA administered three times daily for 1 week. Bleeding stopped in 88% of women who took OCs and 76% of women who took MPA within a median time of 3 days. Other means of hormonal contraception such as the vaginal ring or patches cannot be used for the treatment of acute AUB, since the effective dosage is not predictable.

High dose progestogens can be used as sole agents in acute AUB. Treatment with progestogens is mainly effective in patients with anovulation. Progestogens inhibit further growth of a thickened endometrium and support estrogen primed endometrium. However, if bleeding comes from a denuded endometrium, progestogen treatment will probably be ineffective. MPA can be given up to 20 mg three times daily for a week or norethisterone acetate (NETA) can be given in doses up to 40 mg daily in divided doses until bleeding stops and then tapered down [29]. Another treatment regimen for acute AUB is depo-medroxyprogesterone acetate 150 mg given intramuscularly followed by MPA 20 mg given orally thrice daily for 3 days [30]. This treatment stopped bleeding within 5 days in all 48 women enrolled in a pilot study. Study participants reported infrequent side effects and high satisfaction.

6.2 Abnormal Uterine Bleeding in Adolescents

The aim of AUB treatment in adolescents is to stop bleeding, prevent or reverse anemia and achieve adequate cycle control. The primary cause of AUB in adolescents is anovulation, caused by the immaturity of the hypothalamic-pituitary-ovarian axis. However, prior to any treatment, pregnancy should be excluded.

Bleeding can usually be controlled with combined oral contraceptive pills (OCPs) taken continuously for several months. OCP's containing 20–30 μg of ethinyl estradiol and a relatively androgenic progestogen such as 0.3 mg of norgestrel or 0.15 mg of levonorgestrel can be used cyclically. If breakthrough bleeding occurs, or heavy menstrual bleeding persists and other causes of AUB have been excluded, the dose can be doubled for a short period of time to two pills per day. Since combined hormonal contraceptives can increase levels of coagulation factors such as factor VIII and von Willebrand factor, OCP's might have an additional effect in

cases of an underlying coagulopathies. If estrogen is contraindicated due to a history of thrombosis, migraine, hypertension etc., progestogens alone can be used. Examples are: oral medroxyprogesterone acetate (MPA), or NETA. Oral MPA 10 mg daily or NETA 5 mg can be given for 10–14 days each month to generate a secretory endometrium that induces a withdrawal bleed 1–7 days after stopping the medication. NETA can be aromatised to ethinyl estradiol [31]. Kuhnz et al [32] reported that this conversion resulted in a dose that was equivalent to taking 4–6 μg of ethinyl estradiol for each 1 mg of NETA ingested. The conversion ratio of NETA to EE has been subsequently estimated to be between 0.2% and 0.33% for different doses [33], Chu et al. [34] concluded that a daily dose of 10–20 mg NETA equates to taking a 20–30 μg ethinyl estradilol COC, Conversion to estrogen and the estrogenic effects are of no relevance when these progestogens are taken in low-dose progestogen-only, or combined oral contraceptive pills [35] but probably explains why high-dose NETA is effective at delaying and regulating menstrual bleeding. There are no similar implications for other progestogens in either low or high doses, since conversion to estrogen does not occur [36–38].

It has been reported in other chapters in this book that dydrogesterone binds the progesterone receptor up to 50% more than progesterone itself. However, dydrogesterone stimulates the progesterone receptor alone. It may therefore be appropriate in patients with a thickened endometrium in whom progesterone only effects are required. However, if there is a thin endometrium, estrogen will also be required to provide hemostasis, in addition to dydrogesterone.

The LNG-IUS or etonogestrel/ethinyl estradiol vaginal ring are other possibilities, but may not be acceptable in adolescents. Clomiphene citrate has occasionally been used in anovulatory adolescents. Clomiphene is a selective estrogen receptor modulator (SERM) which blocks the estrogen receptor in the hypothalamus, thus inhibiting the negative feedback. Therefore, estrogen levels can rise to the level required to induce LH release. The use of clomiphene has been reported as a possible therapy in anovulatory adolescents [39]. During the use of clomiphene citrate in adolescents the chance of conception and the rare possibilities of side-effects (headaches, vision changes, ovarian hyperstimulation, etc.) should be taken into consideration.

6.3 Perimenopausal Bleeding

As there is a high incidence of organic disease in perimenopausal women, organic disease must be excluded before progestogen therapy is initiated. As in other age groups, anemia may need to be corrected. Perimenopausal women with AUB may be treated with cyclic progestin therapy, low-dose oral contraceptive pills, the levonorgestrel IUD, or cyclic hormone therapy. Each treatment modality has advantages and disadvantages. The OCP and LNG-IUS provide contraception, in addition to reduction in bleeding volume. Estrogen therapy also provides relief from perimenopausal symptoms, such as hot flushes, night sweats, and vaginal atrophy. The choice

of therapy often is guided by the patient's priorities. Endometrial thickness will also indicate whether estrogen is required, or whether the patient can be managed on progestogen alone. In a study of 120 perimenopausal women, suffering from irregular menstrual cycles, treated by continuous estrogen and cyclic progestin or cyclic progestogen alone [40], 86% of women in the combined treatment group experienced cyclic menstrual bleeding, and reduced vasomotor symptoms. In addition, 76% of the women rated their bleeding as normal in amount and duration.

6.4 Chronic Abnormal Bleeding

Many regimens of progestogens have been used, and there are some comparative studies of different regimens. Dydrogesterone has been compared to micronized vaginal progesterone [41]. 69 women with irregular dysfunctional uterine bleeding were randomly assigned to receive oral dydrogesterone or vaginal progesterone. After three months of treatment, endometrial histology and menstrual cycle characteristics were comparable. However, oral dydrogesterone was far more convenient as it did not require the patient to leave her daily activity, and retire to a clean room to insert vaginal tablets or gel.

NETA and MPA are the two most commonly used progestogens. However, it must be borne in mind that NETA has estrogenic activity, but no glucocorticoid activity, whereas MPA has no estrogenic activity, but does have glucocorticoid activity. NETA seems to have a better effect than MPA in controlling irregular vaginal bleeding.

Depot injectable progestogen (medroxyprogesterone acetate 150 mg IM every 3 months) has been used as in contraception. However, depot MPA can lead to amenorrhea in up to 24% of women, suggesting it is a good option for women with increased bleeding. However, the side effects (irregular bleeding, weight gain, and headache etc. often lead to discontinuation of treatment [42].

A combination of dienogest and estradiol valerate (marketed as Qlair) has been shown to reduce menstrual bleeding [43] and has been approved for such use by the United States Food and Drug Administration (FDA). However, this combination has an anovulatory action, therefore is only indicated in women who have no desire to conceive.

It is convention, that if the contraceptive pill is used, it should be administered cyclically. Cyclic administration is classically for 21 days with a 7 day "pill free interval". Menstruation occurs in the 7 pill free days. More recently, oral contraceptives have been introduced with a 24 day regimen and 4 pill free days. The 24/4 regimen gives better cycle control. However, it is not problematic to take the pill continuously for extended periods, thus allowing the endometrium to recover after heavy menstrual bleeding. Additionally, there is a new contraceptive vaginal ring, containing ethinyl estradiol and segestrol as the progestogen. [44] which provides contraception for 1 year. This contraceptive ring can be left for extended periods in order to lessen the number of menstruations per year.

7 LNG-IUS

The LNG-IUS is particularly useful for the treatment of heavy menstrual bleeding in women who desire contraception. The LNG-IUS has been shown to be the most effective treatment in reducing menstrual blood loss compared with other medical therapies for chronic AUB and can reduce menstrual blood loss by more than 80% and even induce hormonal amenorrhea. However, the LNG-IUS takes time to achieve adequate endometrial quiescence. Initially there may be increased bleeding. The LNG-IUS results in a greater increase in hemoglobin and serum ferritin levels after 6 months compared with oral MPA from day 16–26 of the menstrual cycle [45]. Women also reported higher rates of subjective improvement in their bleeding despite the known initial side effect of irregular bleeding after LNG-IUS insertion. The efficacy of the levonorgestrel IUS was evaluated by Vilos et al. [46] in 56 obese perimenopausal women with AUB. The mean age was 42 years and the mean body mass index was greater than 30. At the 48-month follow-up, the satisfaction rate was 75%; amenorrhea and hypomenorrhea were noted with longer use. Hence, The LNG-IUS is an excellent long-term treatment modality for heavy menstrual bleeding when contraception is also required.

8 Endometrial Hyperplasia

Endometrial hyperplasia, whether simple or complex, with or without atypia has malignant potential. Figure 7.2 shows a sonogram of endometrial hyperplasia. If atypia is present, there is a 29% risk of progression to endometrial cancer [47]. However, in simple hyperplasia, the risk can be as low as 1%. In the perimenopausal or post-menopausal woman, hysterectomy is probably the best treatment option. However, in younger women, endometrial hyperplasia can be found in anovulatory cycles, polycystic ovary syndrome, or obesity. If fertility is desired, progestogens are the mainstay of treatment. There is no need for estrogen as the condition is due to excess stimulation with unopposed estrogen. The role of progestogens is to convert the endometrium to a secretory pattern. Once endometrial hyperplasia has been diagnosed, it is essential to repeat the biopsy 3–6 months later to confirm that regression has taken place. However, the median length of progestin treatment required for regression can be up to nine months. Additionally endometrial hyperplasia is closely related to insulin resistance and metabolic disorder. A low body mass index of <35 kg/m^2 has been reported to be associated with a high resolution rate in patients receiving progestogens [48]. There are case reports which show that if there is no response to progestogens, reversal of hyperplasia could be induced with metformin in addition to progestogens [49].

The main progestogens for treating endometrial hyperplasia are megestrol acetate, medroxyprogesterone 17-acetate [MPA], dydrogesterone and the LNG-IUS. However, there is no consensus on dose, treatment, duration, route of

administration, or randomized controlled trials as to the most effective progestogen [50]. Hence treatment is somewhat empiric, and administered on a trial and error basis. The overall response rate has been reported to be approximately 70%. Moreover, oral progestins are associated with poor compliance and systemic side effects that may limit overall efficacy [51]. Below are some of the advantages and disadvantages of each regimen.

Megestrol has anti-estrogenic and anti-androgenic effects, which may not be acceptable to the patient, or may decrease compliance. Megestrol acetate also has glucocorticoid effects. Symptoms of Cushing's syndrome, steroid diabetes, and adrenal insufficiency, have been reported with the use of megestrol acetate in the medical literature, albeit sporadically [52].

Medroxy progesterone acetate (MPA) however, is an agonist of the progesterone, androgen, and glucocorticoid receptors [53]. Hence there may be side effects of acne and hirsutism in some patients. MPA has glucocorticoid properties, and as a result can cause Cushing's syndrome, steroid diabetes, and adrenal insufficiency. Mesci-Haftac et al. [54] assessed 69 patients with simple hyperplasia, who received MPA. Hyperplasia persisted in 19.7%. Atypia and progression to complex hyperplsia occurred in 3.2% of the patients.

In 1988, Meden-Vrtovec and Hren-Bozic [55] reported on 50 patients with cystic glandular hyperplasia who were treated with dydrogesterone for six cycles in a dose of 20–30 mg. Repeat curettage showed persistence of the hyperplasia in 5 of 18 patients (28%).

Micronized progesterone was introduced in order to provide a bio-identical form of progesterone. Due to metabolism in the liver, it is often administered vaginally, allowing it to by-pass the liver, and have an increased local concentration in the endometrium. Tasci et al. [50] compared sixty premenopausal women with endometrial hyperplasia without atypia in a prospective controlled study. Group I included 30 patients who received lynestrenol in a dose of 15 mg. per day while Group II included 30 patients who received micronised progesterone 200 mg per day for 12 days per cycle for 3 months. After 3 months of treatment no patient in either group showed progression of the hyperplasia. In the lynestrenol group, the rate of resolution was higher than in the micronized progesterone group ($p = 0.045$). Lynestrenol was more effective in inducing resolution in patients more than 45 years ($p = 0.036$). The authors concluded that lynestrenol ensures better endometrial control than micronized progesterone at the above doses in simple hyperplasia without atypia.

The Levonorgestrel Intrauterine device has also been used to treat endometrial hyperplasia. The LNG-IUS has been compared to other progestogens: Dydrogesterone, NETA and MPA. El Behery et al. [56] assessed the results of 138 women aged between 30 and 50 years with AUB and hyperplasia were randomized to receive either LNG-IUS or dydrogesterone for 6 months. The outcome measures were regression of hyperplasia, and side effects or recurrence during the follow-up period. After 6 months of treatment, regression of hyperplasia occurred in 96% of women in the LNG-IUS group versus 80% of women in the dydrogesterone group ($p < 0.001$). Intermenstrual spotting and amenorrhea were more common in the

LNG-IUS group ($p = 0.01$ and 0.0001, respectively). Patient satisfaction was significantly higher in the LNG-IUS group ($P = 0.0001$). Hysterectomy rates were lower in the LNG-IUS group than in the dydrogesterone group ($p = 0.001$). The recurrence rate was 0% in the LNG-IUD group compared to 12.5% in the dydrogesterone group. Gallos et al. [57] reported similar results.

The LNG-IUS has also been compared to NETA [58]. 129 perimenopausal women with non-atypical endometrial hyperplasia were assessed in a randomized controlled trial. Patients received either the LNG-IUS or NETA for 3 weeks per cycle for 3–6 months. A significantly higher regression rate was noted in the LNG-IUS group than in the NETA group (79.7% vs. 60.7%, RR, 1.31 after 6 months). However, no significant difference was found regarding the median time to regression (3 months). The hysterectomy rate during the follow-up period was significantly higher in the NETA group (57.4% vs. 22%, $p < 0.001$).

In a prospective RCT [59] comprising 90 premenopausal women with a histological diagnosis of simple endometrial hyperplasia without atypia, patients were randomly allocated to 3 groups of 30 patients. One group received MPA, 10 mg. per day. The second group received NETA, 15 mg. per day for 10 days per cycle. The third group had a LNG-IUS inserted. Patients were re-evaluated after 3 months of treatment. Patients with regression and persistence were offered the same medication they were using for another 3 months. Patients in the LNG-IUS group showed the highest resolution rate (66.67%). Patients in the MPA and NETA groups had a resolution rate of 36.66% and 40%, respectively. The patients with a LNG-IUS showed a regression rate of 33.3%, whereas patients receiving MPA and NET showed a regression (and persistence) rate of 60% and 56.67%, respectively There was a statistically significant difference between the three groups regarding the proportion of patients requiring further treatment for another 3 months ($\chi^2 = 6.501$; $P = 0.0387$) in favour of the LNG-IUS.

9 Other Forms of Treatment

9.1 Receptor Modulators

Receptor modulators have been used in preliminary trials and have shown promising results. The selective estrogen receptor modulator (SERM), ormeloxifene has an anti-estrogenic effect, which retards endometrial maturation. In a randomized study, Ravibabu et al. [60] reported that ormeloxifene decreases blood loss by 90%, and that the reduction was statistically significant ($p < 0.001$). There was also a significant decrease in the mean endometrial thickness ($p < 0.001$) after treatment with ormeloxifene when compared to mean baseline value. There was significant improvement, 84% of patients had relief from dysmenorrhoea ($p < 0.001$), but anti-estrogenic side effects such as hot flashes and vaginal dryness are a cause for concern.

Ormeloxifene has been compared to medroxyprogesterone acetate. The results were similar in terms of mean duration of bleeding, increased hemoglobin concentration and endometrial thickness [61]

Mifepristone (Ru486), is a selective progesterone receptor modulator (SPRM). Mifepristone induces amenorrhea whilst maintaining endogenous estrogen secretion. Amenorrhea is caused by complete binding of the progesterone receptor, causing atrophy of spiral arteries and hence, anovulatory amenorrhea [62]. Another SPRM is ullipristal acetate. Ullipristal was introduced mainly to shrink uterine myomas. However, reports of liver toxicity have precluded its continued use. If SPRMs are used, it must be borne in mind, that there is an effect on the endometrium known as 'progesterone receptor modulator associated endometrial change (PAEC) [63].

9.2 NSAIDS

A certain amount of relief may be obtained from non steroidal anti-inflammatory drugs (NSAIDs). NSAIDs inhibit the enzyme cyclooxygenase, thereby reducing the raised prostaglandin levels which are found in women with heavy menstrual bleeding. A Cochrane review [64] showed that NSAIDs (mefenamic acid, naproxen, ibuprofen, flurbiprofen, meclofenamic acid, diclofenlac, indomethacin, and acetylsalicylic acid) are more effective than placebo in reducing menstrual blood loss by 25–30% in women with regular menstrual cycles [65], but have a limited effect on the reduction of HMB [65] and are less effective than tranexamic acid, danazol, or the levonorgestrel-releasing intrauterine device (LNG-IUS) [66]

9.3 Antifibrinolytic Drugs

Tranexamic acid is a lysine analogue that allows the formation of stable blood clots by preventing fibrin filament breakdown without influencing coagulation in healthy blood vessels. Data from controlled clinical trials indicate reduced bleeding in AUB by 30–55% [67, 68]. However, there are side effects including headache, nausea and vomiting. Tranexamic acid is the only nonhormonal, noncontraceptive agent approved by the U.S. Food and Drug Administration (FDA) for the treatment of HMB. Concerns have been raised about the potential for thromboses during tranexamic acid treatment. However, population-based data do not indicate an increased risk of thromboses [69]. Tranexamic acid is most often used for women in their reproductive years to reduce heavy menstrual bleeding. One potential benefit is that tranexamic acid is only used during menstruation rather than continuously or for the majority of the menstrual cycle.

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Chapter 8
Progestogens in Contraception

Johannes Bitzer

1 Development and Classification of Protestogens in Contraception

In 1951 C. Djerassi and L. Miramontes converted 3-methoxy-estradiol into a 19-nortestosterone derivate with the help of the Birch reduction. In the next steps this 19-nortestosterone derivate was subsequently transformed by means of several chemical steps into 17α-ethinyl-19- nortestosterone (norethisterone) [1].

Removal of the carbon at the C-19 position of ethisterone changed ethisterone from an androgen to a progestin, resulting in the development of a class of progestins referred to as 19-nortestosterone derivatives. Included in this class are commonly used progestins such as norethindrone, norethindrone acetate, levonorgestrel and ethynodiol diacetate.

In 1951 another progestogene was developed. 19-norprogesterone was synthesized by G. Rosenkranz and C. Djerassi using the same chemical method (Birch reduction). This substance was orally inactive, but it represented a potent progestogen after parenteral administration.

Medroxyprogesterone acetate, megestrol acetate and chlormadinone acetate followed in the years 1957, 1957 and 1959 respectively (all at Syntex). Thus the two parent compounds for the progestogen family were born.

The progestogens used in Combined Hormonal Contraceptives can be classified according to different criteria as follows: [2]

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H. J. A. Carp (ed.), *Progestogens in Obstetrics and Gynecology*,
https://doi.org/10.1007/978-3-030-52508-8_8

1.1 The Time of Introduction into the Market

It has become common use to apply this "historical" classification. According to this first, second, third and fourth generation combined hormonal contraceptives can be distinguished

- First generation: Norethynodrel, Norethisterone Acetate, (NET, NETA)
- Second generation: Levonorgestrel (LNG)
- Third generation: Gestodene, Desogestrel, Norgestimate (GEST, DES, NGM)
- Fourth generation: Drospirenone (DROSP).

Cyproterone Acetate (CPA) and Chlormadinone Acetate (CMA) have never been included in this categorisation as CPA containing pills were originally classified as drugs to treat hyperandrogenism in women who required contraception. CMA was only introduced in some countries and was and is not internationally available. The same was true for Dienogest (DNG) which was developed in Germany and is mainly used in Germany

1.2 Classification According to Molecular Structure

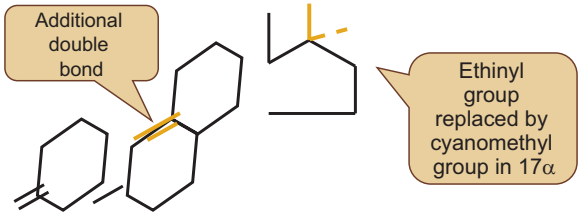
The molecular structure gives an indirect indication about the biologic action of the steroid**.**

Different groups of progestogen can be distinguished. These are described in Fig. 8.1.

1.2.1 Derivatives of Testosterone

The basic molecule is testosterone. Due to elimination at the C 19 position the molecule becomes more progestogenic and the different variations are called C 19 Nortestosterone derivatives (see Fig. 8.2). The structure closest to testosterone can be seen in Norenthynodrel, Norethindrone, Norethisterone Acetate, Lynestrenol (Estranes). Further modification has followed from the original estranes to gonanes such as Levonorgestrel, Gestodene, Desogestrel and Norgestimate

A special molecule in this context is Dienogest.



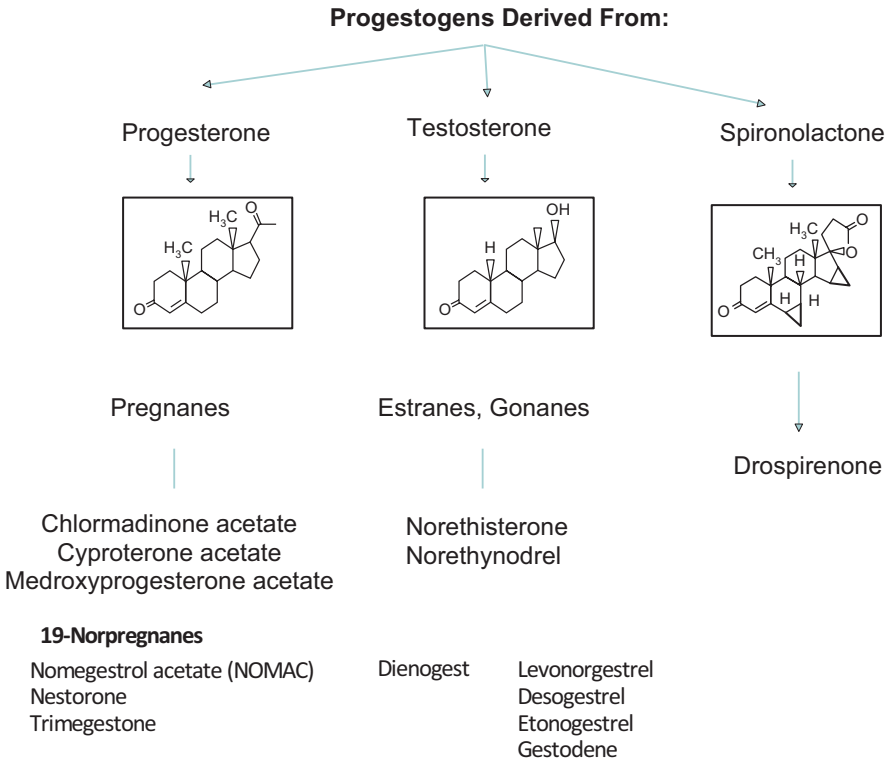


Fig. 8.1 The contraceptive toolbox

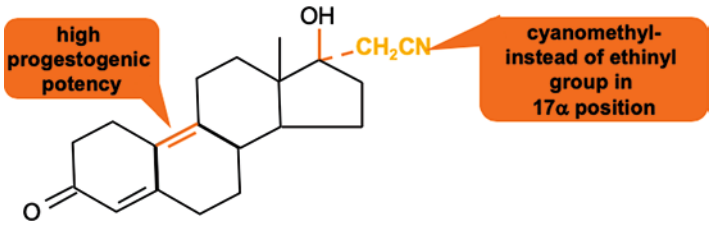


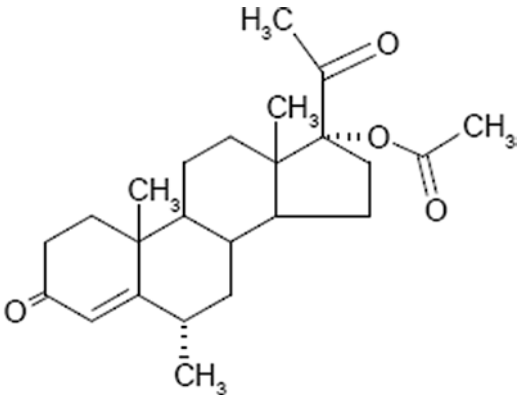
Fig. 8.2 C 19 nortestosterone derivatives

Dienogest is a 19 Nortestosterone derivative with a Cyanomethylgroup instead of the usual Ethinyl Group in in the 17 alpha position.

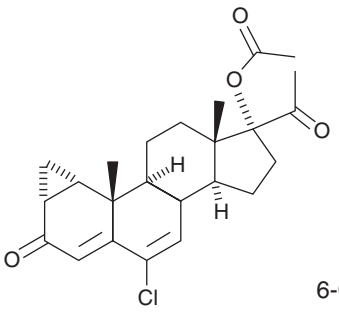
1.2.2 Derivatives of Progesterone

Subgroups can be distinguished depending on the different positions of double bindings between C atoms and the type of C group added.

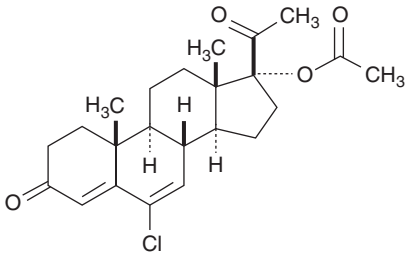
An important molecule is Medroxyprogesteronacetate.



Another important progesterone derivatives in COCs are Cyproterone Acetate and Chlormadinone Acetate.



Cyproterone Acetate

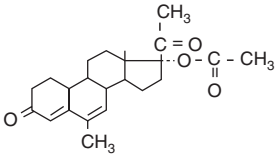


6-Chloro-3,20-dioxopregna-4,6-dien-17-yl acetate

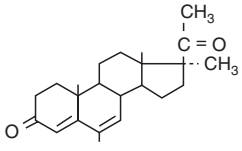
Chlormadinone Acetate

Both have a C-21 structure and are basically different from Testosterone derivates in their action in the body (see below). Both are orally active and suitable for use in combined hormonal contraceptives.

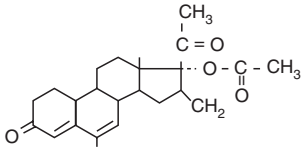
Another subgroup of progesterone derivatives are the 19 Norpregnanes.



Nomegestrol acetate



Demegestone

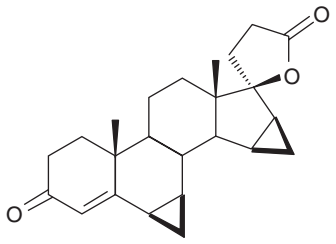


Nestorone

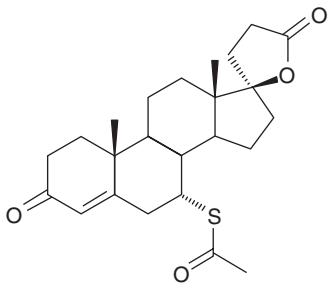
The structure of 19 Nor pregnanes is very similar to progesterone, but the C methyl group is removed from position 19. 19 Nor pregnanes have strong progestogenic activity. Nomegestrol acetate is orally active.

1.2.3 Derivatives of Spironolactone

Drospirenone is the only progestogen which is derived from spironolactone, which is known to counteract sodium retention and has an antiandrogenic action on the skin.



Drospirenon



Spironolacton

The drospirenone Molecule is a " mixture" of the progesterone and the spironolactone molecule.

1.3 Classificiation According to Interaction with Steroid Receptors

Depending on structure progestogens have different interactions with the various steroid receptors in the body. Steroid receptors are located on the membrane of target cells and are linked to the DNA/RNA and Protein production via different messenger systems. Two properties can be distinguished:

(a) The binding capacity, i:e, the intensity with which the ligand of the molecule binds to appropriate receptor sites.
(b) The direction and the intensity of the induced action. The steroid can bind to the receptor but does not induce any activity. The final effect is a reduction of the activity mediated by the receptor by competitive inhibition (the steroid binds to the receptor and thus blocks the binding site for a steroid that would activate receptor activity).

The receptors to which progestogens bind can be the following

- Progestogen receptor: The most important receptor to induce the desired effect.
- Androgen receptor: Activation of androgen receptors mediate androgenic effects on hair growth and activity of the sebaceous glands. Some progestogens bind to this receptor and can either block or activate it (antiandrogenic properties see below).
- Estrogen receptor: This receptor mediates effects in many tissues especially in the endometrial cells.

- Glucocorticoid receptor: The glucocorticoid effect is linked to the activation of the coagulation system.
- Mineralocorticoid receptor: This receptor mediates sodium retention.

Based on this classification of receptor activities several groups of progestogens can be differentiated. The clinical consequences of these differences are still controversial and research is ongoing.

- Androgenic progestogens: Norethyodrel, Levnorgestrel and Norgestimate have androgenic properties. LNG seems to have not only an androgenic but also an antiestrogenic effect. Antiandrogenic progestogens: The most important compounds are the progesterone derivatives CPA and CMA and drospirenone. These molecules bind to the receptor and exert competitive inhibition.
- Mildly antiandorgenic or neutral progestogens: GEST, DES, mainly interact with the progestogene receptor alone. Dienogest has a weak antiandrogenic action.
- Antimineralocorticoid progestogens: Only Drospirenone has antimineralocorticoid action.

The different receptor effects are shown in Table 8.1.

2 Progestogens in Combined Hormonal Contraceptives

Combined hormonal contraceptives (CHC) have proven to be highly acceptable, provide effective protection, have a low health risk profile and provide additional health benefits.

The main concern is the cardiovascular risk which make these preparations unsuitable for women with predisposing risk factors [4]. The increased cardiovascular risk in healthy users of CHC (from 6–12/10,000 women/year) compared to non-users (2/10,000 women/year) is of concern and has led to the development of very low dose EE pills, Estradiol CHC or oral contraceptives without estrogen.

Lowering the dose of estrogen was based on laboratory and epidemiological data indicating that estrogen is responsible for the increased cardiovascular risk and that the progestogens alone do not increase that risk. Laboratory data show that ethinyl estradiol has, due to its action in the liver, a procoagulatory effect by enhancing the factors responsible for coagulation and reducing fibrinolytic factors. It is suspected that estradiol or estradiol valerate do not have such an impact on the liver due to faster metabolism than ethinyl estradiol [5]. Estrogens modify the dynamic balance of hemostasis by enhancing the coagulatory factors (e.g. Factor VII) and the anti-fibrinolytic factors (e.g. PAI-1). The number of D-Dimers rise consecutively due to the higher content of fibrin and its degenerated products in the blood. This balance is also influenced by the amount of ethinyl estradiol that activates the coagulatory site and the dose of progestogen that activates the anti-fibrinolytic factors as e.g. PAI-1 [5].

Table 8.1 Contraceptive progestogens and extra-progestogenic effects

Glucocorticoid activity	Estrogenic activity	Antiestrogenic activity	Androgenic activity	Antiandrogenic activity	Mineralocorticoid activity
Medroxy progesterone acetate	Norethisterone acetate	Levonorgestrel	Levonorgestrel	Cyproterone acetate	Drospirenone
Megestrol acetate			Desogestrel	Chlormadinone acetate	
			Gestodene	Dienogest	

Adapted from, Benagiano et al. [3]

2.1 Health Risks of Combined Hormonal Contraceptives in Relation to the Different Progestogens

Several registry based studies published in the British Medical Journal (BMJ), particularly the Danish registry indicated that there might be an increased risk of venous thromboembolism (VTE) associated with the intake of third and fourth generation COCs compared to preparations containing the progestogen levonorgestrel (LNG) or other first and second generation progestogens [6–10].

The relative risk increase was approximately 2 and absolute attributable risk was given—dependent on the base prevalence rate—between 2 to 8 per 10,000 users. These results contrast with those of published prospective cohort studies, sponsored by Bayer Health Care, at the request of health authorities for a large post marketing survey, which did not find a differences between the various generations of progestogens. The discrepancy led to intensive scientific discussion among epidemiologists about possible confounders and biases in the published studies [11, 12].

These publications with warnings about the increased risk of third and fourth generation contraceptives lead to intense debate among epidemiologists about the limitations of registry based observational studies in comparison to other study designs and about the clinical validity of these results [13–18].

There are some special concerns and considerations regarding progestogens with an antiandrogenic actions.

Cyproterone acetate—In two studies, a higher risk of VTE was seen when compared with contraceptives containing levonorgestrel [19, 20]. In a report from the Danish National Registry the risk was not significantly different from LNG (absolute risk 4.2 and 3.1 per 10,000 woman-years for levonorgestrel and cyproterone acetate, respectively) [21].

Chormadinone Acetate—There are no large epidemiological studies with CMA, but the post marketing studies especially in Germany and Austria did not show an increased risk of VTE in users. The European Authorities however, demand more surveillance studies to be able to determine the cardiovascular risk of chlormadinone which has been shown to be effective in reducing acne and dysmenorrhea.

Drospirenone—Drospirenone, a progestin that also has antiandrogen and antimineralocorticoid properties, has been associated with a greater risk of VTE when compared with levonorgestrel in some, but not all studies (see also above).

Two observational studies have reported that oral contraceptives containing drospirenone were associated with an excess risk of venous thromboembolism (similar in magnitude to the third generation progestins) [6, 7]. Two previous large, prospective, surveillance studies of new users of drospirenone-containing oral contraceptives subsequently reported that the thromboembolism risk was no different from that for other OCs. It has been estimated that 9000 women would need to be treated with a drospirenone OC in order to see one additional case of venous thromboembolism [11, 12]. After the publication of the two surveillance studies, two additional

case-control studies and a registry-based cohort study reported a twofold to threefold increased risk of VTE with OCs containing drospirenone compared with levonorgestrel [4–6]. An FDA sponsored study published after the Drug Safety Communication utilized computerized data files from two integrated medical care programs and two state Medicaid programs to obtain data regarding the risk of several cardiovascular endpoints in combined hormonal contraceptives users. The authors identified a final cohort that included 189,210 person-years of exposure to drospirenone. In adjusted analyses, drospirenone use was associated with a significantly higher risk of VTE relative to low-estrogen comparators (RR 1.74; 95% CI 1.42–2.14) [22]. In 2012, based upon available data, the US Food and Drug Administration (FDA) added revised labeling to all oral contraceptives containing drospirenone, stating that they may be associated with up to a threefold higher risk of VTE compared with OCs with levonorgestrel and some other progestins [23]. The FDA does not advise women to stop drospirenone-containing OCs, but does suggest that an individual's risk of VTE be assessed before starting one in a new OC user, or before considering using one in a woman who has been on an OC not containing drospirenone. Lastly, the warning notes that the VTE risk with drospirenone is small and still lower than the risk of VTE during pregnancy. In 2011, the European Medicines Agency also concluded that drospirenone-containing birth control pills carry a higher risk of venous thromboembolism, but noted the overall risk of blood clot from any birth control method remains small and stopped short of advising women to stop taking pills containing drospirenone [24].

The European Agency has summarized the finding in absolute numbers [25].

Women not using/not being pregnant 2/10,000.

Women using CHC with levenorgestrel, Norethisteron, Norgestiomate 5–7/10,000.

Women using CHC with etonogestrel, norelgesrtromin 6–12/10,000.

Women using CHC with drospirenone, gestodene, desogestrel 9–12/10,000.

Women using CHC with chlormadinoacetate, dienogest, nomegestrol not yet known.

As far as arterial thromboembolism is concerned (Myocardial infarction, ischemic stroke) a systematic review [6] has found a relative risk increase of 1, 6. These diseases are however very rare in the reproductive age group (MI 5/100,000, Stroke 9/100,000) [5] so that the absolute risk attributable risk is between 2 and 3 MI cases per 100,000 users or 4–6 Stroke cases per 100,000.

The risk seems to correlate with the estrogen dosage. There is no definitive answer regarding the differential role of progestogens.

The German BfarM issued in statement indicating that based on a metaanalysis of four observational studies Dienogest is considered to have the same relative risk as other third and fourth generation progestogens [3].

3 Progestogen Only Contraception

There are four types of contraception based on progestogens without an estrogen component:

(a) Progestogen only pills
(b) Progestogen Depot injections
(c) LNG IUD
(d) Progestogens Implants.

3.1 Common Features of Progestogen Only Contraception

3.1.1 General Principle of Action

The basis for progestogen only contraception lies in the specific action of progestogens on reproductive physiology. These actions are type and dose dependent and include:

- Inhibition of ovulation
- Change of the cervical mucus to make it impenetrable
- Endometrial changes which make implantation either difficult or impossible
- Changes in tubal mobility.

The common clinical important features of these methods are:

3.1.2 Very Low or Absence of Cardiovascular Risks [26, 27]

Progestogens have very little impact on the coagulation system (see above). Their effects on blood flow and contractility of vessel walls is very limited. Epidemiological studies do not show any significant risk for thromboembolic venous or arterial disease. Therefore progestogen only contraceptives can be used in women who have a contraindication for combined hormonal contraceptives (WHO MEC Category 4) or where the use is not advised (Category 3).

Contraindications to combined hormonal contraception include:-

- Women postpartum (during the first 21 days, lactating women)
- Women with a combination of cardiovascular risk factors (obesity, smoking, age)
- Women with specific venous thromboembolic risk factors (thrombophilia, antiphospholipid syndrome, family history, long periods of immobilisation, acute DVT with anticoagulant therapy, or past thrombo-embolic events)
- With women with specific arterial risk factors (hypertension, migraine with aura, valvular heart disease, past ischemic heart disease).

3.1.3 Contraindications for Progesterone Only Contraception

There are few contraindications to progestogen only contraception, but these include:- Breast cancer, active liver disease, benign and malignant liver tumours (except nodular hyperplasia).

3.1.4 Side Effects

The most frequent side effects attributed to the action of progestogens are acne, mild hirsutism, depressive mood, sexual pain and weight gain. This is however not based on prospective clinical comparative trials but mainly observational data. The most frequent side effect of continuous use of progestogens is irregular bleeding.

3.1.5 Additional Benefits and Therapeutic Indications

Progestogen only contraception confers several important additional benefits:-

- Lactation: The progestogen only contraceptives can be used in lactating women because there is no reduction in milk production and no negative effect on the newborn.
- Menstrual symptoms: Due to progestogens' antimitotic and transformational action on the endometrial cells progesterone only contraceptives can reduce the frequency and intensity of uterine bleeding. The contraceptive progestogens which inhibit ovulation can reduce dysmenorrhea. Additionally progestogens block the synthesis of prostaglandins in the endometrium by reducing the endometrial thickness.
- Menstrual Migraine: Progestogens in continuous use reduce the intensity of menstrual migraine.
- Endometriosis: Progestogens can reduce the proliferative activity of the endometrium.

3.1.6 High Efficacy in Typical Use

The long acting preparations of contraceptive progestogens (injections, implants, medicated IUDs) have a very high efficacy in typical use due to the fact that the contraceptive effect is largely independent of the compliance of the user.

3.2 Oral Preparations [28]

Older oral preparations containing Levonorgestrel and Norethisterone are unable to inhibit ovulation but their contraceptive effect is based on the action on the cervical mucus which becomes impenetrable to sperm. An additional effect of these progestogen only pills is the effect on the endometrium, desynchronising ovulation and endometrium transformation and preparation for implantation.

These preparations should be taken at more or less the same time every day in order to exert the contraceptive effect. The typical user Pearl Index is between 6–8.

The newer progestogen only pill with 75 µg of desogestrel daily is taken continuously without a seven day break. 75 µg of desogestrel inhibits ovulation and is as effective as combined hormonal contraceptives. This pill is also called an estrogen free inhibitor of ovulation. No major health risks are known.

The efficacy of the low dose non ovulation inhibiting preparations depends very much on the adherence of the user. The pills have to be taken at the same time each day.

The efficacy of the 75-µg desogestrel oral contraceptive is comparable to the efficacy of CHC due to its ovulation inhibiting effect [28].

3.2.1 Health Risks

The ovulation inhibiting progestogen pill can be considered as low dose progestogen based estrogen-free contraceptive. Its wide use provides sufficient information to conclude that there are no major health risks, neither cardiovascular or cancer risks [28].

3.2.1.1 Bone Health

There are no studies indicating that the POPs, have a negative impact on BMD the implant, and the LNG-IUS have a negative impact on bone mineral density (BMD) in users [29].

3.2.2 Side Effects

Due to the daily intake needed for ovulation suppression there is no phase of progestogen withdrawal which is the reason for the bleeding occurring during the pill free interval during the use of combined hormonal contraceptives in the 21/7 regimen. Irregular bleeding is therefore the main complaint which may lead to discontinuation. Other progestogenic side effects such as acne, weight gain, depressed mood are rare.

3.2.3 Special Benefits

There is alleviation of menstrual migraine, pain reduction in patients with endometriosis and reduction of hypermenorrhea and dysmenorrhea.

3.2.4 Contraindications

Current Breast Cancer, active liver disease, benign and malignant liver tumours, (except nodular hyperplasia) unclear vaginal bleeding, continuation after stroke or ischemic heart disease.

3.3 Progestogen Implants [30, 31]

Hormonal implants are subdermally inserted contraceptives that provide reliable contraception for 3–5 years. The matrices are inert or biologically degradable rods or capsules which release the respective steroid continuously over a lengthy period of time. The Population Council in New York has studied long-term contraception with subdermal hormonal implants since 1966. The hormone implants consist of one or several small flexible rods or a capsule inserted under the skin of the upper arm. Depending on the product, they release the progestins megestrol acetate, norethindrone, norgestrinone or etonogestrel for a period of 1 year to 5 years.

Norplant® was composed of six rods. Each rod contains 36 mg of levonorgestrel. The total duration of action of these 6 rods was 5 years. The product has not been marketed since 2002.

Norplant II® (Jadelle®), Norplant®'s successor product, is composed of two flexible silicone rods (43 mm Å~ 2.5 mm) each containing 75 mg of levonorgestrel and also has a duration of action of 5 years. The same product is commercially available in China under the name Sinoplant.

3.3.1 The Etonogestrel Releasing Hormonal Implant Implanon®

Implanon® is an etonogestrel-releasing hormonal implant. The rod is 4 cm long and 2 mm in diameter and is composed of 40% ethylene vinyl acetate (EVA) and 60% (68 mg) etonogestrel (3-keto-desogestrel). The duration of action after subdermal implantation is 3 years.

The main effect is ovulation inhibition, although this inhibitory effect is less at the end of the 3 years. Additional contraceptive effects are the change in the composition of the cervical mucus and making the endometrium less receptive for a

theoretical implantation. The PI is 0.38 pregnancies per 100 women-years of use, which is similar to that of other long-acting methods of contraception.

The concentration falls over time at a rate that depends on body weight. Clinical experience with Implanon® in women weighing more than 80 kg is limited. Available data do not show a decrease in efficacy in obese women.

Accurate placement is crucial to the product's reliability. There are reports of incorrect insertion of the Implanon® rod, possibly making the contraceptive rod impossible to palpate and difficult to find. To make the product easier to use safely and simpler to locate, the system was upgraded with Implanon NXT®. Efficacy may be hampered by drugs affecting the metabolism of etonogestrel like antiviral drugs.

3.3.1.1 Efficacy

The PI is 0.05–0.38 which is similar to that of other long-acting methods of contraception.

3.3.1.2 Health Risks

No major health risks are known. There is no concern regarding bone loss.

3.3.1.3 Side Effects

As with other progesterone only contraceptives, the most frequent side effect is unexpected bleeding, which may lead to various degrees of discontinuation, (approximately 15–18% in USA and Europe and 3–4% in Southeast Asia and Russia. In approximately 5% of users the following side effects were reported: acne, headache, weight gain, mastalgia, vaginal infections and bleeding disorders. Interactions with broad-spectrum antibiotics, St. John's wort, a number of antiepileptic agents and mood-altering drugs have been documented. It should be borne in mind that placement and removal require special training.

3.3.1.4 Additional Benefits

Clinical studies have shown that Implanon® is also effective in treating heavy dysmenorrhea. However, the product is not approved for treating dysmenorrhea. Hence, prescription is off-label. Another possible beneficial effect is the diminution of pain caused by endometriosis.

3.3.1.5 Contraindications

As in other progesterone only contraceptives, contraindications include breast cancer, active liver disease, benign and malignant liver tumours (except nodular hyperplasia).

3.4 Injections (Intramuscular and Subcutaneous) [32]

Medroxy progesterone acetate (DMPA) is available for injection as a depot either in a dose of: 150 mg/1 mL injected intramuscularly or 104 mg/0.65 mL injected subcutaneously. Both preparations last for 3 months. The mechanism of action of DMPA is similar to other progestogens and includes a strong antigonadotropic effect, inhibition of ovulation, inhibition of endometrial proliferation, and changes in the cervical mucus making the mucus impenetrable for sperm. The dose is a standard dose, and no adjustment is necessary for body weight.

There is no reduction in efficacy with concurrent medication.

3.4.1 Efficacy

The Pearl index with ideal use is 0.2. Typical use 4–6. However, due to the need for repetitive injection every 12 weeks adherence failure may occur.

3.4.2 Health Risks

No major health risks have been found. However, long term DMPA use induces an unwanted increase of LDL Cholesterol and reduces peripheral arterial flow-mediated dilatation, which are matters of concern. DMPA does not alter coagulation factors nor increase blood pressure. Some studies have found an increased VTE risk but these studies have methodological weaknesses and only include a small number of cases. Nonetheless the World Health Organization has attributed category 23 to DMPA in women with current VTE, a history of stroke or ischemic heart disease.

The primary concern is the effect of DMPA on bone density. DMPA suppresses endogenous estrogen production from the ovaries by its strong antigonadotropic action. Compared to nonusers, the bone mineral density at the hip and spine of DMPA users decreases by 0.5–3.5% after 1 year and 5.7–7.5% after 2 years of use. There is therefore concern about the use of DMPA in adolescents when the accumulation of bone mass is at its peak, and in premenopausal women as there may be an increased rate of bone loss. However, many studies have shown that the bone loss is reversible and the best evidence available at present indicates that that DMPA does not reduce peak bone mass and does not increase the risk of osteoporotic fractures in later life in women with an average risk of osteoporosis.

At present, DMPA is considered to be contraindicated when pregnancy is planned within the next year, in the presence of osteoporosis and known risk factors for fractures, or in the presence of hypothalamic amenorrhea, anorexia nervosa or chronic glucocorticoid therapy [32, 33].

3.4.3 Side Effects

Side effects include menstrual irregularities (during the first 3–6 months irregular bleeding and spotting, later there may be amenorrhea in up to 75% of users. There may be weight gain of between 3–6 kg (especially in young obese women). Headache, abdominal discomfort and pain, dizziness, nervousness and asthenia have also been described.

3.4.4 Benefits

The benefits include, reduction of heavy menstrual bleeding due to the high incidence of amenorrhea after longer use, a reduced risk of pelvic inflammatory disease, reduction of endometriotic pain, fewer painful crisis in women with sickle cell disease and reduction in vasomotor symptoms

3.4.5 Contraindications

Some studies have found an increased VTE risk but these studies have methodological weaknesses and a small number of cases. WHO has attributed category 2/3 to DMPA in women with current VTE, a history of stroke or ischemic heart disease [4].

3.5 Levonorgestrel Containing Intrauterine Systems [34]

There are three types of Levonorgestrel containing intrauterine systems

(a) LNG 52 (Mirena) containing 52 mg of LNG with an average daily release of 20 μg LNG. Effective for at least 5 years.
(b) LNG 14 (Jaydess) containing 13.5 mg of LNG with an average daily release of 6 μg LNG. Effective for 3 years.
(c) LNG 20 (Kyleena) conatingin 19.5 mg of LNG with an average daily release of 9 μg LNG. Efffective for 5 years.

The progestin secreted by progestin-releasing IUDs thickens cervical mucus and also increase expression of glycodelin A in endometrial glands, which inhibits binding of sperm to the egg [34]. Serum concentrations of progestin can lead to partial inhibition of ovarian follicular development and ovulation. Inhibition of ovulation,

is not the major contraceptive mechanism; one study found at least 75% of women using a levonorgestrel-releasing IUD had ovulatory cycles [35].

3.5.1 Efficacy

The efficacy is very high for all three systems. PI approximately 0.2–0.33.

The cumulative pregnancy rate is 0.5–1.1 after 5 years of continuous use with the LNG 20 IUD. The three year cumulative pregnancy rate is 0.9 with the LNG 14 IUD [34].

3.5.2 Health Risks

There is no indication of an increased risk of cardiovascular diseases [30, 31, 34].

The association between LNG-IUS use and breast cancer has been investigated.

There is some controversy due to contradictory results. Two large retrospective case–control studies of European women showed no increased risk of breast cancer in women using the LNG-IUS for contraception [36, 37].

However, two analyses of a large Finnish cohort suggest a small increased risk (up to 1.3 times) of breast cancer, in particular, lobular and ductal cell cancers, in women using the LNG-IUS for heavy menstrual bleeding [35, 38]. In the Danish Cohort study [39], the authors found a relative risk of 1.21 (95% CI 1.11–1.33).

Taking into account the different results and the type of studies (observational studies), it can be concluded that the use of a progestogen-only method is either not, or to a minor degree (expressed as absolute risk), accompanied by an increased risk for breast cancer [30, 31].

3.5.3 Side Effects

The major side effect is irregular bleeding, which is very common during the first 3–6 months. At 24 months 50% of LNG20 users have amenorrhea, 25% have oligomenorrhea and 11% have spotting. The pattern is similar with the LNG 14 IUD with less amenorrhea (13 versus 24% after 3 years) [34].

Mood changes have recently received special attention. In the Danish Cohort, LNG-IUS users had an increased risk of having to be prescribed antidepressants and a higher risk of hospitalization with depression [40].

The impact of progestogens on the affective state of women is, however, complicated and it seems that the negative impact is limited to a small group of vulnerable women. This may be of clinical importance in those women who suffer from preclinical or undiagnosed perimenopausal depression [41].

Other side effects are rare and include breast tenderness and acne.

3.5.4 Additional Benefits

There are many benefits to using the Levonorgestrel-releasing IUD. The major benefit is reduction in heavy menstrual bleeding and dysmenorrhea in patients without organic pathology and bleeding due to bleeding diathesis including anticoagulation therapy [42]. The effect on reduction of bleeding intensity in women with fibroids and adenomyosis is yet unclear and under investigation. The Levonorgestrel-releasing IUD protects against pelvic inflammatory disease, due to cervical mucus thickening which acts as a barrier towards ascending infections. The Levonorgestrel-releasing IUD can be used to treat endometriosis. There is endometrial protection in premenopausal and menopausal women using estrogen replacement, and a concomitant reduction of the risk of endometrial cancer. The Levonorgestrel-releasing IUD can also be used to treat endometrial hyperplasia and cancer and endometriotic pain.

3.5.5 Contraindications

Contraindications include severe deformity of the uterine cavity, acute sexually transmitted infections, unexplained vaginal bleeding, current breast cancer, Wilson's disease and known or suspected pregnancy.

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Chapter 9
Progestogens and Endometriosis

Matityahu Zolti and Howard J. A. Carp

1 Introduction

Endometriosis is a chronic and recurrent condition in which endometrial-like tissue is found outside the uterus. Endometriotic lesions may be superficial or deeply infiltrating. Lesions are mainly located in the peritoneum, pouch of Douglas, ovaries or sacroiliac ligaments. Endometriosis is defined as deeply infiltrating if penetrating more than 5 mm under the peritoneum [1]. Infiltrating lesions may invade adjacent organs (bladder, bowel or rectum) leading to a wide range of symptoms. Endometriotic lesions may be found in organs as distant as liver, lung or brain. In the ovaries, endometriosis may form endometriomas, with smooth walled cysts filled with a "chocolate" like material. Some women with endometriosis experience extreme pain and/or infertility, while others have less or minor symptoms, or the symptoms appear late in the course of the disease. Endometriotic lesions are surrounded by an inflammatory reaction which may lead to adhesions ranging from filmy synechia to dense adhesions which obliterate all planes of separation between the various organs involved.

The prevalence ranges from 5% to 10% of women of reproductive age, to 50% of infertile women [2, 3]. Laparoscopy and histological confirmation is the gold standard for diagnosis of the disease. However, the condition may be suspected by

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H. J. A. Carp (ed.), *Progestogens in Obstetrics and Gynecology*,
https://doi.org/10.1007/978-3-030-52508-8_9

symptoms, signs, gynecological examination, and imaging techniques such as transvaginal sonography, or magnetic resonance imaging. Both imaging techniques can diagnose ovarian endomeriomas. Adhesions can be diagnosed by transvaginal ultrasound, with the sliding sign technique, which assesses whether the uterus and ovaries move freely over the adjacent organs and tissues. A detailed non-invasive diagnosis of extension within the pelvis can facilitate the choice of a safe and adequate surgical or medical approach. Treatment is dependent on the patient's age and needs. Treatment may be required to reduce pain, enhance fertility, or both or to prevent recurrence. However, except in cases of a solitary nodule which is completely resected or focal lesions such as endometriosis in scar, complete resolution is not possible as yet, all treatment modalities aim to provide temporary relief.

Endometriosis is often associated with comorbidity such as migraine headache anxiety and premenstrual syndrome (PMS) or Premenstrual dysphoric disorder (PMDD) [4]. This chapter describes the role of progestogens in endometriosis.

2 Mechanisms of Progesterone Resistance in Endometriosis

Progesterone acts through a nuclear receptor which regulates transcription (known as the classical pathway. Progesterone also acts on membrane receptors (non classical pathway) [5]. The membrane receptors such as PRa, PRb, PQMR and PGMR1 can trigger different signalling cascades such as P13K, PKC, MAPKPKA which influence ion influx and efflux in sites which have progesterone receptors. Attia et al. [6] first described the concept of progesterone resistance. Resistance was explained by significantly reduced receptor PR-B mRNA and protein levels in endometriosis lesions, whereas PR-A isoforms were generally normal. Subsequently, a series of endometrial gene expression microarray studies indicated that progesterone-regulated genes, e:g glycodelin, N-acetylglucosamine-6-O-sulfotransferase, 17b hydroxysteroid dehydrogenase 2 (17bHSD2)] were downregulated in tissues derived from endometriosis subjects compared with women without endometriosis [7]. 17bHSD2 plays a key role in the conversion of biologically active estradiol to the less potent estrone. As a consequence of decreased expression, of PR-B in stromal cells, estradiol activity is enhanced in endometriotic lesions, even in the presence of progestogens.

Physiologically, luteal phase progesterone secretion downregulates the genes associated with DNA replication, halting endometrial proliferation. Consequently, the genes involved in cell cycle regulation, such as proliferating cell nuclear antigen (PCNA), the cellular marker of proliferation (Ki67), thymidine kinase 1, cyclin E1 protein (CCNE1), forkhead box protein O1 (FOXO1) and mitotic arrest deficient-like 1 protein (MAD2L1), are physiologically downregulated in the early secretory phase, but upregulated in moderate to severe endometriosis [8].

2.1 Environmental Toxicants in Progesterone Resistance

Polycholorophenyls, particularly dioxin have been implicated as causes of progesterone resistance and endometriosis [9]. Dioxin like compounds are persistent organic pollutants which are by products of agricultural pesticides and waste incineration. Dioxins are resistant to degradation, and accumulate, in the environment, particularly in food sources [10]. Dioxin like compounds accumulate in fat-rich tissues in the human body [9]. Nonhuman primates have shown a correlations of endometriosis with dioxin exposure [10]. The biological effects of dioxin are mediated through binding to the arylhydrocarbon receptor (AHR). AHR then binds to specific dioxin response elements to alter the transcriptional activity of specific genes [9]. Dioxins have been implicated in pregnancy loss via disruption of ovarian steroidogenesis and interference with progesterone action in the endometrium [11]. In human endometriosis, the proinflammatory chemokines that are downregulated by progestogens, T-cell expressed and secreted (RANTES) or CCL5 can be directly activated by dioxin-AHR complexes [11]. In mouse models, dioxin exposure leads to the loss of progesterone receptor expression in the endometrium [11].

2.2 Retinoid Resistance

Retinoids are dietary lipids that are paracrine mediators of progesterone action in the endometrium. Altered retinoid production and action may be a deleterious consequence of progesterone resistance in endometriosis [12]. Stromal cells utilize paracrine signaling to elicit genotypic and phenotypic differentiation in response to progesterone. One such retinoid is retinoic acid (RA). RA stimulates 17bHSD2, (see above) Expression of the vitamin A receptor (STRA6) and cellular retinol binding protein 1 (CRBP1), are responsible for the uptake and transport of RA, respectively. These are significantly reduced in the stromal cells of endometriosis patients when compared to controls. Similarly, the expression of retinaldehyde dehydrogenase 1 A2 (ALDH1A2), the enzyme responsible for conversion of retinol to RA, is also decreased. Intracellular shuttling of RA to the nucleus is impaired due to decreased expression of cellular retinoic acid binding protein 2. (CRABP2) and fatty acid-binding protein, epidermal (FABP5), which are responsible for delivery to retinoid receptors RARa/RXRa (retinoic acid and retinoind X receptors a), and PPARb/d (peroxisome proliferator activated receptors b and d), respectively. Levels of CRABP2 are drastically reduced, whereas levels of FABP5 are minimally reduced, leading to preferential shuttling of RA to PPARb/d. Expression of all known RA receptors has been found to be decreased in endometriotic tissue [12]. Conversely, enzymes responsible for the catabolism of RA, such as the RA-metabolizing member B1 enzyme of the P450 superfamily, are significantly increased.

Impaired action of progesterone is likely responsible for altered RA functionality in the stromal cells of endometriotic tissue.

3 Inflammatory Reaction Around Endometriotic Deposits

Neoangiogenesis, fibrosis and hemosiderin accumulation are found in the endometriotic lesion. In endometriosis, peritoneal macrophages are activated, and increased cytokine production is found. However, the macrophages cannot carry out phagocytosis of the lesion [4, 13]. The ectopic endomerium has been reported to contain a protein (Endo 1) [8] which binds to peritoneal macrophages, increasing their production of interleukin 6, and reducing their phagocytic ability [14]. The endometriotic lesions secrete several proinflammatory cytokines which recruit macrophages and T lymphocytes in to the peritoneum such as IL-1, IL-8, TNF-α, IFN-γ. Several angiogenic factors are also expressed by endometriotic lesions such as IL-1, IL-6, IL-8, EGF, Fibroblast growth factor, insulin-like growth factor, vascular endothelial growth factor (VEGF), and Endo I thus explaining the angiogenesis in the peritoneal cavity [15]. As a result, the peritoneal fluid has a high concentration of cytokines, growth factors, and angiogenic factors, derived from the lesions themselves, or from macrophages and other immune cells.

Figure 9.1 shows the intense inflammatory reaction with enhanced angiogenesis around the lesion. Immune cells such as NK cells mediate the inflammatory reaction associated with endometriosis. It has been even been suggested that the peritoneal fluid may be an active promoter of growth of endometrial deposits by lipid peroxidation [16, 17]. These oxidants may stimulate endometrial-cell growth.

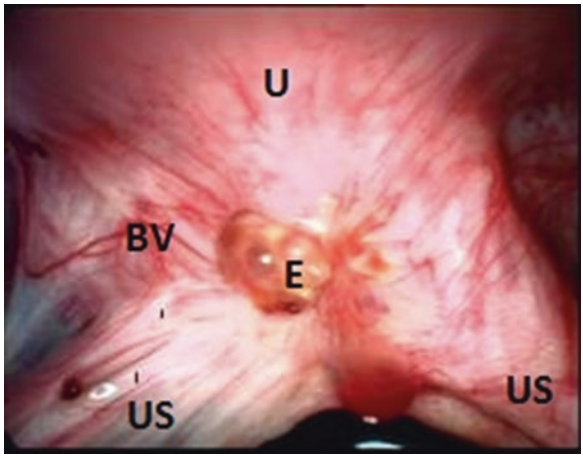

Fig. 9.1 Inflammatory reaction and neoangiogenesis around endometriotic lesion. *U* Uterus, *US* uteroscacral ligaments. These can be seen to be edematous and inflamed. *E* endometriotic lesion, *BV* dilated blood vessels

Endometriosis has many features of an autoimmune disease as there is increased polyclonal B-cell activity, high B-cell and T-cell counts, but with abnormal function [18, 19], and reduced natural-killer-cell activity [19, 20]. High serum concentrations of IgG, IgA, and IgM autoantibodies [21] and anti-endometrial antibodies have been reported [22]. Additionally, there is a high concordance of other autoimmune diseases or phenomena in women with endometriosis such as systemic lupus erythematosus, rheumatoid arthritis, Sjogren's syndrome, autoimmune thyroid disease, allergies, asthma and eczema [23]. Several studies have reported an association between autoimmune thyroid disease and endometriosis associated infertility, as shown by a high prevalence of positive anti-TPO antibodies [24].

Rather than being autoimmune in origin, the inflammatory reaction may be a response to the invasive properties of ectopic endomerium. Ectopic endometrial tissue fragments can attach to, and invade the peritoneal surface. Matrix metalloproteinases (MMP) degrade extracellular matrix. MMP 7 and MMP 11 are normally expressed in the endometrium during menstrual breakdown and subsequent oestrogen-mediated endometrial proliferation. MMP 7 and MMP 11 are normally suppressed by progesterone during the secretory phase [25]. Persistent expression of MMP might enable endometrial tissue to invade the peritoneal surface.

Nuclear factor kappa B (NF-kB) may be crucial for mediating several biochemical processes associated with endometriosis [26]. NF-kB is activated by proinflammatory cytokines and oxidative stress and is increased in endometriotic lesions. NF-kB activation leads to the expression of a number of genes involved in inflammation, such as IL-1, IL-6, IL-8, and cyclooxygenase-2 [27]. Endometriotic tissue has been shown to activate NF-kB [28]. By activating proinflammatory genes, NF-kB perpetuates inflammation and macrophage recruitment. In addition to the inflammatory cascade, NF-kB regulates genes involved in antiapoptosis, tissue invasion, cell proliferation, and angiogenesis. In healthy women, NF-kB-DNA binding is decreased in the secretory phase relative to the proliferative phase, which may be due to the anti-inflammatory action of progesterone [28]. However, in women with endometriosis, NF-kB-DNA binding remains elevated during the secretory phase [29].

3.1 Effect of Sex Hormones on Inflammatory Reaction

Hormonal alterations may influence the ability of endometriotic cells to proliferate, attach to the mesothelium and evade immune mediated clearance [30]. Figure 9.2 shows the effect of various hormones and medications on the development of endometriosis. Endometriotic implants have increased expression of aromatase and decreased expression of 17β-hydroxysteroid dehydrogenase [31], leading to a marked increase in locally bioavailable estradiol. Estradiol stimulates the production of prostaglandin E_2 which further stimulates aromatase activity [32].

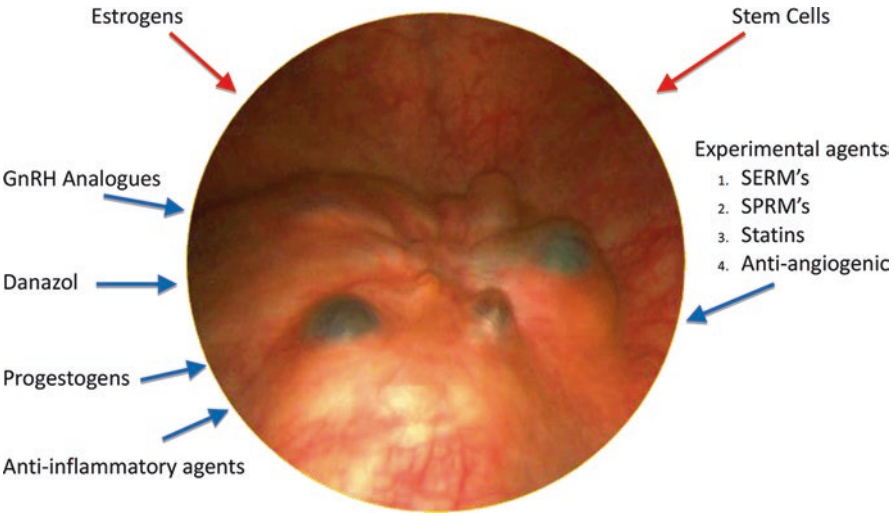

Fig. 9.2 Medications acting on endometriosis. Red arrows = stimulate endometriosis, Blue arrows indicate agents inhibiting endometriosis. *SERM's* selective estrogen receptor modulators, *SPRMS* selective progesterone receptor modulators

4 Genetic Basis of Endometriosis

A genetic basis has been suspected for endometriosis due to the increased incidence in identical twins and first degree family members [33]. Several interesting endometriosis-related genes have been discovered via linkage and genome-wide association methods. There are various candidate genes including genes coding for the estrogen receptor (ER), inflammatory cytokines and adhesion molecules, although an unequivocal consensus genetic "fingerprint" has not been reached thus far [34, 35]. Several polymorphisms have been described in the PR gene. The PROGINS polymorphism may affect ligand-binding and downstream signaling in endometriosis, and has been implicated as a genetic cause of progesterone resistance [36]. Wieser and colleagues [37] first reported an increased frequency of the 306-bp Alu insertion PROGINS polymorphism in patients with endometriosis. A recent meta-analysis pooling 12 studies and involving 1323 cases and 1998 controls found a trend linking the presence of a variant allele and risk of endometriosis, with a conferred risk odds ratio = 1.41–1.43 (p = 0.15–0.17) in homozygous and recessive models [36]; however, the association was only observed in European subjects.

Additionally, downregulation of proapoptotic genes and upregulation of anti-apoptotic genes of the BCL2 and BAX families, have been reported endometriotic lesions [38]. Hence, there may be an intrinsic abnormality in endometriosis that permits ectopic endometrium to attach, survive, invade, and establish a blood supply.

HOX genes, are dynamically expressed in the endometrium, where they are necessary for endometrial growth, differentiation, and implantation. In the human endometrium, the expression of HOXA10 and HOXA11 has peak expression at implantation in response to rising progesterone levels. However, maximal HOXA10 and HOXA11 expression does not occur in endometriosis, due to altered progesterone receptor expression or a dysregulated progesterone response. Consequently, other mediators of endometrial receptivity that are regulated by HOX genes, such as pinopodes, αvβ3 integrin, and IGFBP-1, are downregulated in endometriosis. HOXA10 hypermethylation may silence HOXA10 gene expression and account for decreased HOXA10 in the endometrium of women with endometriosis.

4.1 Epigenomics and Epigenetics

Epigenetic modifications in endometriotic cells silence progesterone target genes by methylation and acetylation which mediate progesterone resistance. These epigenetic changes may modify activators, repressors, enhancers, miRs, and other noncoding RNA. Wu and colleagues first reported the hypermethylation and silencing of the homeobox A10 (HOXA10) promoter in endometrial cells from women with endometriosis compared with controls [39]. HOXA10 is a homeobox gene family member involved in uterine development and function. HOXA10 expression is normally increased in mid-secretory endometrium under progesterone regulation. However, in eutopic endometrium of patients with endometriosis, HOXA10 fails to increase its expression after ovulation. This may be due to HOXA10 promoter hypermethylation and gene-silencing [39]. Additionally, endometrial cells from women with endometriosis exhibit hypermethylation of the PR-B promoter, resulting in decreased expression of the receptor protein [40]. Decreased PR-B expression may lead to degradation of mRNAs [41]. Burney and colleagues found that miR-9 profiling was significantly downregulated in patients with endometriosis compared with controls in the early secretory endometrium [42]. One predicted target of miR-9 is BCL2, a gene encoding the anti-apoptotic protein known to be over-expressed in endometriotic endometrium. Similarly, three members of the miR-34 family, thought to play a role in p53-dependent suppression of proliferation, are also down-regulated in the early secretory endometrium of patients with disease. MiR-196a has been found to be overexpressed in the eutopic endometrium of patients with endometriosis, while its target, PR-B, was significantly decreased [43]. Another miR implicated in progesterone resistance is the increased expression of miR-29c in endometriotic tissue. Its target is FK506 binding protein 4 (FKBP4), a known progesterone regulated protein responsible for decidualization [44]. The exact mechanisms by which miR expression is altered is unclear, but reduced miR expression may be the result of altered methylation of miR gene promoters, as treatment with demethylation agents restores normal expression [45]. The relatively permanent nature of methylation may explain the widespread failure of treatments for endometriosis-related infertility.

5 Evasion of Immune Clearance

Normally, endometrial cells which have been shed into the peritoneum are cleared by the immune system. Failure of the clearance mechanism may predispose to the implantation and growth of endometriosis. Endometrial cells have been found to be resistant to lysis by natural killer (NK) cells when compared to the endometrium from women without disease [20]. Shedding of intercellular adhesion molecule-1 (ICAM-1) by endometrial stromal cells from women with endometriosis may be one mechanism whereby these cells escape NK mediated clearance [46]. Impaired NK cell function (by down regulation of the NK1receptor and compromised macrophage function in endometriosis may further contribute to decreased clearance of lesions.

6 Comorbity of Endometriosis

The prevalence of endometriosis is higher in women with migraine than in control women without headaches [47]. Women with endometriosis are also more likely to have other comorbid conditions affecting mood and pain [4]. Such as PMS\PMDD, Fibromyalgia chronic fatigue syndrome interstitial cystitis and irritable Bowel Syndrome.

7 Progesterone Actions in the Female Brain

In the brain as in other tissues, both estradiol and progesterone act through classical nuclear receptors and non-classical membrane receptors. The various actions of progesterone on the neural system are summarized in Chap. 14.

8 Progestogens as Treatment

No treatment of endomeriosis is curative, but aims at pain relief, or optimising the possibility of fertility. The principles of treatment are summarised in Table 9.1 and include: (1) Debulking and restoration of anatomy, by surgery. (2) Reduction of the estrogen required to maintain endometriosis, by GnRH analogs or Danazol. (3) Reduction of the inflammatory response. The last approach uses progestogens or anti-inflammatory agents. Medical treatment can be used alone or as an adjunct to surgery.

Table 9.1 Management of endometriosis: therapeutic intervention

Debulking	Surgery
Estrogen reduction	1. Danazol, 2. GnRH analogues 3. GnRH antagonists
Reducing inflammatory response	1. Prorogestogens 2. Oral contraceptive pills 3. Anti-inflammatory agents
Endometrial atrophy	1. LNG – IUS 2. Gestrinone

8.1 Mode of Action

Progestogens are effective at a number of different levels. Some progestogens have anti-gonadotropic actions, which inhibit ovarian function to create a hypoestrogenic environment. Progestogens can also reduce the inflammatory response. Hence, progestogens are frequently used as first-line therapy for the treatment of endometriosis. Progestogens do not reduce estradiol levels as much as GnRH agonists. However, progestogens do not induce a medical menopause and are not associated with hot flushes or decreased bone mineral density.

Progestogens most probably acts by reducing the inflammatory response. TNF-α and estradiol induce the proliferation of endometriotic stroma cells via NF-kB, whereas progestogens reduce TNF-α induced NF-kB activation [48]. Progesterone itself is associated with decreased IFN-γ and increased IL-10 in endocervical fluid [49], up regulation of LIF mRNA expression in vitro [50], and inhibits NK cell activity. The progestogen dydrogesterone has been shown to modulate immune responses via suppression of IL-8 production in lymphocytes, inhibition of IFN-γ and increasing levels of IL-4 [51]. The increase in nitric oxide production seen with dydrogesterone may also play an important anti-inflammatory role [52].

8.2 Different Progestogens

Many progestogens have been have been used for the treatment of endometriosis. These include medroxyprogesterone acetate (administered by intramuscular injection), desogestrel, dienogest, cyproterone acetate, dydrogesterone etc. administered orally, either alone, or in combination with oestrogens in the combined oral contraceptive pill or levonorgestrel absorbed from an intrauterine contraceptive device. Progestogens have many beneficial effects. However, the results do not differ very much from the use of the combined estrogen progestin oral contraceptive pill. Some specific progestogens are described below.

8.2.1 Dydrogesterone

Dydrogesterone is a stereoisomer of progesterone manufactured by treating progesterone with ultraviolet light. Dydrogesterone stimulates the progesterone receptor directly without affecting progesterone levels. Dydrogesterone also binds the receptor 50% more than progesterone itself [53]. Dydrogesterone does not stimulate the androgen, glucocorticoid or estrogen receptor.

There are numerous small comparative studies and case reports of dydrogesterone in endometriosis since the 1960's. Schweppe [54] summarized seven control studies of dydrogesterone. Doses between 10 and 60 mg/day, were used for various numbers of days per cycle, and over periods of 3–9 months. The majority of women became symptom-free or experienced a significant reduction in the number/severity of symptoms. These findings have been supported by laparoscopic examination in several of the studies. Dydrogesterome significantly inhibited the proliferation of both epithelial cells and stromal cells and activated apoptosis in endometriotic lesions in a mouse model [55]. The mechanism included endometrial atrophy, However, a Cochrane review published in 2012 [56] which included 13 randomised controlled trials evaluating the use of progestogens, found only one RCT assessing dydrogesterone [57]. There was no significant improvement in objective efficacy at 6 months compared to placebo (OR 0.53, 95% CI 0.14–1.94) nor were there any differences observed in the change in pain score at 12 months of follow up (OR 0.80, 95% CI 0.27–2.37; NS). However, the wide confidence intervals and small number of patients indicates that Schweppe's [54] figures are probably more relevant. Dydrogesterone is especially useful in patients desiring pregnancy. As dydrogesterone does not inhibit ovulation it can be used for symptomatic treatment of pain, reduction of bleeding problems and cycle control.

8.2.2 Dienogest

Dienogest is a synthetic orally active progestogen. As dydrogesterone, dienogest is highly selective for the progesterone receptor and exerts a strong progestational effect. However, dienogest differs from dydrogesterone in having a moderate antigonadotropic effect [58]. The therapeutic dose (2 mg) inhibits ovulation in healthy women with normal menstrual cycles [59]. However, dienogest only moderately reduces oestrogen levels, hence, dienogest does affect bone mineral density [60], and was associated with endometrial glandular hyperplasia in a mouse model [55]. Dienogest does not reduce sex hormone-binding globulin, is bound unspecifically to albumin and does not accumulate using oral doses of 2 mg/day [61].

Two prospective placebo controlled randomized studies assessed dienogest against placebo [58] or versus leuprorelin depot, [60]. Both trials showed a significant improvement in endometriosis-related symptoms, and a similar effectiveness to GnRH agonist therapy. However, the bleeding pattern differed substantially between the two groups. In the leuprolide group most women had infrequent bleeding in the first 90 days and amenorrhea after prolonged treatment. In the dienogest

group prolonged and irregular bleeding were frequent in the first 90 days of treatment. Bleeding problems occurred in up to 80% of patients within the first 3 months of treatment.

In a single-arm extension study of treatment for 15 months and follow-up 6 months after discontinuation of treatment, dienogest was shown to reduce pain symptoms with normalisation and long term relief of symptoms even after treatment discontinuation.

Dienogest has a good safety and efficacy profile, with good tolerability, antiandrogenic action and weak antigonadotropic activity, combined with typical characteristics of 19-norprogestins: strong suppressive action on the endometrium in low doses, a short half-life and high bioavailability [62].

8.2.3 Medroxy Progesterone Acetate (MPA)

MPA can be administered orally in a dose of 15–50 mg orally or injected as a depot form (DMPA). Bergqvist et al [63] compared MPA to placebo. There was a greater quality of life after MPA and pain relief. Telimaa et al. [64] reported the results of a prospective, randomized trial comparing MPA to Danazol. A 50% regression rate of ectopic implants and 13% partial regression with scar formation was reported in the treatment group compared to 12% and 6%, respectively, in the placebo group, and a net reduction in pain symptoms after treatment compared to placebo. (OR = 0.70, CI −8.61 to −5.39; $P < 0.00001$). When Danazol and MPA were compared, both alleviated endometriosis-associated pelvic pain, lower back pain and defecation pain, but they did not differ from each other in these actions. The authors concluded that because of good efficacy and tolerance, high-dose MPA is a useful alternative in the hormonal treatment of endometriosis. However, MPA has glucocorticoid and androgenic effects. Brown et al. [56], have reported significantly more cases of acne (6 versus 1) and oedema (11 versus 1) in the medroxyprogesterone acetate group compared with placebo. The dose is 20–100 mg daily. Harrison and Barry-Kinsella [65] published the results of a placebo controlled trial. Initial and second-look laparoscopy were performed to grade the lesions according to the revised American Fertility Society stages. Surprisingly, both MPA and placebo therapy achieved similar statistically significant reductions in stages and scores at second-look laparoscopy. However, MPA was more effective in improving overall well-being. The authors concluded, that as both MPA and placebo were equally effective in treating endometriosis over a 3-month period, and questioned the role of using MPA altogether.

MPA can also be administered intramuscularly in a depot form (DPMA). DPMA is long acting, and a 150 mg. dose may only need to be repeated after 3 months. Vercellini [66] compared 150 mg of depot medroxyprogesterone (DMPA) every three months with a 20 μg oral contraceptive pill (OCP) and 50 mg danazol. Both the pill and danazol were taken for 3 weeks out of four. Pain reduction with DMPA was as effective as danazol. DPMA has also been compared to leuprolide [67]. Symptoms of dysmenorrhoea were significantly reduced in the DMPA group at 6

months compared with leuprolide (OR 0.19, 95% CI 0.05–0.69; $P = 0.01$) but the effect was short lived, and not present at the 12 months follow-up (OR 0.63, 95% CI 0.37–1.08). At 12 months fewer women in the leuprolide group reported dyspareunia (OR 4.83, 95% CI 2.14–10.93).

Side effects include breakthrough bleeding in approximately 40% of patients, nausea, breast tenderness, fluid retention and depression.

8.2.4 Cyproterone Acetate

Vercellini [68] compared 12.5 mg cytoproterone acetate daily to a continuous monophasic OCP once daily (0.02 μg ethinyl estradiol and 0.15 mg desogestrel). The primary endpoint, as in their previous study, was the degree of satisfaction at the end of therapy. A change in severity of symptoms was also measured by a 100 mm visual analogue score and a 0–3 point verbal rating scale. Cyproterone however, has significant anti-androgenic effects.

8.2.5 Levonorgestrel Intrauterine System (LNG-IUS)

The LNG-IUS is a contraceptive intrauterine device (IUD). As it releases norgesterel in a constant fashion, it lessens the excess bleeding associated with other IUD's, and may even lead to amenorrhea. Endometrial exposure to LNG induces endomerial atrophy. Hence the LNG-IUS can only be used for endometriosis in women who do not desire fertility, and who are prepared to accept amenorrhea.

Several small RCTs have compared the use of LNG-IUS in endometriosis to GnRH agonists and Depot medroxyprogesterone acetate [69]. The mechanism by which the LNG-IUS decreases endometriosis related symptoms is unclear, as the LNG-IUS does not inhibit ovulation nor does it induce a hypoestrogenic state. It has been suggested that the LNG-IUS acts by decreasing the expression of glandular and stromal estrogen and progesterone receptors in the ectopic endometrium [70]. With the LNG-IUS a reduction of the severity of endometriosis has been seen at laparoscopy [71, 72]. The echographic size of recto-vaginal lesions under has also been seen on ultrasound under LNG-IUS treatment [73]. The LNG-IUS has been shown to reduce pain [74]. Tanmahasamut et al. [75] compared a treatment with the LNS-IUS after laparoscopic conservative surgery to expectant management. There was a significant reduction in dysmenorrhoea [5.0 vs 8.1 cm on the visual analogue scale (VAS)] and non-cyclic pelvic pain (VAS 2.2 vs 4.8 cm) but no effect on dyspareunia. The LNG-IUS is as effective as DMPA with no impact on bone mineral density [76]. Bayoglu et al [77] compared the efficacy of the LNG-IUS with the GnRH analogue gosareline on endometriosis related chronic pelvic pain in patients with severe endometriosis during 12 months. Both treatment modalities showed comparable effectiveness in the treatment of chronic pelvic pain related endometriosis.

8.2.6 Norethindrone Acetate (NETA)

Norethindrone (Norethisterone) acetate can be used continuously in a dose of 2.5 mg per day. The evidence for pain relief comes from a study by Muneyyirci-Delale and Karacan [78]. 52 women with endometriosis confirmed by laparoscopy were treated with NETA. Dysmenorrhea and noncyclic pelvic pain were relieved in 48/52 (92.3%) and 25/28 (89.2%) of patients, respectively. Similar results were found in recurrent endometrioma, [79] and adenomyosis. The advantages of NETA include, excellent cycle control, and no harmful effect on the lipoprotein profile [80]. Ferrero et al. [81] has shown that NETA can relieve the pain of endometriosis, particularly when that pain presents as dysmenorrhea.

8.2.7 Effect of Progestogens on Endometriosis Related Infertility

Numerous mechanisms have been proposed to explain the effect of endometriosis in infertility:- altered folliculogenesis reduced preovulatory steroidogenesis of granulosa cells, decreased capability of fimbrial ovum capture, sperm phagocytosis by peritoneal and oviductal macrophages, anti-sperm antibodies and reduced sperm penetration and velocity In addition, altered egg–sperm interaction, defective implantation and impaired early embryonic development have been reported to explain endometriosis related infertility. Consequently it is not surprising that although medical management improves the quality of life for many women with endometriosis, the effect on endometriosis related fertility is not so successful. Many progestational agents inhibit ovulation, precluding their use in patients desiring fertility. A Cochrane database metaanalysis [82] which included 23 trials of over 3000 women found that pretreatment with ovulation inhibiting agents such as oral contraceptives, progestogens, danazol etc. does not improve fecundity, and only delays conception. Pregnancy rates following progestin therapy however, depend on the stage of the disease, and whether medical therapy is an adjunct to surgery. To date, there is no randomized controlled trial that has shown an improvement in fertility after any progestin medication. The situation may be different with progestogens which do not affect ovulation such as dydrogesterone. However, in women with infertility and severe disease, there is little evidence of effect on fecundity. Most of the studies on dydrogesterone were performed before assisted reproduction was available. Surgical treatment is probably preferable, with assisted reproduction immediately after surgery, prior to the recurrence of disease.

8.3 Oral Contraceptive Pill (OCP)

The estrogenic component of the OCP prevents ovulation while the progestogen is continuously supplied. A therapeutic trial is easily performed with either continuous or cyclic OCPs. Continuous administration of OCPs, avoids menstruation and its

associated pain. However, absence of menstruation is not always acceptable to all women. Like progestogen regimens, the progestogen in the pill is believed to produce initial decidualization, followed by atrophy. To-day the OCP is the most commonly prescribed treatment for endometriosis symptoms. A study by Guzick et al [83] compared Lupron and continuous oral contraceptives for the treatment of endometriotic pain; both were found to be equally effective. An advantage of OCP's is that women with endometriosis are at increased risk of epithelial ovarian carcinoma, which may be prevented by OCPs [84].

8.4 Anti-Progestogens (Gestrinone)

Gestrinone is a synthetic steroid with mixed progestogen and antiprogestogen effects, some mild androgenic activity, and some anti-estrogenic activity. The mechanism of action consists of suppression of the release of pituitary gonadotropins. Gestrinone also interacts with the endometrium, inhibiting its growth, enhances lysosomal degradation, leading to a rapid decrease in progesterone receptors. A literature search in the Cochrane database [56] found no RCT comparing gestrinone to placebo or no treatment. The Gestrinone Italian Study Group [85] showed that oral gestrinone was as effective as leuprolide depot injection for pain relief. Visual analog scale pain scores decreased from 4.07 ± 2.86 to 1.23 ± 2.65 at 6 months in the gestrinone group. Two studies have compared gestrinone to danazol [86, 87]. There was no difference in either group regarding pain. Side effects are due to the androgenic and anti-estrogenic effects including, voice changes, hirsutism, and clittoral enlargement. However, most side effects are mild, transient and reversible.

8.5 Choice of Treatment

Progestogens seem to be effective in treating endometriosis-related pain. There is however no evidence for an improved efficacy compared to other hormonal treatment and their use in the treatment of symptomatic endometriosis should be conditional to patient acceptability and tolerance of side effects. There is a problem in that the different progestogens have never been compared to one another. The LNG-IUS has been compared to Depot medroxyprogesterone acetate [69], gestrinone to leuprolide [85], and danazol [86, 87]. Danazol has been compared to medroxyprogesterone acetate [64], but there are few other comparative trials. Therefore the choice of progestogen dependant on the patients desire for conception, and often the treating physician's personal experience and preference.

9 Other Modes of Treatment

GnRH analogues and danazol are widely used forms of treatment. Descriptions of their use are outside the scope of this chapter. We, here describe other forms of non hormonal treatment which may influence the role of progestogens.

9.1 AKR1C3

AKRIC3 has a role in the biosynthesis of prostaglandins, metabolism of progesterone, and biosynthesis of androgens and estrogens, and in the metabolism of isoprenyl aldehydes and retinaldehydes and thus directly or indirectly regulates activation of various receptors. The AKR1C3 gene is expressed in endometriotic lesions, where AKR1C3 might be involved in several pathophysiological processes. Although, there are no published data supporting the importance of AKR1C3 in endometriosis, there is sufficient data available to validate clinical trials. AKR1C3 inhibitors have been shown to reduce endometriotic lesions in a marmoset model. The non-hormonal medical treatment of endometriosis is an unmet need thus further drug development is required.

Interventions to effectively enhance progesterone responsiveness in endometriosis are limited, but new therapeutic approaches targeting the underlying cellular and molecular basis of progesterone resistance may prove efficacious. One study recently reported promising results for fenretinide, a low toxicity retinoid administered to overcome decreased retinoic acid signaling in patients with endometriosis [88]. Furthermore, in mice bearing xenografted human endometriosis tissue treated with fenretinide for two weeks, endometriotic lesion volume was decreased.

10 Conclusions

The World Endometriosis Society published a Consensus on the management of endometriosis. Their recommendations are outlined in Table 9.2. However, there is no panacea for the treatment of endometriosis. The choice of treatment is dependent on the patients age, whether the main symptom is pain or difficulty conceiving, acceptability of the side effects of medical treatment, acceptability of surgery and the results of previous therapy. At present a number of new agents are being assessed for treating endometriosis (Table 9.2). These include Selective estrogen receptor modulators, Selective progesterone receptor modulators, statins anti-angiogenic drugs, and retinoic acid However, it is too soon to judge their efficacy or give recommendations.

Table 9.2 Principles of management of endometriosis (adapted from [89])

Diagnosis	Suspected from clinical features. Diagnosis from non-invasive imaging techniques
Surgical treatment	1. Usually first line approach. 2. Definitive diagnosis can be made 3. Depends on acceptability to patient 4. Improves fertility
Medical treatment	1. Provides symptomatic relief 2. Used for treatment of recurrences 3. Used while awaiting surgery 4. May be treatment of choice in adolescents or after menopause 5. No evidence of improvement of infertility
First line medical treatment	1. NSAIDS, OCP, Progestogens, 2. Choice of progestogen depends on whether ovulation should be inhibited or desire for concurrent fertility.
Second line medical treatment	GnRH agonists with add back therapy, LNG-IUS

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Chapter 10
Progestogens and Breast Cancer

Eitan Pe'er

1 Introduction

Progesterone plays a crucial role in the development of the breast alveolar tissue, and ductal branching. In the normal menstrual cycle, at the mid-luteal phase, corresponding to the peak level of luteal progesterone, mammary epithelial DNA synthesis and mitosis is at its highest. Hence, progesterone can be clearly seen to have a proliferative effect on breast epithelium. As age advances, the amount of progesterone produced by the corpus luteum slowly decreases. The clinical results are shortening of the luteal phase, with concomitant shortening of the menstrual cycle and increasing incidence of premenstrual syndrome (PMS) [1]. In pregnancy, the placenta produces progesterone in increasing amounts. High levels of progesterone induce further lobular-alveolar development of the breast, in preparation for lactation and support the pregnancy. However, if high doses of progesterone are administered before breast surgery, there is less mitotic activity than after treatment with estrogen and progesterone. Therefore, estrogen and progesterone seem to act synergistically in the breast (and not antagonistically as in the endometrium). It has been shown that a single pulse of progesterone has growth stimulatory effect on cultured breast cells, but subsequent effects of further pulses of progesterone are growth inhibitors. There is an arrest of further cell cycles in the late G1stage. Several factors, such as cyclins D1 D3, decrease, or become inactivated by hypophosphorylation and inhibitory factors (such as kinase inhibitors p21, p27) which are induced by progestins. Hence, there is failure to induce further mitosis by frequent progesterone pulses [2].

Progesterone and progestogens have many uses in gynecology:- including treatment of menstrual disorders, combined oral contraception, and progesterone only contraception, hormone releasing IUD's (LNG-IUS) PMS, prevention of premature

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H. J. A. Carp (ed.), *Progestogens in Obstetrics and Gynecology*,
https://doi.org/10.1007/978-3-030-52508-8_10

uterine contractions, hormonal support of implantation after assisted reproduction, prevention of miscarriage etc. The use of progestogens in menopausal hormone therapy (MHT) gained popularity after Ziel and Finkle's [3] report showing that treatment with estrogen alone causes endometrial hyperplasia and subsequent cancer. However, these effects were completely counteracted by the addition of progesterone to the estrogen replacement therapy. All progestogens have the same effect on the estrogen-primed endometrium. They stop the proliferative effect of estrogens and induce a secretory phase that precedes menstruation, if pregnancy does not occur. As hormone replacement therapy (HRT) improved gained popularity, many synthetic forms of progestogen were introduced and are commonly used nowadays. Synthetic progestogens were introduced due to improved oral activity, prior to the advent of micronization, which allowed progesterone to be absorbed both orally and vaginally. Synthetic progestogens differ from one another in their structure, receptor affinity, metabolism and biological effects as described in other chapters.

The Women's Health Initiative (WHI) study examined post menopausal hormone replacement therapy with conjugated equine estrogens (CEE) and medroxyprogesterone acetate (MPA). The study was stopped prematurely because of an increased risk of breast cancer (HR-1.26). However in women treated with CEE alone, there was no increase in the breast cancer risk compared to placebo (HR-0.80) [4]. The lower incidence of breast cancer was also seen in the WHI extended follow-up up study of 11.8 years [5]. Since the publication of the WHI trial there has been additional epidemiological data, all confirming that the addition of progestins to estrogen increases the risk of breast cancer when compared to estrogen treatment alone. Jick et al. [6] reported a case control study of 33,000 women. The reported odds ratio was not significant for developing invasive breast cancer in women using estrogen alone (OR 0.96 95% CI 0.88–1.06) versus a significantly increased odds ratio of 1.44 (95% CI 1.31–1.58); for those women using estrogen and progestogen. A meta-analysis [7] of four randomized trials published up to 2006, found a non significant RR of 0.79 (95% CI = 0.61–1.02) for invasive breast cancer in estrogen only users and a statistically significant increased RR of 1.24 (95% CI = 1.03–1.50) with estrogen and progestogen. Epidemiological studies have reported a non significant RR of 1.18 (95% CI = 1.01–1.38) in estrogen only users, and a significantly increased RR of 1.70 (95% CI = 1.36–2.17) with the use of estrogen plus progestin [7]. In view of the accumulated data cited above, some of the original authors of the WHI report have reiterated their original conclusions, stating that the use of "estrogen-alone treatment" reduces the breast cancer risk and does not substantially interfere with breast cancer detection by mammography [8].

As the molecular understanding of hormonal actions has expanded, with increased clarification of the different modes of action with specific co-activators and co-regulators when attached to their specific receptors, it became clear that not all progestogens have the same actions. The French E3N Study comprised of 80,377 postmenopausal women followed up for more than 8 years, using different regimens of hormone therapy. The use of transdermal estrogen with a micronized progesterone combination was not associated with an increased risk of breast cancer [9]. Indeed, epidemiologic data have not demonstrated a risk relationship with circulating

levels of progesterone [10]. However, all forms of estrogen and synthetic progestogen combinations showed an increased breast cancer risk [9]. A subsequent study by Lyytinen et al. [11] showed that dydrogesterone, unlike other synthetic progestogens carried no increased cancer risk. From a practical and clinical point of view, each progestogen has its own unique biological profile which may, or may not, be shared with other progestogens. The different actions of progestogens are fully described in Chap. 2 on the Pharmacology of the progestogens.

2 Progesterone Receptors

The progesterone receptor is a single gene expressed in two isoforms: PR-A (94 kDa) and PR-B (116 kDa). There are homodimers (A-A), (B-B) and heterodimers (A-B) ocurring naturally, in different proportions in different target tissues in the female body. They exhibit distinct transcriptional regulatory functions targeting various subsets of genes [12]. The inactivated receptor is activated by hormone (ligand) binding. This hormone-receptor complex translocates to the nucleus where it binds to specific DNA sequences in the promoter regions of target genes to activate gene expression.

Alternatively, the expression of specific target genes can be repressed through interaction with various transcriptional factors such as nuclear factor kappa β (NFkβ). Consequently, the clinical significance of activation of the receptor is critically dependent on the transcriptional co-activators and repressors [13]. PR-A is mainly located in the nucleus. This receptor is required for uterine development. PR-B, which is essential for breast development, continuously shuttles between nuclear and cytoplasmatic compartments [14, 15]. Both receptors are co-expressed in the same tissues usually in equal ratios. In humans, normal mammary gland function may rely upon the balanced expression of the two PR isoforms. However, in breast cancer, cells this ratio is often altered. An imbalance between the two isoforms appears to be linked to different cancerous phenotypes in the breast [16]. PR-A receptors are more stable and less active. PR-B receptors undergo extensive cross-talk with mitogenic protein kinases and are therefore heavily phosphorylated (more often via action of growth factors) [17]. The PRs can be phosphorylated (and therefore modified) after ligand treatment in response to local growth factors. Mitogenic protein kinase activity is very high in a cancerous environment. They have been shown to persistently phosphorylate, and thus modify PRs action, even in the absence of a ligand. This causes an inappropriate activation of PRs affecting PR modifying binding partners. Therefore PR action differs in normal breast tissue compared to neoplastic breast tissue. Additionally, the presence or absence of estrogen has a significant modifying effect on the PR. These effects are also organ specific, proliferating in the breast, inhibitory in the endometrium. In summary, the PR has complex and versatile actions which are:- (1) different in normal and neoplastic tissue. (2) Tissue specific. (3) have different biological clinical actions regarding PR-A and PR-B. (4) May be ER dependent or ER independent. (5) Can act

independently without binding ligand (progesterone). (6) Different and specific for each progestogen. (7) Different if given a continuous or cyclic fashion. The above effects clearly indicate the varied effects that progestogens have on breast cancer cells biology, but the mechanisms are still not fully elucidated [18].

Other receptors may also be involved in addition to the ER and PR such as the insulin like growth factor receptor (IGF1R) [19], and steroid receptor crosstalk [20].

3 Breast Cancer -the Progesterone Effect

Breast cancer is the most common tumor in women. The life time risk of developing breast cancer is 12%. Most of the neoplasms present after the menopause and the incidence rises sharply with age. Approximately 5–8% of tumors arise due to a hereditary predisposition of pathogenic mutations in DNA repair genes such as BRCA-1, BRACA -2, CHEK2, ATM, P53 [21]. Most breast cancers are initially hormone dependent with estrogen playing a crucial role in development and progression. The tumor may develop very slowly having a mean doubling time of 90–200 days. After a period that may last several years, many breast cancers become hormone independent [22]. Hormone independence may be due to a mutation in the estrogen receptor.

Breast cancers are subdivided into two major subtypes:- (1) Luminal, estrogen receptor positive (ER+), progesterone receptor positive (PR+) or cytokeratin 18 positive (CK18+), which together account for approximately 80% of all breast cancers. (2) Basal ER negative, PR negative or CK5 positive, which have a much worse clinical prognosis [23, 24]. In the human breast, stem cells are found, which can be defined by expression of epithelial specific antigen (ESA) and α6-integrin. Some believe that ER+ /PR+ luminal cancer cells arise from distinct ER+/PR+ stem cells [25]. However, in recent years it was discovered that in solid ER+/PR+ experimental tumors, CK5+ cells persist that are ER-/PR- stem cells that are actually up regulated by progestins [26]. These colonies, though small in size (up to 100 or less cells) expand and can differentiate into the more common ER+/PR+/CK5-, tumors. Approximately, 2% of cells in these receptor positive breast cancers retain the ER-/PR-/CK5- stem like signature [27]. Treatment of ER-/PR-/CK5+ colonies with progesterone, but not with estrogen, leads to an increase in the ER-/PR-/CK5+ stem cell subpopulation from 1–2% to over 20%. MPA (medroxy-progesterone acetate) has been found to have a more significant effect than other progestins. This increase is independent of estrogen. Hence, it seems that tumor cells can regress from a differentiated state to a more stem-like state, in response to progestins [28]. Reactivation of the CK5+ cells (with strong CK18 expression, proving it to be of luminal origin) by 24 h of MPA exposure is usually more pronounced in the younger recently formed tumors.

Therefore, progestins, acting on the progesterone receptor, may affect the more abundant ER+/PR+/CK5 differentiated cells to reactivate ER-/PR-/CK5+ stem cells. It seems that progestogens have the ability to restore cancer stem cell like

properties to some ER+/PR+ breast cancer cells. Only after this progestogenic effect takes place, estrogen can resume growth and proliferation of the stem cells to expand ER+/PR+/CK5- breast tumors.

4 Local Production and Action of Progestogens

The pharmacological properties of progestogens vary according to the parent molecule from which they are derived (testosterone, progesterone, aldosterone etc.) This, together with their ability to activate or deactivate related glucocorticoid receptors, mineralocorticoid receptors and androgen receptors accounts for the different biological activities specific to each progestogen [29]. The enzyme, 5α-reductase, converts progesterone to 5α-pregnanes (e.g 5α-dihydroprogesterone = 5α-P). 5α-reductase is highly expressed in cancerous cells but not in normal breast cells. 5α-P which is mainly produced by the cancerous cells stimulates cell proliferation and metastasis via activation of the MAP-kinase pathway [30]. In contrast, in normal breast cells, 5α-reductase activity is low. However, other enzymes, namely 3α-hydroxysteroid oxidoreductase and 20α-hydroxysteroid oxidoreductase metabolize progesterone to 3α-dihydroprogesterone (3α-HP) and 20α-dihydroprogesterone (20α-HP). These metabolites exert an anti-mitogenic effect and increase apoptosis and cell adhesions [31]. In tumor tissue, the concentration of mitogenic 5α-pregnane was 14 times that of the anti mitogenic 3α-HP. In contrast, in the adjacent normal breast cells, the concentration of antimitogenic 3α-HP was more than three times that of the mitogenic 5α-pregnane [30]. In view of the fact that 5α-P stimulates cell proliferation and metastasis, while 3α-HP and 20α-HP exert an antimitogenic effect, increasing apoptosis and cell adhesion, treatment with progesterone may stimulate cell growth only in the presence of malignant breast tissue [32].

Both ER/PR positive and ER/PR negative breast tumors are able to convert progesterone to 3α-HP. Specific high affinity membrane receptors exist for both 5α-P and 3α-HP. These receptors are completely distinct not only from each other but also from known ER, PR, AR and corticosteroid receptors [30]. Levels of 5α-P receptors are up-regulated by 5α-P and estradiol, and down-regulated by 3α-HP in both ER/PR positive and negative tumor cells [32]. 5α-P and 3α-HP have opposing effects on initiation and growth of ER/PR negative human breast tumors. These metabolites, which are independently produced by breast cancer cells, underscore the importance of the microenvironment in regulating expression of receptors, adhesion molecules, growth promoters and inhibitors within the breast cancer cells. 3α-HP maintains normal breast tissue. It inhibits proliferation, tumor initiation and tumor growth. 5α-P has exactly the opposite effects. It induces proliferation, tumor growth and detachment and reduces apoptosis.

5 Progesterone and Migration of Breast Cancer Cells

Death from breast cancer is usually due to invasion and metastasis to distant target organs (e.g. brain, bone, lungs). Progesterone and progestins stimulate breast cancer migration and invasion [33]. The migration and invasion of cancer cells involve complex mechanisms. The first step is reorganization of the cells actin/myosin cytoskeleton. This reorganization enables the cells to develop membrane protrusions (called filopodia and lamellipodia). These protrusions are involved in the cell's ability to detach itself from the main tumor body and to enter lymphatic vessels and migrate. There are several 17β-estradiol up-regulating families of actin-binding protein which leads to this phenomenon [34]. Studies comparing the specific effect of different synthetic progestins have demonstrated their individual and different effects on PR+ breast cancer cell migration and invasion in vitro. Activation is mediated in synergy with the activation of the actin-binding protein moesin to increase the formation of membranous structures. These specialized structures interact with the extracellular matrix of nearby cells, allowing locomotion of the breast cells [35]. It has been suggested that an in vitro "invasion index" for the effect of specific progestins on breast cancer cells lines incubated in vitro, with the addition of each progestin, with or without estrogen in the media can indicate the different and variable effects of progestins [36].

6 Protease Activated Receptors and Progesterone

Focal Adhesion Kinase (FAK) is a protein that belongs to the family of cytoplasmic tyrosine kinases. When activated, FAK associates with FA (focal adhesion) proteins allowing autophosphorylation at the docking site of the cells. Mature FA complexes, together with phosphorylated FAK recruit other proteins to allow cell detachment [37]. The protease-activated receptors (PARs) are a family of four vascular receptors that respond to local changes in the proteolytic environment. These receptors are activated by thrombin. PARs are important in tissue repair and response to injuries [38]. In tumor cells, PAR2 is activated by factor VIIa-tissue factor, (TF), regulating proangiogenic growth factor expression. TF is strongly induced by progesterone in breast cancer cell lines [39].

The ability of progestins to regulate PAR expression appears to be cell specific (endometrium, cervix, vascular vessels, breast etc). This action is also progestin specific; levonorgestrel down regulates PAR1 in the endometrium, MPA up regulates PAR1 expression in vascular endothelial cells etc. [37]. Overexpression of PAR1 is a feature of many metastatic cancers. These cancers are more invasive *in vitro* [38]. It has been demonstrated that progesterone regulates PAR1 at the mRNA and protein levels [39]. This regulation is dependent on the presence of PR. Advanced breast cancer is associated with a hypercoagulable state. Progesterone has been shown to enhance the coagulation cascade proteins TF and PAR1 promoting cancer

cells angiogenesis, coagulation, migration and invasion. This may be another mechanism by which progesterone may contribute to the increase in breast cancer incidence in women using continuous combined HRT.

7 RANK/RANKL and Progesterone

Progesterone acts indirectly to promote the proliferation of emerging luminal progenitor cells which do not possess the progesterone receptor. Proliferation has been shown to be a paracrine effect involving the RANK/RANKL system. RANKL—receptor activator of the NF-kβ ligand is highly expressed in human breast cancer cells. RANK and its ligand RANKL are expressed in preinvasive mammary intraepithelial neoplasia and invasive carcinoma of the human breast [40]. The dividing epithelial cells do not contain ER or PR, but are controlled by adjacent resting cells containing sex steroid receptors which secrete various growth factors, of which RANKL emerges as a key paracrine mediator of the progesterone mitogenic signal. It has been shown that progesterone-knock-out (PRKO) mice mammary epithelial cells, when mixed with wild-type (WT) cells, contribute to alveolar and ductal side branching in the pregnant state. This suggests a paracrine factor transmitted from the wild-type breast cells and received by the knock-out cells causing them to proliferate. This paracrine factor was found to be RANKL [41].

Mammary RANKL is induced by exogenous progesterone. In the proliferative phase of pregnancy (in which progesterone is at its peak action driving mammary epithelium expansion and morphogenesis), RANKL is markedly expressed. RANKL expression is confined to ER+/PR+ transmitters' cells [42]. Consequently RANKL may act as the direct link between breast cells via progesterone, inducing side branching and alveolar development [43]. Coexpression of PR is found in nearly 100% of RANKL+ mammary cells [44] .During pregnancy, RANKL expression is upregulated in mammary epithelial cells and is essential for the development of the lobulo-alveolar mammary structures and the formation of lactating mammary glands [45].

Finally, evidence that RANKL has a mediator role in mammary progesterone signaling, came from a study where PRKO mammary epithelial cells were transplanted into the mammary fat pad of WT mice. RANKL triggered mammary side-branching and alveolar budding in the PRKO transplant within the pregnant WT host [45]. It has been demonstrated that the RANKL transduction axis is actually essential for progesterone promotion of mammary tumorigenesis [45, 46]. In these studies MPA (medroxyprogesterone acetate), the progestin used in the WHI study, significantly increased RANKL expression in the ER+/PR+ cell population, in mammary normal epithelium as well as in premalignant and malignant cells. Therefore it is evident that progesterone (as well as MPA) relies on RANKL as a paracrine mediator for its proliferative effects. RANKL also enables the mammary epithelium to evade premature apoptosis [47, 48]. It has been shown that BRCA1 mutation carriers have deregulated progesterone signaling [49] leading to higher

proliferation and DNA damage in progesterone sensitive RANK+ luminal progenitor subsets [21].

Denosumab is a RANKL-neutralizing antibody which disrupts luminal cell/luminal progenitor communication via progesterone, which may therefore control luminal progenitor numbers, hence possibly preventing breast cancer. The role of denosumab has been investigated in breast cancer prevention. Giannakeas et al., [50] reported a 13% decreased breast cancer risk ((HR = 0.87; 95% CI 0.76–1.00). However, when used in established cancer, Coleman et al. [51] have reported denosumab to have no beneficial effect. At present a pilot study of denosumab selectively in in BRCA positive patients has been registered [52]. The results are eagerly awaited.

8 Progesterone and E-Cadherin

E-Cadherin is an epithelial adhesion protein, which is an important, if not the major, component of the tight junctions between mammary epithelial cells. It has been shown that decrease, or loss, of the E-Cadherin protein is associated with tumor cell metastasis and invasiveness, and a poorer prognosis in breast cancer patients [53]. E-cadherin protein is highly expressed in normal epithelial cells adjacent to the breast tumors. In an experimental rat model, treatment with E + R5020 (promegestrone) decreased the levels of E-cadherin precursor and mature E-cadherin protein [54]. E-cadherin decrease was abolished by the progesterone antagonist mifepristone (RU486), implying that the effect is due to the progestin component. In this rat model E + P treatment, as compared with E alone resulted in invasive mammary cancers accompanied by decreased E-cadherin levels and expansion of cells with a basal/myoepithelial phenotype. Similar findings have been observed in invasive primary human breast cancers compared to matched carcinoma in situ. While estrogen alone is sufficient to induce luminal noninvasive tumors, progesterone is required for the expansion of basal-myoepithelial tumor cells that frequently express progesterone receptor B. Progesterone promotes expansion of the more invasive basal/myoepithelial cells via direct activation of progesterone receptor B. It is important to note that only progesterone receptor B mediates the effect of progestins on E-cadherin. The ratio of progesterone receptors A/B is almost equal in normal breast tissue. But this ratio is completely altered, in favor of more B receptors in cancerous breast cells.

Loss of E-cadherin is a hallmark of the invasive behavior of luminal breast cancer cell lines. In vitro studies have found that treatment with E + P leads to complete loss of E-cadherin in 77% of the tumors in the rat model. The data now indicate that Progesterone acting through progesterone receptor B regulates E-cadherin protein expression. Progestins, therefore specifically contribute to the aggressiveness and invasion of breast cancer cells,

9 Bazedoxifene

After the WHI publication, safety concerns regarding the risk of breast cancer in women using the combination of MPA + CEE have stimulated the need to develop safer alternatives.The French E3N has shown that the use of different progestins can eliminate the concerns of higher breast cancer risks. But, with this in mind, the replacement of MPA with Bazedoxifene, a Tissue Selective Estrogen Complex (TSEC), was widely studied [55]. Bazedoxifene was shown to exert an anti estrogenic effect on the endometrium and the breast, thus having the clinical potential of safely replacing progestins in MHT [56]. This effect on the breast was specifically studied in the SMART-5 trial, where 940 women (out of 1843 enrolled) were treated with the combination of BAS/CEE. All had mammography screening before and 1 year after starting the trial. The results were compared to the placebo group. The results of the study showed no increase in breast density or breast tenderness during the active study [57], or a decrease of 38% in breast density in the treated arm whereas the group treated by MPA/CEE had a 60% increase in breast density. After 6 months of BZA/CEE therapy there was a favorable change in multiple risk biomarkers for breast cancer [58].

Endogenous levels of progesterone in post menopausal women have been reported as positively correlated with higher percentage of mammographically dense area [59].

10 Conclusion

The effects of progesterone containing MHT regimens to increase the risk of breast cancer is thought to be a result of promotion of growth of preexisting occult tumors that are too small to be detected by mammography. Clinical studies, such as the WHI, Million Women Study and others have accelerated research to find “evidence” to confirm, or discount the clinical findings. It soon became apparent that progesterone, the *“insignificant hormone”* partner of estrogen in hormone replacement therapy plays an important and complex role in mammary carcinogenesis. Progesterone works synergistically with estrogen on the breast, inducing alveolar proliferation. Progesterone has always been considered as an antiproliferative hormone due to its action on the endometrium, opposing the proliferative effect of estrogen. Hence progestogens were introduced into hormone replacement regimens: to counteract estrogen induced endometrial proliferation. Many laboratories throughout the world are trying to elucidate progesterone dependent mechanisms, in order to clear controversial issues reported in clinical studies. However, it is clear that progestogens have widespread actions on genes, receptors and tissues causing, promoting and inducing breast cancer. In view of the new information gathered so far, new well designed prospective clinical studies are now required.

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Chapter 11
Progestogens in Endometrial Cancer

Oded Raban and Walter Gotlieb

1 Introduction

Endometrial cancer is the most common gynecologic malignancy in affluent countries [1]. Approximately 15% of women will be diagnosed with endometrial cancer before menopause, and 4% will develop the disease before the age of 40 years [2, 3]. The current therapeutic approach for early-stage endometrial cancer includes total hysterectomy with bilateral salpingo-oophorectomy, and lymph nodes assessment (sentinel or pelvic/aortic lymphadenectomy), depending on preoperative or intraoperative pathologic risk profiles. Women with grade 1 endometrial cancer without myometrial invasion (consistent with FIGO 1988 stage Ia) treated by conventional surgery have a disease-specific survival of 99.2% after 5 years, and 98% after 10 years [4]. Many endometrial cancer patients carry an increased burden of medical co-morbidities, such as obesity, diabetes mellitus, and hypertension, conditions that have been associated with a higher risk of surgical adverse events [5, 6]. Some patients carry severe medical co-morbidities that may preclude them from having surgery [7, 8]. This high-risk group of patients with endometrial cancer may sometimes receive progestin treatment as an alternative to surgery, or as treatment until they are deemed fit for surgery.

A second group of patients that might benefit from progestin treatment are young women diagnosed with endometrial cancer who still wish to have children. The excellent cure rates that are attained for well differentiated endometrial cancer have led us to shift the focus from survival towards post treatment quality of life issues. In particular, there has been increased attention focused on fertility preservation, as approximately one of ten patients with endometrial cancer develop the disease during reproductive age. Considering that the average age at first birth has steadily increased in developed nations, going from 1 in 100 women above the age of 35 in

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H. J. A. Carp (ed.), *Progestogens in Obstetrics and Gynecology*,
https://doi.org/10.1007/978-3-030-52508-8_11

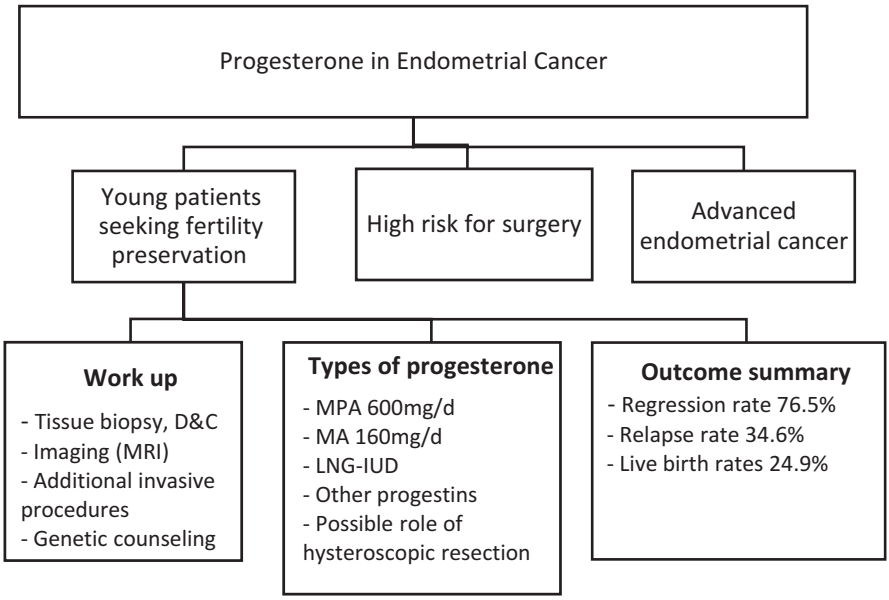

Fig. 11.1 Progesterone in endometrial cancer, chapter summary. MPA, Medroxyprogesterone acetate; MA, Megesterol acetate; LNG-IUD, Levonorgestrel releasing intrauterine device

1970 to 1 in 12 first births today [9]. Hence, it is not surprising that many of these younger women with endometrial cancer desire fertility preserving options. The decision to proceed with conservative management is complex. Many issues related to fertility-preservation for endometrial cancer remain uncertain and warrant further evaluation. The optimal work-up to evaluate the extent of disease in young patients with endometrial cancer who desire to maintain their uterus remains unclear, as is the lack of uniformity in the medical management and surveillance.

In this chapter we will discuss the role of progestin hormonal therapy in the conservative management of endometrial cancer, including the potential risks associated with medical management compared to surgical care, the appropriate candidate selection and work up, the expected outcomes, the variety of progestogens agents that have been used, and the recommended follow-up (Fig. 11.1). We will also describe the role of progestins in advanced endometrial cancer and the foreseeable future of progesterone treatments in endometrial cancer.

2 Progesterone for Fertility Preservation

2.1 Are There Any Risks?

The development of endometrial cancer in young women most often results from a hyperestrogenic state that leads to endometrial hyperplasia. A tissue biopsy of atypical endometrial hyperplasia has been associated with a 29% risk of progression

to endometrial cancer [10], and endometrial cancers have been found in up to 43% of patients with a preoperative diagnosis of atypical endometrial hyperplasia [11]. This high association warrants consideration in management decisions. According to a review of over 2000 women aged 40 years or younger collected from the National Cancer Institute database, the majority of patients (75%) had disease confined to the uterus, but approximately 17% had stage III or IV disease [10]. These younger patients are also at increased risk of other pathological gynecologic conditions, including ovarian tumors. In a review of young women with endometrial cancer by Walsh et al. [11], 26 of 102 women (25%) were found to have coexisting epithelial ovarian tumors (23 synchronous primaries and three metastases). Therefore, any decision to deviate from the standard approach of hysterectomy with oophorectomy and staging should take into account the risk of an undetected, and therefore subsequently untreated, synchronous or metastatic cancer. These studies confirm the need for thorough examination and careful patient selection, while highlighting the risks inherent in conservative management of an unstaged cancer.

Based on these data, the patient's outcome may be adversely affected when choosing to pursue fertility preservation. In the absence of randomized trials, the largest study to evaluate this matter is a retrospective study by Koskas el al [12]. who examined 489 patients aged 40 or younger with grade 1 endometrial adenocarcinoma. The patients were divided into groups who underwent uterine preservation, ovarian preservation, or hysterectomy with oophorectomy. Ovarian and uterine preservation had no effect on either cancer-specific or overall survival. The limitations of Koskas el al's [12]. study include the absence of information on which agents and treatment protocols were used and how they found no evidence for the 17–25% of young patients with concomitant/metastatic adnexal carcinomas published in other reports [12, 13].

2.2 Workup Prior to Treatment

The optimal work-up to evaluate the extent of disease in young patients with endometrial cancer who desire to maintain their uterus has not been established. Every effort should be taken to ensure that the cancer is confined to the endometrium and low grade, and therefore likely to respond to hormonal therapy without compromising curability (Table 11.1). Although most guidelines consider only well differentiated endometroid adenocarcinoma for conservative treatment, there are a few reports on successful progestin treatment of G2 and G3 endometrial cancer [13–15]. As a rule, pretreatment evaluation should consist of a full workup for any signs or symptoms suspicious for advanced/metastatic disease (Table 11.2).

Table 11.1 Suggested criteria for progesterone treatment

I	Absence of frank myometrial invasion
II	Well-differentiated (G1) endometrioid adenocarcinoma
III	No contraindications for progesterone therapy
IV	Potential for fertility
V	Informed consent on the indications and limitations of progesterone therapy

Table 11.2 Suggested procedures for the assessment of a patient with endometrial cancer seeking fertility sparing treatments

Procedure	Purpose
Complete history and physical exam	Look for signs or symptoms suspicious for advanced/metastatic disease, and family history
D&C (hysteroscopy)	– Tumor grading – Possible therapeutic effect
MRI	Assess myometrial invasion and loco-regional disease spread
Diagnostic laparoscopy	Partial surgical staging
Sentinel lymph node biopsy	Value to be determined
Genetic counseling	Risk assessment for patient and family

2.2.1 Tissue Biopsy

Prior to initiating conservative management, dilatation and curettage (D&C) is recommended because it better defines the grade of the tumor compared to office endometrial biopsy. Additionally, there might be value in the removal of most of the endometrial cancer cells by the D&C before starting hormonal treatment [16, 17]. Hysteroscopy is a possible alternative to D&C, by allowing direct visualization of endometrial lesions and accurate diagnosis [18]. Although some studies raise the suspicion for cancer spread secondary to peritoneal spillage via the fallopian tube of the medium used for endometrial cavity distension [19], others have not supported the role of peritoneal spillage [20, 21]. Since discrepancies regarding histologic diagnosis are common [22], pathological review by more than one experienced pathologist can be helpful [23].

2.2.2 Imaging

Attempts should be made to rule out myometrial invasion, adnexal involvement and lymph node metastases, which are regarded as contra-indications for conservative management. MRI has proven to be superior to transvaginal ultrasound or CT for determining myometrial invasion [24]. Pooling of 11 studies, comparing T2-weighted imaging and contrast-enhanced magnetic resonance imaging, revealed similar positive predictive values for myometrial invasion of 0.65 and negative predictive values of 0.85 [25]. MRI is used to assess loco-regional disease spread [26], and Sironi et al. [24] reported a sensitivity and specificity of 74% for MR assessment of

superficial myometrial invasion, although the importance of superficial myometrial involvement on response to progestins is not clear.

2.2.3 Additional Invasive Procedures

There is an increased risk of concomitant adnexal involvement in premenopausal patients with endometrial cancer, reaching up to 25% in the series from Cedars Sinai [11]. Consequently, some physicians perform a diagnostic laparoscopy at the time of D&C [27]. With the evolving data on sentinel lymph node biopsy mapping for endometrial cancer, sentinel lymph node biopsy could be considered in selected cases [28].

2.2.4 Genetic Counseling

Women diagnosed with endometrial cancer at a young age are at increased risk for mismatch repair gene mutations associated with Lynch syndrome [29]. Hence, these women should also be referred for genetic counseling [30], as counseling might reveal important implications concerning the risk of adnexal pathology and colon cancer necessitating screening in these young patients and their families.

2.3 Prognostic Factors

Although the majority of carefully selected patients will respond to progestin therapy, there is at present no way to accurately predict who will respond.

Data remain scarce on clinical or pathologic predictors of response to progestin treatment in premenopausal women with atypical hyperplasia and Grade 1 endometrial adenocarcinoma. Park et al. analyzed 148 patients (age ≤40 years) with stage IA, grade 1, endometrioid adenocarcinoma of the uterus who underwent fertility-sparing management using daily oral medroxyprogesterone acetate or megestrol acetate [24]. 115 (77.7%) showed complete response to progestin treatment, and 35 (30.4%) experienced recurrence after a median follow-up period of 66 months. A body mass index (BMI) ≥25 was the only significant factor associated with a failure to achieve cure (odds ratio [OR], 3.00; 95% CI, 1.35–6.66; $p = 0.007$). A BMI ≥25 was also significantly associated with a higher risk of recurrence (OR, 2.14; 95% CI, 1.06–4.31; $p = 0.033$). The use of MPA (compared to MA) (OR, 0.44; 95% CI, 0.22–0.88; $p = 0.021$), continuing maintenance treatment (OR, 0.22; 95% CI, 0.05–0.94; $p = 0.042$), and a previous pregnancy (OR, 0.25; 95% CI, 0.11–0.56; $p = 0.001$) were significantly associated with a lower risk of recurrence [31]. BMI of 30 or higher was also found to be associated with a higher rate of relapse in a study by Yang et al. which included 88 patients with grade 1 endometrial adnocarcinoma or atypical hyperplasia [32]. Another study found that elevated HbA1C was associated with a higher rate of complete response,

whereas PCOS was associated with a lower response rate [33]. Metformin is a biguanide with a reported anti-neoplastic effect and an association with improved relapse-free survival and overall survival in EC patients with Diabetes. Combination of Metformin with MPA was found to have a high complete reponse rate (96%) of EC within 18 months, and low relapse (17.5%). For this combined regimen, BMI >25 was found to be associated with lower relapse rate [34]. Penner el al. looked at the histopathologic features, using a qualitative abnormal endometrial architecture score, comparing pretreatment and follow-up endometrial specimens to identify predictors of resolution [25]. The score is composed of five features: polypoid, cribriform, papillary, budding and back to back endometrial glands. Resolution rates, expressed as the Standardized Resolution Ratio (SRR), were highest in individuals with a low pre-treatment score and a BMI <35 (SRR = 1.48, $p = 0.03$), lower among subjects with a high pre-treatment score (SRR = 0.37, $p < 0.03$), and lowest in subjects whose first follow-up specimen showed persistent complexity, atypia, or carcinoma with adjacent stromal decidualization (SRR = 0.24, $p = 0.002$) [35]. The presence of progesterone receptors (PR) also predicts response to progestin therapy [36, 37]. In one study the response rate was 8% (seven of 86 patients) for patients who were PR-negative and 37% (17 of 46) for patients who were PR-positive ($p < .001$) [38]. In their meta-analysis, Raffone et al., found that PR status had a moderate predictive value for response to progestin therapy and was influenced by the administration route. PR status had a higher predictive value in the Levonorgestrel releasing intrauterine device (LNG-IUD) sub-group, whereas did not have a significant influence in the oral progestin sub-group [39]. Of note, even in the LNG-IUD group the predictive value was not high, since only 50% of resistant cases were PR-negative and therefore PR-status should not be used as an independent predictive factor. In addition to PR-status, PTEN (Phosphatase and Tensin Homolog, a tumor suppressor gene that is mutated in the majority of type I endometrial cancers) and KRAS (oncogene, GTPase protein, that when mutated leads to cancer) status in combination with the progesterone receptor expression in the tumor seemed promising as biomarkers of response [37], however, a meta-analysis which included 376 patients, concluded that PTEN loss was not significantly associated with response to progestin treatment [40]. Further investigations in predictors of response may ultimately lead to personalized treatments for young women with endometrial cancer.

2.4 Types of Progesterone

At present, there is no consensus on the optimal medication, dose, or length of treatment (Table 11.3). In a 2004 review, the most commonly used agents were medroxyprogesterone acetate 500–600 mg (MPA; 44%) and megesterol acetate 160 mg (35%) for at least 3 months [41]. Both regimens appear to have similar response rates. A meta-analysis that included 370 patients suggested that treatment with megesterol acetate was associated with a higher resolution rate [42], but on the

Table 11.3 Fertility sparing options; advantages and disadvantages

Drug	Dose	Advantages	Disadvantages
Medroxyprogesterone acetate	400–600 mg/day for at least 3 months	Well studied	Known side effects
Megesterol acetate	160–320 mg/day for at least 3 months	Well studied	– Known side effects – Might have higher recurrence rate compared to MPA
Progesterone - intrauterine device	20–65 mcg/day	Low systemic toxicity	– Limited data – Intra-uterine placement required
Natural progesterone	200 mg/d, days 14–25	–	Limited data
Hydroxyprogesterone	500 mg/days	–	Limited data
Norethisterone	5 mg/days	–	Limited data
Progestogens at various doses	–	–	Limited data

other hand, the largest study thus far has shown a higher recurrence rate after megestrol acetate compared to medroxyprogesterone acetate [31].

Additionally, treatment has been reported with the levonorgestrel intrauterine device (LNG-IUD) (Mirena^{TDM}) that releases 20 mcg of levonorgestrel per day [43], in combination with hysteroscopic resection [44], medroxyprogesterone acetate [45] or GnRH analogues [46]. Other treatments used include intramuscular 17-hydroxyprogesterone, oral contraceptive pills, norethisterone, dihydrogesterone, and natural progesterone either utilized alone or in a combination of progestin agents [7, 47].

The choice of progestin should be based on measurable outcomes, including efficacy, side-effects, and patient tolerability. Orally administered progestins are not without side-effects, including mood alterations, headaches, weight gain, breast pain and/or tenderness, and increased risk of thrombus formation. Thrombosis is a serious adverse reaction to MPA. It is caused by the inhibitory activity of MPA against plasminogen activator [48]. Thrombosis can be fatal, especially if leading to cerebral infarction, myocardial infarction, or pulmonary embolism. Clotting factors should be checked monthly, and treatment with MPA should be discontinued on detection of clotting abnormalities. A prospective trial using 600 mg MPA [49] reported that the most common side effects were weight gain and liver dysfunction. There were no cases of thromboembolism. Progesterone therapy is contraindicated in the presence of a history of thromboembolism, breast cancer, or hepatic dysfunction. LNG-IUD might be a means of achieving a localized effect within the endometrium while avoiding the adverse systemic effects, in addition to better compliance compared to a daily pill. Indeed, LNG-IUD was found to have a smaller weight-gain effect when compared to oral progestins [50].

There is no consensus regarding the optimal progestin duration. Progestin therapy has an impact on the endometrial cells as early as 10 weeks after initiation of treatment, but current guidelines recommend a minimum of 6 months of treatment

before assessing the response [23, 51]. Obese and anovulatory women have been shown to require longer periods of progestin therapy to attain a complete response, and are more prone to relapse [31, 35].

2.5 *Outcome*

Although the first publication describing hormonal conservative treatment for fertility preservation was published in 1961 [52], the number of publications describing the outcome is still limited, albeit slowly increasing, (Fig. 11.2) and many questions remain. The possibility of publication bias in the studies analyzed should be borne in mind. Studies showing treatment success are more likely to be reported and published than negative trials, leading to overestimating the success rate. In a

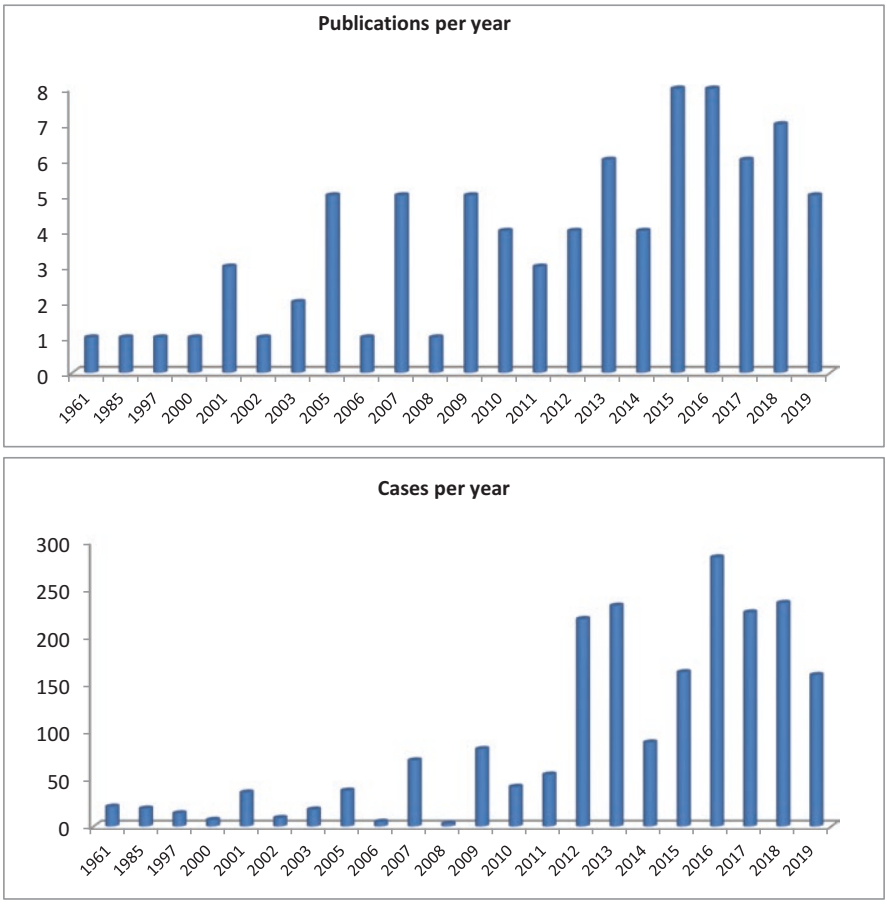

Fig. 11.2 Number of publications and number of reported cases treated conservatively

meta-analysis including 34 observational studies [53], the authors evaluated the regression, relapse, and live birth rates of 408 women diagnosed with early-stage endometrial cancer. The primary studies included the outcome of women with well-differentiated endometrial cancer with 386 women being classified as G1 and 22 women with moderate or poor differentiation (G2 or G3). Half of the studies were prospective cohorts (17 of 34) and the follow-up was more than 5 years in only of the 34 studies. Overall, resolution occurred in 76% (301/408) of reported patients (Table 11.4), and 89 (40.6%) responders relapsed during follow-up. seventy-five-women achieved at least one live birth, yielding a live birth rate of 28%.

Table 11.4 Overview of studies and outcomes of Progestogen Treatment, adapted from Gallos et al. [53] and updated

Study (year, reference)	No. of patients	Regressed (%)	Relapsed (%)	Live births (%)
Bokhman (1985, [92])	19	15 (79)	–	–
Randall (1999, [93])	14	10 (71)	1/10 (10)	3/14 (21)
Kim (2000, [94])	7	4 (57)	2/4 (50)	0/7 (0)
Imai (2001, [95])	14	8 (57)	3/8 (38)	2/14 (14)
Kaku (2001, [22])	12	9 (75)	2/9 (22)	1/12 (8)
Duska (2001, [2])	12	9 (75)	–	–
Wang (2002, [96])	9	8 (89)	4/8 (50)	2/9 (22)
Gotlieb (2003, [97])	13	13 (100)	6/13 (46)	3/13 (23)
Jadoul (2003, [98])	5	3 (60)	0/3 (0)	3/5 (60)
Niwa (2005, [99])	12	12 (100)	8/12 (67)	–
Ota (2005, [100])	12	5 (42)	2/5 (40)	2/12 (17)
Yahata (2005, [101])	8	7 (88)	7/7 (100)	2/8 (25)
Yang (2005, [102])	6	4 (67)	2/4 (50)	2/6 (33)
Le Digabel (2006, [103])	5	3 (60)	1/3 (33)	0/5 (0)
Elizur (2007, [104])	8	8 (100)	3/8 (38)	4/8 (50)
Minaguchi (2007, [105])	18	14 (78)	5/14 (36)	1/18 (6)
Ushijima (2007, [49])	22	14 (64)	8/14 (57)	3/22 (14)
Wheeler (2007, [106])	21	7 (33)	1/7 (14)	–
Yamazawa (2007, [107])	9	7 (78)	2/7 (29)	3/9 (33)
Li (2008, [108])	3	3 (100)	0/3 (0)	–
Eftekhar (2009, [109])	21	18 (86)	3/18 (17)	2/21 (10)
Hahn (2009, [110])	35	22 (63)	9/22 (41)	8/35 (23)
Han (2009, [111])	7	7 (100)	0/7 (0)	5/7 (71)
Signorelli (2009, [112])	11	6 (55)	4/6 (67)	4/11 (36)
Yu (2009, [113])	8	6 (75)	1/7 (17)	0/8 (0)
Mao (2010, [114])	6	4 (67)	0/4 (0)	3/6 (50)
Mazzon (2010, [62])	6	6 (100)	0/6 (0)	4/6 (67)
Minig (2010, [46])	14	8 (57)	2/8 (25)	1/14 (7)
Cade (2010, [7])	16	10 (63)	–	–
Laurelli (2011, [44])	14	14 (100)	1/14 (7)	1/14 (7)
Park (2011, [115])	14	13 (93)	3/13 (23)	13/14 (29)

Table 11.4 (continued)

Study (year, reference)	No. of patients	Regressed (%)	Relapsed (%)	Live births (%)
Perri (2011, [116])	27	24 (89)	9/24 (38)	12/27 (44)
Shirali (2012, [117])	16	10 (63)	0	4/16 (25)
Park (2012, [115])	14	13 (93)	0	–
Dursun (2012 [118].)	43	35 (81)	2/35 (6)	–
Pashov (2012, [119])	11	11 (100)	0	3/11 (27)
Park (2013, [14])	148	115 (78)	35/115 (30)	58/148 (39)
Shobeiri (2013, [120])	8	7 (88)	3/7 (43)	3/8 (38)
Shan (2013, [121])	14	9 (64)	3/9 (33)	1/14 (7)
Parlakgumus (2013, [122])	5	5 (100)	1/5 (20)	1/5 (20)
Wang (2014, [123])	37	30 (81)	15/30 (50)	0
Kudesia (2014, [124])	10	7 (70)	–	2/10 (20)
Pronin (2015, [125])	32	23 (72)	2/23 (9)	–
Hara (2015, [126])	16	11 (69)	9/11 (82)	1/16 (6)
Yang (2015, [32])	51	43 (84)	16/43 (37)	–
De Marzi (2015, [127])	3	3 (100)	1/3 (33)	–
Zhou, (2015, [33])	19	15 (79)	7/15 (47)	2/19 (11)
Wang (2015, [128])	6	6 (100)	0	3/6 (50)
Van Gent (2016, [129])	11	6 (55)	–	–
Baek (2016, [130])	13	7 (54)	4/7 (57)	2/13 (15)
Sato (2016, [65])	32	19 (59)	–	–
Chen (2016, [131])	37	27 (73)	8/27 (30)	5/27 (14)
Reyes (2016, [132])	2	2 (100)	–	–
Mitsuhashi (2016, [133])	19	13 (68)	–	–
Falcone (2017, [134])	28	25 (89)	2/25 (8)	13/28 (46)
Hwang (2017 [15])	5	3 (60)	1/3 (33)	–
Park (2017, [135])	154	111 (72)	43/111 (39)	35/154 (23)
Zhou (2017, [136])	17	15 (88)	1/15 (6.7)	–
Navdeep Pal (2018, [137])	17	5 (29)	–	–
Graul (2018, [138])	18	9 (50)	–	–
Tamauchi (2018, [139])	9	8 (89)	0	–
Giampaolino (2018, [140])	14	11 (79)	2/11 (18)	0
Matsuzaki (2018, [141])	6	5 (83)	2/5 (40)	4/6 (67)
Yamagami (2018, [56])	151	141 (93)	98/141 (70)	–
Mitsuhashi (2019, [34])	42	40 (95)	7/40 (18)	–
Maggiore (2019, [60])	20	16 (80)	8/16 (50)	7/20 (35)
Kim (2019, [61])	35	13 (37)	–	–
Yang (2019, [63])	40	36 (90)	4/36 (11)	–
Total	1511	1156 (76.5%)	363/1048 (34.6%)	219/877 (24.9%)

[a]Only studies that have reported separate outcome for EC and AEH were included

3.6% of patients were diagnosed with ovarian malignancy during follow-up. It is unclear whether these represent concurrent ovarian malignancies or metastatic ovarian involvement from the primary endometrial neoplasm. There were also ten women (1.8%) diagnosed with stage II disease or greater following treatment failure, and there were two deaths reported (0.5%). Another recent systemic review by Gunderson et al. [47] reported oncologic and reproductive outcomes with progestin therapy in women with endometrial hyperplasia and grade 1 endometrial cancer. Forty-five studies with 391 study subjects were identified including 280 women that had grade 1 endometrial adenocarcinoma. The median age for the overall cohort was 31.7 years (range 19–80 years). When stratified by disease type, the durable complete response rate was significantly higher in women with complex atypical hyperplasia (65.8%) compared to those with carcinoma (48.2%; $p = .002$). The rate of initial response in women with complex atypical hyperplasia was also significantly higher (85.6%), than women with carcinoma (74.6%; $p = 0.03$). Disease recurrence was more likely to occur in the carcinoma cohort (35.4%) than the hyperplasia group (23.2%; $p = 0.03$). Further, persistent disease was noted in only 14.4% of women with complex atypical hyperplasia compared with 25.4% of those with carcinoma ($p = 0.02$). Reproductive outcomes did not differ between the cohorts.

In terms of effect on overall survival, Greenwald et al. conducted a population-based study comparing fertility-preservation hormonal treatment to primary surgery in young patients (<45 years) that did not show a significant difference [54].

2.5.1 Repeat Treatment for Recurrence After Complete Response?

Park and colleagues recently published a retrospective multicenter study that shows the safe and effective outcome of re-treating 33 young patients who still wanted to preserve fertility following recurrence after a complete response to progestins [55]. Five of the 33 women failed to respond to a second conservative approach, and another five patients recurred after a second complete response. Three received a third cycle of progestins and two responded again. Five patients delivered six healthy babies following this second conservative approach. The responders were followed for a mean of period of 51 months, no patient died of disease or suffered an adverse outcome. Comparing primary treatment with repeated treatment for recurrence, Yamagami et al. found similar complete response rate for MPA in atypical hyperplasia AH and EC [56]. However, the 5-year recurrence-free survival was lower among the repeated treatment group: 14.0% and 11.2% among patients with AH and G1 EC, respectively, vs. 53.7% and 33.2% among patients with AH and EC, respectively, in the initial treatment group. Pregnancy rates tended to be lower in the recurrent treatment group among AH

patients (11.1% vs. 29.2%; $p = 0.11$), though they were similar for patients with G1 EC (20.8%. vs. 22.7%).

2.5.2 Outcome for Progestin Releasing Intrauterine Devices

Levonorgestrel releasing intrauterine devices (LNG-IUD) are associated with contraceptive efficacy, powerful reduction of menstrual blood volume through suppression of endometrial growth, and accompanying relief of menstrual pain [57]. It has also been shown that the use of LNG-IUD in combination with hormone replacement therapy during or after menopause can prevent endometrial cancer [58]. Evaluating the effect of the LNG-IUD on endometrial hyperplasia, a randomized multicenter trial compared LNG-IUD to oral MPA 10 mg administered for 10 days per cycle or continuous oral MPA 10 mg daily, for 6 months. Regression was observed in all the women in the LNG-IUD group (53/53) and for 96% of the women in the continuous oral group (46/48). Only 69% of the women in the cyclic oral group were responders (36/52).

The efficacy of LNG-IUD in patients with endometrial cancer is presently being investigated. Preliminary data obtained from two separate studies suggests that progestin treatment provided by an IUD in 22 patients with grade 1 Stage I endometrial cancer [44, 59] was followed by a 68% (15/22) complete response after 6 months or longer compared to 72% (73/102) of patients on oral progestin [8]. No relapses or progressions were reported after 6–71 months of follow-up. Fertility outcomes were not reported. In addition, a few more studies, all with a small number of patients suggests that treatment with intrauterine progestin has a similar [45, 46] or slightly improved [60] efficacy when compared to oral progestins. The efficacy of combined MPA and LNG-IUD treatment was evaluated in a small prospective Korean study including patients with G1 EC. Among the 35 patients analyzed, the complete response rate at 6-month was only 37.1%, while partial response was shown in additional 25.7% of cases [61]. Large prospective trials for LNG-IUD are presently underway in order to clarify some of the unresolved issues (Table 11.5).

2.5.3 Combined Progestins Treatment and Hysteroscopy

By allowing direct visualization, hysteroscopic resection of endometrial lesions is considered by some as a more targeted approach. Mazzon et al. have reported a series of six patients who underwent hysteroscopic resection of the tumor along with resection of the adjacent endometrial margins and underlying myometrium. Patients whose tumor was confined to the endometrium and had negative margins were treated by 160 mg/d megestrol acetate starting 5 days post operatively and continued for 6 months. All patients were diagnosed with grade 1 endometrioid adenocarcinoma post hysteroscopy and were PR-positive. At the 12-month follow-up all patients

Table 11.5 Progestin & endometrial cancer, ongoing clinical trials and time of expected results

ClinicalTrials.gov Identifier #	Title	Estimated study completion date
NCT02064725	A Phase II Study of Sodium Cridanimod in Conjunction With Progestin Therapy in Patients With Progesterone Receptor Negative Recurrent or Persistent Endometrial Carcinoma	July 2018
NCT00788671	A Phase II Study of the Levonorgestrel Intrauterine Device (Mirena) to Treat Complex Atypical Hyperplasia and Grade 1 Endometrioid Endometrial Carcinoma	November 30, 2019
NCT02035787	Metformin with the Levonorgestrel-Releasing Intrauterine Device for the Treatment of Complex Atypical Hyperplasia (CAH) and Endometrial Cancer in Non-surgical Patients	March 2020
NCT01686126	A Phase II Randomised Clinical Trial of Mirena® ± Metformin ± Weight Loss Intervention in Patients With Early Stage Cancer of the Endometrium	December 2020
NCT02990728	Mirena® ± Metformin as Fertility-preserving Treatment for Young Asian Women With Early Endometrial Cancer	March 2020
NCT03463252	Value of Levonorgestrel-Releasing Intrauterine System (LNG-IUS) in the Fertility-preserving Treatment of Atypical Endometrial Hyperplasia and Early Endometrial Carcinoma	December 31, 2020
NCT03241914	Megestrol Acetate Plus LNG-IUS to Megestrol Acetate in Young Women With Early Endometrial Cancer	July 3, 2020
NCT02397083	Phase II Study of the Levonorgestrel Intrauterine Device Alone or in Combination with the mTORC1 Inhibitor, Everolimus, for the Treatment of Complex Atypical Hyperplasia and Stage La Grade 1 Endometrial Cancer	September 30, 2027

were free from recurrence [62]. A recent observational study included 120 patients with AH and 40 patients with EC who underwent comprehensive hysteroscopic evaluation and resection followed by progestogen treatment [63]. Megestrol acetate was given in 154 out of 160 patients, and 69 of them also received metformin. At 12-month follow-up, the cumulative regression rate was 89.5% (136/152), including 104 AH patients (88.9%) and 32 EC patients (91.4%). Twenty seven out of sixty (45%) patients that attempted to conceive have achieved at least one pregnancy. BMI <25 kg/m2 and lesion size ≤2 cm were significantly associated with shorter treatment duration to achieve CR.

Comparing various fertility-preservation therapies, a recent meta-analysis found that hysteroscopic resection combined with hormonal therapy in young patients with AH or EC (a total of 95 patients) had a significantly higher regression rate and a lower recurrence compared oral progestogens alone (98.06% and 4.79% vs. 77.20% and 33.38% [64]. Compared to treatment with LNG-IUS alone, there were no significant differences in regression or recurrence rates, however, hysteroscopic

resection had a higher pooled live birth rate. Of note, that one study included seven patients that were treated, in addition to hysteroscopy, with a GnRH analog and not progestogens.

2.6 Follow Up

In view of the high relapse rates (35–41% [47, 53]), the frequency of concomitant adnexal malignancy, and the risk of upgrading of the cancer, close follow up is essential. Current guidelines recommend re-evaluation every 3–6 months [23, 51]. According to NCCN guidelines, in patients with persistent endometrial carcinoma after 6 months of hormonal therapy, pelvic MRI should be performed to exclude myometrial invasion or nodal/ovarian metastasis prior to considering further fertility-sparing therapy [51].

Thinning of the endometrium as seen on transvaginal ultrasound is associated with an increased chance of responding to progestin therapy [49, 65]. However, the predictive value is insufficient to negate endometrial sampling. It is important to note that the diagnostic accuracy of endometrial aspiration biopsy (pipelle) while the LNG-IUD is in place may not be as accurate as dilatation & curettage (D&C) [66].

It is sensible to recommend staging hysterectomy with bilateral salpingo-oophorectomy once the family is completed or if fertility-sparing treatment fails, either due to failure of regression or relapse. When regression is acheived, some recommend assisted reproduction to maximize the chances of a live birth and decrease the time to definitive treatment. Additionally, immediate assisted reproduction avoids prolonged unopposed estrogen stimulation, which could cause relapse.

The need for oophorectomy together with hysterectomy remains debatable in view of the risk of concomitant ovarian involvement [11]. One series based on the database from the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) suggested that there was no increase in cancer related mortality associated with ovarian preservation in young women with early stage endometrial cancer [67]. Other studies reaffirmed this conclusion suggesting that conserving ovaries in early stage endometrial cancer has no effect on either recurrence or survival [68, 69]. Therefore, even though oophorectomy is the mainstay of endometrial cancer management, current guidelines suggest considering ovarian preservation [23, 51].

3 Progesterone in Advanced Endometrial Cancer

Progesterone has long been used for advanced or recurrent endometrial cancer. Kauppila [70] reviewed 1068 patients treated with medroxyprogesterone acetate (MPA), megestrol acetate, or hydroxyprogesterone caproate in different trials, and

found an overall response rate of 34%, with a mean duration of response ranging from 16 to 28 months and a mean survival ranging from 18 to 33 months. However, subsequent clinical studies, based on more stringent criteria for response assessment, reported lower response rates ranging from 11% to 16%, without any significant difference according to the type of progestin used [71, 72]. When MPA 200 or 1000 mg/day was administered to 229 patients with advanced or recurrent endometrial cancer, the low-dose group (200 mg/day group) showed better outcomes (17% complete remission and 8% partial remission) than the high-dose group (1000 mg/day group) (9% complete remission and 6% partial remission) [38]. Median progression free survival and overall survival rates were 3.2 and 11.1 months, respectively, for the low-dose group, and 2.5 and 7 months for the high dose group, with no differences in toxicity noted between the two arms. This GOG (American Gynecologic Oncology Group) trial showed that the response rate was higher in the low-dose group than in the high-dose group with the responses being particularly favorable in G1 and PR-positive cases [38]. A recent meta-analysis found 13 studies in which progestins were used as first-line treatment for advanced endometrial cancer [73]. The response rate was 23.3%, with 12.0% achieving a complete response and 45.8% experiencing any clinical benefit from therapy with a higher response rate for PR-positive tumors compared to PR-negative (35.5% vs. 12.1%, respectively). Median time to progression was 2.9 months and the overall survival was 9.2 months. The combination of progesterone therapy and chemotherapy has been tested in small series and has not shown any clinical advantage compared with either treatment alone [36]. The activity of progestins in this condition is often limited by the frequent down-regulation of PR within the target tissues, resulting in a relatively short duration of response. Tamoxifen can increase PR content in endometrial cancer tissues, but clinical studies on alternating treatment with tamoxifen and progestin have given conflicting results (Table 11.6) [74–76]. A GOG randomized trial found that the addition of megestrol acetate and tamoxifen to Temsirolimus for endometrial cancer did not improve activity, and was associated with increased risk for venous embolism [77]. Therapeutic strategies targeted at enhancing PR expression are currently being investigated worldwide and could potentially improve the clinical outcome of endometrial cancer patients [78, 79].

4 Novel Approaches

4.1 Fourth-Generation Progestins

The fourth-generation progestin dienogest is an effective means of treating endometriosis. At present, its anti-tumor activity is also attracting close attention following a report that dienogest suppresses the proliferation of endometrial cancer-derived cell lines *in vitro* which fail to respond to other progestins such as medroxyprogesterine acetate (MPA) [80]. In vivo, dienogest was found to have anticancer activity

Table 11.6 Hormonal therapy with progestins and tamoxifen in advanced or recurrent endometrial cancer

Study	Hormonal agent	Patients	CR (%)	PR (%)	OR (%)
Thigpen JT [38]	MPA (200 mg/day)	145	17	8	25
	MPA (1000 mg/day)	154	9	6	15
Thigpen JT [142]	TAM	68	4	6	10
Pandya KJ [76]	MA	20	5	15	20
	TAM/MA	42	2	17	19
Whitney CW [74]	TAM/MPA	58	10	23	33
Fiorica JV [75]	TAM/MA	56	21	5	26

CR Complete response, *PR* Partial response, *OR* Overall response, *MPA* Medroxyprogesterone acetate, *TAM* Tamoxifen, *MA* Megestrole acetate

comparable to MPA in a mouse model [81]. The mechanism for antitumor activity of dienogest appears different to conventional progestin preparations. It has been shown to suppress neovascularization [82, 83], the cell cycle [84] and to inhibit PGE2 formation through selective antagonist activity on the PR [85].

4.2 Progesterone Receptor Expression and Reversal of Progesterone Resistance

Many patients with endometrial cancer are resistant to progestin therapy, apparently associated with the absence of the progesterone receptor (PR). Studies aimed at the restoration of PR expression in endometrial cancer have been conducted at the gene and protein levels. Several preclinical studies have been carried out on the control of epigenetic mutations (hypomethylation or hypermethylation) often seen in cases of endometrial cancer for the purpose of stimulating apoptosis and restoration of susceptibility of the cancer to chemotherapy [86]**.** PR gene hypermethylation, responsible for the disappearance of the PR in certain endometrial cancers, can be reversed by DNA methyltransferase (DNMT) inhibitors, shown to stimulate re-expression of the PR at both the mRNA level and the protein level [87–89]. Histone deacetylating (HDAC) inhibitors increase acetylation of histones, thereby unwinding DNA and exposing promoter regions for transcription of genes. Yang et al. have shown that HDAC inhibition using LBH589 was more effective than DNMT inhibition (using 5-aza-deoxycytidine) in restoring PR expression in EC cell lines, while the combination of the drug resulted in contradicting effects depending on the cell line [90]. Epidermal growth factor receptor (EGFR) has also been implicated as a factor involved in progesterone resistance. The EGFR has been detected in histological specimens and cell lines of endometrial cancer and is known to be overexpressed in endometrial cancer, although its role in resistance to progestin has not been clarified. One study analyzed differences in EGFR function and resistance to

progestin in relation to the presence or absence of PR expression in endometrial cancer, reporting that EGFR was detected in 60% of PR positive specimens and 90.5% of PR negative specimens. Furthermore, when further EGFR expression was stimulated in Ishikawa cells (an endometrial cancer cell line), the susceptibility to progestin decreased, accompanied by a reduction in PR expression [91]. AG1478 (a specific inhibitor for EGFR tyrosine kinase) effectively suppressed the proliferation of EGFR overexpression in endometrial cancer cells [91]. On the basis of these findings, it is assumed that excessive expression of EGFR in endometrial cancer cells can reduce the susceptibility to progestin therapy. Therefore, inhibitors specific to EGFR tyrosine kinase may be effective against endometrial cancer resistant to progestin therapy.

According to these data, studying epigenetic modulation in combination with progestins therapy should proceed toward high quality correlative studies in order to further understand the impact of changes in epigenetic regulation on response to treatment.

5 Conclusions

Hysterectomy remains the gold standard for patients with endometrial cancer, but may not be an acceptable option for young women who wish to preserve their fertility or for women with severe co-morbidities compromising surgical survival.

Cohort studies with progestin-based fertility-sparing treatment followed by assisted reproduction show a high chance of disease regression and encouraging live birth rates for patients with early-stage endometrial cancer. The risk of disease relapse during follow-up is significant and women wanting to pursue this treatment need to undergo thorough counseling.

Progestins are probably not curative, because the underlying cause usually persists. Based on our present understanding of the disease, hysterectomy is advocated once family planning is complete. The disease remains confined to the endometrium at the time of hysterectomy in the overwhelming majority of patients, and the outcome and survival are for the vast majority not jeopardized by conservative treatment [53].

Although hormonal management of complex atypical hyperplasia and low-grade, apparent early-stage endometrial carcinoma has been utilized for over 50 years, many questions remain unanswered. Large prospective trials are presently underway to clarify some of the unresolved issues, including the role for levonogestrel containing intrauterine devices (Table 11.5). Combined hysteroscopic resection with progestin treatment might have greater efficacy, though larger studies with longer follow-up period are required prior to changing routine management. Similar to other specific cancer-related populations, an international registry would further advance our understanding. Investigations on novel therapeutic options targeting the underlying causes and molecular pathways are eagerly awaited.

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Chapter 12
Progestogens and the Menopause

Eitan Pe'er

1 Introduction

Progesterone is an essential hormone during the reproductive years. It does-not have any physiological role during menopause. Yet many synthetic progestogens, are in clinical use during the menopause and post menopause, most notably as adjunct to estrogen replacement therapy. Progestogens used in menopausal hormone therapy (MHT) have a common class effect- preventing endometrial hyperplasia induced by by the estrogenic component of MHT [1]. But, the synthetic progestins used nowadays in MHT also have different side effects. This is mainly due to their different actions on gluciogorticoid receptor (GR), mineralocortocoid receptor (MR) and androgen receptor(AR) [2]. In recent years, large clinical trials have shown that the association of progestins with estrogen in HRT might raise the risk of breast cancer [3, 4], but this risk was not confirmed in ongoing clinical studies which have not shown that trend [5]. As shown in other chapters in this book, there is no "class-effect" of progestogens. Each progestin has its own bio-characteristics regarding breast cancer as in other clinical conditions.

In recent years clinical and new basic data have clearly shown that progesterone has many other properties and functions beside reproduction and pregnancy. Progesterone has been shown to have neuroprotective effects [6] and is used in various brain injuries [7].

Progesterone has both genomic and non-genomic actions. The genomic action is the classical reaction whereby the hormone progesterone, acting as a ligand, connects to its receptor in the nucleus and initiates new mRNA protein synthesis. This is a relatively slow process. The rapid actions of progestogens are due to non-genomic actions in which intracellular signaling pathways are activated resulting in alteration of ions fluxes and intracellular calcium concentration within seconds [8].

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H. J. A. Carp (ed.), *Progestogens in Obstetrics and Gynecology*,
https://doi.org/10.1007/978-3-030-52508-8_12

The non genomic actions also induce second messengers, such as cyclic nucleotides and extracellular regulated kinases [9]. These actions are specific to each individual progestogen. As progestogens differ in actions, proper selection is necessary for successful therapy in treating menopausal symptoms or reducing the risk of diseases associated with the menopause.

The new progestogens (by definition progestins) have specific clinical effects related to their interactions not only with the progesterone receptors (PR-A, PR-B), but also with the other steroid receptors: –estrogen receptor (ER), GR, AR and MR. These interaction may induce either activation, or inhibition, of their biological activity. All progestins bind to PR, but each has a distinctive profile of activity on the other steroid receptors [10]. The ideal progestin should not have androgenic glucocorticoid or mineralocorticoid effects, thus preventing water retention, acne, decrease in HDL and breast cell proliferation.

2 Progestogens and Osteoporosis

Progestogens alone have a very limited effect on bone mineral density (BMD). But, pretreatment with estrogen for 4–7 days has been shown to induce progesterone receptors in osteoblasts [11]. Hence, the beneficial effect on BMD, in all clinical trials in which progestogens were added to estrogen.

Medroxyprogesterone acetate (MPA) decreases BMD, both in the hips and spine, in the first 2 years of use, followed by a slight increase [12]. The effect of norethisterone acetate (NETA) with a low dose of 17β-estardiol on BMD has been investigated in a randomized placebo control study [13]. There was a significant increase in BMD both in the lumbar spine (5.2%) and the hip (3.1%) compared to the placebo group (−0.9%). Serum concentration of osteocalcin decreased by approximately 34%, bone-specific alkaline phosphatase decreased by about 30%, and C- terminal propepetide of type I collagen decreased by 20%.Norethisterone is a synthetic progestin derived from 19-nortestosterone. It binds and activates the progesterone receptor twice as much as progesterone itself, with low androgenic and estrogenic activities attributed to its metabolites [14]. NETA alone has been studied in male castrated mice. In this study NETA alone was found to have a slightly protective effect against bone loss [15]. Controversially, inhibition of the nuclear progesterone receptor has been found to augment bone mass, resulting in higher BMD [16].

However, some progestogens may inhibit estrogen's ability to increase serum levels of 1,25-dihydroy vit D. The consequences of this inhibition are to antagoniz estrogen's beneficial effects on the bone [17]. The effects of MHT containing either P4 progestins or MPA appear to be similar om bone mineral density (BMD) both in the spine and hip [18, 19].

3 Progesterone, Hot Flushes and Night Sweats

Nightly micronized progesterone (300 mg) has been shown to cause an overall decrease in the number of daily hot flushes and night sweats by 59% in a randomized controlled study [20]. Extension of this study has shown that micronized progesterone is also effective for severe vasomotor symptoms and that progesterone withdrawal is not followed by a rebound increase in vasomotor symptoms [21]. A similar effect has been shown for medroxyprogesterone acetate (MPA) 10 mg/day. Prior et al. [21, 22] carried out a randomized double-blind trial in women after overiectomy. Patients received either conjugated equine estrogen (CEE), (0.6 mg/day) or MPAfor 1 year. MPA was found to be equivalent to CEE in the control of vasomotor symptoms in women treated immediately following the surgery [21]. Oral progestins have been shown to be effective for vasomotor symptoms in several randomized placebo controlled trial [23, 24].

Estrogen alleviates hot flushes by lowering levels of serotonin and noradrenaline in the brain [24]. Progestogens may act in a different manner. Progesterone acts on the hypothalamus changing the frequency of LH pulses, increasing basal temperature and stimulating respiration. Therefore progesterone and progestins have various effects on the hypothalamus, from which hot flushes are thought to generate [25].

There is now substantial evidence that the kisspeptin, neurokinin B and dynorphin (KNDy) neurons mediate estrogen negative feedback on LH secretion. They also relay progesterone inhibition and modulation of pulsitile GnRH secretion [26, 27]. Recent data have shown that progesterone also can antagonize receptors for neurokinin 3, improving vasomotor symptoms and allievating hot-flushes [28].

4 Progesterone and Venous Thromboembolism

Progestins, when given alone (e.g progestin only contraceptive pills), carry little, if any, risk of VTE. Randomized controlled studies and meta-analyses of observational studies suggest that the risk of venous thrombo-embolism (VTE) is higher among users of combined estrogen and progestogen than among users of estrogen alone. Oral estrogens increase the VTE risk while transdermal estrogens appear to be safe with respect to thrombotic risk [29, 30]. However, MPA, by activating glucocorticoid receptors, potentiate the vascular effects of thrombin [31]. The ESTHER study (Estrogen and Thromboembolism Risk) looked into the risk of VTE in French postmenopausal women treated with HRT. This study was the first to establish a differential association of VTE risk related to progestogen use. The results were irrespective of the route of estrogen administration. Micronized progesterone and pregnane derivates were reported to be safer with regard to VTE risk [32], however, the norpregnane derivatives were associated with a significant increase in VTE risk [33]. The norpregnanes are potent progestogens with antiestrogenic activity. Women

suffering from hyperestrogenic effects, such as breast tenderness or endometrial hyperplasia are more likely to benefit from this norpregnane progestogens.

5 Progesterone and the Brain

Neurosteroids such as pregnenolone, progesterone and estrogen are synthesized de novo in the brain and neural tissue [31]. Enzymes required for the conversion of cholesterol tp pregnenolone are widely distributed in the brain. The neuroactive metabolite is allopregnenolone. Progesterone is metabolized in the brain by 5α-reductase to 5α-dihydroprogesterone. This in turn is further metabolized by 3α-hydroxysteroid dehydrogenase to the neurosteroid allopregnanolone. PR is present in many brain regions, including the hippocampus, frontal cortex, hypothalamus and cerebellum [34].

The neurosteroids are modulatory ligands for a variety of neurotransmitters and nuclear steroid hormone receptors. Allopregnanolone crosses the blood-brain barrier. It has been shown in rodents, that allopregnanolone is an efficacious proliferative agent (both in vitro and in-vivo studies) [35]. It also decreases amyloid protein in human neural stem cells [36]. Allopregnanolone induces neurogenesis that correlates with restoration of learning and memory functions. In a mouse model of Alzheimer's disease' chronic allopregnanolone adminsration was found to promote neurogenesis, oligodendrogenesis and reduced both inflammation and beta-amyloid burden [37]. Allopregnenolone also restores hippocampall-depentend learning and memory, and neural progenitor survival in aging wild type mice [38].

Progesterone metabolites exert considerable sedative effects after binding to the GABAA receptor [39]. GABA-A receptor appears to be primarily responsible for the action of neurosteroids in the brain [40]. The GABA receptor is the principal inhibitory neurotransmittor receptor in the brain. Both progesterone and allopregnenolone are positive modulators of GABA receptors. Fluctuation in the neurosteroid modulation modifes GABAergic signaling. Both have been implicated in a variety of physiological and pathophysiological condition, including stress, sexual behaviours, depression, anxiety, and seizures [41]. Allopregnenolone and P4 progestins significantly improve sleep efficiency and decrease time spent awake after sleep onset. MPA does not improve sleep parameters [42]. Neurosteroids such as pregnanolone affect synaptic functions and myelinization. Their action is mediated through inhibition of the glycogen synthase kinase (GSK-3β) pathway (much as most bipolar mood stabilizers such as lithium) [43]. Neurosteroids are modulatory ligands for a variety of neurotransmitters and nuclear steroid hormone receptors. In a mice model with experimental autoimmune encephalomyelitis it has been shown that both the spinal cord and the brain are sensitive to the protective effects of progesterone. Progesterone has been shown to reduce inflammatory reactions

commonly seen in MS, by the direct effect of progesterone on astrocytes and microglia [44].

5.1 Progesterone in Alzheimer's Disease

Estradiol increases the expression of the progesterone-synthesizing enzymes. Estradiol increases this expression in the hypothalamus, and especially in the astrocytes. Astrocytes are the most active steroidogenic cells in CNS and contribute to neuro-protection [45]. Treatment with different types of progestogens found that these compounds may promote neurogenesis, neural survival, myelinization andincreases memory [46]. There is some data that suggests that allopregnanolone may maintain the regenerative ability of the brain and also can modify the progression of Alzheimer's disease [47]. Progesterone has been shown to improve impaired axonal transport, a key event of the aging brain. Reduced axonal transport has been proposed to play an early and causative role in the development of Alzheimer's disease. In mouse models, reduced axonal transport may lead to aberrant amyloid-β peptide formation and subsequently to neurodegeneration [48]. In a cross-sectional analysis of 271 post menopausal women within 6 years of menopause (mean age 55), concentration of progesterone were significantly and positively associated with composite neuropsychological measures of verbal memory and global cognition' but not with executive functions [49]. In the same study, progesterone levels were unrelated to cognition in 372 postmenopausal women more than 10 years after menopause (mean age of 65).

The timing effect (=Window of Opportunity) of the effect of MHT vs. E2 alone, or placebo, was studied in the ELITE-Cog trial. Compared to placebo, E2/P4 was found to have no negative effects on verbal memory, executive functions or global cognition after 2.5 years of treatment, when treatment was initiated 6–10 years after menopause [50].

Studies in rat models suggest differential actions on brain mitochondrial function of MPA compared with other progestins and progesterone. This is relevant to neurological health in pre and post menopausal women. MPA antagonizes estrogen up-regulation of brain mitochondrial function, whereas progesterone does not [51, 52].

In a large case control Finish study of 84,739 patients with Alzheimer's disease, compared to control taking no MHT. The use of estradiol only, or oestrogen/progesterone therapy was found to slightly increase the risk of Alzheimer's disease. The risk was also increased in womwn starting MHT before the age of 60, but using MHT for more than 10 years. This increase in risk was not related to different progestogens used (norethisterone acetate, medroxyprogesterone acetate 'tibolone or other progestins. They report an excess of 9–18 cases of Alzheimer's disease per 10,000 women aged 70–80 [53].

5.2 Traumatic Brain Injury (TBI)

In TBI, whether post menopausal or not, the use of progesterone was found to be effective in reducing brain damage. This subject is fully discussed in Chap. 14. In brief, data is available showing that progesterone reduces edema, restores blood-brain barrier, protects against secondary neuronal death and promotes behavioral recovery after TBI [54, 55]. A phase II clinical trial, the ProTECT study of 102 patients reported more than 50% reduction in mortality in mortality in severe TBI and a statistically significant improvement in functional outcome in patients with moderate BTI, when treatment was administered no later than 2 h after sustaining TBI [56]. However, these results were no longer positive when the number of patients was increased to 882 in a subsequent multi-center study [57]. Additionally, a metaanalysis of five controlled studies [58] failed to find a beneficial effect. Progesterone has also been described to have antioxidant effects [59] which could contribute to neural survival following injury.

6 Cardiovascular

Progesterone appears to be beneficial in women post myocardial ischemia. Four weeks of treatmeny with E2 (2 mg/day) improved exercise time after myocardial ischemia, coronary artery disease and/or previous myocardial infarction. When transvaginal progesterone was added to the E2, there was further improvement. However, the addition of oral MPA did not show benefit [60].

The established effects of estrogen on lipid profile (i.e increase in HDL and triglycerides' decrease in total cholesterol and LDL) are not affected by the addition of P4 progestins that have no androgenic activity [31].

In the KEEPS substudy on recently post menopausal healthy women who received oral CEE or transdermal E2 combined with oral micronized progesterone, there was no change in endothelial function [61]. Actually, endothelial function was improved and inflammation markers decreased in recently menopausal women who received 3 months of transdermal E 2 plus cyclic oral micronized progesterone compared to non-users [62]. Studies on endothelial nitric-oxide (NO) production, a marker for vasodilation, suggest that MPA has no effect on NO production. However, progesterone and drospirenone did increase NO production [63, 64].

Finally, the subanalysis of the WHI cardiovascular data published in the NEJM had shown the importance of the time interval between commencement of therapy and the time of the menopause. In women receiving HRT less than 10 years from menopause, the hazard ratio for CHD was 0.89 compared to placebo. However, in women starting HRT at more than 20 years from the menopause, there was a hazard ratio of 1.71 [65].

7 Conclusions

Progesterone and progestogens have a significant role in various clinical situations throughout life. As the novel actions of progestogens are elucidated it is clear that these hormones have influence on the outcome of many clinical conditions. It is important to bear in mind that there is no class effect of all these compounds. Each has its own clinical, biochemical and molecular specific effects. In depth knowledge of the physio-pathological effects of each progestogen will enable their better use in many clinical conditions such as hot flushes, brain trauma, sleep disorder and more. The most serious clinical side effects are the raised risk of breast cancer associated with some progestogens (e.g MPA) but not with others such as micronized progesterone. MPA has also been shown to inhibit some of the beneficial effects of estradiol on the CNS. The fact that numerous coregulators affect the end result of ligand-progesterone receptors (both nuclear and membrane) indicates to the complexities of progesterone/progestogens actions. Receptor affinity alone does not determine potency. Our present understanding is that the affinity, potency and efficacy of progestogens are substantiallydifferent between the different types of progestogens and are tissue specific. Prgestogens exhibit considerable variations in their potencies and efficacies as well as the resulting extent of agonist, partial agonists or antagonists responses via other steroid receptors, namely ER, AR, GR and MR.

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Chapter 13
Progestogens and Autoimmunity

Abraham Tsur, Grant C. Hughes, and Yehuda Shoenfeld

1 Sexual Dimorphism in Autoimmunity

The human immune system exhibits sexual dimorphism [1]. Approximately 80% of patients affected by an autoimmune disease (AD) are women [2]. The female to male ratio may vary from 9:1 in systemic lupus erythematous (SLE), Sjögren's syndrome and autoimmune thyroid disease, to 3:1 in multiple sclerosis (MS) and rheumatoid arthritis (RA). Few ADs are more common in males; these include the spondyloarthropathies, autoimmune diabetes and guillain-Barré syndrome [3, 4]. Moreover, men and women show different susceptibilities to allergy and infection, and these differences are influenced by hormonal status [5, 6].

The immunomodulatory effects of sex hormones are a major factor leading to the sexual dimorphism described above [7]. Other non-hormonal factors include genetic differences conferred by sex chromosome complement, specifically genes encoded on the X [8] and Y [9] chromosomes, microchimerism [10], and gender related behavioral factors.

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H. J. A. Carp (ed.), *Progestogens in Obstetrics and Gynecology*,
https://doi.org/10.1007/978-3-030-52508-8_13

The hypothesis that sex hormones are a main player behind the sexual immune dimorphism is supported by the observation that many ADs appear, fluctuate or resolve coincident with major changes in sex hormone status, e.g., at puberty, within the menstrual cycle, during pregnancy and puerperium, and following hormone replacement therapy (HRT) or hormonal contraception treatment [3]. Moreover, the risk of certain allergic diseases and infections is tied closely to hormonal status.

2 The Mechanism Mediating the Immunomodulatory Effects of Progestogens

2.1 Progestogen Signaling

Progestogen receptors are not limited to organs of the reproductive system. Both intracellular (nuclear) progesterone receptors [11] and membrane progesterone receptors are expressed in cells of the immune system [12].

2.2 Innate and Adaptive Immunity

The immune defense responses are grouped under the innate and adaptive immune system. The innate system provides immediate protection against microbial invasion while the adaptive system develops in response to infection and provides more specialized defense against specific infections. Progestogens modulate inflammation, immunity and autoimmunity through direct actions in cells of the innate and adaptive immune systems.

2.2.1 The Innate Immune System

Upon recognizing infection and other triggers, cells of the innate immune system (e.g., dendritic cells and macrophages) release inflammatory mediators and prime the adaptive (memory) response by presenting antigens to immature T cells and B cells. Progestogens appears to program dendritic cells that favor the differentiation of Tregs, but not Th1 or Th17 cells [13]. Importantly, progestogens suppress production of several pro-inflammatory cytokines (e.g., IL-1β, TNF-α, IL-6 and IL-23) involved in the pathogenesis of RA. This modulation, in concert with up-regulation of endogenous inhibitors of IL-1β and TNF-α, could be an important mechanism of pregnancy-induced remission of RA and MS [14, 15]. Concurrently, the anti-inflammatory effects of progestogens may increase the risk of certain infections during pregnancy. Suppression of the innate anti-viral cytokine IFN-alpha by increased serum levels of progestogens may explain why pregnant women, or

women using medroxyprogesterone birth control, have significantly increased risk of acquiring HIV [16, 17].

2.2.2 The Adaptive Immune System

T cells protect against infection by directing the elimination of infected cells and by providing maturation signals to B cells. B cells in turn produce antibodies (Abs), which can neutralize and kill pathogens. In AD, healthy tissues are attacked by T cells and self-reactive auto-antibodies (autoAbs). A subset of T cells, T helper (Th) cells, is strongly regulated by progestogens. During pregnancy, high levels of progesterone appear to suppress the development and functions of Th1 and Th17 cells while facilitating the development and functions of Th2 cells. Th1 cells are important for defense against intracellular pathogens, but one of their effector molecules, interferon-gamma, may be harmful to the developing fetus [18, 19]. Th17 cells, characterized by production of the pro-inflammatory IL-17 cytokine, are believed to be involved in the chronic inflammation of RA and MS [20], and their activation is associated with recurrent pregnancy loss [21]. During pregnancy, progesterone appears to foster the induction of regulatory Th cells (Tregs) [22], which may prevent harmful maternal immune responses against the feto-placental unit [23]. Thus, during pregnancy, progesterone contributes to a shift in the maternal immune system toward increased Treg/Th2 activity and reduced Th1/Th17 activity. While this shift may contribute to the remission of RA and MS during pregnancy, it also appears to mediate the increased risk pregnant women have for infection with select intracellular pathogens such as *Listeria monocytogenes*, HSV-2 and HIV [24, 25].

Progesterone and estrogen have important effects on B cell differentiation and effector functions. Progesterone suppresses immunoglobulin class switch recombination and somatic hypermutation. These two processes are required for B cells to produce potent protective Abs potent pathogenic autoAbs. This mechanism may also mediate progesterone protection from certain autoAb related ADs. Interestingly, estrogen enhances these same pathways, suggesting that progesterone-estrogen balance is an important determinant of outcomes in Ab-mediated responses [26]. An animal study has demonstrated that the nuclear progesterone receptor suppressed the emergence of class switched IgG autoantibodies in aged female lupus-prone mice [27]. In addition, during pregnancy, progesterone and other pregnancy hormones might induce remission of RA by altering post-translational glycosylation of autoAbs, rendering them less capable of inducing inflammation [28, 29].

3 Progestogens Effect on Specific Autoimmune Diseases

Progesterone levels vary at different phases in a woman's life. An initial rise occurs at puberty with the start of ovulation. Progesterone levels fluctuate with each subsequent cycle, peaking in the mid-lutea phase, and returning to basal levels during the

estrogen-dominant follicular phase. A more prominent and prolonged rise in progesterone (and estrogen) levels occurs during healthy pregnancy. After menopause, progesterone levels decline back to pre-puberty levels. These physiological changes represent an opportunity to examine the effects of progesterone levels on immunity and autoimmune diseases.

3.1 Rh Rheumatoid Arthritis (RA)

Several studies have shown that pregnancy leads to improvement of RA in half to three quarters of patients, followed in many cases with a postpartum flare [30, 31]. A systemic review and metanalysis of ten studies including 237 patients corroborated that disease activity improved in 60% of patients during pregnancy, followed by a postpartum flare in 46.7% of women [32]. In addition, it has been demonstrated that during the high progesterone state of the luteal phase of the ovulatory cycle there is a subjective improvement in morning stiffness and pain of RA patients [33]. As mentioned above, high systemic progesterone levels during pregnancy may contribute to remission of RA via enhancement of Th2 and Treg activity, decrease of Th1 and Th17 production, and inhibition of the inflammatory cytokines IL-1β, IL-6, TNF-α and IL-23. Interestingly, it has been demonstrated that the described improvement of RA during pregnancy occurred more frequently (75%) in women without anti-citrullinated protein antibodies (ACPA) and rheumatoid factor (RF) than in women with these autoAbs (39%) [34].

3.2 Multiple Sclerosis (MS)

During the second and third trimesters of pregnancy, there is a decrease in the frequency of MS relapses, followed by an increase in the relapse rate for up to 6 months postpartum. Reported effects of the menstrual cycle on MS are inconsistent [35]. Like with RA, progesterone may contribute to pregnancy remission of MS through suppression of Th1 and Th17 activity. In addition, progestogens may protect against neuronal damage via non-immunologic mechanism, such as promoting myelin repair [36]. In the POPART'MUS study high doses of progestin were given immediately after delivery and continuously during the first 3 months post-partum. In 2009, 126 patients had been enrolled and 107 patients had completed the protocol, although results have not yet been published [37]. However, other hormonal changes during pregnancy may also modulate the immune responses to MS. Human chorionic gonadotropin (hCG) significantly increases in pregnancy altering dendritic cell activity, reducing T-cell activation and cytokine production, and stimulating Treg cell recruitment to the fetal–maternal interface [38].

3.3 Systemic Lupus Erythematous (SLE)

In contrast to RA and MS, SLE disease does not appear to remit during pregnancy; in fact, disease flares are common during pregnancy, ranging between 25–60% [39–41]. Some non-hormonal factors have been shown to increase risk of SLE flare during pregnancy, such as active disease during the 6 months prior to conception and a history of lupus nephritis [42]. Animal studies suggest that estrogens promote disease development, while progestogens may have a protective role [7, 22, 43]. A protective role for progestogens is supported by a study observing a reduced incidence of lupus flares in women treated with progestogen only pills [44]. However, a previous study published in 2005 does not support a disease promoting effect for estrogens nor a disease protective effect for progestogens. The authors observed a similar incidence of flares as well as time to first flare in women with SLE who were randomly assigned to combined oral contraceptives, a progestin-only pill, or a copper intrauterine device (IUD) [45, 46].

3.4 Autoimmune Thyroid Disease (AITD)

Both Hashimoto's thyroiditis (HT) and Graves's disease show very high female-to-male prevalence ratios, which may reflect sex hormone effects. Interestingly, in a mouse model of autoimmune thyroiditis [47], estrogen had a protective effect while progesterone appeared to augment the levels of autoimmunity. Furthermore, in a cross-sectional study among healthy female relatives of patients with autoimmune thyroid disease, estrogen use was associated with a lower risk of autoimmune thyroid disorders [48]. Therefore, it appears that, in contrast to SLE, estrogens may protect against the development of AITD. The roles of progestogens in this regard have not been clearly elucidated.

4 Immunomodulation and Prevention of Preterm Birth

As described in detail in other chapters of this book, micronized vaginal progesterone is currently the standard of care for women carrying a singleton pregnancy diagnosed with a mid-trimester short cervix who do not have a history of preterm birth. RCT's and meta-analyses have established that among these women micronized vaginal progesterone decreases the risk of spontaneous preterm birth (sPTB) and improves perinatal outcomes [49]. In a manuscript entitled "Interdisciplinary exchange of ideas: progestogens for autoimmunity, biologics for pregnancy complications" [50] the authors speculated that some of these obstetric effects of progesterone may also be mediated by immunomodulation. Therefore, the authors suggested that biologic autoimmune modulators may provide more specific and

more potent effects, and possibly better results than micronized progesterone, in preventing sPTB.

5 Progestogen Hypersensitivity

Another aspect of the interaction of progestogens with the immune system is progestogen hypersensitivity, also known as autoimmune progesterone dermatitis, or cyclic urticaria. This condition is characterized by a hypersensitivity reaction to endogenous or exogenous progestogens [51–54].

The clinical presentation of progestogen hypersensitivity is mainly dermal but may also by systemic. Table 13.1 summarizes the different manifestations [53, 54]. Exacerbations occur whenever endogenous or exogenous levels of progestogens rise, as detailed in Table 13.2. The most classic manifestation is cyclic—appearing at the end of luteal phase of the ovulatory cycle when progesterone levels are high, resolving a few days after menses [53, 54]. This condition has recently been described also following vaginal progesterone exposure during pregnancy [55]. The disease is clinically suspected based on the cyclic manner and/or exacerbation due to external progestins. Confirmation of the diagnosis can be achieved using progesterone skin tests [56, 57].

The classic first line treatment is inhibition of endogenous progesterone secretion by suppression of ovulation. Ovulation can be suppressed pharmacologically with estrogens, or by continuous GnRH agonists. Administration of unopposed estrogens may increase the risk of endometrial carcinoma, thus limiting their use [56]. GnRH agonists induce a medical menopause due to estrogen suppression, which is also undesirable. A novel treatment approach is desensitization with small doses of progesterone [52, 58]. This approach is also relevant for non-dermal manifestations such as dysmenorrhea and premenstrual syndrome [52, 59]. The use of high dose systemic steroids is controversial both because of inconsistent data regarding their benefit [56] and the many side effects. In a few patients with refractory symptoms,—bilateral oophorectomy has been used. This option may succeed in controlling hypersensitivity symptoms but should be considered as a treatment of last resort [56, 60].

Table 13.1 Clinical manifestations of progesterone hypersensitivity

Dermal manifestations	Systemic manifestations
Erythema multiforme	Progesterone induced anaphylaxis
Eczema	Premenstrual syndrome
Urticaria	Dysmenorrhea
Pruritus	Mastalgia
Angioedema	Headache
Dermatitis	Arthralgia
Acne	Asthma/rhinitis

Table 13.2 Timing of exacerbations of progestagens hypersensitivity

Due to endogenous progesterones rise
Luteal phase of menstrual cycle when progesterone levels are high (resolve during pregnancy)
Due to exogenous rise in progestins
Contraceptive pills
Postmenopausal hormone replacement therapy
Vaginal progesterone during pregnancy for prevention of preterm birth

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Chapter 14
Progestogens in Non Gynecological Indications

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1 Introduction

Progesterone is the most basic of all steroid hormones. All other steroid hormones are produced physiologically by modifying the progesterone molecule into glucocorticoids, mineralocorticoids, estrogens and androgens (see Fig. 14.1). Progesterone acts by agonizing the progesterone receptor. It is known that numerous body organs have estrogen or androgen receptors, explaining the differences between both sexes. Sex differences are seen in behavior patterns, cyclic responses of the hypothalamus, hair production and distribution, in addition to the differences in the sex organs. Glucocorticoid receptors are also found in virtually all organs of the body. Therefore, it should be no surprise that progesterone receptors are widely distributed and that progesterone affects numerous organs in both sexes as well as the effects of progesterone on the uterus and reproduction.

Men are also dependent on progesterone. When a lack of progesterone is found in men, many conditions can be improved with progesterone supplementation. Two

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H. J. A. Carp (ed.), *Progestogens in Obstetrics and Gynecology*,
https://doi.org/10.1007/978-3-030-52508-8_14

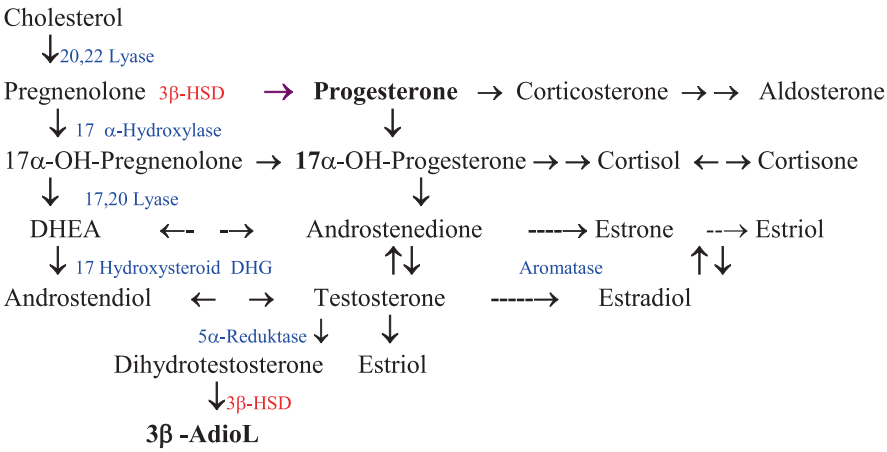

Fig. 14.1 Biosynthesis of sex hormones

examples are prostate hyperplasia and erectile dysfunction [1]. In addition to progesterone being produced by the Leydig cells, it is also produced in the central nervous system by the glial cells of the brain and spinal cord [2] and in the Schwann cells [3] of peripheral nerves. Therefore, progesterone supplementation has been used successfully in depression, sleep disorders, multiple sclerosis, spinal cord neurodegeneration, brain trauma, CVA and epileptic seizures.

Both, a gene defect or an age-related decrease may lead to a lack of progesterone.

Adequate substitution of progesterone for both, prophylaxis and the correction of several deficiencies in the male and also in children and for a number of neurological disorders is both justified, and desirable. However, as progesterone is not widely used outside of the accepted gynecological indications (described in other chapters in this book), appropriate control studies are urgently required.

2 Progesterone in Evolution

Since life first evolved on this planet, steroids have been present in both animals and plants as sterols. In animals, progesterone receptors have been found at the dawn of evolution, predating the Cambrian period (543 million years ago) [4]. The receptor has been found in the Rotifer, Brachionus manjavacas [5], where it may regulate the response to the foreign tissue introduced in sexual reproduction. Hence progesterone had a dual role, both reproductive and anti-inflammatory or immune. As evolution proceeded, glucocorticoids evolved from progesterone. Glucocorticoids have a more profound anti-inflammatory or immune effect than progesterone, and have therefore taken over the anti-inflammatory effects. However, some anti-inflammatory effects remain, as shown later. With the development of multicellular organisms, progesterone was secreted in all tissues, and its receptors were found in all tissues

e.g. skin, brain, Mullerian duct, gonads etc. Even to-day in humans, progesterone is secreted in the brain as a neurosteroid, and its effect is seen in the skin as well as other organs.

As evolution proceeded, estrogen and testosterone took over gametogenesis, in the same way as cortisol took over anti-inflammatory actions. However, progesterone has maintained its gametogenic function as a "Maturation Inducing Steroid" in certain fish [6], and induces meiotic division in amphibian oocyte [7]. In retiles Progesterone acts on the oviducts inducing expression and deposition of egg-white proteins, decreases myometrial contractility, facilitates processing of eggs, formation of eggshell, and deposition of egg-white proteins [8]. In birds, egg shell quality has been related to preovulatory plasma progesterone concentrations [9]

Hence, the name progesterone, (for pregnancy steroid ketone) may be a misnomer, as the mammals adapted the action on the oviduct for the needs of viviparity and used the anti-inflammatory actions to allow the development of a semi-allogeneic embryo.

3 Progesterone in Males

In men progesterone is produced in the Leydig-cells of the testes, suprarenal gland, glial-cells of the brain and the spinal cord [2] and in the Schwann'cells [3] of peripheral nerves. Progesterone, pregnenolone and their metabolites have been found in all the above tissues. In the kidney progesterone is metabolized to androgens, and then secreted into the circulatory system.

Although relatively little is known about normal values of hormones in men, A reference range of 0.13–0.97 ng/ml has been published [10]. Zurnoff et al. [11] reported a mean serum progesterone level of 0.18 + 0.03 ng/ml for men compared to 0.21 + 0.05 ng/ml for women in the follicular phase. Additionally, diminished levels of testosterone and DHEA can be corrected with human-identical hormones or even DHEA (Dehydroepiandrosterone) [12]. However, the restoration of a healthy balance of hormones however rarely achieves attention. The highest levels of progesterone are found in the saliva of newborn infants. During the first month of life, progesterone falls to one third of its previous level, and after 2 years the circadian rhythm has become established with high morning peaks and low evening values [13]. Progesterone displays a wide spectrum of biological activities in multiple tissues. These effects can be stimulating or restraining, depending on the respective tissue, dose, point in time of application and progesterone-receptor-distribution.

A question arises as to whether or how progesterone levels should be supplemented. Lee used 5–8 mg progesterone transdermally and considered a level of 400 pg/ml in saliva as optimal [1]. Unfortunately, at present, few if any laboratories use saliva levels to determine hormone levels. Rimkus [14] has used a progesterone level of 4–10 ng/ml in serum, and supplemented progesterone levels if under 4 ng/ml, and an increase to 10 ng/ml, is considered normal. However, Nadjafi reported in the first edition of this book that she considers a progesterone level of 12 ng/ml to

be better. Progesterone levels may be age dependant, decreasing with increasing age [15]. Nadjafi reported in the first edition of this book that a dose of 100 mg oral micronized progesterone (Utrogestan, Bessins International, Belgium) at night from day 6 of the month until the end of each month, (with a break from day 1–5 of the month, to prevent down-regulation of the receptor) [1] increased serum values of progesterone from 3.5 ng/ml to 12 ng/ml. With substitution of DHEA or testosterone, the progesterone level increased to 6 ng/ml.

3.1 Progesterone Receptors

The prostate is the male equivalent of the uterus and Skene's glands, (both develop from the distal part of the Mullerian duct and the surrounding glands). Hence it is not surprising that the prostate has progesterone-receptors as well as estrogen and testosterone-receptors [16]. There are two isoforms of progesterone receptors: PR-A and PR-B. The PR-B isoform is a full-length-receptor, the PR-A isoform has 164 amino acids less than the PR-B receptor. The ratio of PR-A and PR-B levels in the target cells determine the type and extent of the progesterone effect [17]. PR-A is responsible for progesterone-dependent reproduction and the BR-B for normal differentiating effects, e.g. the breast [18]. PR-B is more active than PR-A and is cell-specific. PR-A suppresses transcription activities of other steroid hormone receptors, including ERα and PR-B. Progesterone has similar effects as 3β-Adiol, the agonist of ER β, which should rather be renamed the 3β-Adiol-Receptor, since 3β-Adiol is not an estrogen. Progesterone receptors have been identified in the heart, liver, sperm, epithelial-cells of the eyes, brain and nerves and in the prostate. There are also two isoforms of 3β-hydroxysteroid-dehydrogenase. Type I in the placenta and skin and Type II in the suprarenal gland, ovaries and testes. The progesterone receptor mediated suppression of gonadotropin reduces LH, FSH and thus testosterone in the male. It reduces pulsatile frequency and has little affinity for the androgen receptor [19].

The non-genomic effects of progesterone are regulated in men by intracellular receptors in the membrane. High concentrations influence the membrane liquid directly. Membrane-dependent progesterone effects are cell capacitation, LH receptor expression, leading to testosterone synthesis in the Leydig cells and interactions with the GABA receptor complex for sedation and anesthesia. Interactions also occur in fatty tissue and the kidneys.

3.2 Effects of Progesterone in the Male

Progesterone is needed for fertility in men, since progesterone increases the volume of the ejaculate and improves sperm-motility. The addition of progesterone to the Percoll-medium in cases of assisted reproduction significantly increases sperm-

motility [20]. Hence, progesterone may have retrained some of its evolutionary function as the Maturation inducing substance (MIS) seen in fish. Progesterone improves sexual performance in rats [21]. Consequently, progesterone should also improve sexual performance in men due to similarities to human neuro-endocrine mechanisms. Indeed, patients receiving progesterone reported more frequent morning-erections and distinctively improved sexual performance.

The sleep-inducing effects of progesterone are mediated by allopregnanolone and pregnanolone, the metabolites of progesterone [22]. Hence, sleep is improved with progesterone supplementation. Part of the mechanism whereby progesterone improves sleep patterns may be due to progesterone increasing pulmonary gas exchange and reducing alveolar CO_2 pressure, leading to improved respiration and therefore to undisturbed sleep [23–25]. Indeed drowsiness is a side effect when progestogens are used in supraphysiological doses therapeutically. Under progesterone therapy, many patients snore less or not at all. Whether sleep apnoea is also due to progesterone deficiency requires further investigation.

Other important effects of progesterone are anesthesia, immune-suppression, mild diuretic, antihypertensive [26] anticonvulsive [27], antioxidant actions [28] and bone formation [29]. Progesterone enhances thyroid function, normalizes blood-sugar-, zinc-and copper-levels.

3.3 Progesterone and Prostate Cancer

Progestogens may be used to prevent and treat benign prostatic hypertrophy, and cancer of the prostate. Progestogens have three main actions, an antiandrogen effect, a antigonadotrophic effect and a cytotoxic effect.

3.3.1 Anti-Androgenic Effect

Hereditary prostate cancer is associated with a defect in the 3 β-Hydroxysteroid-Dehydrogenase gene [30], which codes for the enzyme needed to metabolize progesterone from pregnenolone leading to a progesterone-deficiency. The same enzyme is needed to metabolize 3 β-Adiol from Dihydrotestosterone (DHT), hence the DHT level remains in excessive concentrations DHT stimulates proliferation of prostate cells significantly more than testosterone does, enlarging the prostate gland and narrowing the urethra causing symptoms of BPH [31]. Additionally the age-related decrease of progesterone leads to a gradual decrease of testosterone and an increase of cell-growth-promoting estradiol [1, 30]. When hormone levels diminish in the aging male, markers of inflammation increase. These markers include C reactive protein (CRP), interleukin-1β, interleukin-6, TNF-α and PSA total and free PSA levels and the quotient of both. In patients with prostate hyperplasia receiving progesterone these parameters of inflammation decrease steadily back to the normal range. Micturition problems improve or even disappear with progesterone

supplementation as the prostate shrinks. However, the process takes approximately 9–12 months.

Progesterone as the natural 5α-reductase-inhibitor controls the metabolism of testosterone to DHT. The deficiency of 3β-hydroxysteroid -dehydrogenase (whether hereditary or age-related), leads to a decrease of progesterone, thus leaving DHT unopposed. Since 3β-Adiol cannot be metabolized from DHT, an excessive amount of DHT remains to promote prostate hyperplasia [32]. Consequently, progesterone or progesterone derivatives may be used in future to treat BPH and prostate cancer, as progesterone inhibits 5α-reductase enzyme [33]. Progesterone is also the natural aromatase-inhibitor controlling the effect of growth-promoting estradiol. The luminal cells of prostatic epithelia show high amounts of ERβ, whereas ERα is found primarily in the basal cells. In prostate cancer ERβ is down-regulated and ERα is spread to the luminal cells. ERβ is reduced ten times and PR-A and PR-B are deficient, whereas ERα remains unchanged, leaving 17β-Estradiol-functions unopposed [34]. These mechanisms may explain why progesterone-deficiency triggers the development of prostate cancer.

Progesterone derivatives such as 6-ethyleneprogesterone, megestrol and medroxyprogesterone acetate [35] are potent inhibitors of 5α-reductase, could play an important role in conversion of testosterone to dihydrotestosterone. Megestrol acetate is used in the treatment of prostatic cancer [36]. Thus, progesterone replacement therapy (PRT) in men (8–10 mg daily), along with 1–2 mg/day of testosterone, has been advocated to protect against prostate cancer [37]. Studies have shown a marked decline in elevated prostate-specific antigen (PSA) in patients treated with progesterone [38].

3.3.2 Anti-Gonadotrophic Effect

Progesterone also inhibits pituitary LH release and leading to an antiandrogenic effect.

3.3.3 Cytotoxic Effect

Progesterone derivatives also have cytotoxic effects. When patients with progressive prostatic cancer were administered sequentially alternating high-dose oral medroxyprogesterone acetate 1 g for 26 days followed by intravenous epirubicin 25 mg/m^2 weekly for 4 weeks, there was more than a 50% reduction in the size of measurable lymph node and skeletal metastases. The normalization of serum acid phosphatase and 50% reduction in serum alkaline phosphatase correlated with the improvement of subjective response, with a marginal objective effect in prostatic cancer [35]. Another study showed that medroxyprogesterone acetate in hormone refractory cases alone seems superior to estramustine or prednisolone treatment [39].

The prostate is the equivalent of the uterus and Skene's glands in the female. Progesterone stops further proliferation of the endometrium in the luteal phase of

the menstrual cycle. The effect of progestogens in the prostate is similar to the effect of progestogens on the endometrium, preventing endometrial hyperplasia and carcinoma.

4 Progesterone, the Neurotrophic Hormone

Progesterone is not only produced in the Leydig-cells of the testes, the ovary and adrenal cortex, but also in the glia-cells in the brain and spinal cord [2] and in the Schwann-cells of the peripheral nerves [3], (with especially high levels in the sciatic nerve).

In the brain as in other tissues, estradiol, testosterone and progesterone act through classical nuclear receptors and non-classical membrane receptors. In the classical pathway, progesterone diffuses into the cell and binds to its receptors (PRa and PRb), acting through specific progesterone response elements (PREs) within the promoter region of target genes, thus regulating transcription. Progesterone, and some of its neuroactive metabolites, such as allopregnanolone and dihydroprogesterone (DHP), also act through the non-classical pathway. The non-classical pathway includes membrane receptors such as PRa, PRb, PQMR, and PGMR1 [40]. The membrane receptors lead to the activation of signaling cascades [PI3K, PKC MAPK, protein kinase A (PKA)], second messengers, ion influx and efflux, and the transcription of different genes [41].

Progesterone also regulates the glial cells which are responsible for myelination. In the CNS oligodendrocytes synthesize myelin. Oligodendrocytes originate as oligodendrocyte progenitor cells (OPC), which migrate toward unmyelinated axons, where they mature and form myelin sheaths [42, 43]. Progesterone promotes intracellular signaling, proliferation of oligodendrocyte progenitors [43] and transcription of key components such as myelin basic protein and 2′, 3′-cyclic nucleotide-3′-phosphodiesterase requires for myelin synthesis [43]. Oligodendrocytes in return produce high amounts of progesterone and metabolize progesterone. The progesterone metabolite DHP also regulates oligodendrocyte function and myelination [43]. Furthermore, allopregnanolone modulates GABA-A receptors, inducing the proliferation of OPC in an autocrine/paracrine loop [44].

In the Peripheral nervous system, neurons and Schwann cells produce progesterone and, and metabolize progesterone to DHP and allopregnanolone. Through the classical pathway, progesterone and DHP tetrahydroprogesterone, dihydrotestosterone and 3α-diol stimulate the expression of two important proteins of the myelin of peripheral nerves [45] glycoprotein P0 (P0) and peripheral myelin protein 22 (PMP22). Allopregnanolone, acts via GABA receptors, promoting the production of GABA, which induces the proliferation of Schwann cells [46]. Hence progesterone and its metabolites modulate the myelination and remyelination in the peripheral nervous system.

4.1 Multiple Sclerosis (MS)

(MS) is an autoimmune inflammatory disease affecting the central nervous system, with demyelinization and neurodegeneration. The effect of progestogens is discussed in Chap. 13, progestogens and autoimmunity. As stated above, oligodendrocytes produce myelin. Progesterone also suppresses matrixmetalloproteinases [47], which maintain the inflammatory plaques in multiple sclerosis. Consequently in pregnancy under high progesterone-levels exacerbation of MS does not occur. Postpartum relapses are considered to be induced by the decreased levels of these steroids [48]. Progesterone has been shown to reduce the inflammatory reactions commonly seen in MS due to the direct effect of progesterone on astrocytes and microglia [49]. However, trials are urgently required to investigate whether progestogens may ameliorate MS or prevent recurrences after delivery.

Unfortunately, no results are available from such trials. In the previous edition of this book, Dr. Nadjaafi reported one anecdotal case of a female patient, aged 62 with MS, who received progesterone supplementation (200 mg microniced progesterone from day 6 until the end of the month) for the previous two years. She had improvement of her symptoms and regained the ability to use stairs more easily.

4.2 Brain Trauma and Stroke

Many early studies, on both animals and humans suggested that progesterone may improve the prognosis in traumatic brain injuries. In rats, receiving progesterone for 3–5 days, both cerebral edema and behavioral abnormalities were prevented when progesterone was administered [50]. In humans, both Wright et al. [51], and Xiao et al. [52] reported protection against necrotic damage and behavioral abnormalities caused by traumatic brain injury in 77 of 100 patients receiving progesterone and a Glasgow Coma Score less than or equal to 8 within 8 h respectively Additionally, progesterone treated patients had a statistically significant lower 6-months mortality an reduced mean intracranial pressure 72 h and 7 days post trauma.

These and other publications led to further large multi-center-trials in patients with brain trauma or stroke, and metaanalyses. Unfortunately, the metaanalyses have not supported the initial enthusiasm. Ma et al [53], reported a meta-analysis on three single-center studies, and identified a 39% lower mortality rate and 23% more favorable outcome in the patients who received progesterone treatment. However, a later metaanalysis of eight RCTs, .the results demonstrated there was no evidence that progesterone has a protective role in patients with TBI [54]. When Ma et al. updated their metaanalysis [55] to five trials, their previously reported benefit was no longer apparent. As in all metaanalyses, the question remains as to whether there is a subgroup who may respond, or whether a secondary data analysis, assessing long term follow up or neurological imaging studies broken down into the location and type of lesion may show different results.

4.3 Peripheral Neuropathy

In addition to the brain, progesterone acts on peripheral nerves, where both classical and non-classical steroid receptors are present. As stated above, progesterone synthesized by Schwann cells promotes new myelin sheath formation and increases the myelinated axons In an interesting experiment, bone marrow stromal cells (BMSCs) were induced to differentiate into Schwann-like cells (SLCs) using progesterone. These SLC's were then transplanted in a rat model of sciatic nerve injury with 1-cm gaps. A sciatic function index (SFI), histological, immunohistochemical and ultrastructural studies were used in evaluating the improvement in the nerves regeneration. The results showed significant differences in the SFI between the control and the treated groups ($P < 0.05$), and electron microscopy showed myelination in the transplanted cells [56]. Sarabia Estrada et al [57], published a study where rats were implanted with progesterone-loaded chitosan, unaltered chitosan, or silicone tubes, after surgical removal of a 5-mm segment of the proximal sciatic nerve. In order to evaluate the progesterone and chitosan effects on sciatic nerve repair and ipsilateral hindlimb function. Progesterone-impregnated chitosan tubes enhanced innervation of the affected muscles, which allowed partial recovery of gait locomotion.

4.4 Epilepsy

As long ago as 1995, Herzog [58] published, that progesterone reduces complex partial seizures (CPS) and secondary generalized motor seizures (SGMS) in women with low serum mid luteal progesterone levels (less than 5 ng/ml). Since 1995, there have been an additional publications regarding the effect of progesterone in epilepsy [59–61]. However, two more recent trials [62, 63] have not shown evidence of effect, but have suggested that there may be an effect in subgroups of patients.

4.5 Parkinson's Disease

The Dopamine neurons of hemi-Parkinson-rats express increased progesterone receptors A and B and a decrease in the estrogen receptor ERα, compared to initial pluri-potency during differentiation. Ninety two per cent of the dopamine neurons had progesterone receptors [64]. Reduced progestogen levels are associated with the development of Parkinson's disease (PD) [65], and in 6-hydroxydopamine (6-OHDA)—treated male rats pregnenolone and dihydroprogesterone levels are lower in the striatum and cortex, respectively [66]. Intrastriatal injection of the toxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) can induce PD in rats. Progesterone administrated before MPTP treatment prevents MPTP toxicity in male mice [67], and when given one, day after MPTP stimulation, it decreases astrocyte

activation and restores dopamine levels [68]. However, no improvement of dyskinesia was observed after treatment with progesterone in MPTP-treated female monkeys [69]. However, different progestogens have different actions. In a double-blind trial in women progesterone, administration had an rather anti-dopaminergic effect [70], while medroxyprogesterone acetate co-administration with estrogen improved dyskinesia in female PD patients [71].

4.6 Progesterone and Spinal Cord from Neurodegeneration.

Progesterone–pellets were given to Wobbler-mice with pronounced neurodegeneration. Progesterone led to reduction of the cell-vacuoles and the mitochondrial structure remained intact. In treated mice the grip-strength was improved and survival was-prolonged compared to the untreated mice [72].

In a standardized rat contusion mode Thomas et al. [73] was able to show that progesterone significantly improved neurologic recovery after spinal cord injury that resulted in incomplete paraplegia. Rats treated with progesterone had significantly better outcomes after progesterone treatment compared with dimethylsulfoxide treated or non-treated control groups The improvement was corroborated in histologic analysis by relative sparing of white matter tissue at the epicenter of the injury in the progesterone-treated group ($P < 0.05$). Yang et al. [74] observed that progesterone significantly reduces neuronal death in mice following spinal cord injury. This was mediated via down-regulation of inflammatory cytokines, including NOS2, MCP-1, and IL-1β as well as activated caspase-3 and GFAP. Myelin basic protein was also upregulated. In view of the large numbers of patients with spinal cord injury, additional studies are urgently needed.

4.7 Progesterone in ADD, and ADHD

It has been reported, that when progesterone is given to progesterone-deficient pregnant women, the children are more advanced at 1 year and have greater academic achievement at 9–10 years and 17–20 years. The best academic results were found when the mothers received progesterone before the 16th gestational week, for longer than 8 weeks and over 5 g in total [75]. In 2001 Trotter et al. [76], reported that progesterone and estradiol supplementation in premature babies to the levels they would have received in utero, led to normal psychomotor development, higher bone mineralization and reduction of lung diseases.

Platt [77] is of the opinion that progesterone deficiency is the cause for attention deficit disorder, and that progesterone deficiency together with insulin excess and noradrenalin excess leads to ADHD, as lack of Progesterone, too much Insulin and noradrenalin. Platt has reported on a 9-year old boy with ADHD who lost his behavior-disturbances when progesterone was administered. In 6 months he became

one of the best pupils in class. In the previous edition of this book, Naadjafi reported having treated a 12-year old boy with low values of progesterone and testosterone (according to the Tanner classification [78]. Administration of 100 mg Progesterone before bedtime from day 6 until the end of each month was accompanied by serum levels of progesterone, estradiol, testosterone, DHEA-S and noradrenalin becoming normal according to the Tanner classification. After 3 months this youth received better grades at school. Nadjafi also reported treating ten more patients with similar good results. One patient with ADHD was treated with Ritalin, could stop Ritalin immediately after progesterone supplementation. The lack of progesterone is due to a gene-defect of 3 β-hydroxy-steroid–Dehydrogenase [1], the enzyme needed to produce progesterone from pregnenolone, hence the progesterone deficiency. These children not only have too low values of progesterone, but also very low values of testosterone. With progesterone therapy, the testosterone level increases as seen by penile growth. With these changes, height increases, as does self-esteem.

4.8 Anxiety

The anxiolytic effects of oral Progesterone have been examined in a double-blind crossover-study in 38 men. The Hamilton's-Anxiety-Scale was significantly reduced 4 h after progesterone administration and remained lower when examined after nine and 24 h. Patients in the placebo arm of the trial only had a mild and non-significant reduction of the anxiety scale [79]. Additionally, in a cross-over RCT of women with premenstrual symptoms, oral micronized progesterone was associated with a significant improvement in anxiety [80]. Hence the addition of progesterone may allow the dose of antidepressants to be gradually reduced or stopped completely. Conversely, Bristot et al. [81] have suggested that antidepressants or antipsychotics may exert their effects by normalizing the levels of progesterone. However, there is also literature that progesterone is associated with increased anxiety levels.

5 Other Therapeutic Effects of Progesterone

5.1 Progesterone and Statin Use

Progesterone supplementation reduces cholesterol and returns the lipoprotein levels to normal. Normalization of cholesterol levels takes place especially quickly if in addition to progesterone supplementation, hypothyroidism is also corrected with a thyroid preparation. Indeed long-term simvastatin intake reduces serum testosterone, estradiol, and progesterone levels in male rats [82]. Indeed the peripheral neuropathy associated with statin use in those individuals who are susceptible could conceivably be due to reduction of progesterone levels to sub-optimal levels.

After correction of the hormone profile, there may be patients who could stop Statins completely.

5.2 Asthma

It has been reported that patients with asthma can stop their medication if substituted with Progesterone [77]. Indeed exogenous progesterone induces paradoxical downregulation and desensitization of β_2-adrenoceptors in asthmatic women, compared with non-asthmatic subjects [83]. Some studies have reported that estrogen and progesterone improve total lung capacity and reduce the exacerbation of asthma symptoms, such as coughing, wheezing and dyspnea [8, 84, 85].

5.3 Arthritis

The anti–inflammatory effects of progesterone have been reported in men with active arthritis [86]. Intra-articular injection to one knee in 12 men produced a local anti-inflammatory effect, which lasted for three months. An especially impressive good effect was observed in two men with Polyarthritis, who did not respond to other therapies. The authors suggested either an immuno-suppressive effect on lymphocytes or induction of immuno-suppressive glycoproteins or binding of the glucocorticoid-receptor, to explain the anti-inflammatory effect.

In rodent models of rheumatoid arthritis, pregnancy-associated amelioration of disease can be mimicked in nonpregnant arthritic animals by achieving pregnancy-like levels of oestrogen or progesterone [87]. Thus, the antirheumatic effects of oestrogen and progesterone might require the high circulating hormone concentrations occurring during pregnancy.

5.4 Carpal Tunnel Syndrome

Ginanneschi et al. [88] have treated sixteen women with carpal tunnel syndrome by local injection of 17α-hydroxyprogesterone-caproate. Progesterone therapy was compared to corticosteroids. Corticosteroid therapy was followed by a 1 month-pain-free period. However, the pain free period was 6 months after injection of the long acting progesterone-derivative. In a randomized controlled trial of local progesterone vs corticosteroid injection for carpal tunnel syndrome in 78 patients [89], it was reported that he efficacy of progesterone local injection was superior to corticosteroid injection for relieving symptoms and improving functional and electrophysiologic findings at long-term follow-up.

6 Conclusions

Progesterone is a widely distributed hormone with numerous anti-inflammatory effects. The anti-inflammatory effects have developed in parallel to the reproductive effects through millions of years of evolution. Many of the anti-inflammatory effects may have clinical and therapeutic applications which have only started to be investigated. Trials are sorely needed to determine the therapeutic implications both in an evidence based approach for the majority of patients and in a personalized approach for the minority of patients who may benefit.

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