Ovarian Stimulation Protocols

Gautam N. Allahbadia
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Foreword

The book by Gautam N Allahbadia and Yosiharu Morimoto is a "state of the art" textbook in the field of assisted reproductive technologies (ART).

The 11 chapters on ovarian stimulation protocols give the reader a comprehensive understanding of the regulatory principles of follicular development and the physiology of ovarian stimulation as well as updated information on a broad range of subjects related to ovarian stimulation. The chapter on practical guidelines to monitor treatment is useful for physicians as well as fertility nurse coordinators and serves as an excellent guide to every day practical aspects of monitoring during ovarian stimulation.

The reader will find a superlative review on the long-standing discussion on agonists versus antagonists in controlled ovarian hyperstimulation (COH) and updated chapters on more specific issues and novel drugs such as recombinants versus biosimilars and long-acting gonadotropins.

Modern trends and changes in the concepts of ovarian stimulation by using mild stimulation and treatment protocols, aiming to prevent ovarian hyperstimulation, are thoroughly summarized. There are two chapters, which describe groups of patients, such as poor ovarian responders and polycystic ovary syndrome (PCOS) in which stimulation of the ovaries is a challenge and who need more attention and individualization. Finally, all aspects of luteal phase support in COH are critically described and discussed.

This textbook is a fantastic theoretical and practical guide on a very important topic and a useful resource to professionals in the field of ART.

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Preface

Controlled ovarian stimulation (COS) is the first step for in vitro fertilization (IVF) treatment, a treatment often described and experienced as stressful to patients and their partners. Current controlled ovarian stimulation (COH) for assisted reproductive techniques (ART) pursues three main objectives: hypophyseal activity suppression, multiple follicle growth stimulation, and ovulation induction. By suppressing hypophyseal activity, it is possible to prevent an untimely luteinizing hormone (LH) surge and allow the appropriate development of the leading follicle. The classical GnRH agonist long protocol is the most widely used in COH for ART. However, an alternative regimen, based on gonadotropin-releasing hormone (GnRH) antagonist was next introduced in clinical practice. As competitive antagonists, these drugs display an immediate and quickly reversible effect and they avoid hormonal withdrawal side effects. Moreover, this protocol shows undeniable advantages, including a shorter duration of treatment, lower amount of gonadotropins required, shorter hormonal and ultrasound monitoring of patients, milder physical and emotional stress, and a lower risk of ovarian hyperstimulation syndrome (OHSS). The use of GnRH antagonists was traditionally restricted to selected patients, as poor responders and women at high risk of developing OHSS such as those with as polycystic ovary syndrome (PCOS) and patients who had previously experienced OHSS. Several practical aspects of implementing a GnRH antagonist-based stimulation protocol are described in the subject chapter; selection of the correct dose, choosing when to start the antagonist, programming of cycle starts, selection of the appropriate gonadotropins, and triggering of final oocyte maturation are elucidated.

The prediction of extremes of ovarian response to stimulation and the irreversibility of reduced ovarian reserve remain important clinical and basic science research issues of IVF treatment. Recommending commencement of ovarian stimulation, using any of the available exogenous compounds without knowledge of individual ovarian potentials, is simplistic and dangerous because of the possible adverse consequences for the woman. The identification of groups of patients likely to benefit from one protocol than another is central to the work-up process of IVF. Determining the agents for ovarian stimulation as well as their combination, the daily dose and duration, according to some background information, should be seen as the way to enhance safety and cost-effectiveness.

It should be stated that no single approach is successful for all patients, and that, there is currently, no firm clinical consensus regarding the relative

efficacy of the different stimulation protocols. Personalized IVF offers several benefits; it enables clinicians to give women more accurate information on their prognosis, thus facilitating counseling, especially in cases of extremes of ovarian response. The main objective of individualization of treatment in IVF is to offer every single woman the best treatment tailored to her own unique characteristics, thus maximizing the chances of pregnancy and eliminating the iatrogenic and avoidable risks resulting from ovarian stimulation. Personalization of treatment in IVF should be based on the prediction of ovarian response for every individual. The starting point is to identify if a woman is likely to have a normal, poor, or a hyper-response and choose the ideal treatment protocol tailored to this prediction. The subject chapters outline that antral follicle count (AFC) and anti-Mullerian hormone (AMH), the most sensitive markers of ovarian reserve identified to date, are ideal in planning personalized COS protocols. These sensitive markers permit the prediction of the whole spectrum of ovarian response with reliable accuracy, and clinicians may use either of the two markers as they can be considered interchangeable. Following the categorization of expected ovarian response to stimulation, clinicians can adopt tailored therapeutic strategies for each patient. Two important chapters in this monograph summarize the predictive ability of ovarian reserve markers, such as AFC and AMH, and discuss the therapeutic strategies that have been proposed in IVF after this prediction.

Controlled ovarian stimulation directly influences ART outcomes. Indeed, several studies have shown that the total International units (IU) of gonadotropins, used for ovarian stimulation, inversely correlates with pregnancy rate. Nowadays, two main gonadotropins are used in ART protocols, human-derived follicle-stimulating hormone (h-FSH) and recombinant FSH (r-FSH). The difference between these two hormones is dramatic. Indeed, the human-derived FSH is an acidic isoform of the hormone, while r-FSH is a less acid one. In particular, during a physiological menstrual cycle, the acid isoform is produced during the follicular phase (probably, it is more effective in recruiting follicles), while the less acidic isoform is produced during the mid-follicular phase (preovulatory). The two most commonly used gonadotropin forms are urinary human menopausal gonadotropin (hMG) and recombinant FSH in combination with GnRH agonists or GnRH antagonists. Cycles stimulated with recombinant FSH appear to have a higher risk of premature progesterone rise in the late follicular phase if not triggered on time. Recently, Corifollitropin alfa, a new long acting recombinant FSH was introduced, which sustains multiple follicular growth for 7 days in women undergoing ovarian stimulation using GnRH antagonists. Future trials should aim to eliminate OHSS and multiple pregnancy rates by performing a single stimulation in a simplified Corifollitropin alfa/GnRH antagonist cycle, triggered by a GnRH agonist followed by cryo-thawed single embryo transfer (SET) in consecutive natural cycles. With this approach, the two major complications of COH for IVF could be eliminated without jeopardizing the outcome.

The human chorionic gonadotropin (hCG) trigger, used for final follicular maturation in connection with assisted reproduction treatment, combines ovulation induction and early luteal phase stimulation of the corpora lutea.

The use of a GnRH agonist (GnRHa) for final follicular maturation has, however, for the first time allowed a separation of the ovulatory signal from the early luteal phase support. This has generated new information that may improve the currently employed luteal phase support. Combined results from a number of randomized controlled trials, using the GnRHa trigger suggest an association between the reproductive outcome after IVF treatment and the mid-luteal phase serum progesterone concentration and these have been covered ably in this monograph.

One of the most vexing challenges in the practice of Reproductive Medicine is the management of the "poor responder," specifically the patient manifesting an inadequate follicular response to ovarian stimulation. Poor response predicts a reduction in the number of mature oocytes retrieved, with the consequences of fewer embryos available for selection and transfer, reduced pregnancy rates, and a markedly decreased likelihood of residual embryos for cryopreservation. This topic has been covered threadbare in this book.

The two main complications associated with the use of assisted reproduction techniques, ovarian hyperstimulation syndrome and multiple pregnancies, could be eliminated by milder ovarian stimulation protocols and the increased use of a SET policy. In contrast to current approaches, the aim of mild stimulation is to develop safer and more patient-friendly protocols in which the risks of the treatment as a whole are minimized. This monograph attempts to present the current status of milder protocols to its readers. Gentle ovarian stimulation protocols, such as "mini-IVF" and "IVF Lite," have several potential advantages over conventional IVF protocols, including less medication and fewer injections, producing fewer eggs, but eggs of higher quality. The IVF Lite protocol, described in this monograph, requires a reliable and cheap method for embryo cryopreservation, such as vitrification, because of the negative impact of Clomiphene citrate on the endometrium and since cryopreserved embryo transfers with this protocol have yielded much higher pregnancy rates than fresh transfers.

We have attempted to include the A–Z of current knowledge in this dynamically changing field of controlled ovarian stimulation. This book will benefit not only postgraduate students and new entrants into the field of ART but also the senior consultants by helping them to update their clinical skills

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Regulatory Principles of Follicular Development

1

Takahide Mori

Abstract

Controlled ovarian stimulation (COS) is one of the key issues for the successful outcome of in vitro fertilization (IVF). Although retrieval of multiple oocytes is aimed at in COS, the regulatory principle governing the sequential program of follicular development in natural cycles is likely to be similar to those in stimulated cycles. In addition to the conventional pituitary-ovarian axis, the oocyte itself has now become a novel regulatory factor in folliculogenesis. As the entire process of follicular development proceeds stepwise from preantral to preovulatory stages under the influence of a functional interplay among these regulators, belonging to the hypothalamo-pituitary-ovarian axis, each with specific roles, the author intends to describe fundamental principles governing folliculogenesis first and then to propose a rational and realistic idea of selecting the most appropriate stimulation protocol of the indicated ones, tailored to meet the patients ovarian reserve.

Keywords

Follicular development • Follicular growth • Follicular maturation • Ovarian stimulation • Regulatory principles • Gonadotropins • Gonadal steroids • Inhibin • Activin • Oocyte factors • Individualization

Introduction

From a cohort of antral follicles, only one is selected for further maturation to preovulatory stage under follicle-stimulating hormone (FSH) and luteinizing hormone (LH) regulation. In in vitro fertilization (IVF) cycles, multiple oocyte aspiration has become possible owing to the

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development of artificial ovarian stimulation with purified gonadotropin preparations under pharmacological pituitary desensitization with gonadotropin-releasing hormone analogs (GnRHa) and is called controlled ovarian stimulation (COS) [1]. Despite differences in dynamics of follicle development between natural cycle and COS, the intervening principle seems in common. As illustrated in a pyramid-shaped diagram (Fig. 1.1), COS is likely to follow the principles that govern follicular development in a stage-related mode. Adopting the terminology of follicular growth for the stage of follicular development from preantral until dominant follicle selection and of follicular maturation for the stage beyond selection until the preovulatory stage, functional systems of regulation in the whole process of follicle development are described. Based on the rationale, practical selection of the most appropriately indicated COS protocol is constructed to make it compatible with individual patient's acceptability of differing ovarian reserve.

Preantral Follicle Growth

Initial and Cyclic Recruitment (Figs. 1.1 and 1.2)

It appears that preantral follicle growth consists of two developmental steps: initial recruitment being a transition from the primordial to primary follicles followed by cyclic recruitment, which is gonadotropin (Gn)-sensitive but not a dependent stage since mRNA of receptors for FSH (FSHR) can be identified in primary follicles [2]. The primordial follicle pool before initial recruitment stays strictly in a dormant state until activated with positive regulators coming from the oocyte and/or from surrounding cells. Against heavy constraint by negative regulators, such as anti-Müllerian hormone (AMH), a Müllerian-inhibiting substance (MIS), cyclic recruitment can take place under the joint action of oocyte factors and Gns, to which both, secondary follicles with two-layered granulosa cells and preantral follicles with differentiated

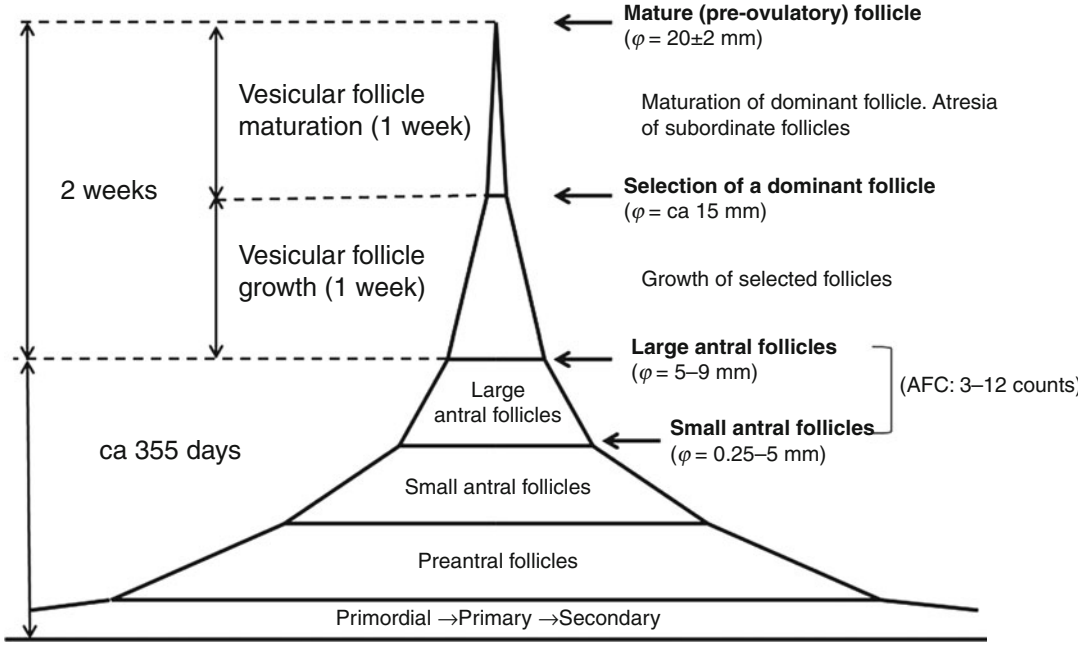

Fig. 1.1 Pyramid-imitative shape of dominant follicle selection in the human ovary. Since terminology and size of each stage of follicles are not uniformly standardized among authors, these are arbitrarily defined by the author (Reproduced with permission from Mori T et al. *Horm Front Gynec*. 2009)

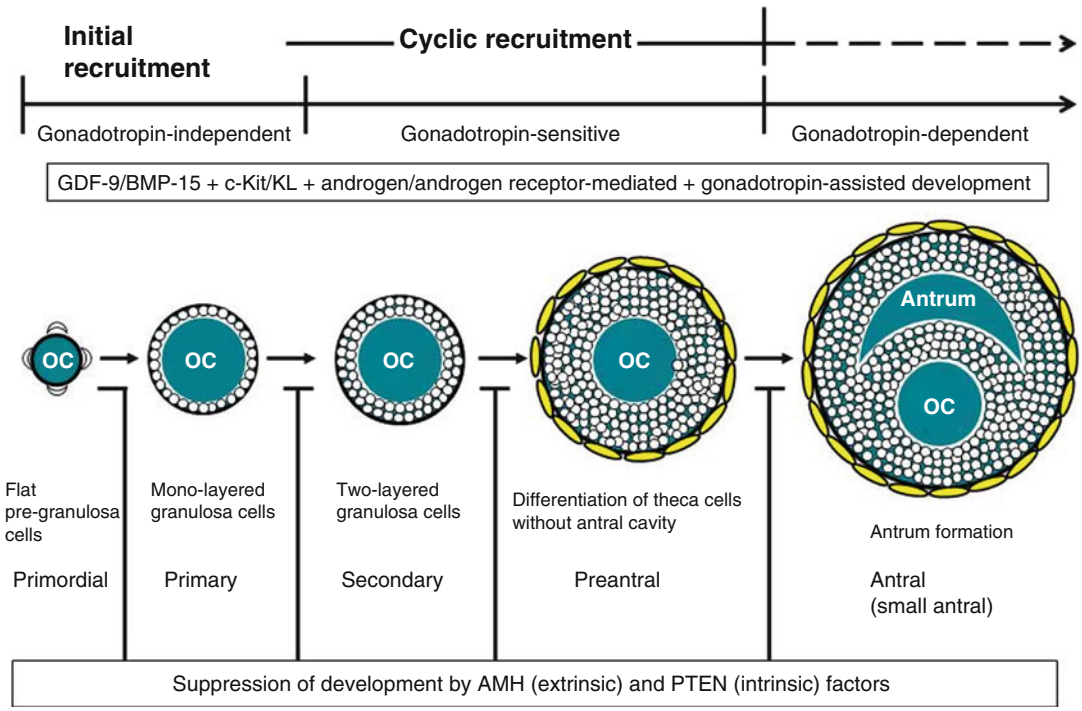

Fig. 1.2 Regulation of preantral follicle growth by stimulatory and inhibitory factors working at different stages of the preantral follicular development (Reproduced with permission from Mori T et al. *Horm Front Gynec*. 2009)

theca cells (TCs) outside the granulosa cell layer have become sensitive; until the antral stage, when Gn dependency is established with the expression of both kinds of receptors for FSH (FSHR) and LH (LHR); preantral follicles now enter Gn-dependent stage of follicular development.

Growth Differentiation Factor-9 (GDF-9) (Fig. 1.3)

GDF-9, a member of transforming growth factor beta (TGF-β) superfamily, is expressed by primary oocytes through ovulatory follicles in mammals including humans [3, 4]. Since primordial follicles of GDF-9 null mice are able to progress to primary stage, GDF-9 may not be required for transition from primordial to primary follicles, namely, for initial recruitment [5]. Although the molecular mechanism of differential function of GDF-9 and androgen (A) is ambiguous [6], both of which enhance antral folliculogenesis through insulin-like growth factor-1 (IGF-1), commitment and co-ordination of GDF-9 with A has become evident [7]. Intriguing enough is that GDF-9 alone enhances progesterone (P) production by cultured granulosa cells (GCs) via prostaglandin (PG) E2/receptors for PGE2 (EP2) pathway, though the physiological significance remains to be elucidated. Thus, GDF-9 contributes to preantral follicle growth directly by promoting A synthesis by the TCs or indirectly, by enhancing FSHR expression on GCs.

Bone Morphogenetic Protein-15 (BMP-15) (Fig. 1.3)

BMP-15 (GDF-9B), another member of oocyte-derived TGF-β superfamily, is an additional critical factor for primordial follicle development [8, 9], playing as a strong inducer of kit ligand (KL) in GCs. KL acts to produce oocyte BMP-15 through its receptor c-Kit. Production of KL in

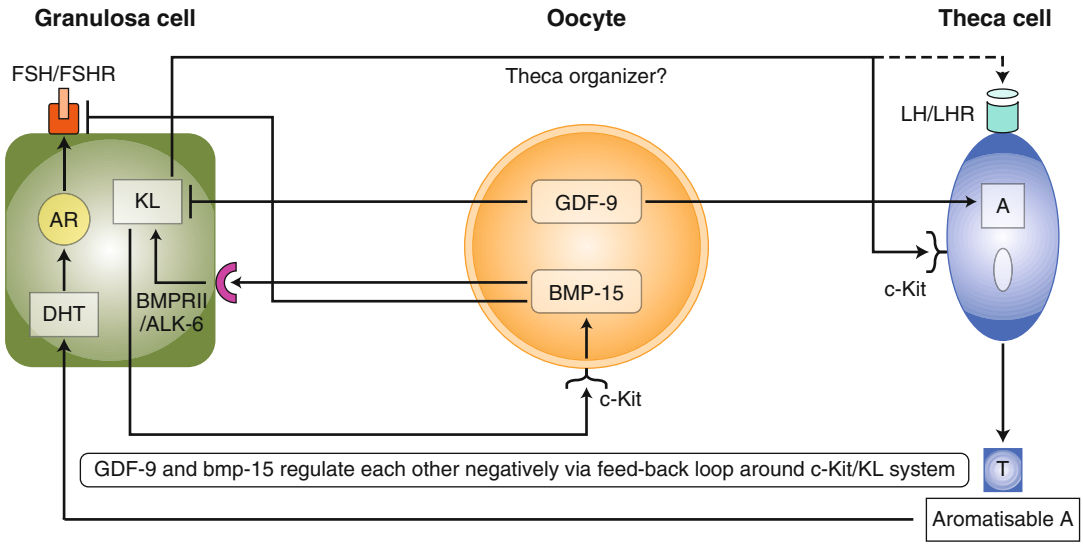

Fig. 1.3 Regulatory interplay of oocyte-originated GDF-9 and BMP-15 among oocytes and granulosa and theca cells for preantral follicle development. Testosterone converts to non-aromatizable androgen, 5α-dihydrotestosterone (5α-DHT) by 5α-reductase for acquisition of binding capacity to receptors for androgen (*AR*) in granulosa cells

GCs is inversely regulated by GDF-9 exhibiting a negative feedback on KL expression in GCs through c-Kit. c-Kit is also expressed in TCs, which KL can stimulate to produce A. The produced A converts to 5α-dihydrotestosterone (5α-DHT) in GCs to be able to bind to nuclear receptors for A of GCs. It is of note that both oocyte factors act synergistically with each other for follicular growth at least through the antral stage [10] via theca A production, even if the signaling pathways are different (Fig. 1.3).

Androgen

Evidence accumulates to indicate that A plays a role of a sort of growth factor in preantral as well as in antral folliculogenesis [11, 12]. As androgen receptors (ARs) are expressed in GCs and c-Kit on theca cells, either GDF-9-stimulated or BMP-15/KL-mediated theca A is capable of converting to non-aromatizable, receptor-binding 5α-DHT to bind to AR in GCs which, in turn, enhance expression of FSHR on GCs [13], resulting in acceleration of GC proliferation (Fig. 1.3). It is therefore, probable that GDF-9/BMP-15-dominated preantral folliculogenesis is not restricted to the Gn-sensitive stage but is extended to a much later stage of development, in the sense that the growth factor-mimicking action of A may be taken over successively to the antral follicle stage.

c-Kit/Kit Ligand (KL) System

KL, also termed as stem cell factor (SCF) or steel factor (SF), discovered originally as a factor of regulating stem cell growth and differentiation, acts through c-Kit tyrosine kinase receptor [14]. Two important roles have been attributed to c-Kit/KL system. First, GC-derived KL acts on oocytes to enlarge and initiate transition from primordial to primary follicle. Second, KL/c-Kt system is involved in differentiation of TCs from stroma cells (SCs) as a system entitled to be theca organizer [14] (Fig. 1.3).

Anti-Müllerian Hormone (AMH), Müllerian-Inhibiting Substance (MIS) (Fig. 1.2)

Anti-Müllerian hormone is a member of TGF-β superfamily and is the only strong negative regulator of initial and cyclic follicle recruitments. Critical roles are assigned to AMH at two steps of

follicular growth: at primordial follicle recruitment and dominant follicle selection [14]. The highest expression is observed immunohistochemically in GCs of preantral and small antral follicles (<4 mm), declining in larger follicles (4–8 mm) in humans [15], and is likely to be produced by GCs of growing follicles. AMH suppresses primordial to primary follicle transition, though is not expressed in primordial follicles. The hormonal mechanism for this inhibition is considered via reduction of aromatase and LH receptor (LHR) expression [16]. AMH signaling is mediated by activin (Act) receptor-like protein kinases (ALKs).

Anti-Müllerian hormone is clinically relevant to stimulation protocols because it is widely known as an excellent marker of ovarian reserve (OR) that is indispensable to estimate the quantitative and qualitative capacity of primordial follicle pool. Since measuring primordial follicle number is impossible, alternatively, the growing follicle number is usually employed as an indirect clinical marker, as indicated by the close correlation of AMH value with primordial follicle stock. Serum AMH declines with age to undetectable levels in menopause. As a matter of fact, both antral follicle count (AFC) and serum AMH are equally valuable for the prediction of ovarian response. According to Bologna criteria [17], poor ovarian response (POR) is defined as described in Table 1.1.

Table 1.1 Bologna criteria for poor ovarian response (POR)

Two of the following three criteria should be met for diagnosis of POR
1. Maternal age: ≥40 years of age or presence of any risk factor
2. Anamnesis for POR (number of oocytes recovered in a conventional ordinary ovarian stimulation cycle ≤3)
3. Presence of at least one of the following clauses is encountered by ovarian reserve test (ORT):
Antral follicle count <5–7 or AMH <0.5–1.1 ng/mL

Determined and recommended by ESHRE Consensus Workshop, March 19–20, 2010 [17]
NB: Baseline FSH level: FSH >10–15 IU/I is not adopted due to inadequate accuracy
AMH: The strongest expression has been reported in granulosa cells of antral follicles of φ=4–6 mm. It has two critical roles in follicle development: one is firm suppression of primordial follicles and the other raising up FSH threshold for dominant follicle selection

PTEN (Phosphatase and Tensin Homolog Deleted on Chromosome 10) (Fig. 1.2)

Preantral growth is strictly suppressed by AMH and PTEN to keep the primordial follicle pool dormant against growth stimuli. It is of note that the AR can be observed histochemically in TCs and GCs earlier than receptors for estrogen (ER) in these cell types [11], suggesting earlier involvement of A than estrogen (E) in preantral follicle growth.

Antral Follicle Growth

Gonadotropins (FSH and LH)

Principles of Dominant Follicle Selection

The specific role of Gn in antral follicle growth is selection of a single dominant follicle among a cohort of large antral follicles (5–9 mm) (Fig. 1.1) that start growing in response to gradual rise of FSH around the perimenstrual period (also termed as first FSH window) in natural cycles (Fig. 1.4). Dominant follicle selection is a fundamental event for mono-ovulatory species including humans and is primarily regulated by the FSH threshold theory [18], along with LH ceiling hypothesis [19] (Table 1.2).

According to the FSH threshold theory, tonic FSH stimulation accelerates growth of a cohort of follicles, not uniformly but differentially, depending on the intensity of FSHR expressed in each of the selected follicles. Accordingly, the follicle with the highest density of FSHR should have priority to be chosen for growth, being given the opportunity to grow up in response to the lowest level of FSH [18]. This asynchronous follicular growth is exaggerated as follicle development proceeds until selection of a single dominant follicle because graded increase of serum FSH levels cause exclusion of non-eligible follicles with lesser expression of FSHR [19, 20] (Table 1.2).

Timing of Dominant Follicle Selection

Another issue is the timing of dominant follicle selection. There is a theoretical reason, indicat-

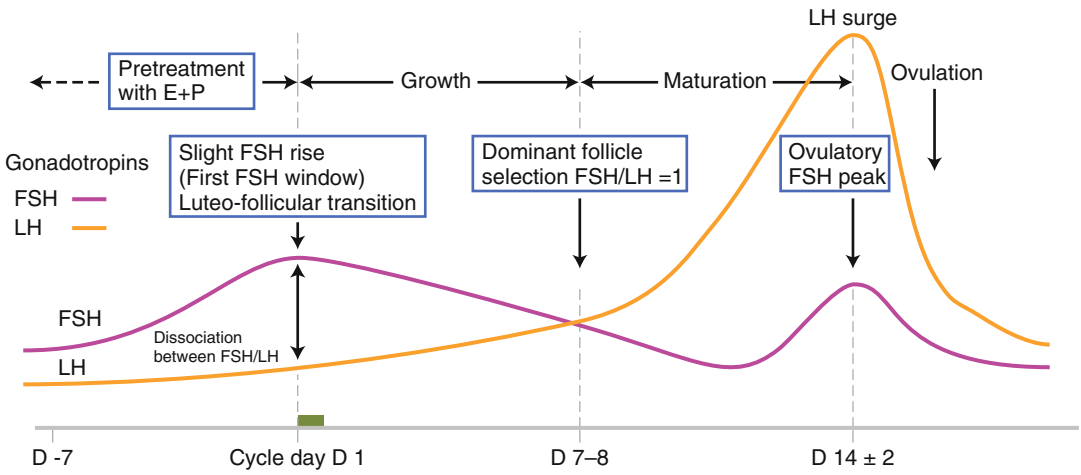

Fig. 1.4 Changes in serum follicle-stimulating hormone (*FSH*) and luteinizing hormone (*LH*) throughout the human menstrual cycle: Pretreatment with estrogen (*E*) plus progesterone (*P*) ensures dissociation between basal FSH and LH levels, resulting in perimenstrual rise of FSH

Table 1.2 Principles of dominant follicle selection by FSH and maturation of selected follicle by LH

FSH threshold hypothesis (Brown 1978) [18]	LH ceiling hypothesis (Hillier 1993) [19]
1. Ovarian follicles have development-related requirements for stimulation by FSH	1. Ovarian follicles have development-related requirements for stimulation by LH
2. FSH, beyond a certain "threshold" level, stimulates granulosa proliferation and functional maturation (expression of aromatase, luteinizing hormone receptors, inhibin synthesis, etc.)	2. LH, beyond a certain "ceiling" level, suppresses granulosa proliferation and initiates atresia (non-dominant follicles) or premature luteinization (preovulatory follicle)
3. Follicles become increasingly sensitive (lower threshold) to FSH as they mature	3. Mature follicles are more resistant (higher ceiling) to LH than immature ones
4. During ovulation induction, FSH dose should exceed the threshold of the most mature follicle	4. During ovulation induction, LH dose should not exceed the ceiling of the most mature follicle

ing that it should be the day when the descending FSH curve crosses with the ascending curve of LH in the mid-follicular phase of the cycle (day 7–8) on the basis that the baseline FSH level rises up to its highest value around the perimenstrual period, and then declines due to suppression by increasing E and inhibin-B (Inh-B) coming from the growing selected follicle cohort of the corresponding cycle (Figs. 1.4 and 1.5). If suppressive activity is strengthened too much, all the follicles belonging to the cohort will stop growing due to FSH threshold hypothesis [18]. An intervening principle has been reported that BMP-15 has the potency to suppress FSHR expression [20], a mechanism by which excess stimulation of FSH can be avoided so as to keep the FSH value below the threshold level [18] as illustrated in Fig. 1.5. Without this protection mechanism, all the growing cohort of follicles shall die by atresia.

Since the day of dominant follicle selection can, in theory, be monitored by the decrease in the FSH/LH ratio below 1.0 in terms of comparable bioactivity units, the ratio should have stayed above 1.0 until the day of selection (Fig. 1.4). Subsequently, the ratio declines below 1.0 as a result of rising levels of LH after the dominant follicle enters maturational stage. This principle might also be valid in multifollicular stimulation cycles, if one assumes plural dominant-equivalent follicles (DEFs) being selected for further maturation instead of monofollicular growth. Thus, it is reasonable to conceive that the day of Inh-B peak should coincide with the day of dominant follicle selection (Figs. 1.4 and 1.5). Based on this concept, the

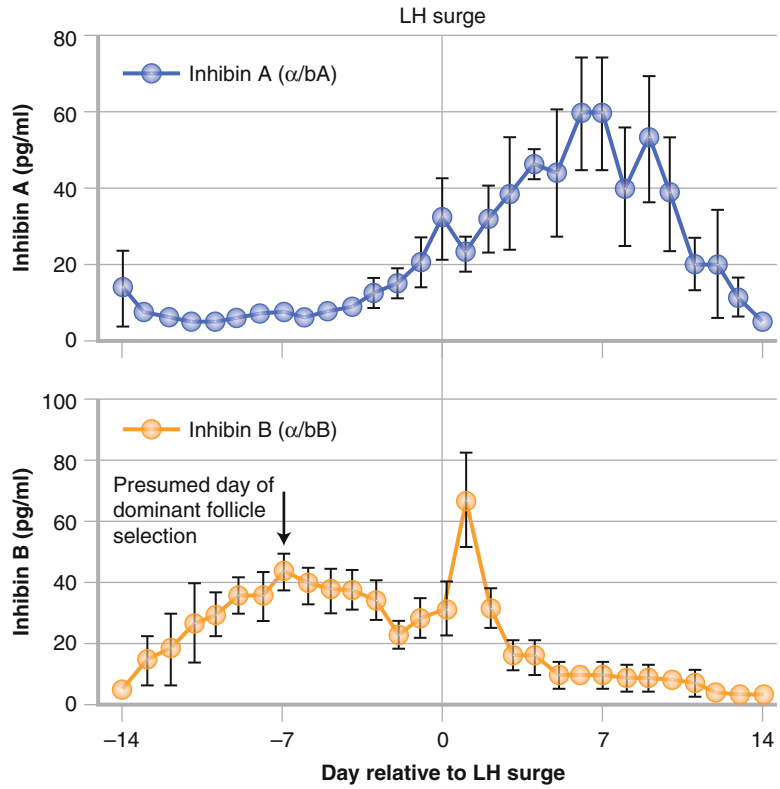

Fig. 1.5 Peripheral blood concentrations of inhibin A (Inh-A) and inhibin B (Inh-B) during human menstrual cycle: Inh-B increases to reach the highest level around on D-7 in the mid-follicular phase, the day when descending FSH curve just crosses with ascending LH curve, signifying the day for dominant follicle selection (Tajima K et al. 2006, revised and adapted from the original Figure by Groome et al. [29])

FSH/LH ratio could be utilized as a good indicator for assessing the terminal point of follicular growth and/or the initiating point of follicular maturation in ovarian stimulation protocols [21].

Atresia of Subordinate Follicles

There are at least two initial origins of atresia inside of follicles: the first one is of the oocyte and the second of follicle cell origin, [21] which occurs via an apoptotic mechanism [22]. In the preantral stage of the follicles, the first appears predominant, being replaced by the second as follicular growth proceeds towards the vesicular stage (Fig. 1.1). All the subordinate follicles except for the selected one are destined to undergo atresia due to shortage of FSHR density outside the aptitude zone of FSH/LH levels, expressed in the course of follicle development (Fig. 1.6). It is also probable that subordinate follicles are ready to undergo atresia when exposed to excess FSH- and/or E-induced LHR [23, 24], expressed on GCs than those of the ceiling value [19]. At the same time, E is shown to inhibit C17A enzyme activity to prevent A overproduction by a sort of product inhibition mechanism; otherwise A may exhibit atresia-inducing action.

Gonadal Steroids

Androgen (A)

Although regulation of steroidogenic function by two types of follicular cells with differential regulation through FSH and LH has elegantly been defined [25], the significance of theca A synthesis is pointed out with a changing profile of steroidogenesis in human follicular development [26]. Theca A contributes to follicular growth at least in two distinct ways [7, 19, 27]. First, it enhances FSH-stimulated follicle growth via intensifying FSHR expression on GCs, as was observed with the preantral follicles [7, 21]. Since FSHR expression on GCs is likely to be mediated by GC-expressed AR [11, 12], growing follicles should be prepared with the intensifying density of FSHR until dominant follicle selection since FSH works as the major driving force

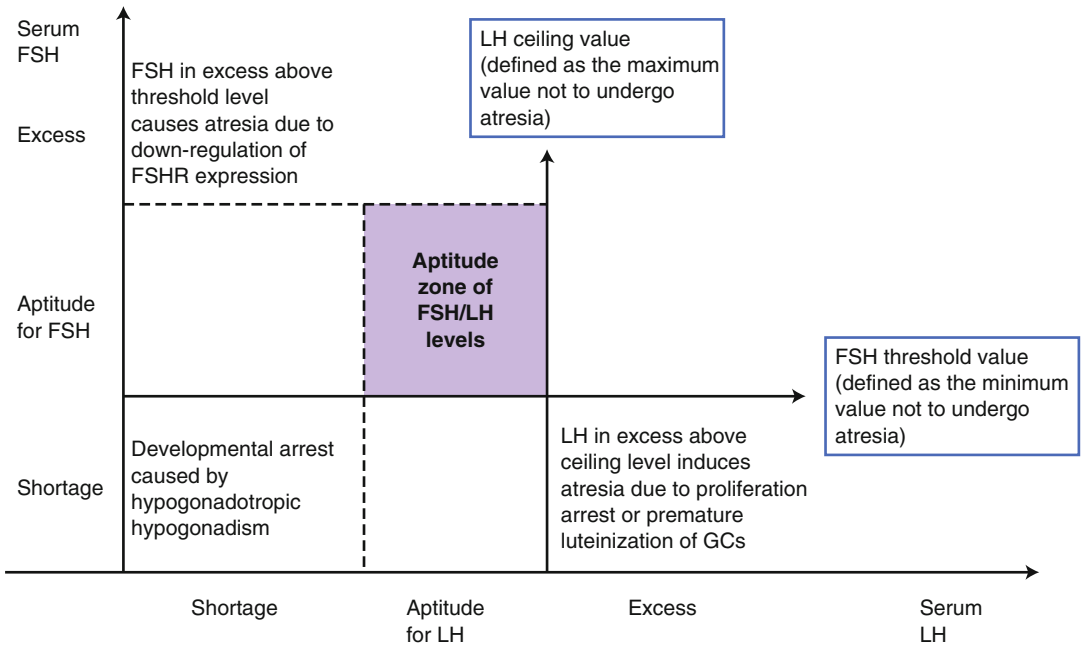

Fig. 1.6 Aptitude zone of FSH and LH levels for dominant follicle selection and subsequent maturation until preovulatory stage: Depicted by the author based on FSH threshold theory (Brown [18]) and LH ceiling hypothesis (Hillier SG 1992)

of GC proliferation. Second, utilizable substrate A should be provided in order to meet the increasing demand of E for GC proliferation in this rapidly growing stage (Fig. 1.7). Given that the AR expression is the highest in preantral and antral follicles with a subsequent gradual decrease along with follicular development towards the preovulatory stage [27], it is reasonable to interpret that the converted E from A is to be utilized for GC proliferation through cyclin D2 activation to support mitotic activity at this stage of development [28], rather than for GC differentiation (Fig. 1.7). Even if the two events are supposed to be regulated simultaneously by ARs in GCs, the expression of ARs is gradually downregulated as the developmental stages proceed [27], and another mechanism must come into existence to sustain substantial amount of E needed for the maturation process to proceed without falling into atresia. This will be discussed later. Taken together, FSH/androgen-tonic stimulation is the major stream of follicular growth, up until dominant follicle selection (Fig. 1.8).

Estrogen (E)

Granulosa cell proliferation is apparently induced by direct E action during the growing stage and E is an indispensable driving force. Since E is expected to serve for GC proliferation and not for differentiation in this rapidly growing phase, caution must be taken not to induce premature production of E because excess E may result in premature GC differentiation, an unfavorable condition if occurs prior to dominant follicle selection due to excess LHR expression on GCs [23, 24] that culminates in follicular atresia.

Theca A is involved in E production in GCs in two ways: one is as a substrate for aromatase and the other as an FSHR inducer. Accordingly, theca A must convert to 5α-dihydrotestosterone (5α-DHT) by 5α-reductase before binding nuclear AR of GCs under strict control of FSH. Considering a co-operative relation between the two enzymes, it is tempting to hypothesize that there must be some proper timing for the two enzymes to be activated even more strongly, simultaneously under both FSH and LH control.

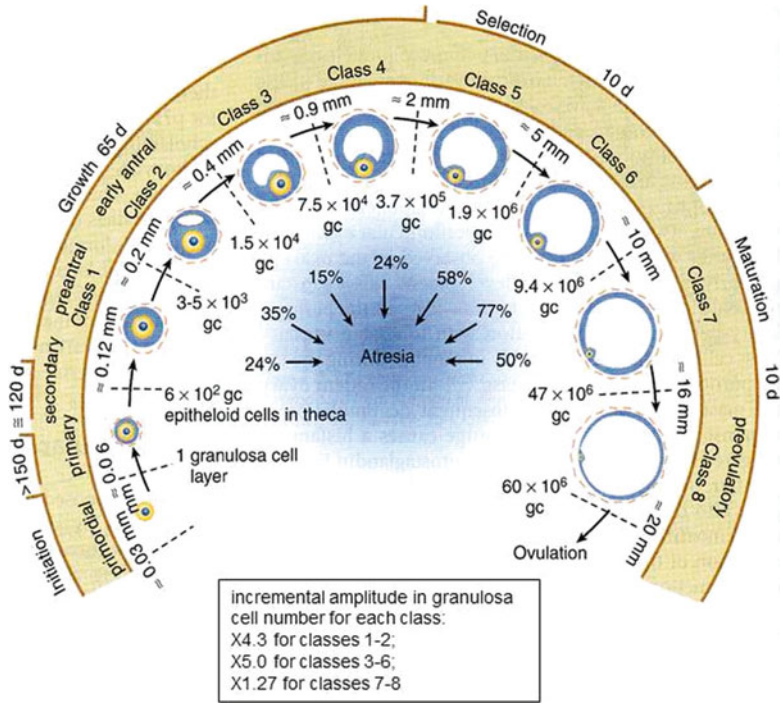

Fig. 1.7 Stages of follicular development in the adult human ovary and extent of atresia in the eight classes of growing follicles (Reproduced with permission from Gougeon [30]). Chapter author (T. Mori) notifies that incremental amplitude in granulosa cell number for each class is ×4.3 for classes 1–2, ×5.0 for classes 3–6, and ×1.27 for classes 7–8, respectively. Suppose that the differential amplitude may reflect velocity of granulosa cell division in each class of follicles; follicles of classes 1–2 are growing at relatively rapid rate, those of classes 3–6 most rapidly and those of classes 7–8 quite slowly, signifying that each of the three categories may reflect each of the large antral, the growing vesicular, and the maturing vesicular follicles, respectively

Progesterone (P)

There is no indication to suggest any active participation of P in follicular growth. Rather, P could participate in follicular atresia among subordinate follicles in competition for dominancy at this stage of development, though concrete evidence is still lacking.

Activin (Act) and Inhibin (Inh)

Act and Inh, both being produced by GCs under stringent control of FSH, belong to TGF-β growth factor superfamily. Act has a homodimeric composition of two subunits, βA and βB, forming active mature forms of Act A (βAβA), Act B (βBβB), and Act AB (βAβB). Inh is a heterodimer composed of α and β subunits. Accordingly, the subunit structure of Inh-A is αβA and Inh-B is αβB, respectively. Act exerts its intensive amplifying effect on GCs to synthesize E in an autocrine fashion, whereas Inh stimulates theca A synthesis in paracrine fashion during antral follicle growth until around the time of dominant follicle selection (Figs. 1.5 and 1.9). Considering the modifying function of Act and Inh in conjunction with follicular steroidogenesis during antral growth, Act serves as the principal contributor for E biosynthesis because this steroid is expected to act as a potent mitogen for GC proliferation rather than for differentiation in antral follicle growth (Fig. 1.7). The predominant autocrine activity of Act as an enhancer of production of E, a powerful factor of GC mitosis, has well been documented on the one hand; the turning point of dominancy from Act to Inh remains to be defined on the other hand.

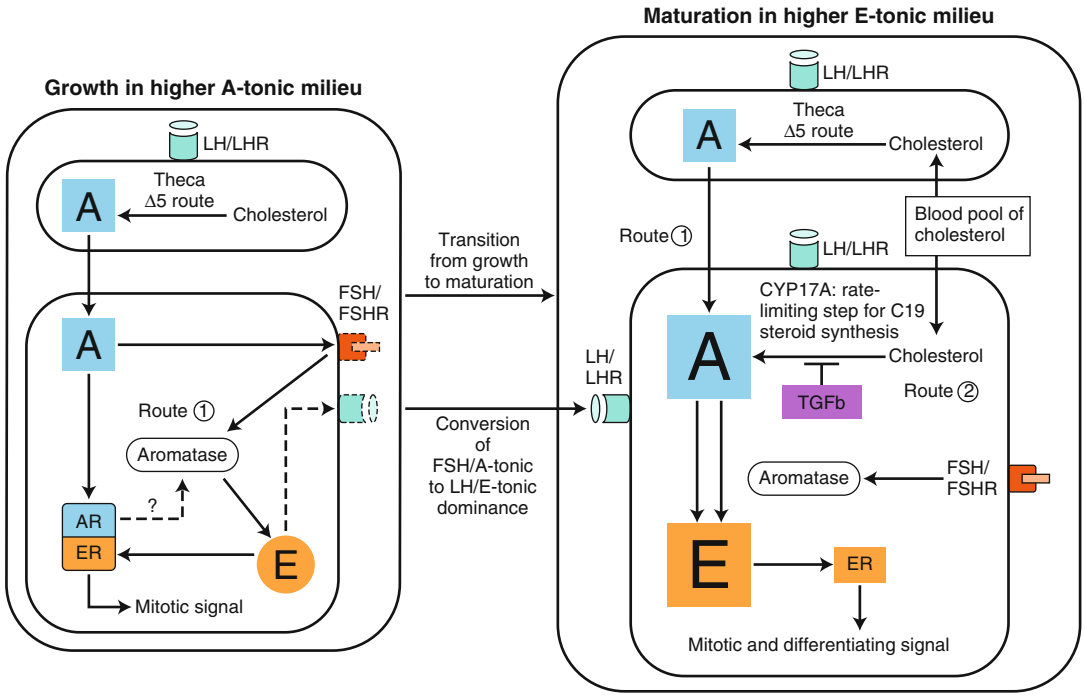

Fig. 1.8 Functional shift in regulatory system of Gn-gonadal steroids during transition from growing to maturing follicle development. While FSH/androgen system controls predominantly via single route of E synthesis in the stage of follicle growth, FSH + LH/estrogen govern follicle maturation via double routes until the stage of preovulatory development

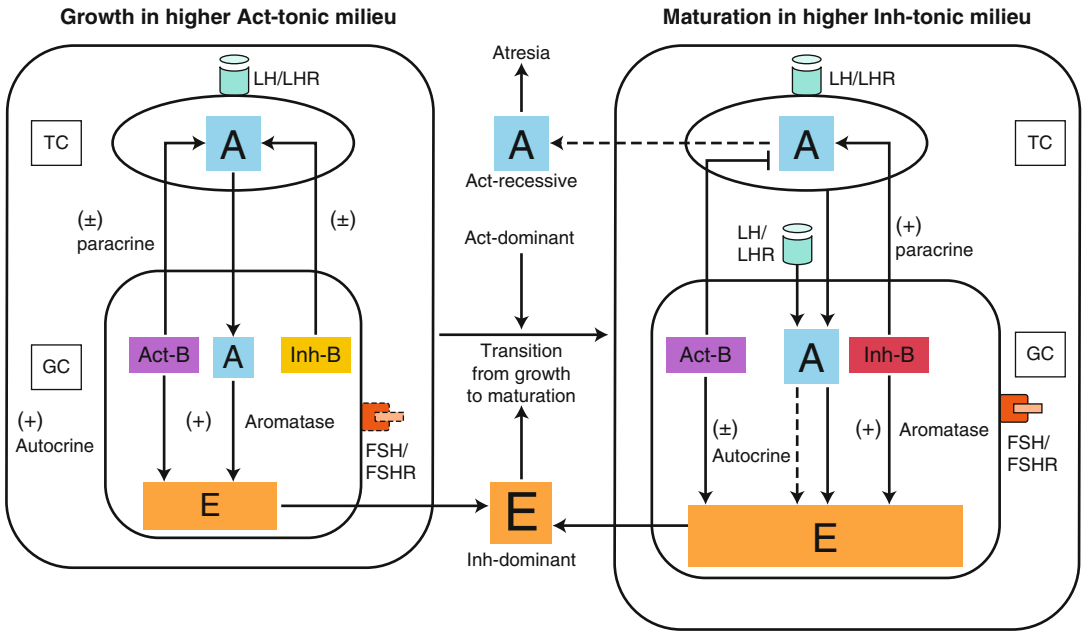

Fig. 1.9 Functional shift in Act-Inh regulatory system during transition from growing to maturing follicle development. Act tonus is relatively higher in antral to small vesicular follicles than in large vesicular follicles. In addition to suppressive action on FSH secretion at the pituitary level, Act-B stimulates growth of antral follicles by enhancing granulosa cell E production in an autocrine fashion. As follicular development proceeds, Inh-B exerts its paracrine action toward theca cells to accelerate androgen (*A*) production, the provision of which, in turn, accelerates granulosa cell E synthesis in a paracrine fashion

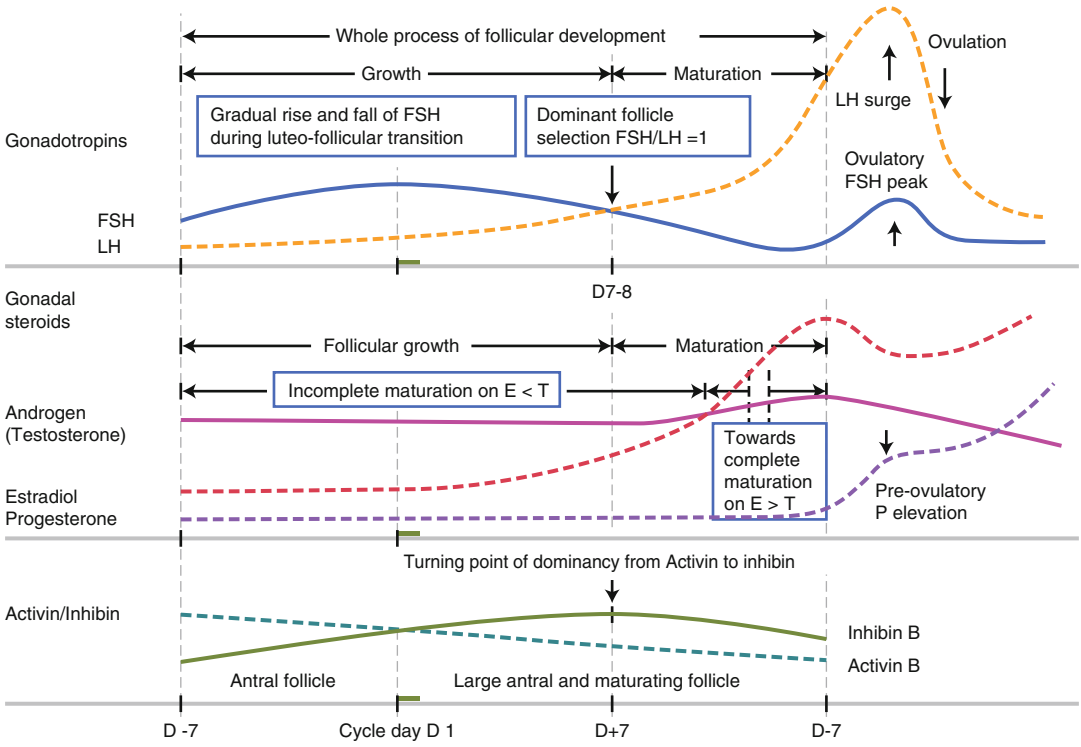

Fig. 1.10 Putative three-dimensional regulations with gonadotropins, gonadal steroids, and activin-inhibin systems of follicle development during the natural menstrual cycle. It is important to notice that α-subunit formation is the rate-limiting step to determine the direction of producing Act and/or Inh during process of follicular development

At the time when Inh-B reaches its highest level, the suppressive potency of Inh-B on pituitary FSH secretion becomes maximal, and this time point should coincide with timing of dominant follicle selection, as already described. Once a dominant follicle is selected, the suppressive potency of Inh-B declines to prevent further growth of subordinate follicles and should be replaced by the continual rising of E from the selected dominant follicle that enters the maturion stage afterwards [29] (Figs. 1.9 and 1.10).

Follicular Maturation

Gonadotropins (FSH and LH)

Dividing Velocity of GCs

At a glance of the classified diagram of follicular development (Fig. 1.7) in which eight distinct classes are discriminated depending on the dividing velocity of GCs in each class [30], it is notable that the last two classes of 7 and 8 show a drastic slowing down in the multiplication index of GC number (×1.27) in contrast to those of class 3 through 6 (×5.0), signifying that GCs of the final two classes undergo cellular differentiation rather than proliferation to suggest that follicles in these classes are maturing rather than growing. If LH in addition to FSH must have commitment to a certain extent in the maturational stage of development, LH could respond to an increasing demand of E for final maturation of GCs, cumulus cells (CCs), and OC itself [19, 31] as well. Notice must be taken that the term "follicle maturation" means full differentiation of the constituent follicle cells, and not of oocyte itself, which is induced by the LH surge.

LH-Assisted Maturation

Upon the turning point of dominant follicle selection, the selected follicle enters the next and final stage of maturation when LH is asked to play a supplementary but critical role to FSH action in

terms of accelerated E production. Therefore, LH activity is thought to be required for proper maturational processes to proceed [32]. The possible role of increasing LH for final maturation is presumed to supply the additionally required E that is actively produced in joint action of GCs and TCs, both for triggering positive feedback action on the pituitary (Fig. 1.8) and possibly, towards the oocyte for initiating meiosis [31].

It is proven that human chorionic gonadotropin (hCG) activity is contained as an intrinsic constituent of highly purified human menopausal gonadotropin (HP-hMG, Menotropin) preparations. While E exerts its feedback action negatively on FSH during the growing phase of large antral (vesicular) follicles (Fig. 1.1), since increasing concentrations of E trigger LH surge during maturational and preovulatory stages, it seems critically important to judge an accurate timing for hCG injection so as to harvest oocytes of good quality in COS that usually accommodates multiple follicles, as described with the term of DEFs. It has been reported that excess FSH relative to LH could cause premature P elevation via enhancement of TFGβ expression (Fig. 1.11). For this purpose, monitoring the serum E value per maturing follicle, the relative ratio of E/A and size of mature follicles point toward full maturity prior to hCG injection (Figs. 1.4 and 1.10).

LH-Induced Atresia

LH looks to exhibit dual actions in the maturational stage [21]. In contrast to LH-assisted maturation, LH may accelerate atresia when exposed to 5α-DHT, which inhibits GC proliferation by decreasing cyclin D2 mRNA expression and cell cycle arrest at G1 phase [33], leading to apoptosis followed by atresia. According to the LH ceiling hypothesis [19] (Fig. 1.6), it is also probable that excess LH above ceiling level could arouse premature luteinization of GCs, culminating in GC apoptosis [22] or TCs necrosis [26].

Gonadal Steroids

Androgen (A)

Since the dominant follicle must switch to maturing from growing in order to respond to the acutely increasing demand for E for its three subsequent targets to work on, endometrial preparation for implantation, positive feedback on the pituitary, and oocyte maturation [21, 31], the single maturing follicle and/or DEFs become endowed with an additional route of E biosynthesis, which can be called granulosa Δ5 route (Fig. 1.8). Cholesterol is now provided directly from circulation through a carrier protein into GCs of maturing follicle and fully matured GCs are well equipped with enzymes relevant to

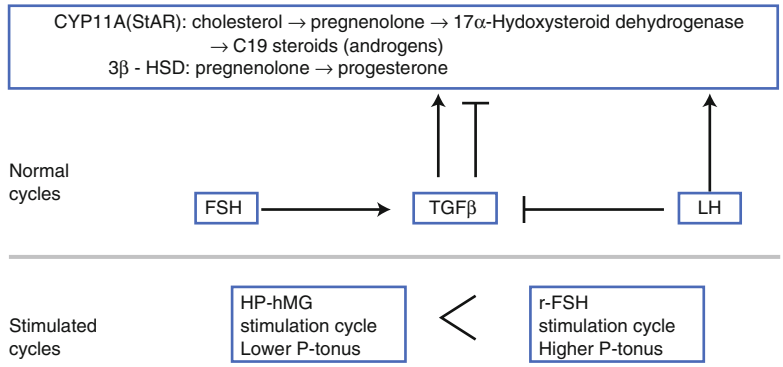

Fig. 1.11 Regulatory mechanism for appearance of preterm P elevation. TGF-β is upregulated by FSH but downregulated by LH in normal cycles, As proven, a limited amount of human chorionic gonadotropin (hCG) is reported to contain as an intrinsic constituent in terms of biological activity in highly purified human menopausal gonadotropin preparations (HP-hMG) (Menotrophin). In HP-hMG-stimulated cycles, intrinsically contained hCG-derived LH activity counteracts against the enhancing action of FSH/TGF-β-induced P production, resulting in protection of preterm progesterone (*P*) elevation

steroidogenesis from cholesterol to A. In other words, another Δ5 route is available in GCs of maturing follicles. Thus, substrate A is robustly supplied for aromatase activity via double routes in response to the principal E demand.

Estrogen (E)

Estrogen exerts its obligatory function on the three targets at the terminal point of follicular development, as already described [31–34]. Instead of negative feedback action of E on the pituitary in the growth phase of development, E action turns around to positive feedback on the pituitary in this maturing phase of development. The triggering signal for the LH surge is induced by acutely increasing E, secreted from the fully matured follicle in natural or COS cycles if pituitary desensitization is not induced.

Progesterone (P)

Premature Luteinization

When premature luteinization, defined as a P/E_2 ratio of more than 1.0 on the day of hCG injection [35], was observed, it was pointed out that this group of patients have a worse outcome than those with a lower P/E_2 ratio in a Gn-agonist protocol, an evidence suggesting poor ovarian reserve. Three factors are pointed out to be causally related to premature P rise independently of initiating premature LH surge: follicle number, FSH drive, and LH activity [36]. Out of the several cut-off values proposed, ranging from 0.8 to 2.0 ng/mL (2.5–6.4 nmol/L) on the day of hCG, 1.5 ng/mL can be adopted as a standard value in terms of the most frequently quoted figures [37].

Preterm P Elevation

On the other hand, early rise of P can sometimes be encountered independently of premature luteinization and/or premature LH surge prior to hCG injection in stimulated cycles under pituitary downregulation. This rise has once been correlated with the premature LH surge or early luteinization, induced by relative excess of LH activity contained in gonadotropin preparations. There has been much confusion concerning the concept and terminology of this kind of irregular P elevation because the conventional term of premature luteinization appears mixed up with premature LH surge, each of which may occur independently from each other. Accordingly, it is unclear whether each of the two terminologies has its own discrete concept. In order to clear any ambiguity, it is the author's proposal that early rise of P during the phase of follicular maturation should be termed as "preterm P elevation," indicating an untimely elevation of circulating P that may occur in stimulation cycles, independently of a premature LH surge in order to determine the best timing for hCG injection in the course of monitoring full maturation of follicles.

Mechanism of Preterm P Elevation

It has become evident that FSH rather than LH activity is causally related to preterm P elevation with the involvement of TGF-β [38, 39], whereas TGF-β normally inhibits ovarian 17α-hydroxylase activity, a rate-limiting enzyme leading to A synthesis from C21 steroid to avoid excess production of P. Conversely, its activity is strictly downregulated by LH and upregulated by FSH under normal non-stimulated condition. Once stimulated with Gns, FSH acts to stimulate Δ5 pathway and 3β-hydroxysteroid dehydrogenase (3β-HSD) activity in GCs, resulting in increase in P synthesis (Fig. 1.11). The pharmacokinetic nature of gonadotropin preparation, employed for stimulation, should be taken into account on the basis of the choice of Gn preparations so as to tailor it to individual's ovarian reserve.

Activin (Act) and Inhibin (Inh)

A gradual but drastic switch from Act-B to Inh-B takes place during the maturational stage of development. In contrast to strong expression of Act in GCs of the preantral and antral follicles, the intensity decreases as follicular growth proceeds. There are three points of evidence to be noted with a link to switching from growth to maturation stages [40] (Figs. 1.9 and 1.10). First, the transition coincides with the time of appearance of the second pathway in the substrate A synthesis for aromatase in GCs. Second,

with regard to theca A production, Inh-B over-rides Act-B in driving potency via the positive feedback action of Inh-B produced on GCs in a paracrine fashion towards the TCs in the growth stage of large antral (vesicular) follicles before dominant follicle selection. Thirdly, Act-B acts as an autocrine enhancer for GC aromatase activity in the growth phase, while Act-B activity is almost completely replaced by Inh-B production, which, in turn, serves as a paracrine factor for A production in the TCs. Summarizing the role of Act-Inh system in maturing follicles, higher Inh/E tonus is characterized by paracrine and autocrine action of Inh-B over Act-B tonus owing to paracrine action of Act (Fig. 1.9). Thus, there is a functional turnover from Act to Inh during the growth to maturation stage of follicular development.

Consideration of Individualization of Stimulation Protocols

Indications and Acceptability

There must be at least two factors to be considered upon final selection of the most appropriate stimulation protocol; those are indication in terms of patient's OR and acceptability in terms of medical as well as non-medical reasons. OR is the first and most fundamental factor to be considered for selection of first-line protocols. Any patient with proven sufficient reserve deserves any kind of COS protocol with a broad spectrum of indications. Along with diminishing OR, the range of indications usually becomes narrower, depending on the actual ovarian response.

There is a discrepancy between the ovarian reserve and actual ovarian response that is expected from the selected stimulation protocol for indication. This discrepancy may come from an inappropriate selection of the protocol, incorrect estimation of the individual's OR, pharmacokinetic properties of the Gn preparation used [41–46], and non-medical circumstantial situations. Since each patient has her own receptive range that might be determined by the patient's ovarian reserve, the chosen protocol should match or must be placed at least in the spectrum of receptivity to the patient in question. Even if an individualized protocol were chosen in terms of the indication, it may or may not guarantee the expected clinical outcome in patients with declining ovarian reserve, such as aging women and other limiting conditions, as described in the Bologna criteria [17] (Table 1.1). Notice must be taken in understanding that the baseline (b) FSH level (bFSH >10–15 IU/l) is not incorporated in this criteria due to inadequate statistical accuracy. The description appears somewhat skeptical because bFSH value is informative to evaluate ovarian response in terms of a given stimulation cycle, in other words, in terms of responsiveness to FSH at the onset of starting the indicated stimulation and not in terms of ovarian reserve that consists mostly of preantral follicles. The reason for this is that diminished number of selectable follicles at the beginning of stimulation could be recovered to a certain extent if supplementary medication with the androgen, dehydroepiandrosterone sulfate (DHEA-S) or corticosteroid is given over prolonged cycles ahead of the treatment cycles until bFSH goes down to levels comparable or somewhat higher to those seen in younger patients (<10–15 IU/l).

Setting up of an Individualized Protocol

Upon setting up of an individualized protocol, acceptability of the protocol may be influenced not only by medical but by extra-medical factors such as cost, job work, the spouse's thought toward family planning, and so on. It is therefore, necessary for the doctor in charge to keep these social factors in mind when making a final decision to choose the most appropriate protocol. The concept of individualization can therefore, be termed as selection of the most appropriate stimulation protocol from the indicated candidate protocols that may be applied to the patient, as described extensively in several elaborate reviews including risk [47–51].

Conclusion

Ever since clinical application of human IVF has started, the fundamental methodology of ovarian stimulation keeps evolving year by year to establish a wide variety of systematized modalities for IVF treatment. Owing to our understanding and knowledge obtained in the past three decades, we have reached a certain goal of handling ovarian stimulation, assisted by endocrine, ultrasound, and pharmacologic preparations.

In constructing stimulation protocols, it is important to keep in mind that both indication and acceptability of the individual patient should be taken into account while choosing the most appropriate protocol from the indicated ones so as to meet the demands of her own OR.

However, our current medical managements have proven quite limited for women approaching the terminal stage of reproductive cycles, that is, oocyte aging makes contemporary medical technologies almost impossible for a successful outcome. A new era, with novel medical technologies with innovative ideas is expected to come.

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Monitoring Ovarian Stimulation: Current Perspectives

2

Madhuri Patil

Abstract

In the normal ovulatory cycle, the recruited cohort of antral follicles can be identified by cycle day 5–7, the dominant follicle emerges by day 8–12, grows approximately 1–3 mm per day thereafter (most rapidly over the 1–2 days immediately preceding ovulation), and measures approximately 20–24 mm in mean diameter when the luteinizing hormone (LH) surge occurs; lesser follicles rarely exceed approximately 14 mm in diameter. In 5–10 % of spontaneous cycles, two preovulatory follicles may develop. The ultrasound examination enables the follicle diameter and endometrial thickness to be measured, which evaluates the fecundity function by using blood-flow assessment and the combined three dimensional (3D) and blood-flow investigation.

Ovarian ultrasonography defines the size and number of follicles contributing to the measured estradiol (E2) level. Thus, in an ovulation induction cycle, ultrasound can tell us about the ovarian reserve and adequately monitor the process of downregulation, follicular and endometrial development, and timely administration of human chorionic hormone (hCG), with an increase in the overall pregnancy rates and decrease in the incidence of ovarian hyperstimulation syndrome (OHSS) and multiple pregnancy rate.

Baseline follicular stimulating hormone (FSH), antiMullerian hormone (AMH), and inhibin B levels on day 2 or 3 on menstrual cycle and dynamic tests can give information about the ovarian reserve. Monitoring LH, E2, and progesterone during ovulation induction can determine the follicular growth and its competency, predict poor and hyper-response, and diagnose premature LH surge, premature luteinization and luteal phase adequacy.

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Keywords
Ovarian stimulation • Controlled ovarian stimulation • Transvaginal ultrasound monitoring • Hormonal monitoring • Follicular growth • Endometrial thickness

Abbreviations

2 D	2 Dimensional
3 D	3 Dimensional
4 D	4 Dimensional
AFC	Antral follicle count
AMH	Antimullerian hormone
ART	Assisted reproductive technology
B	Blood flow
BMI	Body mass index
CC	Clomiphene citrate
CCCT	Clomiphene citrate challenge test
CL	Corpus luteum
COS	Controlled ovarian stimulation
E2	Estradiol
EFORT	Exogenous FSH ovarian reserve test
EP	Ectopic pregnancy
ET	Embryo transfer
FET	Frozen embryo transfer
FI	Flow index
FSH	Follicular stimulating hormone
FVQ	Flow vessel quotient
GnRH	Gonadotropin-releasing hormone
GT	Gonadotropins
hCG	Human chorionic hormone
HRT	Hormone replacement treatment
ICSI	Intracytoplasmic sperm injection
IM	Intramuscular
IR	Implantation rate
IUI	Intrauterine insemination
IUP	Intrauterine pregnancy
IVF	In vitro fertilization
LH	Luteinizing hormone
LPD	Luteal phase deficiency
LUF	Luteinized unruptured follicle
MTX	Methotrexate
NNT	Numbers needed to treat
NPV	Negative predictive value
OHSS	Ovarian hyperstimulation syndrome
OI	Ovulation induction
ORT	Ovarian reserve test
P4	Progesterone
PCOS	Polycystic ovarian syndrome
PD	Power Doppler
PDA	Power Doppler angiography
PE	Elevated progesterone
PFBF	Perifollicular blood flow
PG	Prostaglandin
PI	Pulsatility index
POD	Pouch of Douglas
PPV	Positive predictive value
PSV	Peak systolic velocity
PUL	Pregnancy of unknown location
RI	Resistance index
SC	Subcutaneous
TAS	Transabdominal scan
TVS	Transvaginal ultrasound scan
USG	Ultrasonography
VEGF	Vascular endothelial growth factor
VFI	Vascularization flow index
VI	Vascularization index

Introduction

The aim of ovulation induction (OI) is to induce follicular growth. Pharmacological agents initiate, augment, or modulate the hormonal and gametogenic response of the ovary to overcome the natural follicular selection process to increase the number of oocytes available for fertilization. The OI protocol is different for a non-assisted reproductive technology (ART) cycle, which aims at mono-follicular development as compared to an ART cycle where we desire multifollicular development. It is the ART cycle, which requires more specific protocols and stringent monitoring.

It is also important for the clinician to know, whether OI is being performed for anovulation or for superovulation in a normal cycle.

Why Monitor OI Cycles?

The monitoring process is intended to enable the physician to choose the most suitable protocol to obtain best possible outcome, and to try to avoid complications. It is done in three stages.

1. Studying the patient's initial parameters
   - Base line scan – to rule out ovarian (ovarian cysts, hydrosalpinx) or uterine pathology (myomas, adenomyosis, polyps, intrauterine adhesions, endometrial abnormalities, congenital anomalies) and determine the ovarian reserve by performing the baseline antral follicle count (AFC)
   - Baseline hormonal profile – ovarian reserve, FSH: LH ratio, androgen excess, thyroid profile and hyperprolactenemia to predict the response to ovarian stimulation
   - Choose an appropriate stimulation regimen to prevent ovarian hyperstimulation syndrome (OHSS), multiple pregnancy, or poor response
2. Monitoring the ovarian response to ovulation induction, which includes
   - Confirm downregulation after gonadotropin-releasing hormone (GnRH) agonist administration
   - Determine response to the drug
   - Determine the dose and length of gonadotropin therapy
   - Determine optimal time for human chorionic gonadotropin (hCG) administration
   - Detect ovulation
   - Time oocyte retrieval
   - Identify patients who are poor responders and women at risk of developing OHSS
3. Completion of therapy
   - Diagnose complications of OI
     (a) Premature luteinization
     (b) Luteinized unruptured follicle (LUF) syndrome
     (c) Endogenous LH surge
     (d) Retention/functional cyst
   - Confirm pregnancy
   - Rule out multiple pregnancy
   - Diagnose late onset OHSS

How Do We Monitor the OI Cycle?

When we consider the monitoring process, we have to take into account the patient's comfort by simplifying treatment protocols and reducing the time and cost of monitoring. Before we embark on any OI therapy, it is important to assess the ovarian reserve. The monitoring involves transvaginal ultrasound for antral follicle count (AFC) and ovarian volume and hormone evaluation, which includes FSH, LH, estradiol, progesterone, and beta hCG.

Assessment of Ovarian Reserve

Ovarian reserve testing is required to identify women of relatively young age with diminished reserve and those around the mean age (41 years) at which natural fertility on average is lost but still have adequate ovarian reserve.

Assessment of ovarian reserve by ultrasound is done by measuring the AFC (Fig. 2.1), ovarian volume (Fig. 2.2), and stromal blood flow (Fig. 2.3), which will help us in predicting the ovarian response to controlled ovarian stimulation (COS). The number of antral follicles correlates well with the woman's age, ovarian reserve, and ovarian response to gonadotropin stimulation. As the ovary ages and the ovarian reserve decreases, there is a noticeable reduction in the ovarian volume and the number of antral follicles. An AFC of less than 5 and/or ovarian volume of less than 3 cm^3 is a good marker to predict poor ovarian response to COS in assisted reproduction programs (ART). There could be some intercycle variability in the AFC. Seventy-one percent of variation is due to intra subject examination and only 29 % is due to individual cycle variation.

The AFC can be evaluated either using the 2-dimensional (2D), 3-dimensional (3D), or 4-dimensional (4D) ultrasonography (USG) (Sono AVC – hypoechoic aspect of the ultrasound display

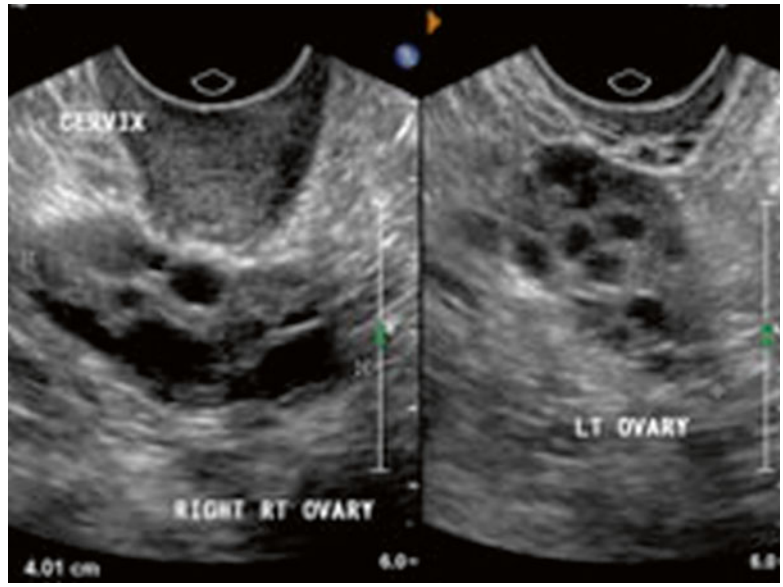


Fig. 2.1 Antral follicle count

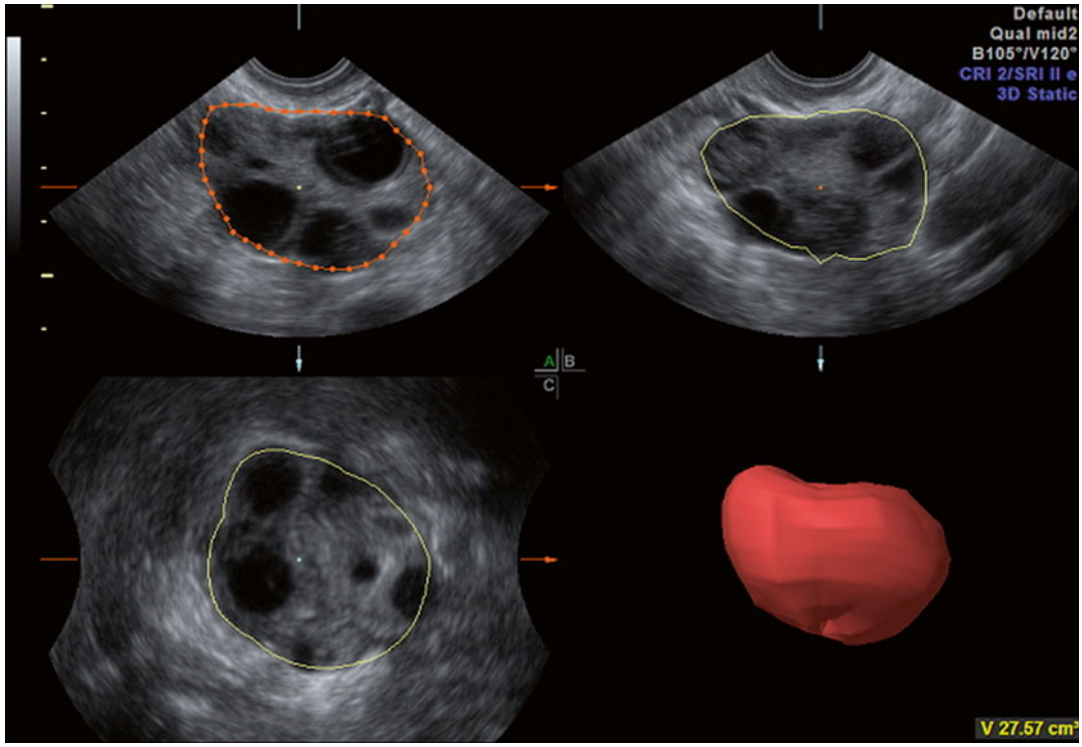


Fig. 2.2 Ovarian volume

is inverted to demonstrate fluid-filled areas within the 3D dataset). Sono AVC (Fig. 2.4) is the best model for predicting the number of oocytes retrieved with a retrieval rate of 60 %. AFC is a good predictor of response but not of pregnancy. The optimum cut-off value of AFC for poor response is ≤10 but the post-test probability was reported to be the highest at cut-off levels of <8 [1].

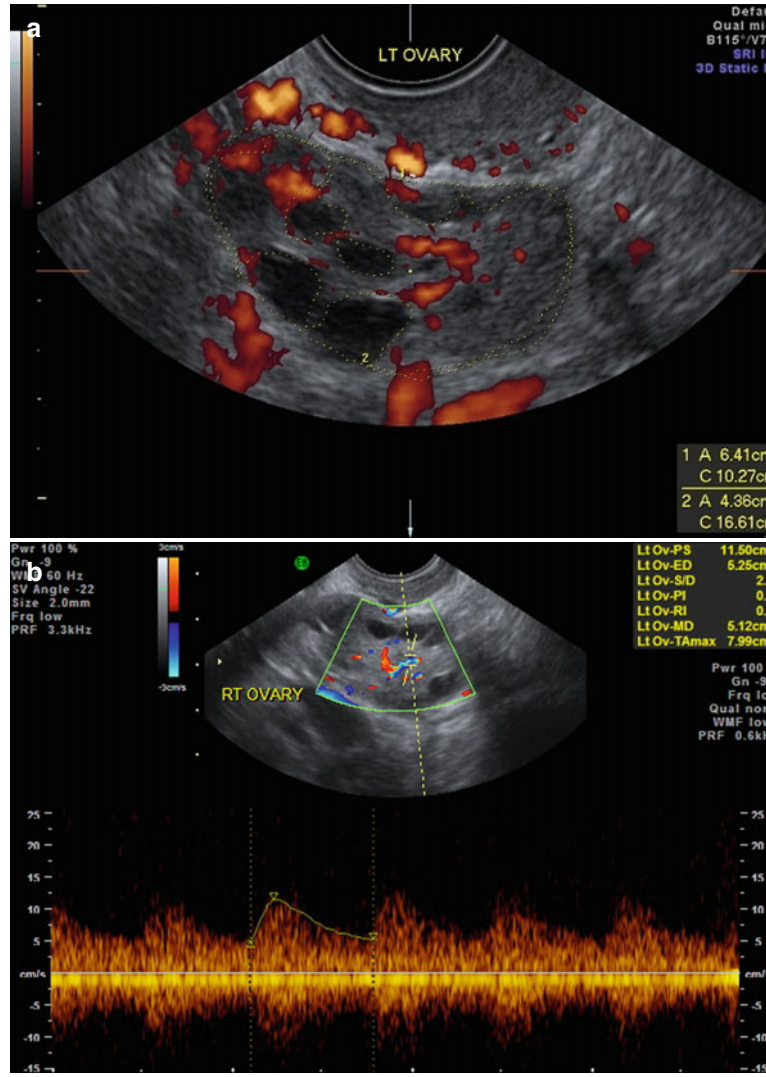


Fig. 2.3 (**a**, **b**) Stromal blood flow

The optimum cut-off value for hyperresponse of AFC is ≥14 with a sensitivity of 82 % and specificity 89 % to predict ovarian hyperstimulation syndrome (OHSS). The ovarian volume also correlates with the number of growing follicles, but not with the number of oocytes retrieved [2]. It was also observed that women with small ovaries with a volume of less than 3 cm^3 have a very high cancellation rate of in vitro fertilization (IVF) [3]. 3-dimensional ultrasound allows more precise calculation of ovarian and stromal volumes (Fig. 2.2). However, yet again, the predictive value for pregnancy by measuring the ovarian and stromal volume is limited (1.0–1.4) [4, 5].

Stromal Blood Flow (Fig. 2.3a, b)

- Stromal Flow Index (FI) [6]
  - <11 low responder
  - 11–14 Normal responders
  - >15 risk of OHSS
- Stromal Peak Systolic Velocity (PSV)
  - Low stromal PSV in the early follicular phase predicts poor responders

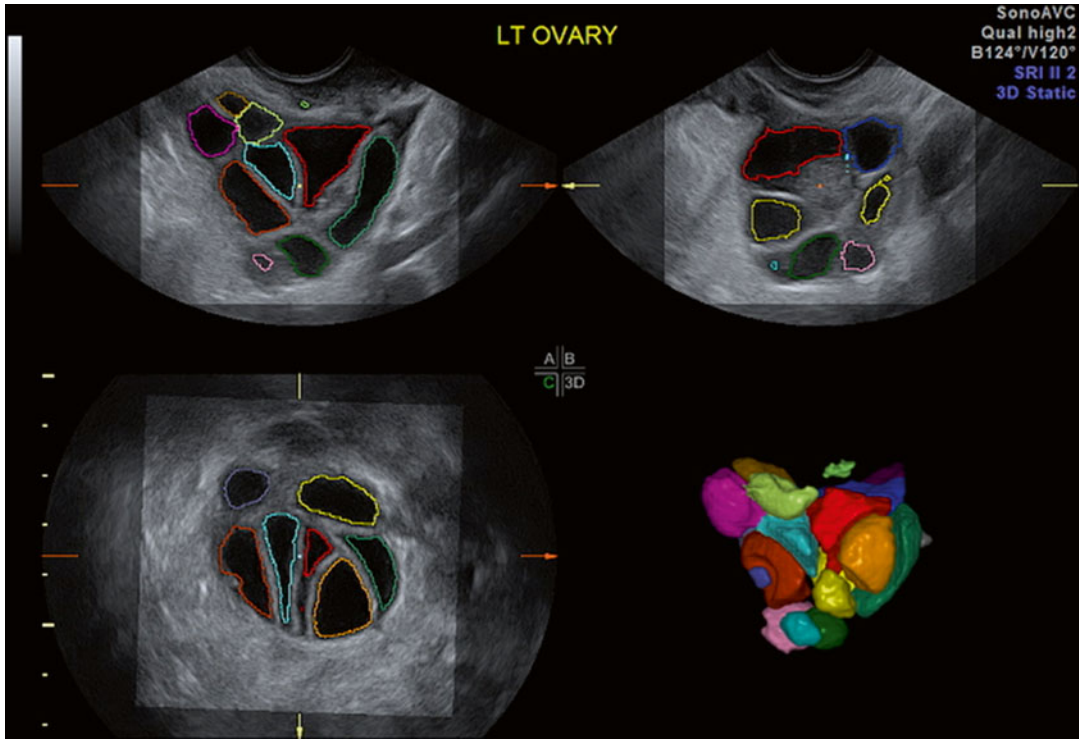


Fig. 2.4 Sono AVC

  - Increased stromal PSV with unchanged resistance predicts increased risk of OHSS
- Uterine Artery Blood Flow (Fig. 2.5)
  - Lower uterine artery resistance index (RI) and higher PSV has a high incidence of OHSS.
  - Uterine artery RI >0.79 is indicative of poor response and higher requirement of gonadotropin dose.

Ovarian reserve can also be assessed by performing hormonal tests like day 2 serum FSH, estradiol (E2), antiMüllerian hormone (AMH), and inhibin B levels.

Determination of ovarian reserve can predict response to tailor the correct stimulation regimens for adequate response so as to prevent complications and improve pregnancy outcomes. It also helps in improving the efficacy, safety, and cost-effectiveness of treatment.

Ovarian reserve testing allows us to choose individualized COS protocols, based on the age, AFC, and AMH. The dose can be further amended according to the body mass index (BMI).

Monitoring the Ovulation Induction Cycle

Follicular monitoring is today a pivotal investigation in both infertility evaluation and treatment. It is the gold standard to document ovulation, follicular development and growth, corpus luteum integrity, and endometrial growth and character.

Ovulation induction involves administration of either oral ovulogens or gonadotropins to enhance fertility. These drugs cause a supraphysiological increase in serum FSH, either indirectly as with oral ovulogens or directly as with gonadotropins, leading to the recruitment of a larger cohort of follicles. To time the intercourse, intrauterine insemination (IUI) or oocyte retrieval, we need to trigger ovulation at a particular diameter of the growing follicle for an optimal outcome. Moreover, ovulation induction can be associated with multiple gestation, OHSS, and torsion of ovary. Monitoring ovarian function, especially when women are administered ovulation induction drugs, becomes mandatory. The monitoring can be done either by ultrasound or by hormone bioassays.

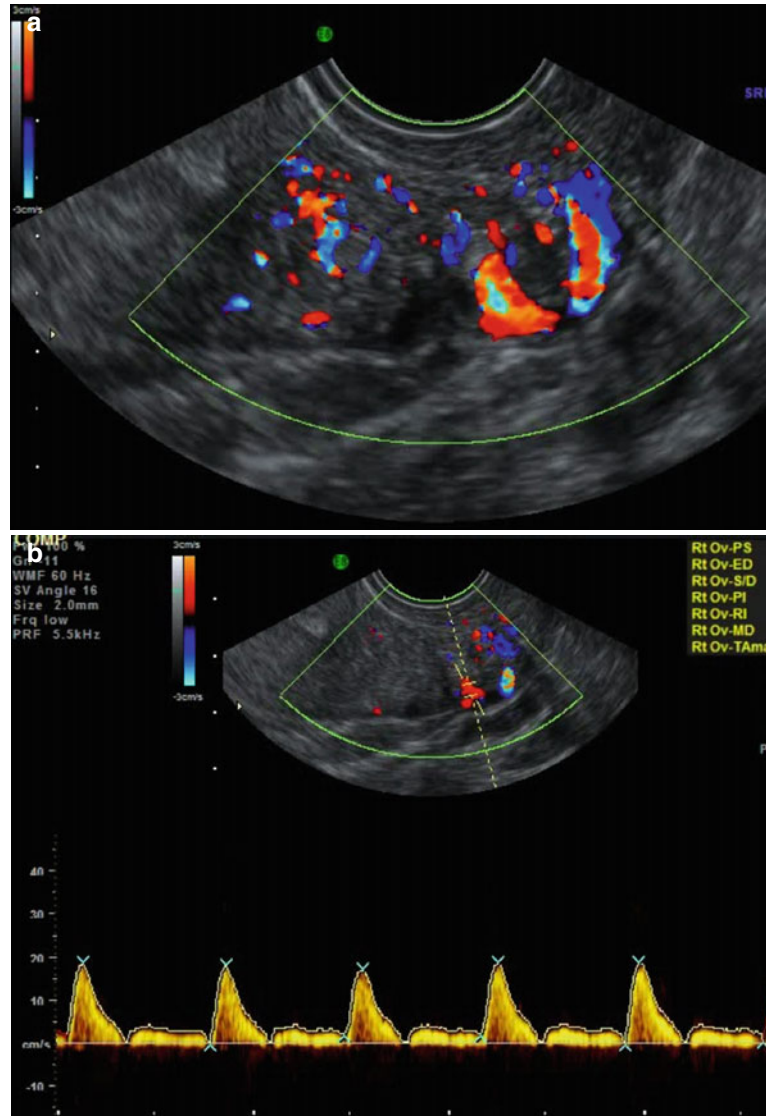


Fig. 2.5 (**a**, **b**) Uterine artery and venous blood flow

Ultrasound Monitoring

Ultrasound provides information on uterine and adnexal pathology, ovarian morphology, ovarian reserve and blood flow, endometrial thickness, morphology, and blood flow, follicular growth and timing to trigger ovulation, and feasibility of oocyte retrieval.

Color Doppler provides qualitative information, while the power Doppler (PD) signal can provide quantitative information [7]. In combination with 3D ultrasound, PD offers a tool with which one may not only demonstrate but also quantify total endometrial and regional uterine blood flow [8, 9].

Monitoring Ovarian Response to Ovulation Induction Agents

Ultrasound assessment of follicular growth was first introduced in 1978 when Hackelöer and Robinson [10] described a linear relationship between follicle size and circulating E2 levels. Since then, transvaginal ultrasound scan (TVS) has been used routinely to monitor follicular growth in natural cycles in ovulation induction programs, and during controlled ovarian hyperstimulation (COH) for ART cycles. TVS is the method of choice because of better visualization and accuracy though at times a transabdominal scan (TAS) may be required in special situations,

especially if the ovary is placed high in the pelvis and not visualized on a TVS.

The monitoring is different for a timed intercourse, IUI, and ART cycle and also for a natural, COS, and oral ovulation induction cycle [10].

During the natural cycle, a cohort of small antral follicles (2–5 mm in diameter) appears in the ovary early in the proliferative phase, which are selected 80–90 days prior from the primordial follicular pool. As FSH levels rise in the early follicular phase, further growth of the follicles occur. Decline of FSH in the late follicular phase, which is physiological, allows the selection of the single most sensitive follicle to continue to develop. The follicle, which will be selected to become dominant, will depend on the FSH and LH receptor content of the granulosa cells. The follicle, which has developed maximum receptors for FSH and LH in response to FSH will continue to grow, while the other follicles will undergo apoptosis and atresia. Once the leading follicle reaches a diameter of approximately 14 mm, the daily growth rate is between 1.5 and 2.0 mm until a diameter of 22–25 mm is reached, when ovulation occurs (Fig. 2.6).

Thus, in a natural cycle, monitoring provides information on whether the menstrual cycles is ovulatory or anovulatory. It can also identify delayed ovulation despite normal cycle length (28–30 days), short luteal phase as well as to assess hormonal (progesterone – P4) competence and support. It also provides information about the endometrial growth and morphology. It can also diagnose luteal phase abnormalities like luteinized unruptured follicle.

In natural cycles, serum E2 levels correlate with the follicle size, while the contribution of small atretic follicles to the steroidal milieu is negligible.

In a natural cycle, the first scan can be done either on day 9 or 10 of the menstrual cycle after the baseline scan on day 2 or 3. The scans can be repeated every 48 h till the follicles reach 14 mm, but once dominance is established and the follicle is 14 mm, the scan is repeated every 24 h. This is essential to determine the exact time of ovulation as the follicle can rupture at any time once the follicle becomes more than 16 mm. In these patients, pregnancies have been reported if IUI is done once follicular rupture is documented, hence, the importance of daily monitoring.

Characteristic ultrasound appearance at the time of ovulation includes diminution in the follicle size or sudden collapse of the follicle, blurring of the follicle borders, which become crenated, and appearance of intrafollicular echoes, which are more isoechogenic with respect to surrounding ovary (Fig. 2.7) and presence of a small amount of free fluid in the pouch of Douglas (POD) (Fig. 2.8). Thereafter, an irregular, slightly cystic structure representing the corpus luteum shrinks throughout the luteal phase of the cycle until luteolysis occurs before menses.

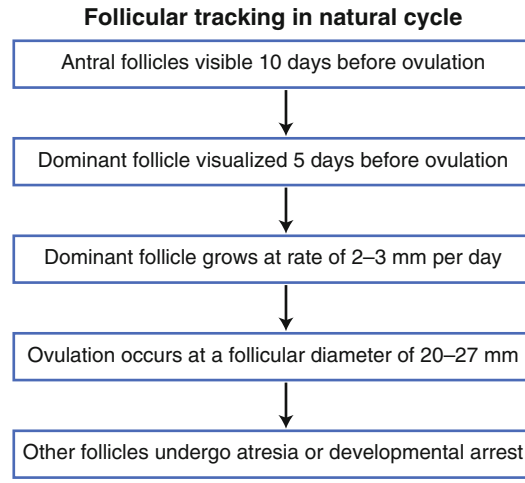


Fig. 2.6 Monitoring a natural cycle

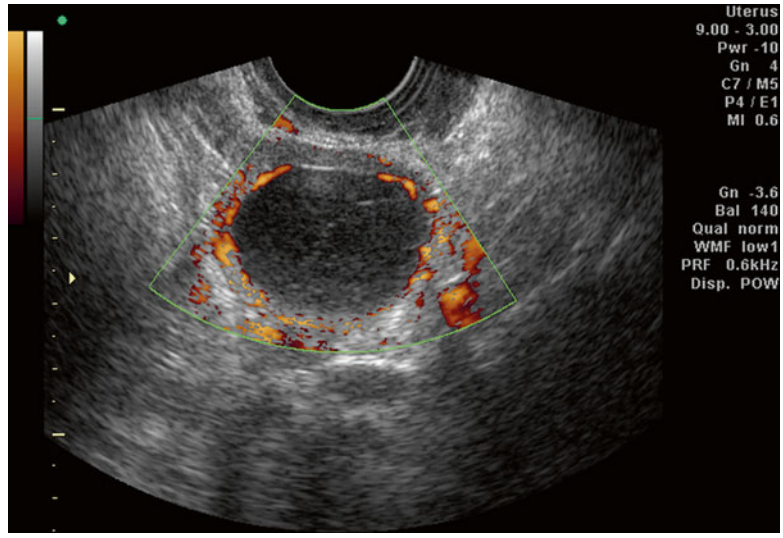
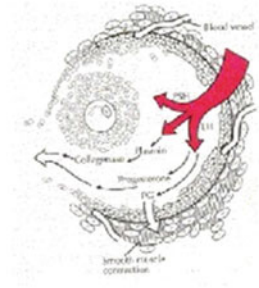
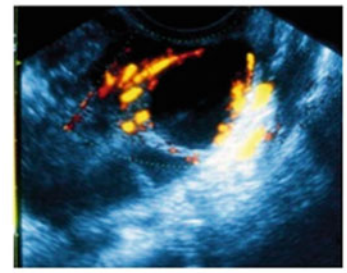


Fig. 2.7 Corpus luteum with increased peripheral blood flow

Sonographic indicators of ovulation

- **Sudden collapse of growing follicle**
- **Central echoes within the follicle**
- **Crenation of the follicular wall**
- **Decreased follicular size**
- **Appearance of follicular fluid in cul-de-sac**
- **Formation of CL - internal follicular area becomes isoechogenic with respect to surrounding ovary**





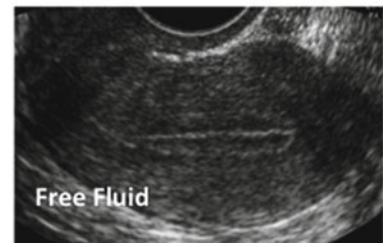


Fig. 2.8 Free fluid in the POD

The role of ovulation-inducing agents for IVF is to disturb this normal relationship by increasing the amounts of FSH available to follicles other than the dominant follicles and thus, to increase the total number of follicles that reach the preovulatory stage. When oral ovulation agents are used, we have fewer dominant follicles as compared to gonadotropin cycles.

Baseline scan on day 2 or 3 is essential before initiation of any ovulation induction therapy to (Fig. 2.9):

- identify the morphology of the ovary and adnexal abnormalities – ovarian cyst and hydrosalpinx
- assess the ovarian reserve
- identify uterine abnormalities – myomas, adenomyosis, polyps, intrauterine adhesions, endometrial abnormalities, and congenital anomalies
- decide the stimulation protocol for adequate response

As selection of dominant follicle occurs early in the follicular phase, OI drugs are initiated within 3 days of the menstrual cycle if (Fig. 2.10)

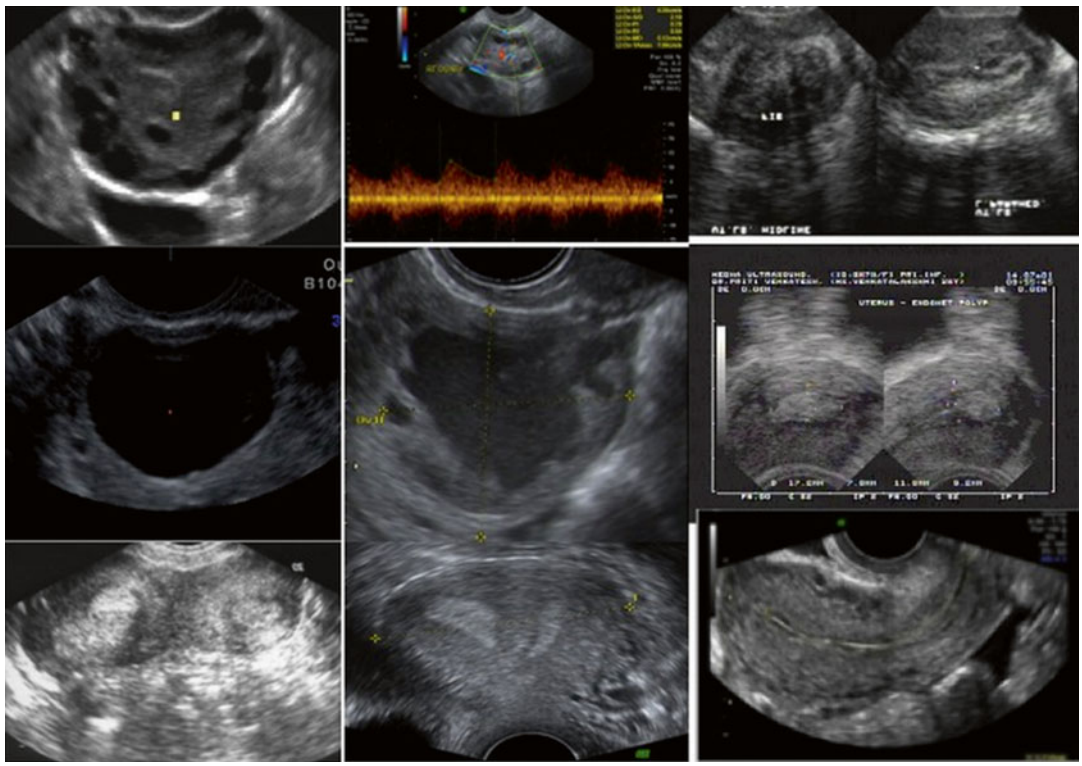


Fig. 2.9 Baseline scan before ovulation induction, to rule out (OI TRO) pathology

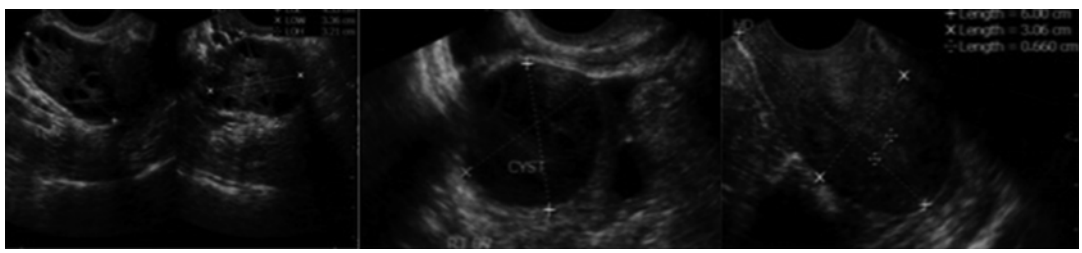


Fig. 2.10 Criteria for initiation of ovulation induction drugs

the follicular size is <10 mm, there are no ovarian cysts, endometrial thickness is <6 mm, estradiol levels are less than 50 pg/mL, and progesterone level is less than 1.5 ng/mL. The dose of drugs used should be tailored to each individual.

Ultrasound scanning is useful in monitoring the response to oral ovulation agents like Clomiphene citrate, Tamoxifen, and aromatase inhibitors in anovulatory women. TVS is usually performed 4–5 days after the last dose of the oral ovulation agent and then, every other day till the follicle is 14 mm, and then daily until a follicle of approximately 20 mm in diameter is seen. Ovulation trigger is given with either recombinant-hCG, 250 μg subcutaneous (SC) or urinary hCG, 5000 IU intramuscular (IM) or GnRH agonist, 1 mg SC (Fig. 2.11).

Ovulation induction with gonadotropins overcomes the normal feedback mechanism that allows for physiological unifollicular ovulation, causing growth of a cohort of follicles at various stages of development. For an IUI cycle, only a maximum of two or three follicles are required to prevent OHSS and multiple pregnancies. To prevent these complications, gonadotropin use requires close monitoring with ultrasound and E2 levels. In ovulation induction cycles, a baseline ultrasound scan is performed to exclude functional ovarian cysts, as well as other pelvic pathologies. Monitoring is usually carried out using TVS on day 4 of treatment and then on day 7 and then depending on the follicular diameter, the scans are repeated either daily or on alternate days. The dose of exogenous gonadotropins is adjusted according to the response. If two leading follicles (>18–20 mm) are seen, human chorionic gonadotropin (hCG) should be administered. IUI is done 36 h after the hCG injection.

When gonadotropins are used for COS in ART (Fig. 2.12), the monitoring is more stringent due to multifollicular development. When measuring large number of follicles, the interobserver variation in measurement is larger than the intraobserver variation and therefore, follicular tracking is more accurate when each scan is performed by the same clinician [11].

The gonadotropins are initiated after a baseline scan on day 2 or 3 is normal, and the first scan after initiation of gonadotropins is done on the 4th day. Further adjustment of the gonadotropin dose depends on serial USG findings and E2 levels as follows.

Change in the dose depending on the USG follicular tracking:

If on Day 4

- Number of follicles <4, dose increased by 37.5/75 IU
- Number of follicles >8 dose reduced by 37.5/75 IU

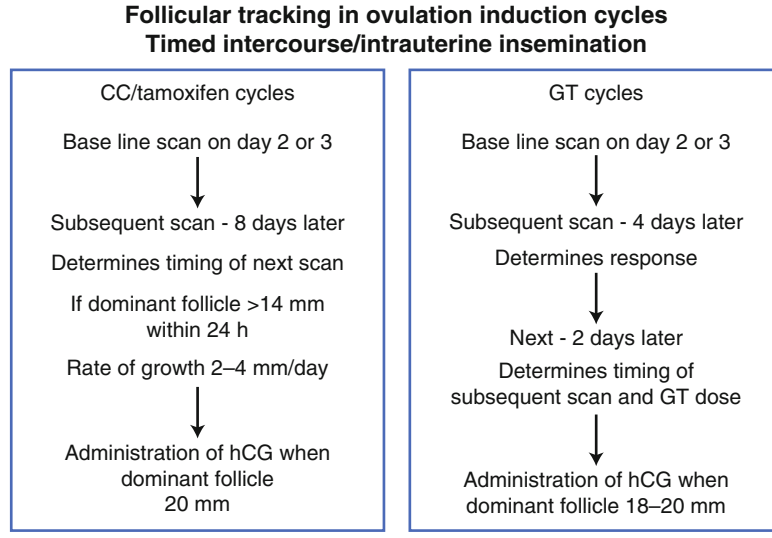


Fig. 2.11 Monitoring a TI or IUI cycle

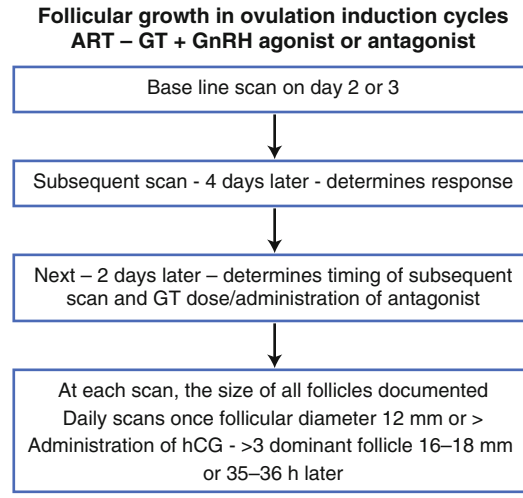


Fig. 2.12 Monitoring an ART cycle

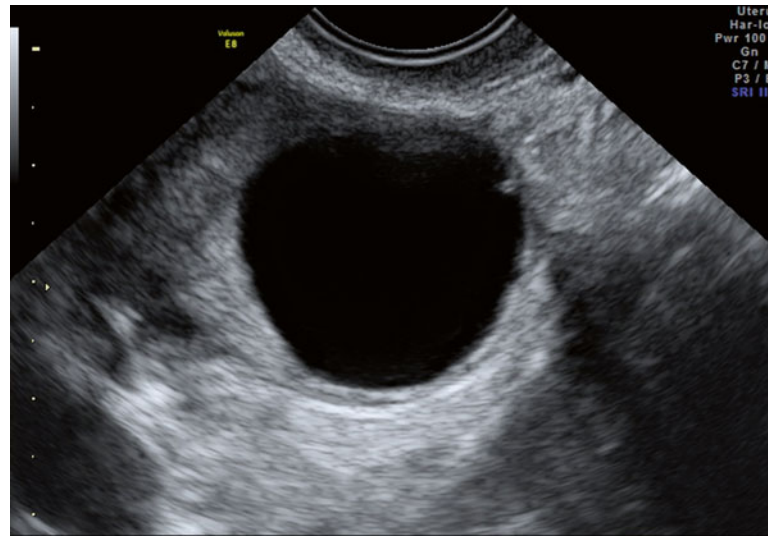


Fig. 2.13 Preovulatory follicle

If on Day 7

- Rate of growth <2–3 mm/day and <4 follicles, which are <12 mm in size dose increased by 37.5/75 IU
- Rate of growth >2–3 mm/day and number of follicles >10, which are > 12 mm in size the dose is decreased by 37.5/75 IU

Once dominance is achieved, the follicular growth is approximately 2–3 mm per day. Hence, we continue the same dose if follicular growth is 2–3 mm/day. Thereafter, the dose is increased or decreased depending on the rate of growth and number of dominant follicles along with E2 levels.

Monitoring preovulatory follicles with follicle diameters (Fig. 2.13) has limitations to predict oocyte quality. Although the follicle growth pattern may be a predictive indicator of the oocyte quality [12], it is difficult to identify individual follicle changes in multiple ovarian follicle growth induced by gonadotropin stimulation.

Transvaginal sonography, performed by an experienced operator, and the daily measurements of serum E2 concentrations may have limited value in predicting the success of the cycle or the risk of OHSS. Probably, hormonal monitoring along with ultrasound is required only in

cases where there is a poor response or hyperresponse. It is also required in those cases who are undergoing frozen embryo transfer (FET) in a natural cycle. Usually, the serum E2 concentrations are proportional to the amount of LH in the gonadotropin preparation used; it is lower in only FSH cycles as compared to those where human menopausal gonadotropin (hMG) is administered.

Color Doppler Studies of Ovarian Circulation

Using color Doppler, one can detect the vascularity of the ovarian stroma, follicular surface, and corpus luteum. PD analysis is an indirect indication of "health" of the follicle and possibly developmental competence of the corresponding oocyte. We know that initiation and maintenance of follicular growth depends on the development of perifollicular microvascular network and intrafollicular hypoxia can have an effect on mitochondrial function and chromosomal organization in oocytes and early embryos [13].

Thus, quantitative and qualitative assessments of perifollicular flow allow more accurate assessment of follicular competence (Fig. 2.14). Follicles that have more than 75 % of their surface perfused, ovarian stromal PSV of more than 10 cm/s, and RI of less than 0.4–0.48 contain mature oocytes of satisfactory quality and result in better grade of embryos.

Perifollicular Blood Flow (PFBF) Grading

- Grade 1: Blood flow (BF) <25 % of the follicle's circumference
- Grade 2: BF ≥25 % but <50 %
- Grade 3: BF ≥50 % but <75 %
- Grade 4: BF ≥75 %

The perifollicular blood flow characteristics, measured by color Doppler images, are related to the intrafollicular oxygen content and vascular endothelial growth factor (VEGF) concentration, and oocytes from severely hypoxic follicles were associated with high frequencies of abnormalities in the organization of the chromosomes on the metaphase spindle [14].The best predictors of IVF outcome are the ovarian flow index (FI) using 3D ultrasound and power Doppler angiography (PDA) on the hCG day and the transfer of grade 1 embryos [14].

Follicles having a perifollicular blood flow of >50 % have increased oocyte retrieval rate with

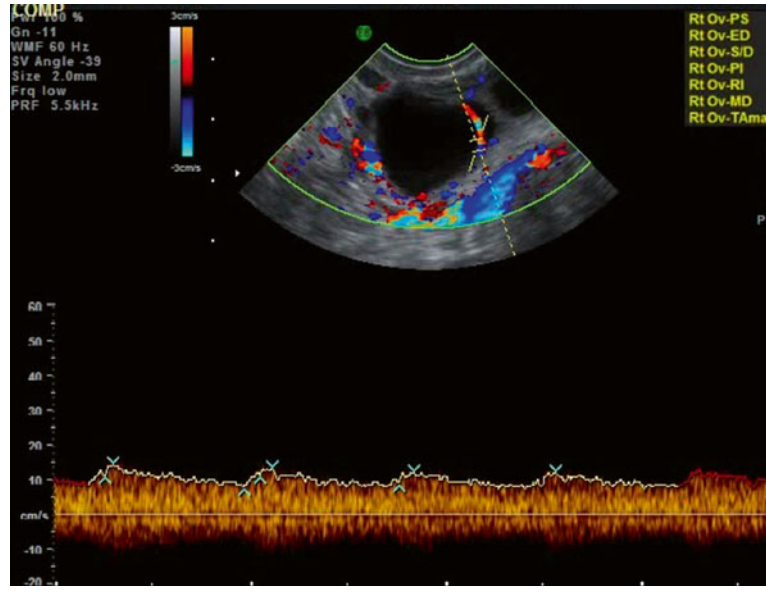


Fig. 2.14 Perifollicular blood flow

more number of mature oocytes with high fertilization rate and lower triploidy rates.

Rising PSV with steady low RI suggests that the follicle is close to rupture (follicular PSV goes as high as 45 cm/s an hour before ovulation), whereas steady or decreasing PSV with rising RI suggests that the follicle is proceeding towards LUF. It was also observed that fertilization of a follicle with PSV of less than 10 cm/s has high chances of the embryo being chromosomally abnormal.

Doppler in the secretory phase gives an idea about the function of corpus luteum (CL). Usually, the RI of the corpus luteum (Fig. 2.15) is between 0.35 and 0.50. In luteal phase deficiency (LPD), RI is 0.58 ±0.04, PI is 0.70–0.80, and PSV is between 10 and 15.

Luteal Phase Doppler

In the mid-luteal phase, the spiral artery RI is 0.48–0.52, uterine artery PI is 2.0–2.5, and uterine artery PSV is 15–20 (Fig. 2.16). Increased resistance to uterine blood flow in the mid-luteal phase is an important contributing factor in some cases of infertility. When pulsatility index (PI) is used as the measure of impedance, it was found that a PI of <3.0 [15] or <3.34 [16] was more favorable for pregnancy. No difference was found in uterine or ovarian artery PI between pregnant and non-pregnant women, but there was a non-significant increase in uterine receptivity when the uterine artery PI was in the range of 2.0–2.99 on the day of embryo transfer [17]. It was also seen that RI was found to be significantly lower at the time of oocyte collection in women who achieved a pregnancy [15]. In a recent study, Ng and colleagues [18] performed 3D ultrasound power Doppler 1 day after the LH surge in women undergoing frozen embryo transfer in natural or Clomiphene-induced cycles. These investigators found that endometrial thickness, endometrial volume, endometrial pattern, uterine PI, uterine RI, and endometrial and subendometrial 3D power Doppler flow indices were similar between the non-pregnant and pregnant groups [18]. They concluded that measurement of uterine artery blood flow should not be part of routine IVF practice. It was also emphasized in this study that the age of women was the only predictive factor for pregnancy. Early secretory transformation of endometrium is a feature of LPD [18].

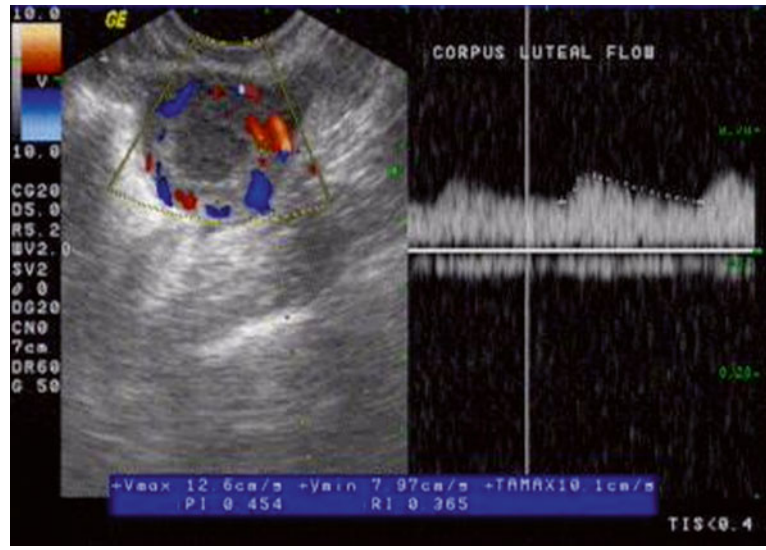


Fig. 2.15 Doppler of corpus luteum

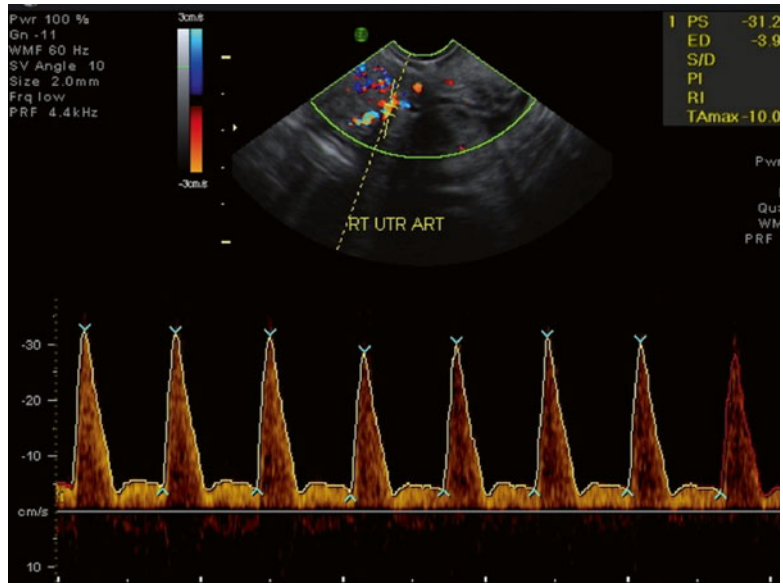


Fig. 2.16 Luteal phase uterine artery blood flow

Effect of Ovarian Stimulation Drugs on PFBF

(a) Clomiphene citrate (CC): CC administration reduces the ovarian and, specifically, the perifollicular vascularization [19]. In one study it was noted that in polycystic ovary syndrome (PCOS) patients who ovulate on Metformin treatment, the ovarian blood flows are similar to those observed in healthy women [19].
(b) Letrozole: Does not alter perifollicular blood flow.
(c) Gonadotropins: LH activity in urinary gonadotropins is associated with > PFBF as compared to recombinant FSH. In the group with high perifollicular blood flow, the clinical pregnancy rate was higher in women treated with urinary gonadotropins as compared to those treated with recombinant FSH ($P < 0.05$), although the numbers were small [20].

PFBF and ART Results

High grade ovarian PFBF in the early follicular phase during IVF is associated with both high grade PFBF in the late follicular phase and a higher clinical pregnancy rate. In an oocyte donation cycle, women who received embryos originating from oocytes developed in well-vascularized follicles had a statistically higher pregnancy rate (34 % vs. 13.7 %) than women who received embryos derived from oocytes grown in more poorly vascularized follicles [21]. It was also observed that poor responders had significantly higher uterine and perifollicular Doppler flow resistances. Moreover, it was noted that the pregnancy rate per cycle was significantly higher in normoresponders (26 %) than poor responders (6 %).

Ultrasound Assessment of the Endometrium

Synchronization between endometrial and embryo development is an essential prerequisite for successful implantation and therefore, monitoring endometrial changes during ovulation induction is important. Monitoring endometrial changes when tracking follicular growth is a reliable bioassay of the patient's estrogenic status. The changes correlate with plasma E2 and P4 levels. The endometrium undergoes cyclic morphological as well as histological changes throughout the menstrual cycle. During

menstruation, the endometrium appears as a thin echo that gradually thickens throughout the proliferative phase to reach the typical periovulatory trilaminar appearance. After ovulation, the rise in circulating progesterone induces stromal edema and growth of spiral arterioles, resulting in increased echogenicity of the thick secretory endometrium.

Various ultrasonographic indicators have been investigated for the evaluation of endometrial receptivity in spontaneous and stimulated cycles, including endometrial thickness, endometrial pattern, uterine artery, and endometrial blood flow.

The morphology of the endometrium in the different phases of menstrual cycle is illustrated below (Fig. 2.17).

- Early proliferative phase – translucent and thin on either side of mid-line echo
- Late proliferative phase – increase in thickness with a hyporeflective area in the center
- Following ovulation – shrinks in thickness, becomes dense echogenic on either side of mid-line echo

Endometrial Receptivity Markers

Conventional Markers

- Thickness
- Morphology
- Uterine artery flow
- Peristalsis

Newer Markers

- 3D Endometrial volume
- 3D Endometrial configuration
- 3D Endometrial vascularity quantification

Many clinicians have reported no difference in endometrial thickness between pregnant and non-pregnant women [22, 23], while others have observed a positive correlation between endometrial thickness and pregnancy outcome [24, 25]. Zhang and coauthors [26] found that increased endometrial thickness was associated with improved treatment outcome, but the association was dependent on patient age, duration of ovarian stimulation, and embryo quality [26]. On the contrary, Richter and colleagues [27] found that the higher clinical pregnancy and live birth rates associated with increasing endometrial thickness were independent of the effects of patient age and embryo quality [27]. A meta-analysis demonstrated that endometrial thickness is a better negative than positive predictor of implantation [28]. Different studies have proposed different endometrial thickness cut-off levels for successful implantation to occur: ≥6 mm [22], ≥10 mm [25], and ≥13 mm [29]. There have been no reports of adverse effects of a thickened endometrium on implantation, pregnancy, or miscarriage rates in IVF [30].

An association has also been noted between the ultrasound endometrial texture, echogenic patterns, and serum hormonal (estradiol and progesterone) levels. In IVF cycles, a preovulatory,

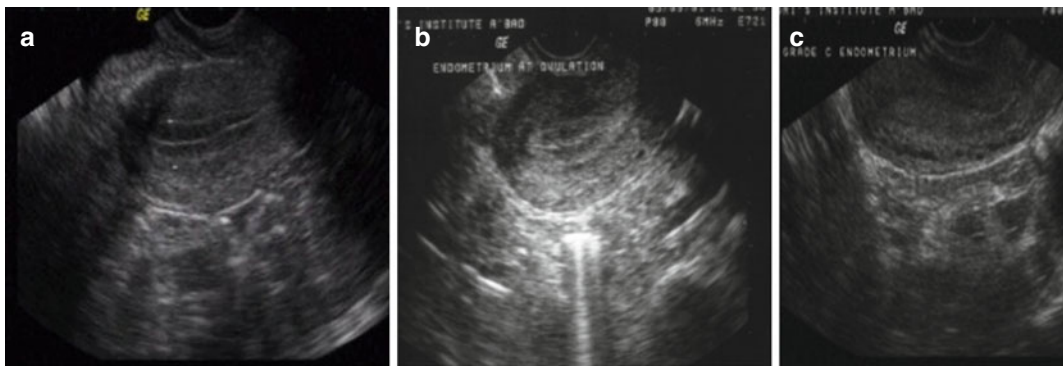


Fig. 2.17 Morphology of endometrium in the different phases of menstrual cycle. (**a**) Preovulatory, (**b**) at ovulation, (**c**) post ovulation

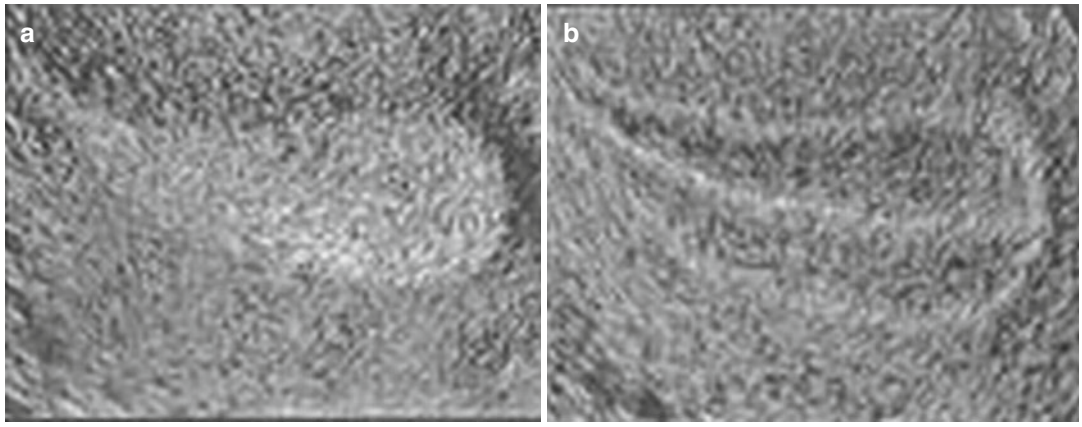


Fig. 2.18 (**a**) Hyperechogenic endometrium, (**b**) triple-line pattern

echogenic, and homogeneous pattern has been associated with premature rise in progesterone levels, probably due to high LH levels in the early proliferative phase or premature secretion of LH, especially in the antagonist flexible protocol. This is due to the presence of high estradiol levels, which are related to multifollicular development. Endometrial hyperechogenicity prior to ovulation is a poor prognostic factor for pregnancy (Fig. 2.18a). On the other hand, women with a triple line pattern on the day of oocyte retrieval conceived in 80.0 % of the cases (Fig. 2.18b).

The endometrial pattern with an outer hyperechogenic and inner hypoechogenic layer on the day of oocyte retrieval had predictive value of IVF treatment [31]. Homogenous and hyperechogenic sonographic endometrial pattern had a predictive value of 100 % for a nonconceptional cycle, whereas multilayered endometrium was visualized in conception cycles [32].

The endometrial thickness and pattern also provide useful information in FET or oocyte donation cycles in which the endometrium is supplemented with estrogen and progesterone [15]. A minimal endometrial thickness of 6 mm is required before embryo replacement for pregnancy to be achieved [33, 34]. In a study published by El-Toukhy et al. [35] an endometrial thickness of 9–14 mm on the day of progesterone supplementation in an FET cycle was found to be associated with higher implantation and pregnancy rates compared with an endometrial thickness of 7–8 mm [35]. They reported lowest pregnancy rates when the endometrial thickness was either less than 7 mm or more than 14 mm.

Endometrial Volume (Fig. 2.19)

The minimum endometrial volume, which is associated with pregnancy, is 1.59 mL when calculated by 3D ultrasound, but most pregnancies occur in volumes of 2–13 mL. The calculation of endometrial volume is particularly useful in cases of synechiae, adenomyosis, and uterine anomalies to predict the outcome of treatment.

Endometrial and subendometrial volume increase rapidly during the follicular phase and then remain almost unchanged during the luteal phase [36].

Ultrasound Parameters, which Indicate a Good Receptive Endometrium Include:

- Endometrial morphology, which shows a "triple line" pattern.
- Endometrial thickness of 8–14 mm.
- Uterine vascularity – mean uterine artery PI between 2 and 3 and uterine artery PSV 15–20 cm/s.
- Presence of subendometrial and endometrial flow.
- Higher subendometrial vascularization index (VI), FI, and vascularization flow index (VFI)

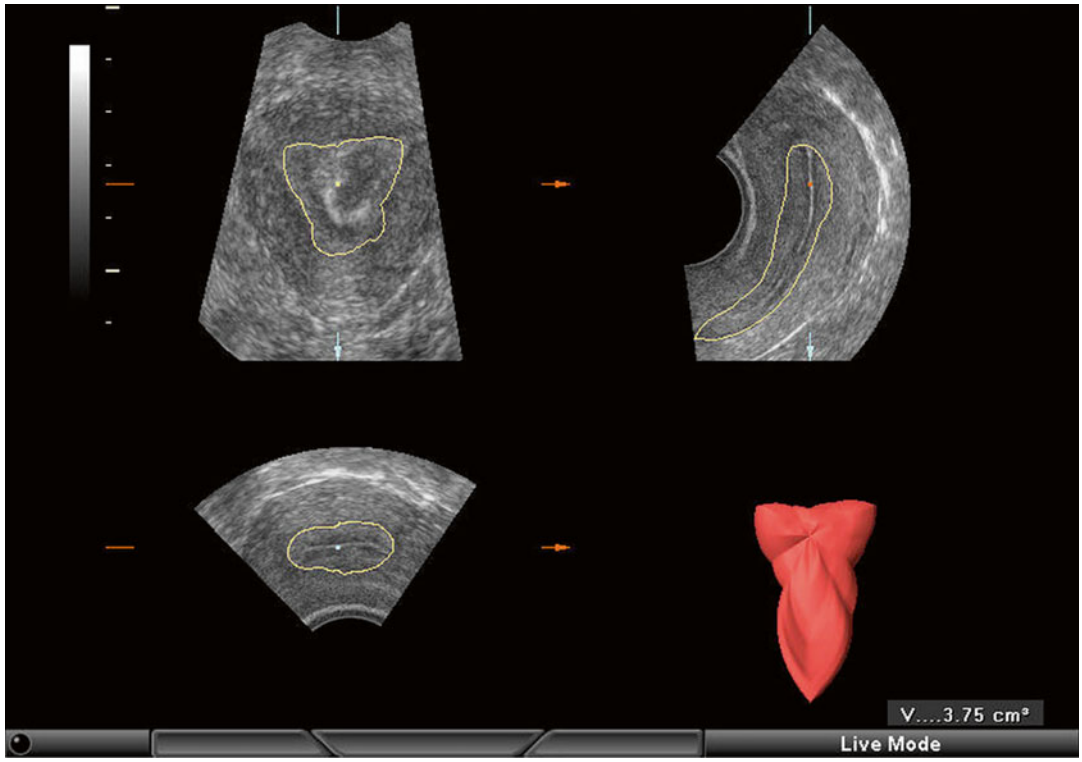


Fig. 2.19 Endometrial volume evaluation

were observed on the day of hCG in the conception group.
- Endometrial volume of > 2 mL yields a significantly higher pregnancy rates.

The persisting presence of endometrial fundocervical waves after hCG administration results in lower pregnancy rates. Normally, these are seen till administration of hCG and later, the wave direction switch occurs to cervico-fundal. With this pattern of endometrial wave switch, there is a higher likelihood of pregnancy. Less than three peristaltic contractions of the subendometrial myometrium at every 2 min interval on the day of hCG administration is associated with a poor implantation rate (IR). High estradiol levels in COS cycles are associated with higher peristalsis, which negatively correlates with implantation.

Number and Type of Waveform During the Menstrual Cycle

- Follicular phase: 4–5 uterine contractions per minute – retrograde
- Luteo-follicular transition: 2–3 uterine contractions per minute – antegrade
- Luteal phase: <2.5 uterine contractions per minute

The presence of high frequency uterine contractions on the day of embryo transfer negatively affects IVF–ET outcome. If frequency of contractions is less or falls, the clinical pregnancy rate rises [37].

Recently, pulsed Doppler and three-dimensional color and power Doppler studies have been applied to evaluate endometrial receptivity by the uterine and endometrial blood flow status.

Endometrial and Subendometrial Vascularity

Endometrial and subendometrial vascularity indices (Fig. 2.20) are high throughout the follicular phase; peak value is reached for 3 days before ovulation and reduces to a nadir 5 days after ovulation and then increases again during the luteal phase [38]. Relative endometrial hypoxia during the implantation phase aids blastocyst implantation. Patients who get pregnant have a lower RI (0.53 vs. 0.64) and it was observed that the hyperechoic endometrium had a higher incidence of absent subendometrial blood flow [39] and in these cases, no pregnancy was reported [40].

Endometrial Vascularity Zones by Applebaum (Fig. 2.21)

Zone I – Myometrium surrounding the endometrium
Zone II – Hyperechoic endometrial edge
Zone III – Internal endometrial hypoechoic zone
Zone IV – Endometrial cavity

Conception rates are very low when vascularity is not seen in Zone III–IV.

Endometrial Blood Flow Quantification

Endometrial blood flow quantification is done using the 3D power Doppler (Fig. 2.22) with VOCAL™ (Virtual Organ Computer-aided Analysis). It is shell imaging, which is used to define and quantify the power Doppler signal within the endometrial and subendometrial regions, producing indices of their relative vascularity.

Endometrial Vascularization Using 3D Power Doppler (Fig. 2.23)

Endometrial vascularization is calculated by measuring the vascular index, flow index, vascular flow index, and flow vessel quotient.

- Vascularization index (VI) reflects number of vessels in volume of tissue and is calculated by dividing the number of color voxels by total number of voxels.
- Flow index (FI) reflects the amount of blood flow and is calculated by dividing the sum of color intensities by number of color voxels.

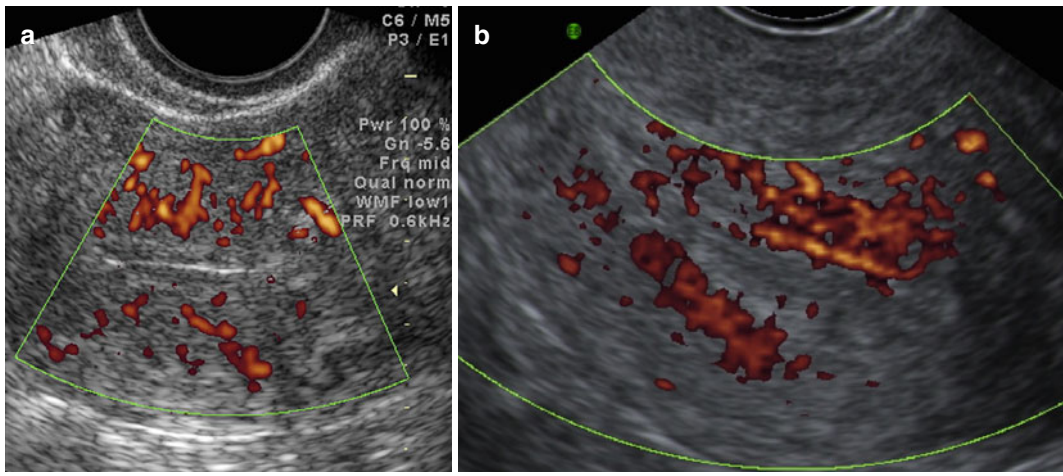


Fig. 2.20 (**a**, **b**) Subendometrial blood flow

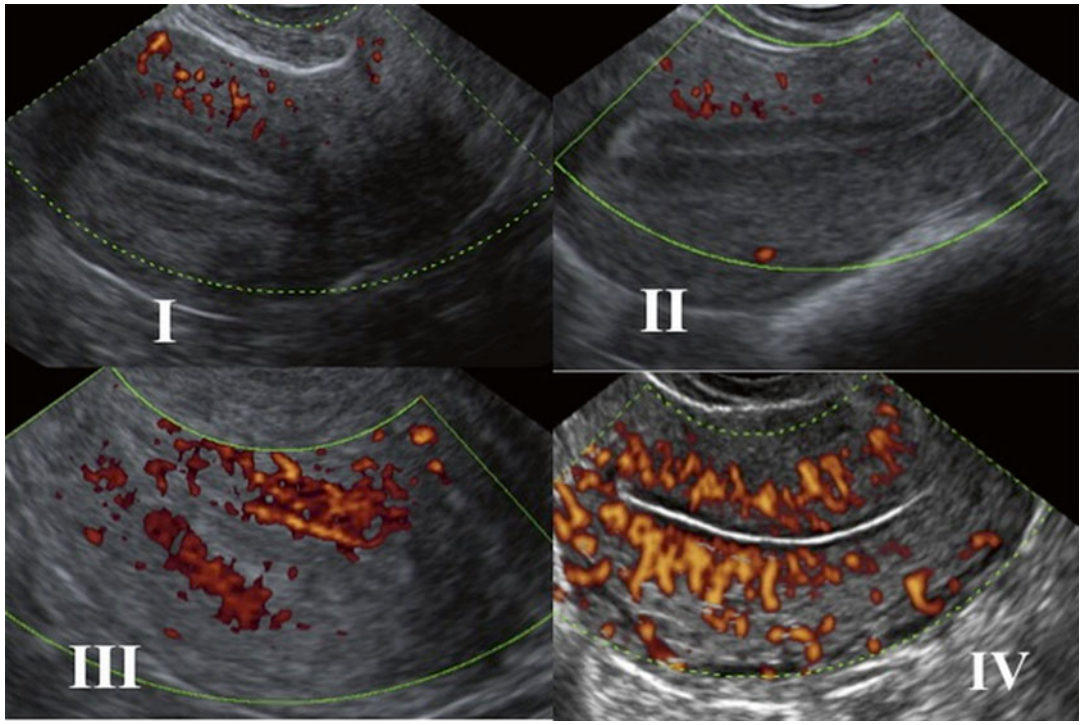


Fig. 2.21 Endometrial vascularity zones

Fig. 2.22 Endometrial blood flow quantification

- Vascularization flow index (VFI) reflects vessel presence and blood flow and is calculated by dividing the sum of color intensities by total voxels.
- Flow vessel quotient (FVQ) is calculated by dividing flow index by vascular index (FI/VI)

We can use these indices in predicting the occurrence of pregnancy.

- No pregnancy if VI <1.0
- No pregnancy if FI <31
- No pregnancy if VFI <0.25

Endometrial and subendometrial vascularity (VI/FI/VFI) is significantly less ($P \leq 0.003$) in patients with low volume endometrium, but not in those with thin endometrium [41]. It is also significantly lower in stimulated cycles than that in the

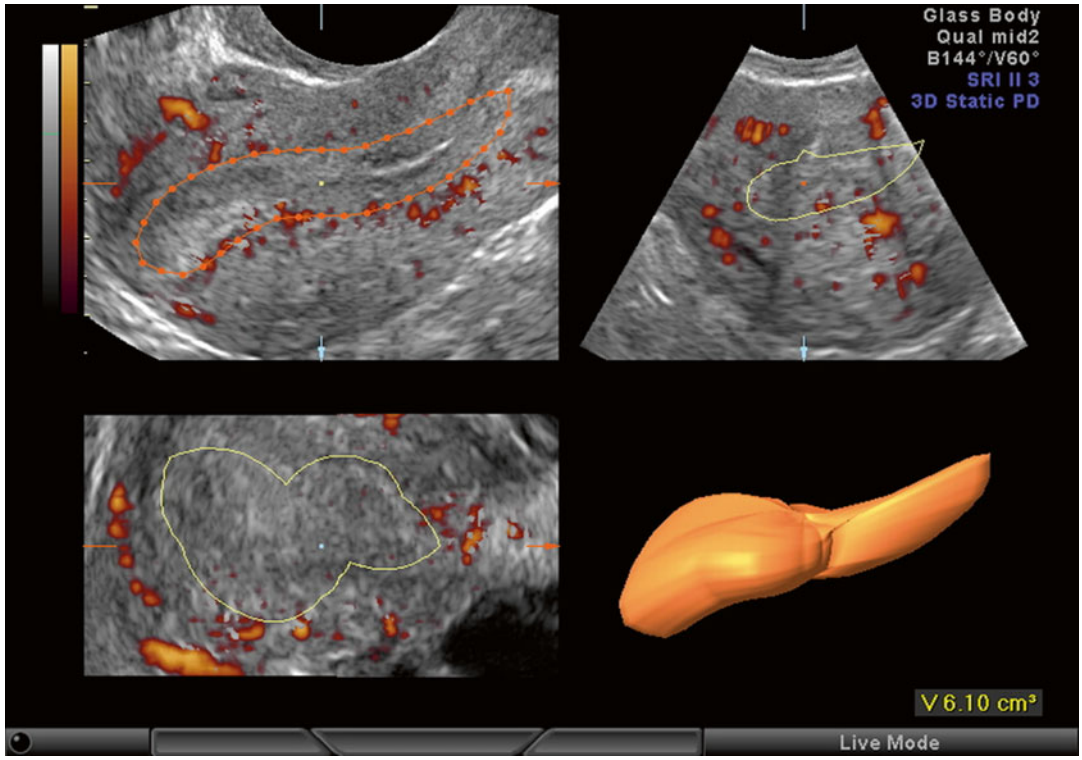


Fig. 2.23 Endometrial vascularization using 3D power Doppler

natural cycle [42]. CC reduces endometrial vascularity as compared to Letrozole. In COS cycles, endometrial blood flow was negatively affected by E2 concentration [43] and hyper-responders tended to have low VI/VFI 2 days after hCG administration and also had a higher incidence of absent endometrial and subendometrial blood flow. Luteal phase vascularity was also altered in high responders [42].

Endometrial Vascularity in Special Situations

Fibroids: Endometrial and subendometrial 3D power Doppler flow indices were similar in patients with and without small intramural fibroids [44].

Hydrosalpinx: Patients in the hydrosalpinx group had significantly lower endometrial and subendometrial VI and VFI.

Unexplained infertility: Endometrial and subendometrial vascularities are significantly less during mid to late follicular phase irrespective of E2 or P concentrations and endometrial morphometry [38].

Repeated miscarriage: Patients with live births had significantly higher endometrial VI and VFI and subendometrial VI, FI, and VFI, when compared with those who had a miscarriage. Of all the vascular indices, only endometrial VI was significantly associated with the chance of live birth with an odds ratio of 1.384 [95 % confidence interval (CI) 1.025–1.869, $P=0.034$]. In FET cycles, patients with live births had significantly higher endometrium VFI, subendometrial VI, and VFI than those with miscarriages. Hence, one can conclude that endometrial and subendometrial vascularity was significantly higher in pregnant patients with live births following stimulated IVF and FET treatment than in those who suffered a miscarriage [45].

Correlation of Endometrial and Subendometrial Blood Flow to Pregnancy Rate in ART

Endometrial and subendometrial blood flow on the days of hCG and on the day of embryo transfer and the percentage change in endometrial and subendometrial blood flows between these 2 days were not predictive of pregnancy in ART cycles [41]. It is just prognostic and not a predictive index in ART cycles.

Maintenance of Records During Monitoring of an Ovulation Induction Cycle

For optimal outcome of infertility treatment, monitoring of the ovarian response in COH cycles should be plotted in a chart. Follicular growth, recorded on these specially designed charts (Fig. 2.24) allows us to see all the relevant characteristics of the cycle at a glance.

These include

- Date and day of cycle
- Number of developing follicles in each ovary
- Dynamics of follicular growth
- Endometrial thickness
- Type of ovulation regimen
- Quantity of medication used
- Baseline hormone levels
- E2, if required, in the proliferative phase
- E2 and P4 on the day of hCG
- Any change in the dose and hormonal evaluation done must also noted
- Date and time of administration of hCG

Follicles can occasionally be confused with other pelvic structures, but they can be differentiated by rotating the transducer 90°. If the structure is a vessel, it will then elongate, acquiring a tubular shape. The internal iliac artery can easily be identified by its arterial pulsations, while a hydrosalpinx generally has a less regular shape.

Ultrasound, after oocyte retrieval and before embryo transfer, can also identify fluid in the endometrial cavity (Fig. 2.25) and is usually associated with a poor prognosis. It could be present due to excessive cervical mucus that ascends into the endometrial cavity, fluid reflux from a hydrosalpinx, subclinical uterine infection, and abnormal endometrial development.

The presence of persistent fluid accumulation at the time of embryo transfer warrants freezing of all embryos and transfer in a subsequent cycle.

Name Age

LMP Attempt No

Treatment: IVF/ICSI/TESA-ICSI/IUI/Planned relations/Others

Baseline scan: Uterus:

Ovaries: Normal Abnormal

Right Ovary 1 2 3 4 Left Ovary 1 2 3 4

Note: 1. Cyst 2. Multiple Follicles 3. Not located 4. Poorly seen

Drugs Used: Clomephene/Tamoxifen/Letrazole/FSH/hMG/Rec FSH/Rec LH/hCG/GnRH agonist/GnRH antagonist/ Dexamethazone
Serial Ultrasound scan

Date	Day of cycle	Right Ovary	Left Ovary	Endometrial thickness	Cul de sac fluid	GnRHa	GnRHA	FSH	hMG	hCG	FSH mIU/ml	LH mIU/ml	E2 pg/ml	P4 ng/ml

Fig. 2.24 Follicular monitoring chart

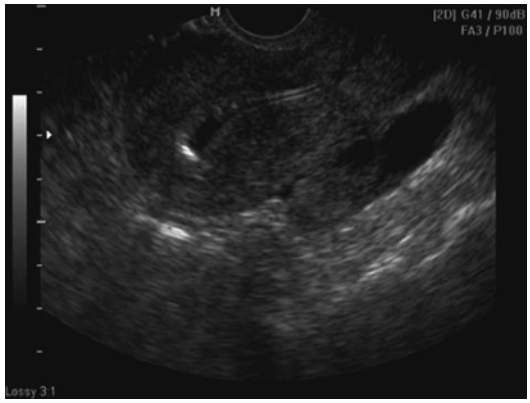


Fig. 2.25 Fluid uterine cavity

Uterine Artery Blood Flow

There have been conflicting reports in the literature regarding the usefulness of the application of color Doppler ultrasound for monitoring and predicting pregnancy outcome of IVF cycles. It was observed that uterine blood flow is a poor reflector of subendometrial blood flow during stimulated and natural cycles, and its measurement cannot reflect endometrial blood flow during stimulated cycles [18].

Several studies used the pulsatility index (PI) as the measure of impedance and determined that a PI of <3.0 [46] or <3.34 [22] was more favorable for pregnancy. More recently, Steer and coauthors [16] found similar results in women undergoing FET in a downregulated hormonally prepared cycle [16]. In contrast, other researchers found that uterine artery PI did not significantly change until the mid-luteal phase. No difference was found in uterine or ovarian artery PI between pregnant and non-pregnant women, but there was a non-significant increase in uterine receptivity when the uterine artery PI was in the range of 2.0–2.99 on the day of embryo transfer [17]. Other investigators used resistance index (RI) and found that it was significantly lower at the time of oocyte collection in women who achieved a pregnancy [15]. In a recent study, Ng and colleagues [18] performed 3D ultrasound power Doppler 1 day after the LH surge in women undergoing FET in natural or Clomiphene-induced cycles. The age of women was the only predictive factor for pregnancy. Endometrial thickness, endometrial volume, endometrial pattern, uterine PI, uterine RI, and endometrial and subendometrial 3D power Doppler flow indices were similar between the non-pregnant and pregnant groups [18]. Currently, measurement of uterine artery blood flow should not be part of routine IVF practice.

Uterine arterial blood flow was lower in CC–stimulated cycles during the periovulatory period than those in the spontaneous menstrual cycles [47], and also demonstrated that uterine vascular impedance on the day of ovulation was lower in the conception cycles, while there were no differences between conception and non-conception cycles in the luteal phase [48].

Tridimensional Automated USG for Monitoring Controlled Ovarian Stimulation Cycles

Two-dimensional USG is difficult and less reliable in the presence of numerous follicles of different sizes during COS and is also relatively arbitrary. Accurate assessment of follicular size is required for timing and oocyte collection as significantly less mature oocytes are recovered from follicles with a mean diameter of <15 mm. Three-dimensional ultrasonography-based automated volume count (SonoAVC) can individually identify and quantify the size of any hypoechoic region within the 3D data sets (Fig. 2.26), providing an automatic estimation of their absolute dimension and volume. It estimates the volume of follicle to within ±0.5 cm^3. This enables the quantification of an unlimited number of volumes that arise in a COS cycle, as it eliminates the possibility of measuring the same follicle more than once. Thus, Sono AVC is a quicker and more reliable method of measuring follicles in a COS cycle, but its effect on the pregnancy rate has not yet been studied. The number of the mature oocytes, fertilized oocytes, and clinical the pregnancy rates (42 % vs. 43 %) were similar with both 2D ultrasound and Sono AVC methods [49].

Three-dimensional ultrasound with Sono AVC significantly improves the interobserver

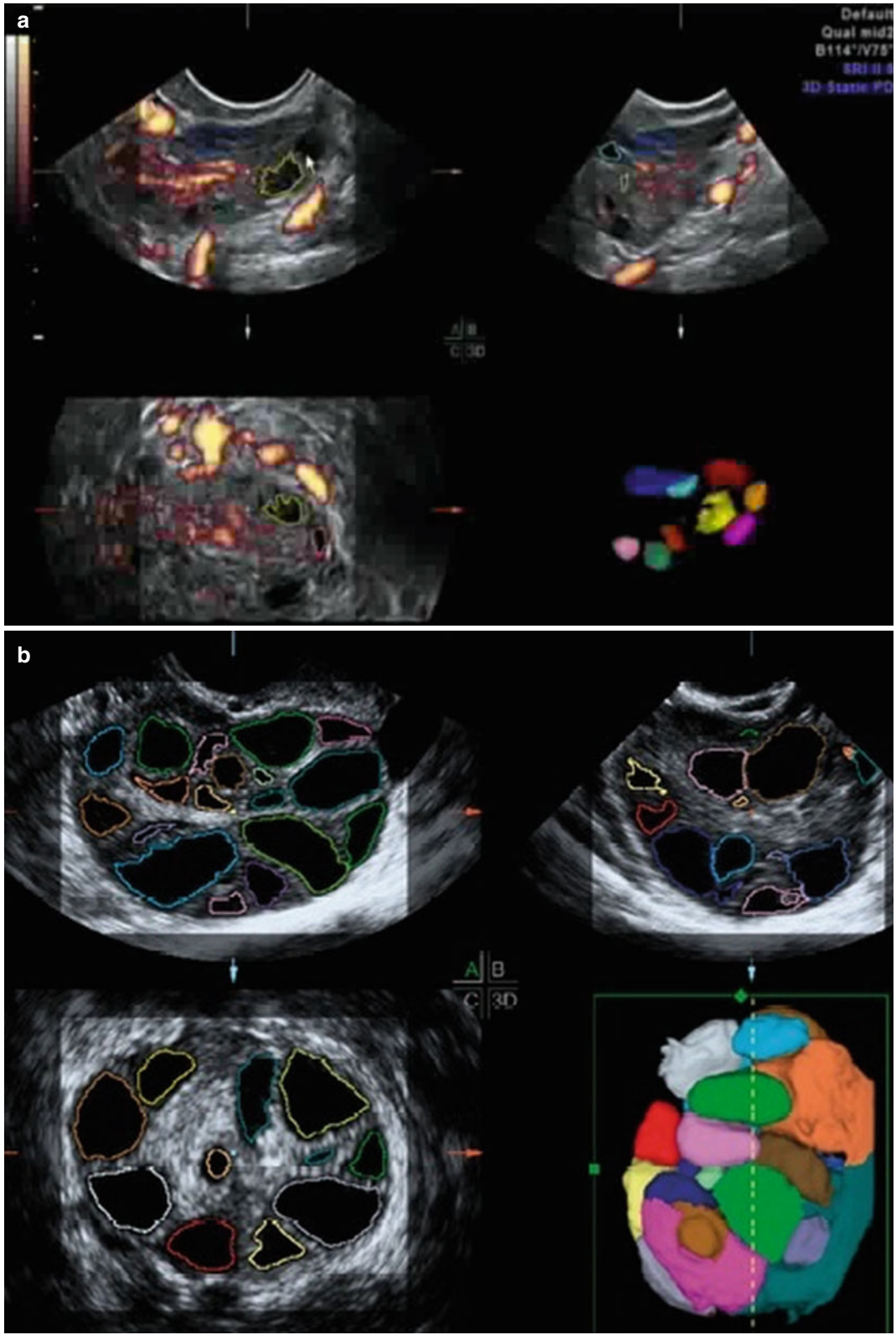


Fig. 2.26 (**a**, **b**) SonoAVC for follicular monitoring

reliability of antral follicle counts and allows quicker assessment of follicle size and number, making it an important tool in the assessment of ovarian reserve.

It however, has the following disadvantages:

- Increases time of ultrasound as a lot of time may be spent postprocessing.
- If two or three follicles are close by, it measures them as one, and it is the operator who needs to identify and separately count these follicles using the snipping tools.
- At times, certain follicles may not be measured at all and the operator needs to scan the ovary in *X*-, *Y*-, and *Z*-axis to identify the left out follicles.
- The clinical outcome of assisted reproduction treatment also did not show any improvement with the use of SonoAVC, and so we need to determine whether it is cost-effective to be used routinely in all IVF cycles [50].

Monitoring Abnormal Response (Figs. 2.27, 2.28, and 2.29)

Ultrasound is also useful in monitoring abnormal response to ovulation induction, which includes premature luteinization, LUF, endogenous LH surge, poor response, hyperstimulation, presence of retention or functional cysts, and ovarian torsion.

Premature Luteinization (Fig. 2.27a)

Follicles <15 mm with echoes are seen and these correlate with high P4 levels in the fol-

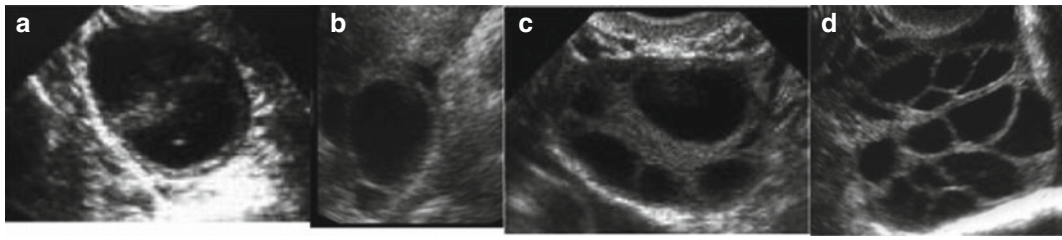


Fig. 2.27 Abnormal response to ovarian stimulation. (**a**) Premature luteinization, (**b**) LUF, (**c**) Poor response, (**d**) Hyper-response

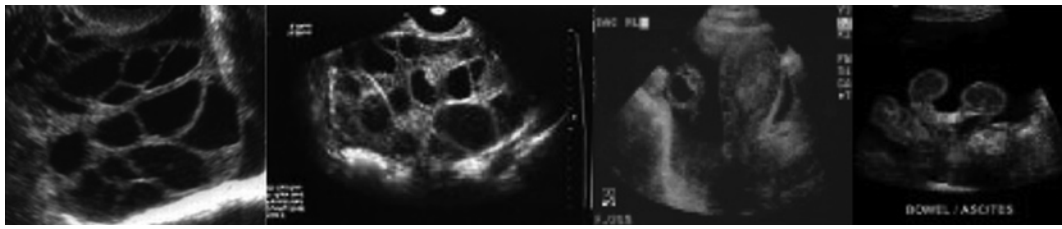


Fig. 2.28 Ovarian hyperstimulation syndrome

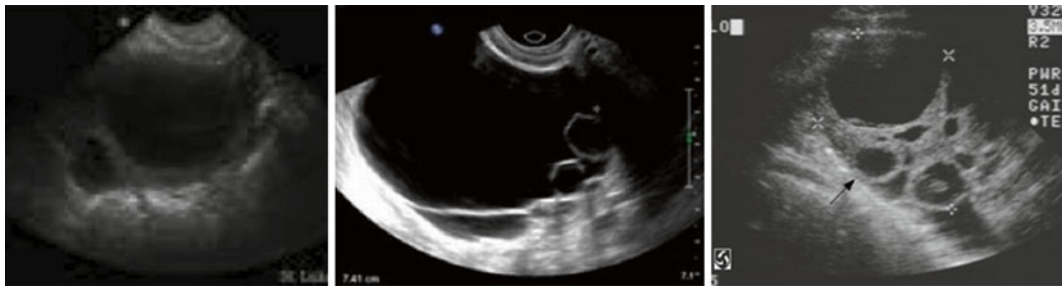


Fig. 2.29 Functional and retention cyst

licular phase. A premature and suboptimal LH surge results in progesterone production but no ovulation, and oocyte maturation without follicular rupture. It is associated with poor quality oocytes and embryos with an out of phase endometrium thus, reducing the implantation rate.

Luteinized Unruptured Follicle (LUF) (Fig. 2.27b)
Luteinized unruptured follicle is diagnosed when the dominant follicle is still apparent 48 h after administration of hCG or LH surge. The size of the follicle may reach 34–36 mm and has thick walls and may have internal echoes. The endometrium is thick and echogenic with no fluid in the pouch of Douglas. It is due to insufficient strength of the LH surge to induce follicular rupture but sufficient to induce oocyte maturation.

Endogenous LH Surge
Endogenous LH surge is seen on ultrasound as a premature rupture of follicles at a diameter of less than 16–17 mm. It is associated with compromised oocytes and embryo quality as a result of exposure to inappropriate LH levels. This requires extensive endocrine monitoring and can be prevented with the use of GnRH agonists or antagonists.

Poor Response (Fig. 2.27c)
Poor response can be predicted by estimating the baseline AFC and ovarian volume (<3–4 AFCs and volume <3 mL). At times, the AFC may be normal but the women may not respond to gonadotropins for various reasons, so the presence of less than two to three follicles on ultrasound on day 7 of ovulation induction with gonadotropins also suggests poor response.

Ovarian Hyperstimulation Syndrome (OHSS) (Figs. 2.27d and 2.28)
Ultrasound is essential for the prevention, diagnosis, and monitoring of OHSS. Judicial use of TVS for follicular monitoring while inducing ovulation with gonadotropins remains critical for the prevention of OHSS. TVS is also used to monitor the ovarian volume, keep record of number of follicles and corpus luteum and their size, diagnose ascites and pleural effusion when monitoring for the progress of OHSS. Ultrasound can also be used to guide paracentesis of the ascites or pleural effusion in cases, which develop severe respiratory distress to avoid trauma to ovaries or other abdominal structures.

Functional Cyst (Fig. 2.29)
Functional cyst is diagnosed by the presence of cyst at prestimulation baseline scan on day 2 or 3 of the menstrual cycles following GnRH agonist stimulation for downregulation. It is characterized by sharp edges and anechogenic contents and is due to the initial FSH surge, which occurs after commencement of GnRH agonist in a long downregulation cycle. The presence of a functional cyst requires either cancellation of cycle or an ultrasound-guided aspiration of the cyst before commencing ovulation induction.

Persistent/Retention Cyst (Fig. 2.29)
The presence of cyst at baseline scan suggests a follicle from previous cycle or a persistent corpus luteum. It may result due to growth of the smaller follicles following the hCG trigger. No drugs are administered for ovulation induction in the presence of a retention cyst. It is followed ultrasonographically and if persistent may require medical or surgical treatment.

Hormonal Monitoring

Fertility is associated with marked daily changes in hormone output, especially estradiol, LH, and progesterone. Any pattern that shows no changes from day to day denotes infertility, and estimation of these hormones during ovulation induction may prove useful. Evaluation of hormonal values can predict both ovarian reserve as well as ovarian response.

Prediction of Response

Accurate prediction of ovarian response enables the clinician to choose the right protocol opti-

mizing the outcome and preventing complications like OHSS and multiple pregnancies. At one extreme of the response spectrum, we can identify women who are at risk of OHSS and can adjust our stimulation strategy to incorporate GnRH antagonists [51]. We thereby minimize the risk of this potentially fatal complication, but potentially even more importantly, we have the ability to completely eliminate it by adopting a GnRH agonist trigger before oocyte retrieval [52]. For potential poor responders, we currently use a flare strategy because of its reduced treatment burden and ability to capitalize on endogenous LH activity, in accordance with recent studies supporting a beneficial role of LH in older women [53].

Achieving an appropriate ovarian response without cycle cancellation or adverse events related to under- or overstimulation to antiestrogens or exogenous gonadotropins is important. To predict ovarian response to ovarian stimulation and to individualize the starting dose of exogenous gonadotropins or the need for exogenous luteinizing hormone, various hormonal tests have been suggested. These include FSH, AMH, and estradiol levels. The standard first-line investigation to assess ovarian function is measuring FSH though it has a much lower correlation with primordial follicle counts and follicular recruitment rates and has limited ability to diagnose ovarian dysfunction, including PCOS. Today AMH is considered to be the best marker for the prediction of ovarian response with a strong linear relationship of AMH with AFC in predicting ovarian reserve.

The ROC regression analysis demonstrated a high accuracy for AMH and for AFC in predicting poor response, but only a moderate accuracy for FSH. In predicting pregnancy after IVF, all three ovarian reserve tests (ORT) had only a very small or no predictive effect.

Basal FSH

Basal FSH is an indirect measure of the size of follicle cohort [54]. Basal serum FSH concentrations increase on day 2, 3, or 4 of the menstrual cycle with advancing reproductive age. FSH is commonly used as a measure of ovarian reserve, and high values have been associated with, but do not necessarily predict both poor ovarian stimulation and the failure to conceive. Its role is limited in the evaluation of young healthy women [55].

Multiple cut-off values above 10 IU/L (10–20 IU/l) demonstrate high specificity (83–100 % range) but poor sensitivity (10–80 %) for predicting poor response to stimulation (<2–3 follicles or <4 retrieved oocytes) [56]. Using similar cut-off values, the sensitivity for predicting pregnancy is very low. High FSH levels have not been associated with an increased risk of aneuploidy in pregnancies resulting from IVF [57, 58]. Although FSH rises with increasing reproductive age, it remains unknown whether high FSH levels in women of reproductive age predict an earlier onset of menopause [59]. Elevated day 3 FSH is a heterogeneous group, which could be either due to true reduced ovarian reserve, presence of heterophylic antibodies or FSH receptor polymorphism in patients with otherwise normal ovaries [60]. Pregnancy rates are significantly higher ($P<0.05$) in women with normal FSH in those aged <36 years compared to those aged ≥36 years [61]. Consistently elevated FSH concentrations confer a poor prognosis [62], a single elevated FSH value in women <40 years of age may not predict a poor response to stimulation or failure to achieve pregnancy [63]. It does not diagnose poor ovarian reserve until high thresholds are reached [62].

Limited evidence suggests that women with fluctuating FSH levels should not wait for the "ideal" cycle, wherein the FSH concentration is normal, to undergo IVF stimulation [64, 65]. Thus, a basal FSH level has limited utility as a screening test [56, 62, 64–66]. A single FSH value has very limited reliability because of inter- and intra-cycle variability (particularly, if it is not elevated). Elevated day 3 FSH/LH ratio due to low LH concentrations predicts reduced ovarian response and is associated with an inferior outcome in IVF treatment cycles and may be used as an additional predictor for decreased ovarian response.

Estradiol

As a test of ovarian reserve, basal estradiol on day 2, 3, or 4 of the menstrual cycle has poor inter- and intra-cycle reliability [67]. Very low

predictive accuracy, both for poor response or excessive response and therefore, basal estradiol alone should not be used to screen for ovarian reserve. The test has value only as an aid to correct interpretation of a "normal" basal serum FSH value. Elevated day 2 estradiol values (>75–80 pg/mL) indicate an inappropriately advanced stage of follicular development, consistent with ovarian aging or simply reflect the presence of functional ovarian cysts. No relationship has also been found between serum E2 levels and pregnancy rates [68]. Thus, the use of day 2 estradiol value for the prediction of ovarian reserve is still debatable [69].

Inhibin B

Normal day 3 inhibin B value is > 45 pg/mL. Using 45 pg/mL as the threshold for low ovarian reserve, has specificity between 64 and 90 % and sensitivity between 40 and 80 %. The positive predictive value (PPV) of inhibin B is generally low (19–22 %), and the negative predictive value (NPV) is high (95–97 %) in general IVF populations [70–72]. In populations at high risk for decreased ovarian reserve, PPV can be as high as 83 % [72]. The odds ratio for a clinical pregnancy (basal serum inhibin >45 pg/mL versus <45 pg/mL) was 6.8 (CI 1.8–25.6). It is a better predictor for cycle cancellation than ovarian response and is influenced by the amount of fat in an individual, with lower levels in obese women [73].

AntiMullerian Hormone (AMH)

Serum concentrations of AMH, produced by granulosa cells of early follicles, are gonadotropin-independent and therefore, remain relatively consistent within and between menstrual cycles in both normal young ovulating women and in women with infertility [67, 74–76]. The true individual cycle fluctuation of AMH is about 11 %.

For hyper-response, the optimal cut-off value of 3.36 ng/mL has a sensitivity of 90.5 % (95 % CI 69.6–98.5) and specificity of 81.3 % (95 % CI 75.8–86.0) [77]. Sensitivity and specificity of AFC and AMH for the prediction of high ovarian response were 89 % and 92 % for small AFCs and 93 % and 78 % for AMH at the cut-off values of ≥16 and ≥34.5 pmoL/L, (4.86 ng/mL), respectively. On the other hand, for prediction of poor response, the optimum cut-off value for AMH is 0.99 ng/mL and the post-test probability was highest at cut-off levels of 0.59 ng/mL [1].

Dynamic Tests: Clomiphene Citrate Challenge Test (CCCT) and Exogenous FSH Ovarian Reserve Test (EFORT)

Inhibin B increment in the EFORT has best discriminative potential for hyper-response (ROC-AUC 0.92). E2 increment in EFORT, CCCT, and bFSH, at different cut-off levels, was of less clinical relevance compared with inhibin B increment in the EFORT at the cut-off level of 130 ng/L for the prediction of hyper-response [78].

CCCT appeared to have the best discriminative potential for poor response, as expressed by the largest ROC-AUC (0.88) followed by inhibin increment in EFFORT. E2 and inhibin B increment in EFORT and bFSH at different cut-off levels were of less clinical relevance compared with CCCT at the cut-off level of 18 IU/l, which has a 85 % positive predictive value [78].

Hormonal Monitoring in an Ovulation Induction Cycle

Ovulation induction without the use of gonadotropins (GT) and GnRH analogs is easy and occasionally requires measurement of E2 levels depending on the response, endometrial thickness, and number and size of follicles. Estimation of LH in these cycles allows us to precisely identify the time of ovulation and therefore, is used in natural cycles and oral ovulation-inducing cycles. However, with the use of GT and GnRH analogs in ART cycles, both E2 and LH are monitored more often. We very well know that premature LH surge can impair the development of the oocyte and affect its fertilizing ability and it needs to be detected. A premature LH surge can occur with high levels of E2 in the mid-follicular phase. This could be due to the use of estrogen in the early part of follicular cycle and development of large number of follicles resulting in high E2, especially in cycles where GnRH analogs are not used.

Serum LH

The relationship between follicle size and the serum E2 level is not sufficiently strong to predict the LH surge confidently on the basis of only one variable, but it has been observed that LH surge is unlikely to occur before the follicle diameter has reached 15 mm and/or the serum E2 level has reached 164 pg/mL. LH levels should be measured daily once the follicle reaches 15–16 mm to determine the LH surge and the exact time of ovulation. The mean peak value of LH is 97 U/L/24 h with a standard deviation =/−78 U/L. The LH surges that result in ovulation are extremely variable in configuration, amplitude, and duration.

LH Surge Can Be Detected by Measuring

1. Serum LH levels.
2. Metabolites of LH in urine using urinary LH detection kits. Urinary hormone metabolites accurately reflect LH and correspond to serum patterns and thus, a high predictive value for detecting ovulation. Detection of the LH surge by a urinary LH test may have false-negative results.
   - When peak levels are 40 IU/L
   - When women have surges of 10 h in duration
   - When diluted urine is tested

A study by Lloyd et al. showed that when LH kits alone were used to time IUI

- 36 % of inseminations were timed incorrectly
- 15 % of women had already ovulated

Serum Estradiol Levels

By day 5–8 of the menstrual cycle, aromatase activity begins in granulosa cells of follicles larger than 6–8 mm, with the dominant follicle producing more estradiol-17b than other follicles in the cohort [79–83].

To best reflect the ovarian response to stimulation and provide for an efficient flow of information, gonadotropins are generally administered in the evening, typically between 5:00 p.m. and 8:00 p.m., and serum estradiol measurements are obtained early in the morning. Results are usually available for review by mid-day, and change in the dose and duration of gonadotropins can be made. Follicles less than approximately 10 mm in mean diameter produce relatively little measurable estrogen and larger follicles secrete progressively more as they grow and approach maturity. Usually, estradiol levels rise at a constant exponential pace, doubling approximately every 2–3 days over the days before peak follicular development is achieved. A shallower or steeper slope of increase suggests the need to increase or decrease the level of stimulation. In contrast to a natural cycle, the linear relationship between follicle size and E2 measurements is lost due to the presence of many developing follicles that contribute to the circulating E2. In the natural ovulatory cycle, estradiol levels peak between 200 and 400 pg/mL, just before the LH surge. Comparable levels of estradiol should be expected in gonadotropin-stimulated cycles, for each mature follicle observed. In a COS cycle, one must also consider the number and size of smaller follicles and their lesser but collective contributions to the serum estradiol concentration apart from the large follicles when measuring estradiol levels. Cycle fecundability increases with serum estradiol levels; unfortunately, so do the risks of multiple pregnancy and ovarian hyperstimulation and this is due to multifollicular development, making more oocytes available for fertilization. With existing COS regimens, best results are generally obtained when estradiol concentrations peak between 500 and 1500 pg/mL; pregnancies are uncommon at levels below 200 pg/mL.

Normal follicular growth correlates with E2 measurements and therefore, it can be measured to modulate the dose of gonadotropins in the following manner.

The initial dose changed after 4–5 days depending on the E2 levels

- If a rise >100 % is observed, then the dose is reduced by 75 IU.
- If a rise <50 % is observed, the dose is increased by 75 IU.
- If a rise between 50 and 100 % is observed, same dose is maintained.

Plateauing or decreasing levels require cancellation of the cycle.

Progesterone

Progesterone levels are estimated on day 2 of the menstrual cycle before COS is initiated and on the day of hCG. Elevated progesterone (PE) is associated with endometrial asynchrony and subsequently, low pregnancy rates though the pathophysiology of pre-hCG progesterone rise and its impact on pregnancy outcomes remains inconclusive as no randomized controlled trials (RCTs) are available. But Chu-Chun Huang et al. [84] studied 1784 IVF/ICSI cycles and concluded that the clinical pregnancy rate was significantly decreased in women with longer durations of serum P elevation, independent of the protocol used and the ovarian response [84].

Despite the use of GnRH analogs, a subtle preovulatory rise in the serum P4 concentration before the administration of hCG for final oocyte maturation still occurred in 5–30 % of COS cycles [85–87].

An inverse correlation was observed between the clinical pregnancy rate and the duration of preovulatory progesterone elevation and not the absolute progesterone value on the day of hCG administration. It was also noticed that these patients tend to be younger with better reserves and have lower baseline FSH levels [84].

PE on the day of hCG administration is associated with a significantly decreased probability of pregnancy after fresh embryo transfer in women undergoing ovarian stimulation using gonadotropins and GnRH analogs for IVF but not after transfer of frozen–thawed embryos originating from that cycle. The corresponding numbers needed to treat (NNT) is ~10, which means that for every ten patients with PE, three instead of four pregnancies should be expected [88].

It was also observed that E2 levels on the day of hCG appear to be increased in the presence of PE. In addition, there was some evidence that PE is associated with an increase in the total amount of FSH used for ovarian stimulation but not the length of stimulation. Freezing embryos and transferring them in a subsequent frozen–thawed cycle (the "freeze-all" strategy) has been proposed as a way to bypass impaired endometrial receptivity [89, 90], and it is also considered to be the most frequently used method for managing PE [91].

It was also observed that prolongation of follicular phase is associated with a higher incidence of premature secretory changes on the day of oocyte retrieval in cycles stimulated with recombinant FSH (r-FSH) and GnRH antagonists [92].

Cancellation of Ovarian Stimulation Cycles

The definite indication for cancellation of cycle is poor follicular growth and E2 levels of less than 100 pg/mL on day 5–6 of COS. The possible indication for cycle cancellation may be the presence of an adnexal cyst secondary to GnRH agonist used in A long protocol, risk of OHSS, occurrence of an endogenous LH surge, or a steady decline in E2 levels and poor ovarian response.

Monitoring the Luteal Phase

What women want to know after treatment with ovulation induction medication taken either for a timed intercourse, IUI or ART cycle is whether there is a pregnancy, whether it is in the right place, is it normal, and is it going to continue normally.

The most important hormones monitored for this in the luteal phase are progesterone and beta human chorionic gonadotropin (β-hCG).

Luteal Phase

During the luteal phase, the increased values of progesterone and E2 play an important role in the maintenance of the low FSH and LH levels. During the luteal phase, the frequency of GnRH pulses decrease, while the amplitude increases [93] due to the high progesterone and E2 concentrations [94, 95]. Gonadotropin

secretion is also suppressed by E2 and progesterone, and this action is possibly mediated via an increase in β-endorphin activity in the hypothalamus [96].

What we measure normally in the luteal phase is day 21 progesterone levels in a 28-day menstrual cycle, which will detect ovulation and adequacy of the luteal phase. In irregular cycles, the test may be performed later in the cycle and repeated weekly until the next menstruation. Progesterone has a pulsatile release; thus, a single level may not be useful unless elevated. Values of 10 ng/mL or more are suggestive of normal progesterone production.

The capacity of the CL to produce progesterone is closely related to the extent of its vascular network [97–100]. CL angiogenesis is controlled by local secretion of growth factors [101], namely, vascular endothelial growth factor (VEGF) [102–104].

The relation of blood flow indices in the corpus luteum, measured by transvaginal color Doppler ultrasonography and hormone profiles were studied; the velocity and the impedance indices of the blood flow were both associated with the P4/E2 ratio in spontaneous and CC cycles, while the blood flow indices and the P4/E2 ratio were not correlated in COH cycles [105].

Progesterone is responsible for endometrial decidualization, decrease in smooth muscle contractility, decrease in prostaglandin (PG) formation and immune responses (inhibits T-lymphocyte-mediated tissue rejection).

Luteal-Follicular Transition

During the passage from the luteal to the next follicular phase, an increase, or "intercycle rise," in serum FSH concentrations occurs. FSH starts to increase 2–3 days before the onset of the menstrual period [106], remains elevated during the early follicular phase, and returns to the basal value in the mid-follicular phase [107, 108].

In the absence of a pregnancy, there is a gradual but significant decline in the levels of inhibin A, E2, and progesterone [109, 110], which is responsible for the intercycle rise of FSH that starts in late luteal phase.

The controlled ovarian stimulation cycle, which aims to mature several FSH-sensitive antral follicles during IVF/ICSI (intracytoplasmic sperm injection) treatment using gonadotropins in a GnRH agonist or antagonist protocol results in multi-folliculogenesis. After the hCG trigger, all these follicles are converted into corpora lutea after the release of oocytes in a timed intercourse or IUI cycle or after oocyte retrieval in an ART cycle. Several corpora lutea created produce large amounts of progesterone and E2. If there is a pregnancy, the hCG produced by the chorionic villi will rescue the corpus luteum to support early pregnancy.

Luteoplacental shift occurs at the 7–8 pregnancy week (Fig. 2.30). The dominant ovary volume and vascularization decrease throughout the first trimester placenta and the gestational sac grows continuously [111].

It was seen that the luteal activity significantly increased for the first weeks of pregnancy in a COS cycle. It was also observed that the placental development may be delayed/disturbed after COH and probably, this is the cause for the adverse outcome after fresh ET following a COS cycle. Therefore, today many clinicians are freezing all the embryos to be transferred in the subsequent natural or hormone replacement treatment (HRT) cycle to improve the outcome.

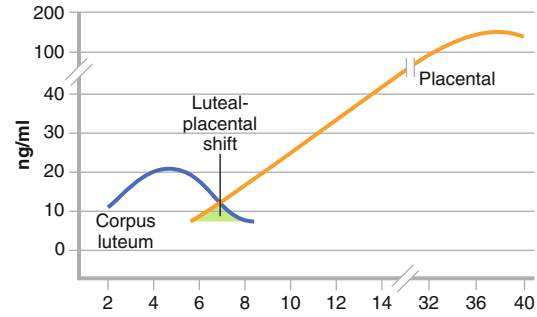


Fig. 2.30 Luteal–placental shift

Monitoring Early Pregnancy After Ovulation Induction

Monitoring early pregnancy after ovulation induction can be done by measuring the progesterone and beta hCG levels along with transvaginal ultrasound. A single progesterone measurement in early pregnancy is a useful test for discriminating between viable and non-viable pregnancies. A low progesterone value early in the pregnancy, especially in the presence of a positive beta hCG in patients presenting with bleeding, pain, and inconclusive ultrasound results can rule out a viable pregnancy [112].

At times, despite a positive beta hCG, the pregnancy cannot be located on ultrasound. This could be because the ultrasound is either performed too early (before beta hCG is 1000 mIU/mL) or too late in cases where the pregnancy has failed or due to altered anatomy, it is too difficult to visualize the pelvic structures or the pregnancy is too bad to be seen.

In such cases, it is important to diagnose an ectopic pregnancy as early as possible in order to initiate treatment. A slow rise in beta hCG and steady decrease in the progesterone levels are suggestive of an ectopic pregnancy.

An absolute single serum hCG level had the lowest diagnostic value, while strategies using serum hCG ratios, either alone or incorporated in logistic regression models, showed reasonable diagnostic performance for EP.

In the presence of pregnancy of unknown location (PUL), one has to balance fears of mistakenly treating intrauterine pregnancy against missing a "life-threatening ectopic." In such instances, it inevitably leads to overdiagnosis of ectopic pregnancy and employment of "preventative" management strategies. This may, at times, harm a normal intrauterine pregnancy.

The majority of women with PUL (50–70 %) have a spontaneously resolving pregnancy with serum hCG levels declining to undetectable levels. Such a pregnancy can either be a failed intrauterine pregnancy (IUP) or a resolved ectopic pregnancy (EP), as the location of the pregnancy remains undetermined. In some women, the pregnancy duration is simply too short to allow its visualization on the initial scan. Follow-up scans in combination with rising serum hCG levels will eventually demonstrate an intrauterine pregnancy (IUP). In 7–20 % of women with a PUL, an EP is eventually diagnosed and these women can be treated either with laparoscopic surgery or medical therapy with systemic methotrexate (MTX). Only a minority of women will have a persisting PUL, defined as an inconclusive TVS in combination with a rise or plateau in serial serum hCG levels. The optimal management for persisting PUL is not known. Systemic MTX as well as expectant management is reported to be successful [113].

We also need to differentiate patients with pathological pregnancy that will resolve spontaneously form those with pathological pregnancy necessitating active therapeutic intervention and those with an early normal intrauterine pregnancy.

Future research involving progesterone test to explore its relation with beta hCG may help in predicting outcomes and calculating post-test probabilities for the whole range of progesterone and beta-hCG values.

Discussion

To ensure safe clinical practice, and prevent OHSS and multiple pregnancies, it is important to monitor treatment response carefully by serial ultrasound scans and serum E2 levels. Evaluating serum progesterone may help in improving the success rate of ART treatment.

Baseline ovarian ultrasonography is prudent between consecutive cycles of stimulation with exogenous gonadotropins. In the absence of any significant residual ovarian cysts or gross enlargement, treatment can begin again immediately without the need for an intervening rest cycle. Higher cycle fecundability and cumulative pregnancy rates have been observed in consecutive treatment cycles than with alternating cycles of stimulation and no treatment [114, 115]. When baseline ultrasonography reveals one or more residual ovarian cysts, it is usually best to briefly postpone further treatment. Stimulation cycles in

the presence of ovarian cysts are less often successful [116], possibly because newly emerging follicles can be difficult to distinguish from regressing cystic follicles, leading to errors in interpretation. Although many believe that suppressive therapy with a cycle of oral contraceptives helps in the regression of residual ovarian cysts, there is no evidence that such treatment is more successful than observation alone.

Studies of endometrial growth in exogenous gonadotropin-induced ovulatory cycles suggest that ultrasonographic measurement of endometrial thickness has great value. Cycle fecundity increases with endometrial thickness, which correlates with serum estradiol concentrations [117]. Few pregnancies result from cycles in which endometrial thickness is less than approximately 7 mm on the day of hCG when treated with ovulation induction drugs [118–120].

Previously, monitoring of ovarian function was based mainly on measuring serum estradiol concentrations, and results were interpreted in relation to the success rate and development of OHSS. Moreover, previously it was thought that complications were not dependent on monitoring but on the stimulation protocol [121]. Today, we know that monitoring as a whole cannot prevent the complications but helps us identify patients at risk of developing these complications and thus, modify our protocols. In ART cycles, the goal is to retrieve mature oocytes and this goal cannot be reached by measuring estrogen only, since the maturity of the oocyte is closely associated with the size of the follicle, a parameter, which can accurately be measured by ultrasound. In addition to the size of the follicles, it has been shown that the best marker for serum estrogen concentrations, and also a major factor in the implantation process is the endometrial thickness and its ultrasonographic texture, a parameter which, again, can adequately be measured by ultrasound. Thus, ultrasound can be used alone to accurately monitor. OI therapy for both in vivo and in vitro fertilization by successfully measuring endometrial thickness and size of ovarian follicles. Follicular growth, uterine measurements, and endometrial thickness correlated strongly with E2 concentrations ($P<0.0001$). Endometrial thickness on the day of hCG administration was significantly higher ($P<0.01$) in conception compared with non-conception cycles, whereas no significant differences were observed in serum E2 concentrations.

The chance of achieving a pregnancy, predicted by uterine artery Doppler and perifollicular blood flow in women whose PI values were higher than 3.26 and 1.08 was very low, with a sensitivity of 1.00 and specificity of 0.59 and 0.82, respectively. The data provided evidence for an association between utero-ovarian perfusion and reproductive outcome following IVF treatment [122]. The ovarian volume, follicular volume, vascularization index, flow index, and vascularization flow index were significantly greater in the pregnant group. 3D ultrasonography and power Doppler angiography allow for an easier ovarian assessment in IVF cycles [123].

Estradiol measurement may provide additional information in predicting OHSS or poor response, which requires cycle cancellation, though avoidance of estradiol and LH assay may simplify the IVF protocols. Monitoring by both ultrasound and estradiol levels is important in those women, who are at risk of developing OHSS. Evaluation of estradiol level during monitoring will help us in deciding between hCG and GnRH agonist for triggering ovulation.

Apart from the selection of the appropriate trigger, we could use certain preventive measures like coasting, intravenous albumin or hydroxyethyl starch solution (36 %), and cryopreservation of all embryos (33 %) with transfer in the subsequent cycle.

In ovulation induction cycles with gonadotropin, which do not use GnRH analogs, premature LH rise or luteinization may occur. These cycles require more stringent monitoring with ultrasound, serum LH, and progesterone along with estradiol in order to accurately time hCG administration or detect ovulation in a non-ART cycles.

In ovulation induction cycles, which use GnRH analogs, monitoring by ultrasound alone is sufficient and will simplify the treatment and its cost and also increase the patient's convenience. Measuring serum estrogen levels will not add significantly to efficacy or safety of the treatment.

Summary

- Monitoring helps the physician to choose the most suitable protocol, to obtain best possible outcome, avoiding complications.
- Baseline USG provides valuable information on ovarian morphology and allows the most appropriate stimulation regimen to be chosen to prevent OHSS and multiple pregnancies and helps in predicting patients response to ovarian stimulation.
- AFC and AMH are equally accurate predictors of high ovarian response to COS and allow us to identify the patients who are at increased risk for OHSS.
- The relationship between AFC and AMH concentrations is more reliable than that observed with FSH, inhibin B, and estradiol on cycle day 3.
- Basal FSH should not be used as a screening tool but instead used to counsel patients appropriately regarding the realistic chance of conception and aiding in the determination of appropriate GT dose.
- Induction of ovulation and IVF protocols can be monitored successfully by measuring endometrial thickness and size of ovarian follicles.
- USG monitoring of follicular growth is the most important tool in the assessment of progress in ovarian stimulation and improves the chance of safe and effective treatment with various ovulation induction agents.
- USG also enables the diagnosis of disorders and complications of ovulation induction.
- Ultrasound can alone be used to accurately monitor OI therapy for both in vivo and in vitro fertilization by successfully measuring endometrial thickness and size of ovarian follicles and correlates strongly with serum estradiol concentrations.
- Estradiol measurement may provide additional information in predicting OHSS or poor response.
- Evaluation of estradiol along with ultrasound monitoring in women with a risk of developing OHSS helps in choosing the trigger for ovulation and luteal phase support.
- Monitoring the luteal phase helps confirm ovulation, luteal function, and pregnancy.
- Pregnancy can be documented by evaluation of beta hCG 15 days after ovulation or by ultrasound 20 days post-ovulation when beta hCG is 1000 mIU/mL, an end point desired by tracking ovulation.
- Monitoring ovulation induction cycles adds to the common pool of information, which increases our knowledge and understanding of human reproduction.

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LH and hCG: Their Distinct Physiological Roles and Use in Ovarian Stimulation Protocols

3

Johan Smitz

Abstract

Luteinizing hormone (LH) and human chorionic gonadotropin (hCG) have been used in diagnostics and therapeutics from biologically purified sources. Though both hormones function via the same receptor (LHCGR), mostly hCG has been used due to its widespread availability. Hence, in the mind of the practising physician, both molecules have been considered equal. The recent availability of recombinant LH has led us to reconsider the specificities of both hormones in terms of actions on the body.

LH and hCG play essential roles in the reproductive cycle. LH plays a key role in follicular maturation and the ovulation process, and hCG is the "pregnancy hormone."

LH and hCG are different in terms of structure, expression, regulation, and function. LH and hCG fundamentally differ in their expression patterns and have complex and unique aspects. LH and hCG should be considered as hormone mixtures, the composition of which fluctuates during the course of the ovarian cycle and pregnancy and throughout the lifespan of men and women. Diverse isoforms have distinct functions, reflected by their relative abundance in normal and aberrant physiologic processes. Quantitative and qualitative distinctions in signaling cascades, activated by LH and hCG have been recently discovered; furthermore, the extragonadal activities are currently under exploration. Availability of recombinant LH and hCG as new therapeutic tools for use in specific clinical pro-fertility conditions could lead us to reconsider the specific indications for each of both molecular entities. The first part of this chapter reviews the current knowledge on both parent molecules, emphasizing their specificities and the consequences at the receptor level.

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Introduction

Luteinizing hormone (LH) and human chorionic gonadotropin (hCG) have been used in diagnostics and therapeutics from biologically purified sources. Though both hormones function via the same receptor (LHCGR), mostly hCG has been used, due to its widespread availability. Hence, in the mind of the practising physician, both molecules have been considered equal. The recent availability of recombinant LH has led us to reconsider the specificities of both hormones in terms of their actions on the body.

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LH and hCG are different in terms of structure, expression, regulation, and function. LH and hCG fundamentally differ in their expression patterns and have complex and unique aspects. LH and hCG should be considered as hormone mixtures, the composition of which fluctuate during the course of the ovarian cycle and pregnancy and throughout the lifespan of men and women. Diverse isoforms have distinct functions, reflected by their relative abundance in normal and aberrant physiologic processes. Quantitative and qualitative distinctions in signaling cascades, activated by LH and hCG have been recently discovered; furthermore, the extragonadal activities are currently under exploration. Availability of recombinant LH and hCG as new therapeutic tools for use in specific clinical pro-fertility conditions could lead us to reconsider the specific indications for each of the molecular entities. The first part of this chapter reviews the current knowledge on both parent molecules, emphasizing their specificity and the consequences at the receptor level.

Molecular Structure

Luteinizing hormone and hCG are heterodimeric glycoproteins, comprised of α and β subunits. hCG retains the full 145 – amino acid complement in its β-subunit. As for LH, the β-subunit undergoes cleavage of its 24-amino acid leader sequence to generate its final 121-amino acid sequence.

Due to structural differences and post-translational modifications, hCG is more stable and has a longer circulating half-life than LH. Due to the heterogeneity of circulating isoforms, the half-lives of these molecules should be expressed as a range: minutes for LH and hours for hCG. The shorter half-life of LH is physiologically relevant, as it allows production of LH pulses. The longer half-life of placental hCG and its greater receptor binding affinity make it more bioactive than hLH [2, 3].

Molecular Forms in the Circulation

Luteinizing Hormone

Gonadotropic cells of the adenohypophysis produce LH, which regulates ovulation. In a normal menstrual cycle, a surge in LH induces ovulation from the dominant follicle. In the first part of the follicular phase, LH stimulates androgen production in thecal cells. These androgens are

aromatized by the granulosa cells to estrogen under influence of FSH. Prolonged, exponentially increasing estrogen levels induce a positive feedback on the pituitary gland and the subsequent LH surge results in ovulation [4]. LH promotes progesterone production, supporting development of the corpus luteum [5]. Variations in LH isoform composition are observed during the reproductive life cycle. In general, LH isoforms with shorter half-lives but increased biopotency are present in younger postpubertal women, whereas longer-lived LH isoforms prevail in postmenopausal women [6, 7]. Women with polycystic ovarian syndrome (PCOS)—an endocrine condition associated with altered folliculogenesis and anovulation—appear to predominantly secrete LH isoforms that have a high ratio of biological-to-immunological activity [8, 9].

Human Chorionic Gonadotropin

Four physiologically important isoforms of hCG have been described: regular hCG, hyperglycosylated hCG (h-hCG), hyperglycosylated free hCG β subunit, and pituitary hCG. Different cell types produce these isoforms, which display disparate half-lives and biologic functions. Isoforms have unique, although sometimes overlapping, functions: the four variants share a common protein sequence (β subunit), but each is modified differently.

After an initial early surge of h-hCG regular hCG is secreted by differentiated syncytiotrophoblasts. It is the prevailing hCG species measured in serum during pregnancy [10]. Historically, it was believed that hCG induced promotion of progesterone secretion by the corpus luteum in early pregnancy. However, hCG has other functions during pregnancy as its levels continue to increase after hCG is no longer needed for progesterone production [11].

Human chorionic gonadotropin is additionally involved in placentation: maintaining angiogenesis of the uterine vasculature and promoting differentiation of cytotrophoblasts into syncytiotrophoblasts [12–14]. Proposed roles for regular hCG comprise fostering implantation, preventing fetal rejection, co-ordinating uterine and fetal growth, and, potentially, growth and development of fetal organs [10].

During the implantation process, extravillous cytotrophoblasts and h-hCG concentrations peak early in the first trimester [10]. The structural difference between regular hCG and h-hCG is the complexity of the oligosaccharide side chains; h-hCG tends toward oligosaccharides with a greater number of sugar residues. The percentage of hCG in the form of h-hCG subsequently declines, becoming a minor component of total hCG measurement during the last two trimesters.

The association between pregnancy loss and low h-hCG levels supports a key role for this variant in implantation [15, 16].

Pituitary hCG is secreted by gonadotropic cells of the anterior pituitary. Pituitary hCG has a shorter half-life than its placental counterpart due to the higher number of sulfonated side chains [17]. The concomitant temporal appearance of hCG with LH during the menstrual cycle and their common receptor suggest that it functionally may mimic LH and support the progesterone production during the luteal phase [10].

Metabolism

One of the initial steps in hCG degradation is proteolytic cleavage of the β subunit (possibly by human leukocyte elastase), which generates a "nicked" form of the protein. Nicked hCG rapidly dissociates into its component α and β subunits. h-hCG dissociates more readily than regular hCG. The nicked β subunit is further degraded, predominantly in the kidneys, resulting in a predominant β-core fragment in urine [11, 18, 19].

The extent of gonadotropin glycosylation dictates molecular charge determining clearance rate. The more acidic isoforms have a longer half-life in vivo [20]. The grade of sialylation of LH positively correlates with the metabolic clearance rate [21].

The Common Luteinizing Hormone/Choriogonadotropin Receptor, Its Polymorphisms and Mutations

Luteinizing Hormone and hCG bind to and activate a common receptor, the LH/choriogonadotropin receptor (LHCGR), also known as the LH receptor (LHR) [22, 23]. LHCGR is expressed by multiple cell types in the ovary: thecal, luteal, interstitial, and differentiated granulosa cells. Expression of LHCGR in these cell types during the ovarian cycle is dynamic, depending on changes in the hormonal milieu [24]. LHCGR is downregulated transiently after the preovulatory LH surge, reaches a maximum at the mid-luteal phase, and decreases with corpus luteum regression. This pattern is the inverse of what has been observed for bioactive LH isoforms, where biologic-to-immunologic activity is maximal at mid-cycle and reaches a nadir during the luteal phase [25]. Temporal changes in LHCGR expression involve transcriptional as well as post-transcriptional regulation [24].

Mutations in LHCGR are associated with developmental and reproductive abnormalities, including psuedohermaphrodism, micropenis, hypospadias, and infertility [22, 24]. More recent suggestions state that polymorphisms in the LHCGR sequence contribute to risk for conditions associated with infertility, including PCOS. A genome-wide association study detected a link between a polymorphic marker in the region of LHCGR and PCOS in Han Chinese women (confirmed in a subsequent case-control cohort) [26]. Interestingly, the specific polymorphic marker, associated with PCOS in the Han population, failed to correlate with PCOS in Caucasian [27, 28].

Signaling Pathways Linked to LHCGR

LHCGR is capable of binding αβ dimeric LH, hCG, and h-hCG. Also, nicked hCG binds LHCGR, but with a much lower affinity [29, 10, 11]. LHCGR signaling pathways are a subject of active investigation: it is generally accepted that the cyclic adenosine monophosphate/protein kinase A (cAMP/PKA) pathway drives the downstream events inducing ovulation and the steroid biosynthesis processes.

Many other signaling pathways are however, triggered: LHCGR stimulation also activates the phospholipase C/inositol phosphate (PLC/IP) signaling pathway [30], but it has been suggested that PLC-based signaling only occurs during the preovulatory LH surge and during pregnancy when levels of its ligands are high. Investigators have recently reported PLC to be the mediator of final granulosa cell differentiation in response to LH [31].

In addition, extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) and AKT have been identified as major downstream players in LHCGR-mediated signaling [32, 33]. The ERK1/2 and AKT pathways participate in the regulation of oocyte and follicle maturation [34, 35].

As LH and hCG were considered to be functionally equivalent, the post receptor effects were traditionally presumed to be similar. New data, however, suggests that this is not true. In recent experiments, phospho-ERK1/2 levels were reported to be greater in cultured human granulosa cells after long-term exposure to LH compared with hCG [36].

The Evidence for Extragonadal LHCGR

Detection of LHCGR in regions other than ovarian cell types, including the decidua, uterine vasculature, umbilical cord, fetal organs, cytotrophoblast cells, and adrenal cortex, has fueled a debate regarding the potential role for gonadotropins in extragonadal locations [10, 37, 38].

Emerging new data propose that extragonadal LHCGR has functional significance; further study is needed to clarify its role in normal and aberrant cellular processes and to characterize the individual effects of various LH and hCG isoforms.

Physiology

By understanding how LH and hCG affect normal and abnormal human development and reproduction, current treatments for reproductive disorders may be improved while uncovering other medical disciplines where diagnostic and therapeutic measures of these gonadotropins may be of use, e.g., in cancer. The physiology of LH and hCG throughout the reproductive lifespan and the impact of disrupting their normal expression profiles and functionality are concisely summarized as follows.

The Basal LH Secretion Pattern

The increase in gonadotropin activity during puberty drives gonadal steroidogenesis—primarily testosterone and progesterone by thecal cells in females [39]. Most aromatizable androgens, generated by thecal cells, are converted to estradiol by the granulosa cells. Testosterone, estradiol, and adrenal androgens produce the physical changes associated with puberty. As estradiol production increases, stimulation of the endometrium occurs, eventually leading to menarche (approximately 2–3 years after the first signs of puberty). The reproductive axis matures in middle-to-late puberty, with the establishment of estrogen positive feedback leading to the LH surge [40]. Ovulatory cycles do not become established until some years after menarche.

After puberty, the pattern of LH secretion during the menstrual cycle becomes more regular in normo-ovulatory women. In most women with PCOS, however, higher basal levels of LH are the result of increased pulse frequency and amplitude throughout the cycle [41–43]. This lack of variation in LH secretion pattern contributes to the anovulatory cycles, often found in women with PCOS.

The Roles of LH and hCG During Ovulation and Pregnancy

In the first part of the follicular phase, estradiol exerts a negative feedback effect on LH; however, LH activity stimulates thecal cell production of androgens for estradiol production by the granulosa cells. As estradiol levels rise exponentially in the second part of the follicular phase, a positive feedback effect is induced, leading to the LH surge and subsequent ovulation [44]. With ovulation, the ovulatory oocyte reinitiates meiosis I and progresses through to meiosis II. LH drives progesterone production and secretion from the corpus luteum until, if pregnancy occurs, hCG will induce its survival. Initially hCG is mostly the hyperglycosylated isoform (h-hCG) [45].

What is less well known is that low levels of pituitary hCG parallel the dynamics of LH secretion during the menstrual cycle [46]. The role of this pituitary hCG is unclear, but the pattern of expression suggests an overlapping one to that of LH [10]. A suggestion is that hCG expression may elevate the peak range of LH, thereby aiding in the promotion of ovulation and the early secretion of progesterone [11].

Roles of LH and hCG on Fertilization and Implantation

The presence of LHCGR in human Fallopian tubes and sperm suggests a role for LH and hCG in fertilization [47, 48]. Expression of LHCGR is greater in Fallopian tubes during the luteal phase compared with the proliferative phase of the menstrual cycle or Fallopian tubes from postpartum or postmenopausal women [47].

Secretion of hCG by the blastocyst may elicit a cross talk with endometrium to allow implantation [49, 50]. Endometrial LHCGR expression increases in mid-luteal phase at a time where the endometrium is receptive to implantation (i.e., the implantation window) [50]. Some researchers claim that a blastocyst, producing locally high levels of hCG could extend the implantation window [49].

Human choronic gonadotropin is also believed to support implantation and placentation by remodeling endometrial tissue, promoting maternal immunotolerance of fetal tissue, inducing neoangiogenesis, and increasing the natural killer (NK) lymphocyte population [10, 49, 50]. As the

effects of fostering endometrial receptivity were initiated even before embryonic hCG expression, researchers found epithelial hCG is expressed and produced in the human endometrium biopsy specimens during the early to mid-secretory phase of the menstrual cycle [51]. Further studies on the role of hCG on endometrium, related to implantation, should be conducted.

Pathology of LH, hCG, and LHCGR

Mutations at the level of LH, hCG, and their common receptor (LHCGR) have taught us the extent to which LH and hCG signaling is required for the formation and development of the reproductive organs throughout life and its importance in fertility regulation. Observed gene alterations may be naturally occurring, as is the case for human mutations/polymorphisms, or induced in mouse knock-out models. A description of human phenotypes associated with changes in LH, hCG, and LHCGR function or expression are summarized in Table 3.1.

Human Mutations/Variants

LHβ

Naturally occurring mutations, resulting in inactive LH, are rare in women. Two cases in female patients with inactivating LHβ mutations have been described [52, 53]. Characterization of one of these individuals revealed normal development and appropriate pubertal milestones followed by secondary amenorrhea and infertility [52]. The reproductive findings in these women confirm that adequate LH is not absolutely needed for normal sexual differentiation and puberty, but essential for ovulation and corpus luteum functionality.

hCG

It has been hypothesized that mutations with a significant effect on hCG would not be compatible with successful pregnancy and are thus not found [54]. It seems logical that polymorphisms in the hCG β-subunit (i.e., the CGB gene) are associated with an increased risk of recurrent miscarriage [54].

LHCGR

Activating and inactivating mutations have been described in the LHCGR gene. Women with activating LHCGR mutations display no functional reproductive abnormalities. On the other hand, patients with inactivating mutations of LHCGR have a similar phenotype to that of inactivating LHβ mutations, including oligomenorrhea and infertility [55]. An LHCGR mutation that is believed to reduce receptor expression and binding capacity has been implicated in empty follicle syndrome [56]. In general, the loss of

Table 3.1 Phenotypes associated with mutations in human *LHβ*, *CGB*, and *LHCGR* genes

Gene and type of mutation	Phenotype	Effect on fertility
LHβ		
Inactivating	Oligomenorrhea, secondary amenorrhea	Infertile
Polymorphisms	Endometriosis, hyperprolactinemia, luteal insufficiency, menstrual disorders, PCOS	Reduced fertility
CGB		
Polymorphism	Recurrent miscarriage	Reduced fertility
LHCGR		
Activating	Leydig cell adenoma	Reduced fertility
Inactivating	Oligomenorrhea/amenorrhea, empty follicle syndrome	Infertile

LHβ luteinizing hormone β-polypeptide, *CGB* chorionic gonadotropin β-polypeptide, *LHCGR* luteinizing hormone/choriogonadotropin receptor, *PCOS* polycystic ovary syndrome

function of LHCGR mutations had only generated overt reproductive pathology in the homozygous state (i.e., autosomal recessive inheritance).

Pharmacological Uses of LH and hCG

Actions of LH Bioactivity on Follicle and the Relation with Oocyte Quality

In humans, physiological follicular growth is driven by a delicate interplay between FSH and LH that affects theca and granulosa cells, leading to the selection of a single dominant follicle through a series of feedback mechanisms [57]. Effects of LH are mediated via LH receptors, which are expressed as soon as theca cells are present on secondary follicles. Theca cells play a unique role in the generation of androgens and growth factors, which influence growth and differentiation of granulosa cells that are under endocrine control of FSH via the presence of FSH receptors. FSH drives the development of the granulosa cell compartment and is essential for follicle survival and differentiation. Effects of FSH are amplified via several paracrine loops, including the products of aromatization that depend upon the provision of androgens by the theca cells. Multiple follicular recruitment can be obtained by applying supraphysiological amounts of FSH alone [58, 59]. While supraphysiological amounts of FSH can increase survival of many follicles in one cycle and provide an increased oocyte production, serum LH concentrations seem to determine a favorable outcome only when kept within certain limits. The exact amounts of administration of LH and/or hCG to administer in combination with FSH to obtain successful pregnancies have been under recent scrutiny [60–63]. With the progression of follicle growth, LH receptors are expressed on the granulosa cells. It has been shown that in human, follicles of 10 mm diameter are becoming responsive to LH action [64, 65]. Receptors for LH are highest in the mural granulosa cells closest to the basement membrane and their density decreases centripetally. Demonstration of expression of LH mRNA and receptor protein in cumulus-corona might be species specific and is influenced by differences in assay specificity, type of follicles from which the cumulus-oocyte complexes (COC) are isolated, the hormonal supplements used in the incubation medium and timing of the analysis after isolation and culture of the cumulus cells [66–68]. LH action on the oocyte itself is indirect: there is an upregulation of EGF-like substances in the mural granulosa cells that have their receptors in the cumulus cells [69, 70]. LH activity in the follicular environment positively influenced early embryonic development in primate, bovine, and ovine [71–73] and has also previously been associated with conception cycles in patients undergoing COH for ART [74–76].

Ovulation Induction and Ovarian Stimulation: A Modulatory Role by LH Bioactivity

In a minority of female patients consulting for anovulation, the origin of the defect is in the central nervous system at the hypothalamic or pituitary level (anovulation WHO type I). These women have no measurable FSH and LH. Restoration of a cycle can either be obtained by pulsatile lueinizing hormone-releasing hormone (LHRH) treatment or by gonadotropin administration. In this case, a direct gonadotropin substitution is preferred and there is an absolute need to administer LH bioactivity. As human menopausal gonadotropins (hMGs) contain equipotent LH and FSH amounts, there is a constant LH supply always available in ovulation induction (OI) schemes. In the case that treatment with recombinant follicle-stimulating hormone (r-FSH) is considered, there is a need to co-administer recombinant LH (r-LH) or recombinant hCG (r-hCG).

For all other indications for ovulation induction therapy (WHO type II or type III), there is sufficient endogenous LH background concentration present to allow follicle growth and endometrial preparation. In principle, OI could be performed with r-FSH only, however, in patients with polycystic ovary disease, there are now good indications that highly purified hMG (HP-hMG) is equally effective and has a reduced number of

complications, such as multiple pregnancy and early onset hyperstimulation syndrome, which is accompanied with a lot of discomfort for the patients [77]. According to the ceiling theory, the LH level present in the daily injections may prohibit the transition of small to medium-sized follicles in the cohort to grow further up to the preovulatory stage [57]. Hence, it was proposed to consider a biphasic type of treatment for ovulation induction, wherein phase 1 FSH is used, followed with LH (or hCG) when the largest follicle has reached 13–14 mm (a stage at which it has acquired the LH receptor). The administration of LH would then take over the function of FSH in those follicles expressing LH receptors. In the smaller follicles, where LH receptor on granulosa is still insufficiently expressed, the LH support would not be functional, leading the follicles into atresia.

In summary, regarding the role of LH, its dose and time of administration at a particular stage of follicular growth are very important; its presence is essential to drive known theca cell functions such as steroidogenesis and provision of paracrine factors to granulosa. However, LH is a double-edged sword: an excess of LH would drive the small follicles into atresia due to accumulated androgens which remain unconverted (in small follicles aromatase is still inactive). Depending on whether mono- or multiple folliculogenesis is desired, timely administration of LH in combination with FSH is important to modulate the ovarian response.

Use of LH or hCG in Ovarian Stimulation for ART

Conditions in Need of LH or hCG Supplementation

In circumstances, where patients had a profound desensitization prior to stimulation (e.g., in the "long" GnRH protocol), an iatrogenic state of shortage of LH activity can be induced. When the gonadotropin preparation used for stimulation does not contain LH activity, an LH shortage might result in these women [78, 79]. The degree of gonadotropin suppression is dependent on the type of GnRH analog used and on their route and frequency of administration [80–82]. Regarding the serum LH concentrations measured after gonadotropin treatment, there was significantly more circulating LH present in patients treated with an hMG preparation than those exposed to r-FSH alone [79]. FSH stimulation of the gonads provokes signals back to the hypothalamic-pituitary axis via ovarian steroids and gonadal peptides that suppress the endogenous gonadotropin secretion [83]. A retrospective analysis of serum hormone profiles in 71 patients downregulated with a GnRH agonist (Decapeptyl) revealed a surprisingly high incidence of 50 % of the patients with low LH (≤1 IU/L) when treated with r-FSH after pituitary desensitization. Compared to the HP-hMG patients, estradiol concentrations produced by granulosa cells from the r-FSH only treated group were significantly lower. The difference in estradiol (E2) output can be explained by the responsiveness of theca cells to the constant exposure to hCG in the HP-hMG group. Increased E2 levels in HP-hMG are the reflection of an increased production of E2 per follicle through a higher provision of androgen precursor molecules. Serum androstenedione and total testosterone concentrations are significantly elevated throughout the last days of stimulation in HP-hMG cycles. The occurrence of pregnancy in relation to steroid exposure levels over the last 8 days of stimulation treatment was inversely correlated with progesterone (≤0.71 μg/L), androstenedione (≤2036 ng/L), and the free androgen index (FAI) (≤0.013) in HP-hMG treatments. Values over the median value for these two parameters for the entire population reduced the occurrence of a pregnancy by a factor 2 to 3, emphasizing the existence of an endocrine profile which when exceeded was associated with a negative outcome. Similar relationships between steroid levels in circulation during the preovulatory period and the prevalence of conception by IVF treatment have been previously documented [84].

Ceiling Doses for HCG and LH

Using the current HP-hMG preparations throughout the entire stimulation phase in GnRH agonist

downregulated patients does not induce premature luteinization of the large follicles as long as the daily administration dose remains under 100 IU, which is a dose well above the hCG provision, which would be administered by regular treatment [63]. It is expected that daily administration of equivalent doses of recombinant LH from the beginning of the stimulation is also safe [85]. Also, another team, who studied GnRH agonist-suppressed infertile women treated with different FSH preparations, demonstrated no correlation between rising preovulatory progesterone concentrations and LH activity [86, 87], but rather, a strong positive correlation with serum FSH. Moderate progesterone increments have been observed in severely downregulated patients treated exclusively with recombinant FSH [88, 89]. Supraphysiological doses of FSH are able to induce progesterone elevations and to increase thecal androgen production. Supraphysiological FSH levels mobilize factors from granulosa cells that promote the production of progesterone by the theca cells [90].

Routine LH or hCG Bioactivity in Combination to FSH

Large clinical trials, comparing the use of HP-hMG with r-FSH in GnRH agonist downregulated and in GnRH antagonist suppressed patients (700 and more patients per study) demonstrated higher live birth rates in the hCG containing HP-hMG preparation. The mechanisms behind the positive effects of low hCG levels on gamete quality still remain largely enigmatic. The studies with HP-hMG suggest that constant background of LH bioactivity in the form of hCG during the preovulatory phase has a major impact upon the steroid environment with potential downstream effects on gamete competence. In large prospective randomized studies comparing the use of HP-hMG to r-FSH in combination with GnRH analog for IVF, HP-hMG yielded higher live birth rates, despite a lower oocyte recovery rate, compared to r-FSH. In the Merit® trial, part of the explanation for superior results with HP-hMG could be attributed to the higher embryo quality parameters and higher implantation rates in HP-hMG top-quality embryos [75]. The reason for better embryological outcomes in HP-hMG is not known; beneficial effects from a paracrine environment, induced by LH bioactivity on oocyte cytoplasmic maturation might involve androgen action, epidermal growth factor (EGF)-like factors, or factors from the transforming growth factor-beta (TGFβ) superfamily, linked to developmental competence [69, 70, 91]. Larger prospective studies are needed to evaluate the significance of LH exposure at the molecular level in oocytes and embryos and to clarify suggested differences in hCG or LH effects.

Conclusions

The availability of recombinant products with very specific and distinct bioactivity allows further study of the actions of LH and hCG. Clinical data suggest that the effects of the two molecular entities, working via the same hLHCG receptor might be different. Many reasons for the observed differences have already been provided, but the high molecular complexity of the two hormones and their interaction on the reproductive organs need further study.

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Recombinants versus Biosimilars in Ovarian Stimulation

4

Gautam N. Allahbadia and Akanksha Allahbadia

Abstract

Biosimilars, also known as follow-on biologics, are biologic medical products whose active drug substance is made by a living organism or derived from a living organism by means of recombinant DNA or controlled gene expression methods. Recombinant human follicle-stimulating hormone (r-hFSH) was one of the early biologic drugs to be approved and is used in assisted reproductive technologies (ART), sometimes known as in vitro fertilization. The drug was first marketed as Gonal-F by Merck Serono but has lost its European patent some time ago, US patent extends to 2015. In 2014, two FSH biosimilars obtained marketing authorization by the European Medicines Agency. Biosimilar FSH preparations are expected to be biologically and clinically "non inferior" to the originator product. However, prescribing a biosimilar to a patient calls for certain basic understanding by physicians of the scientific factors associated with the safety and efficacy of these products. Substituting an innovator brand by a biosimilar brand calls for caution in terms of quality, safety, and efficacy aspects due to clear differences between biosimilars and their reference products. The impact of FSH and human chorionic gonadotropn (hCG) biosimilars on cost and outcomes of ART is far from being established, since insufficient information is available to demonstrate the pros and cons in the long-term application.

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Introduction

Limited access to high-quality biologics due to the cost of treatment constitutes an unmet medical problem globally. The term "biosimilar" is used to designate a follow-on biologic that meets the extremely high standards for comparability or similarity to the originator biologic drug that is approved for use for the same indications [1]. Biosimilars (or follow-on biologics) are terms used to describe officially approved subsequent versions of innovator biopharmaceutical products made by a different sponsor following patent and exclusivity expiry on the innovator product [2]. Biosimilars are also referred to as subsequent entry biologics (SEBs) in Canada [3]. Reference to the innovator product is an integral component of the approval.

Use of biosimilar products has already decreased the cost of treatment in many regions of the world, and now a regulatory pathway for approval of these products has been established in the USA. The Food and Drug Administration (FDA) led the world with the regulatory concept of comparability, and the European Medicines Agency (EMA) was the first to apply this to biosimilars. Patents on the more complex biologics, especially monoclonal antibodies, are now beginning to expire and biosimilar versions of these important medicines are in development. The new Biologics Price Competition and Innovation Act allows the FDA to approve biosimilars, but it also allows the FDA to lead on the formal designation of interchangeability of biosimilars with their reference products [4]. The FDA's approval of biosimilars is critical to facilitating patient access to high-quality biologic medicines, and will allow society to afford the truly innovative molecules currently in the global biopharmaceutical industry's pipeline [4].

Unlike the more common small-molecule drugs, biologics generally exhibit high molecular complexity, and may be quite sensitive to changes in manufacturing processes. Follow-on manufacturers do not have access to the originator's molecular clone and original cell bank, nor to the exact fermentation and purification process, nor to the active drug substance. They do have access to the commercialized innovator product. Differences in impurities and/or breakdown products can have serious health implications [1]. This has created a concern that copies of biologics might perform differently than the original branded version of the product. Consequently, only a few subsequent versions of biologics have been authorized in the USA through the simplified procedures allowed for small-molecule generics, namely, Menotropins (January 1997) and Enoxaparin (July 2010), and a further eight biologics through the 505(b) [2] pathway [5].

Biosimilars can only be authorized for use once the period of data exclusivity on the original "reference" biological medicine has expired [1, 5]. In general, this means that the biological reference medicine must have been authorized for at least 10 years before a similar biological medicine can be made available by another company. For biosimilar medicines, the company needs to carry out studies to show that the medicine is similar to the reference medicine and does not have any meaningful differences from the reference medicine in terms of quality, safety, or efficacy. As information on the reference medicine is already available, the amount of information on safety and efficacy, needed to recommend a biosimilar for authorization, is usually less than the amount needed to authorize an original biological medicine. As with all medicines, the European Medicines Agency continues to monitor the safety of biosimilar medicines once they are on the market [1, 5].

None of the biosimilars were reported to have evidence of significant clinical variation relative to reference compounds in the absence of corresponding differences in biophysical properties

[5]. A recent study provides evidence that current EU guidelines have resulted in the approval of biosimilar therapeutics with comparable efficacy and safety profiles for the recommended indications of their respective reference originator biologics. It is anticipated that these precedents will serve as a starting point in the development of a process for approving biosimilars worldwide to provide efficacious and tolerable biotherapeutics with a significant cost advantage for national health care programs and consumers [5].

Discussion

Follicle-stimulating hormone (FSH) is a glycoprotein hormone essential for reproduction, both in females and males, and it is physiologically produced by the anterior pituitary gland in several isoforms. This heterogeneity is also typical of FSH-containing compounds, both urinary-derived and recombinant products. These compounds are widely used in assisted reproductive technologies (ART), to induce multifollicular development. Recently, the increased cost pressure on healthcare systems and the patent expiration date of widely used biotechnology-derived, recombinant FSH prompted the pharmaceutical interest in FSH biosimilars.

Recombinant human FSH (rh-FSH) is an important drug in Reproductive Medicine. Thorough analysis of the heterodimeric heavily glycosylated protein is a prerequisite for the evaluation of production batches as well as for the determination of "essential similarity" of new biosimilars [6]. The concerted application of different liquid chromatography-mass spectrometry methods enabled the complete depiction of the primary structure of this pituitary hormone. Sequence coverage of 100 % for the α- as well as the β-chain was achieved with tryptic peptides. Most of these peptides could be verified by tandem mass spectrometry. Quantification of the glycoforms of each glycopeptide was accomplished with the software MassMap®. The currently marketed product Gonal-F™ and a potential biosimilar were compared with the help of these procedures [6].

Ruman et al. [7] evaluated the efficacy of two novel long-acting rhFSH analogs, rh-FSH-N2 and rhFSH-N4, in stimulating murine folliculogenesis. Recombinant hFSH-N2 and -N4 were administered via single IP injection to 3-week-old female mice ($n=10$) that were killed 48 h later for dissection and histologic examination of reproductive organs and serum inhibin A. Results were compared with other groups of mice that received either single or q 12 h injections for 48 h of commercial rhFSH, or a single injection of pregnant mare serum gonadotropin (PMSG). A subgroup of the mice receiving rh-FSH-N4 was supplemented with daily injections of small doses of hCG to simulate LH add-back. Recombinant human FSH-N2 and -N4 administration induced a statistically significant increase in ovarian weights, uterine weights, and inhibin A levels compared with single and twice-daily injection of rhFSH. PMSG induced the greatest increases in all three measured parameters. There was no statistical difference between rhFSH-N2 and rhFSH-N4 for any parameter analyzed. A single injection of rhFSH-N2 or -N4 induced a greater number of antral follicles than did either single or q 12 h injections of rhFSH. The addition of small doses of hCG to rhFSH-N4 increased inhibin A levels and antral follicle number to reach statistical equivalence to PMSG treatment. The authors concluded that addition of a synthetic polypeptide containing two or four N-linked glycosylation sites to rhFSH increases in vivo bioactivity of the hormone compared to commercial rhFSH. After a single injection, both rhFSH-N2 and rhFSH-N4 effectively induced a greater follicular response in the mouse than did rhFSH [7].

Kim et al. [8] formulated a study to develop efficient Chinese hamster ovary (CHO) cells that express rhFSH in serum-free conditions and to investigate the effect of this newly synthesized rhFSH on folliculogenesis and ovulation. A stable single CHO cell that expresses rhFSH at a high level was obtained by introducing the human chorionic gonadotropin (hCG) alpha-subunit and FSH beta-subunit genes. After purification processing, they investigated the effect of this newly synthesized rhFSH on folliculogenesis in

hypophysectomized rats and ovulation in androgen-sterilized mice. The ovary weight, uterine weight, number of follicles, and ovarian morphology were evaluated in immature hypophysectomized rats. The number of ovulated oocytes and ovarian morphology were examined in androgen-sterilized mice. After purification processing, they analyzed the new rhFSH using matrix-associated laser desorption ionization-time of flight and found that this new rhFSH increased both ovarian weight and uterine weight in hypophysectomized rats and induced ovulation in androgen-sterilized mice. The study concluded that the newly synthesized rhFSH can be safely used in anovulatory infertile woman as well as in ovulation induction protocols for subfertile women [8].

Moon et al. [9] compared the efficacy and safety of a new rhFSH (FSH; DA-3801) with Follitropin-alpha (Gonal-F) in women undergoing controlled ovarian hyperstimulation (COH) for ART. This was a phase III, multicenter, randomized, non-inferiority study. A total of 97 women were randomized to receive COH using DA-3801 (DA-3801 group, $n=49$) or Gonal-F (Gonal-F group, $n=48$). All subjects underwent COH using a gonadotropin-releasing hormone (GnRH) antagonist protocol. The primary efficacy endpoint was the number of oocytes retrieved, and the secondary efficacy endpoints included the total dose of FSH, the duration of stimulation, the serum estradiol levels on the day of hCG administration, and the fertilization, implantation, and pregnancy rates. Safety was evaluated using pre- and post-treatment laboratory tests and all adverse events were recorded. The number of oocytes retrieved was 13.0 ± 6.2 (DA-3801) versus 10.6 ± 6.7 (Gonal-F) in the intention-to-treat (ITT) population, and 12.7 ± 6.4 (DA-3801) versus 11.0 ± 7.1 (Gonal-F) in the per-protocol (PP) population. The non-inferiority of DA-3801 was demonstrated with differences of 2.3 ± 6.5 (95 % confidence interval [*CI*] = 0.13, infinity) and 1.7 ± 6.7 (95 % *CI* = −0.74, infinity), respectively, in the ITT and PP populations. The total dose of FSH used (1789.8 ± 465.5 vs 2055.6 ± 646.7 pg/mL, $P=0.027$) and duration of stimulation (8.3 ± 1.4 vs 9.1 ± 1.9 days, $P=0.036$) in the ITT population were significantly lower in the DA-3801 group. Other secondary efficacy endpoints, including pregnancy and implantation rates and the incidence and severity of adverse events, were comparable between the two groups. The results of this study demonstrate that DA-3801 is not inferior to Follitropin-alpha in terms of its efficacy and safety in women undergoing COH for ART [9].

Choi [10] presented the first clinical results of biosimilar rhFSH in 2006 on the efficacy and safety of LG rhFSH (LBF0101) versus Follitropin-alpha (Gonal-F) in IVF/ICSI cycles. The number of oocytes retrieved by LG rhFSH was not significantly different to that by Gonal-F (12.3 ± 5.9 vs $4.2 =/- 8.6$). LG rhFSH was equivalent to Gonal-F in terms of secondary efficacy parameters. LG rhFSH and Gonal-F did not show significant difference regarding adverse events and local reactions to injection [10].

A new recombinant Follitropin was developed as a mixture of heterodimeric alpha and beta subunits by LG Life Sciences (Seoul, Korea). Koong et al. [11] conducted a study to evaluate the efficacy of the new recombinant Follitropin (LBFS0101) in an in vitro fertilization-embryo transfer (IVF-ET) program. Between April and July 2005, 28 cycles of COH-IVF were included in this prospective study. The patients were randomly divided into LBFS0101 ($n=15$) and Gonal-F ($n=13$) groups. COH was performed with GnRH agonist, and ovarian response was monitored by transvaginal sonography (TVS) and estradiol (E2) concentrations. Outcomes of fertilization and pregnancy were compared. Production of anti-FSH antibodies by injected recombinant FSH was monitored in the patient's serum before and after COH-IVF using ELISA. There was no statistical difference between two groups in the ovarian response, such as duration of stimulation, number of follicles, and serum E2 concentration on the day of hCG injection. There was no statistical difference in pregnancy and fertilization rates [11].

Lee et al. [12] analyzed the efficacy and tolerability of Follitropin-heterodimeric alpha-beta subunit mixture (LBFS0101; LG Life Sciences Ltd Seoul, Korea), new rhFSH preparations,

for superovulation in patients undergoing IVF-ET. One hundred and three infertile women undergoing IVF-ET in 2005 were enrolled into the study. After downregulation with Buserelin acetate, patients were randomized to receive LBF0101 ($n=52$) or Gonal-F ($n=51$). rhFSH was administered at 150–300 IU/day for 3–5 days, and then dosages were adjusted according to the ovarian response. A total of 15 cycles were cancelled, and 88 cycles (LBFS0101 = 47 cycles; Gonal-F = 41 cycles) were studied. There were no statistically significant differences in any clinical profiles of patients between the two preparations. Also, cumulative dose of rhFSH (2371.3 =/− 728.4 mIU for LBFS0101 vs 2409.8 ± 769.4 mIU for Gonal-F), duration of rhFSH treatment (9.3 =/− 4.6 days vs 10.6 =/− 5.8), and number of retrieved oocytes (14.2 =/− 9.1 vs 14.4 =/− 8.8) were not different. Clinical pregnancy rates and implantation rates were similar [44.7 % (21/47), 22.1 % (38/1690 for LBFS0101 and 56 % (23/41), 29.55 (941/139) for Gonal-F]. Anti-FSH antibody was not detected in all samples. The authors concluded that the results of their clinical study indicate that LBFS0101 may be suitable for use in ovarian stimulation for human IVF-ET programs [12].

FINOX Biotech (Finox AG) announced in 2012 that the pivotal phase III study (FIN3001) with Afolia, a biosimilar recombinant follicle-stimulating hormone (r-FSH), in patients undergoing ART, has met its primary endpoint [13]. Afolia is a new "biosimilar" medicine, an almost exact copy of the originator product that was produced using recombinant DNA technology. Both Afolia and the reference product Gonal-F are formulations of the naturally occurring hormone FSH, which plays a key role in human reproduction. Afolia is the result of a targeted drug development process, aimed to replicate as closely as possible the reference product. Afolia demonstrated clinical and statistical equivalence to the reference product Gonal-F. Equivalence was defined by retrieving similar numbers of oocytes during standard treatment duration of 10–16 days with a fixed dose of r-FSH. The equivalence margins required that the difference in the number of oocytes retrieved not exceed ±2.9 oocytes. Results prove that Afolia is "biosimilar" to Gonal-F: the number of oocytes retrieved was 11.3 in the AFOLIA group, compared to 10.8 in the Gonal-F group. The treatment difference was 0.52 with a 95 % CI of −0.81 to 1.79. The predefined equivalence margin was met. FIN3001 was an assessor-blinded, multicenter, phase III study, including a total of 410 patients in a 2:1 randomization scheme in favor of Afolia. The treatment effect and the safety profile of Afolia in controlled ovarian stimulation were compared to the widely used reference medicine, Gonal-F. Secondary endpoints included the number of days treated with FSH, the total dose of FSH received, the quality of oocytes retrieved, the quality of embryos transferred, and other important clinical parameters for ART. The results from the secondary endpoints were also similar in both treatment groups [13].

Recombinant hCG (r-hCG) had been initially manufactured by transfecting non-human cell lines (Chinese hamster ovary cells) with genetic material capable of replicating identical amino acid sequences to the human compound and developed as a pharmaceutical product named Ovidrel® (Merck Serono, Switzerland). Today, you have biosimilar molecules available in India (Triggerix®, Lupin Pharma, India).

Human chorionic gonadotropin is a therapeutic protein used for ovulation induction in women with infertility. Dong-A Pharm. Co. has developed r-hCG [product code DA-3803], produced in Chinese hamster ovary cells, and evaluated its biologic properties, such as biologic potency, efficacy, and pharmacokinetic profile, compared with a reference product, Ovidrel® [14]. The purpose of a recent study was to evaluate the efficiency of the purification process of Dong-A rhCG (DA-3803) and its bioequivalence from a biosimilar perspective. To confirm bioequivalence, the in vivo/in vitro biologic potency, ovulation induction rate, and pharmacokinetic profile of DA-3803 were compared with those of Ovidrel®. DA-3803 showed equivalent potency with Ovidrel®, and similarity between DA-3803 and Ovidrel® was observed in an efficacy evaluation that measured ovulation induction [14].

Conclusion

A similar biological or "biosimilar" medicine is a biological medicine that is similar to another biological medicine that has already been authorized for use. Biological medicines are medicines that are made by or derived from a biological source, such as a bacterium or yeast. They can consist of relatively small molecules, such as human insulin or erythropoietin, or complex molecules such as monoclonal antibodies. Owing to affordability and easy accessibility, biosimilars have established a good reputation among healthcare professionals. Though biosimilars are gaining popularity in national and international markets, it is important to remember that the biosimilars are not biological generics. These are rather unique molecules which are supported by only limited clinical data at the time of approval [15]. Therefore, there are concerns regarding their efficacy, long-term safety, and immunogenicity. At present, India is one of the leading contributors in the world biosimilar market. India has demonstrated the greatest acceptance of biosimilars, which is reflected from over 50 biopharmaceutical brands getting marketing approval [16]. The Indian biotechnology industry is also gaining momentum, with revenues of over US $2.0 billion reported in 2006, 70 % of which were biopharmaceuticals [17, 18]. According to a report, published in May 2014, by Visiongain, a business information publisher and consultancy in London, the world market drug revenues for biosimilars and related follow-on biologics will reach $9.2bn in 2018, and multiply in size to 2024, the fastest growth expected to be experienced within the submarkets for biosimilar monoclonal antibodies (mAbs) and insulins [19].

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Agonists versus Antagonists in COH

5

Aygul Demirol and Suleyman Guven

Abstract

The gonadotropin-releasing hormone (GnRH) analogs suppress the pituitary follicle-stimulating hormone (FSH) and luteinizing hormone (LH) secretion and enable the control of ovarian folliculogenesis to yield high pregnancy rates in IVF/ICSI cycles. The GnRH analogs have many advantages such as high potency, increased half life, and increased binding capacity to pituitary GnRH receptors, compared with the GnRH molecule. The two types of GnRH analogs in clinical practice are the GnRH agonist and GnRH antagonist. The efficacy of these two GnRH analogs is still under debate. Recent studies have failed to reveal the superiority of one molecule over another. In normo-responders, the implantation rate, clinical pregnancy rate, and miscarriage rates were similar in the GnRH antagonist regimens as well in the GnRH agonist long protocol. However, a significantly higher number of oocytes and higher proportion of mature MII oocytes was retrieved per patient randomized in the GnRH agonist group compared to the GnRH antagonist group. In poor responders, the duration of stimulation with the GnRH antagonist was smaller than the GnRH agonist cycle, although there was no statistical difference in the number of oocytes retrieved, the number of mature oocytes retrieved, the cycle cancellation rate, and clinical pregnancy rate between the GnRH antagonist and GnRH agonist protocols.

Keywords

GnRH analog • GnRH antagonist • GnRH agonist • IVF • Controlled ovarian stimulation

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Abbreviations

COH	Controlled ovarian hyperstimulation
IVF	In vitro fertilization
ICSI	Intracytoplasmic sperm injection
GnRH	Gonadotropin releasing hormone
GnRH	a Gonadotropin releasing hormone agonist
GnRH	ant Gonadotropin releasing hormone antagonist
FSH	Follicle stimulating hormone
LH	Luteinizing hormone
OC	Oral contraceptive
P	Progesterone
OHSS	Ovarian hyperstimulation syndrome

Introduction

In the early twentieth century, the function of gonadotropin-releasing hormone (GnRH) was first elucidated. Researchers discovered that any lesions involving the anterior pituitary may lead to genital atrophy. This fact gives us to understand the hypopthalamo-pituitary gonadal axis [1]. The GnRH decapeptide and its amino acid sequence was discovered in 1971 [2]. Modifications at amino acid positions 6 and 10 gave rise to GnRH analogs. The GnRH analogs have many advantages, such as high potency, increased half life, and increased binding capacity to pituitary GnRH receptors, compared with the GnRH molecule [1].

Two types of GnRH analogs, GnRH agonists and antagonists, have been discovered (Table 5.1). These peptides that mimic the action of GnRH are of potential clinical interest because they can be used to suppress gonadotropin secretion and subsequent sex steroid production [3]. The main function of these molecules is to prevent the endogenous LH surge in controlled ovarian hyperstimulation. By this function, the cycle cancellation rate is decreased and the in vitro fertilization (IVF) cycle outcomes improved. Furthermore, these molecules give clinicians the flexibility to schedule the oocyte retrieval time [1].

Table 5.1 GnRH analogs

GnRH agonists	GnRH antagonists
Triptorelin	Nal-Glu-GnRH
Leuprolide	Antide
Buserelin	Azaline B
Goserelin	Cetrorelix
	Ganirelix

Derived from reference Ortmann et al. [3]

GnRH Agonists

The amino- and the carboxy-terminal sequences of the native GnRH molecule are critically important for binding to the receptor, whereas the amino-terminal domain plays a critical role in receptor activation. Several GnRH agonists have been synthesized: they are characterized by changing the amino acid on position 6. This new property gives new advantages to the resulting molecule. The agonist analogs become more resistant to enzymatic degradation and possess higher affinity to the GnRH receptor [3].

The GnRH agonist initially stimulates pituitary secretion (flare effect), subsequently inhibits its pituitary gonadotropin secretion due to reduction of GnRH receptors on the cell membrane of the gonadotropic cell (downregulation). As the receptors remain bound to the agonist for a while, the resumption of pituitary secretion usually begins 2 weeks after interruption of treatment. Full restoration of ovarian function takes place in more than 6 weeks [4].

GnRH agonists induce profound suppression of endogenous release of gonadotropins during the early follicular phase, allowing the early antral follicles to grow synchronously in response to exogenous gonadotropins to accomplish simultaneous maturation. This leads to an extended widening of the FSH window, an increased number of recruited mature follicles, and a higher number of retrieved oocytes [5].

There are two most commonly used GnRH agonist protocols. The first one is long agonist downregulation GnRH agonist protocol (Fig. 5.1). In this protocol, GnRH agonist is given in the mid-luteal phase of previous cycle. The

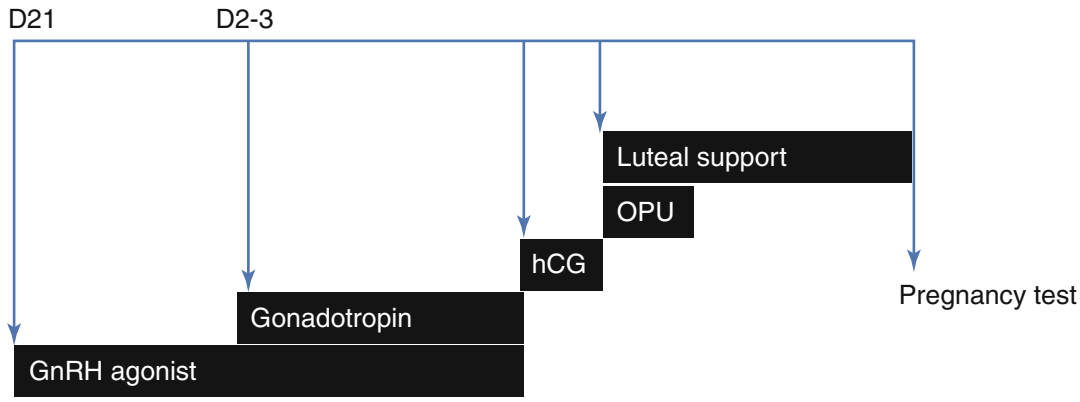

Fig. 5.1 GnRH agonist downregulation protocol

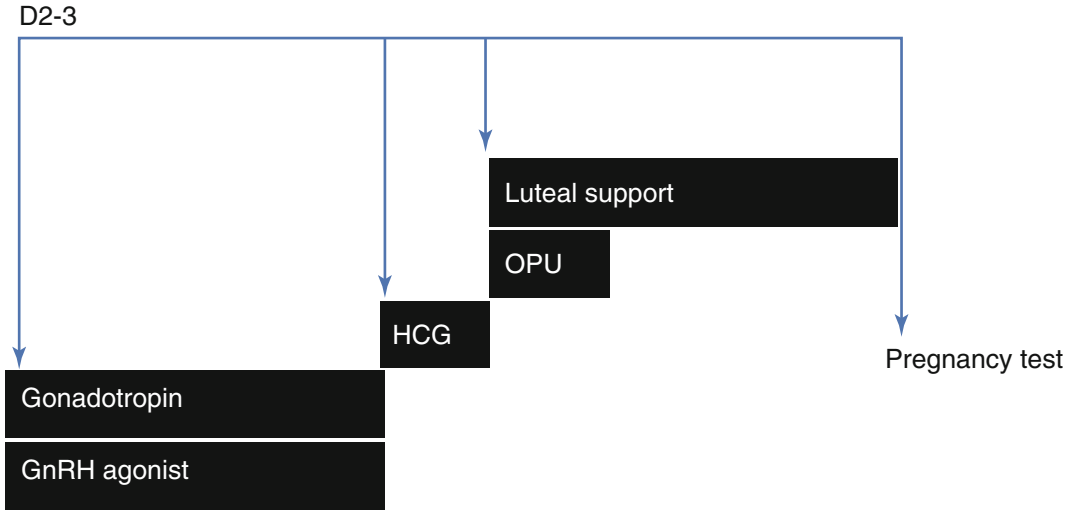

Fig. 5.2 Short flare GnRH agonist protocol

second one is the short flare agonist downregulation protocol, where the GnRH agonist is given on day 2 of menstruation (Fig. 5.2) [1].

In the long protocol, a GnRH agonist depot preparation or daily injections are initiated during the mid-luteal phase of the preceding cycle. Today, this protocol, which aims at complete desensitization of the pituitary gland before starting stimulatory therapy, is still the most used worldwide for assisted reproductive techniques. Despite its effectiveness in preventing the premature LH surge and allowing strict cycle control, it has some disadvantages such as the following.

(a) Duration: treatment cycle at least 14 days longer than the normal menstrual cycle.
(b) Administration of the agonist in the presence of a possible early pregnancy.
(c) Cyst formation: the flare-up effect might interfere with ovarian function.
(d) Hormonal withdrawal symptoms.
(e) Gonadotropin use: more gonadotropins are used as compared with cycles without the use of GnRH agonists.
(f) Ovulation induction only possible by human chorionic gonadotropin (hCG) or LH, but not by GnRH due to desensitization of the pituitary gland.

(g) Need of luteal phase support due to desensitization of the pituitary gland.
(h) Increased incidence of moderate and severe OHSS as compared with cycles without the use of GnRH agonists.
(i) Disturbance to subsequent menstrual cycles due to prolonged pituitary suppression after desensitization [6].

The GnRH agonist protocol may result in stable and low LH and progesterone (P) levels throughout the stimulation phase and may also cause suppression of endogenous FSH levels, leading to a follicular cohort of all small follicles at the initiation of FSH stimulation resulting in a synchronized follicular development. The advantages of this protocol are increased number of oocytes collected, additional pregnancy chances from cryopreserved embryos, and improvement in patient scheduling [7].

GnRH Antagonists

Antagonistic analogs of GnRH are derived from multiple amino acid substitutions at positions 1, 2, 3, 6, 8, and 10 in the decapeptide. These compounds have to be administered at high doses to result in the counteraction of endogenous GnRH activity. The first generation of GnRH analogs may cause anaphylactic reactions, due to stimulation of histamine release. The new generation compounds – Ganirelix and Cetrorelix – are free of such side effects. These analogs suppress gonadotropin (FSH, LH) secretion immediately and the levels of sex steroids decline. GnRH antagonists do not induce an initial increase of gonadotropins. Apart from their action to compete with GnRH for receptors on gonadotroph cell membranes, recent data indicate that prolonged treatment with GnRH antagonists leads to down-regulation of GnRH receptors. GnRH antagonists act mainly through competition with native GnRH for the specific membrane receptors [3].

Three GnRH antagonist protocols have been described:

(a) Fixed day 6 protocol: 0.25 mg GnRH antagonist/daily until hCG administration.
(b) Single dose protocol: 3 mg GnRH antagonist on day 7 of stimulation.
(c) Flexible dose protocol: 0.25 mg GnRH antagonist when follicles reach >14 mm [7] (Fig. 5.3).

One of the most promising aspects of introducing GnRH antagonists into ovarian stimulation is

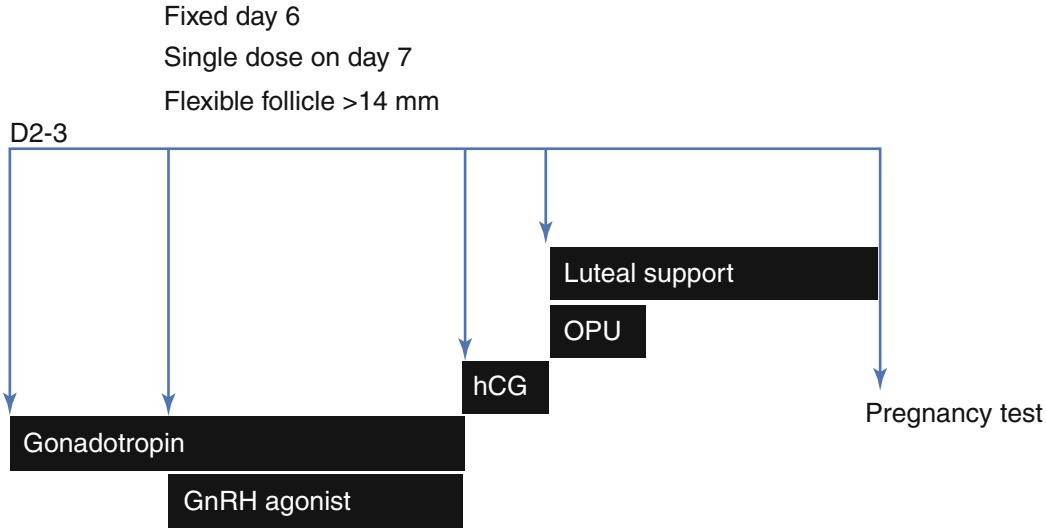

Fig. 5.3 GnRH antagonist protocol

the theoretical possibility of making this treatment less aggressive and much "softer" than an agonistic long protocol.

Based on review results of Felberbaum et al. [6], antagonist protocols have clear advantages, such as a shorter stimulation period, no sex steroid withdrawal symptoms, a lower rate of OHSS, and the possibility of finding more comfortable ways of ovarian stimulation. The pharmacological mode of action of GnRH antagonists is more physiological than that of GnRH agonists. GnRH antagonists allow immediate suppression of gonadotropins while preserving pituitary responsiveness to endogenous GnRH, which means enormous flexibility within therapeutic options. The GnRH antagonists may be given only when needed. The same publication suggested using GnRH antagonist protocol in selected patients, especially in poor responders [6].

In 2013, Depalo et al. [7] reported that the GnRH antagonist regimen is effective in preventing a premature rise of LH and therefore, results in a shorter and more cost-effective ovarian stimulation protocol compared to the long agonist protocol [7]. However, there is a difference in the synchronization of follicular recruitment and growth in the GnRH agonist and GnRH antagonist regimens, with better follicular growth and oocyte maturation seen with GnRH antagonist treatment [7, 8]. The main advantages of antagonist protocols are that it gives immediate, reversible suppression of gonadotropin secretion, which avoids the adverse effects related to the initial flare up and subsequent downregulation. The clinicians may initiate the IVF treatment in a normal menstrual cycle. Antagonist protocols result in endogenous inter-cycle FSH rise rather than FSH suppression, thus resulting in a significant reduction in the effective dosage and shorter treatment, than with the GnRH agonist. The main disadvantages of antagonist protocols are that it may cause high inter-cycle endogenous FSH concentrations, inducing secondary follicle recruitment and leading to asynchronous follicular development [7].

GnRH Antagonist versus GnRH Agonist Protocol in Good Responders

In a recent retrospective study, the outcome parameters of the patients anticipated to have a good response to stimulation, based upon baseline characteristics using either a GnRH agonist or antagonist protocol in their first IVF cycle were compared. The authors clearly reported that clinical pregnancy (43.6 % vs. 48.6 %) and live birth rates (34.9 % vs. 40.1 %) were similar in good responders utilizing either a GnRH agonist or antagonist during their first cycle of IVF [9].

In another prospective randomized study, the cycle outcomes of oral contraceptive (OC) pill pretreatment in recombinant FSH/GnRH-antagonist versus recombinant FSH/GnRH-agonist stimulation in IVF patients were reported. In both the protocols the patients had similar number of two pronuclei (2PN) oocytes, cryopreserved embryos, embryos transferred, implantation, and pregnancy rates [10].

The positive effect on reduction of OHSS rates in antagonist protocols was elucidated in a recent meta-analysis [11]. Based on 45 randomized controlled trials (RCTs) study results, the authors failed to reveal any statistically significant results in terms of ongoing pregnancy rates and live birth rates. However, the authors clearly reported that the use of antagonist compared with long GnRH agonist protocols was associated with a large reduction in OHSS [11].

The study questions whether a GnRH agonist and a GnRH antagonist protocol for the same patient undergoing IVF have different cycle outcomes was answered in a recent study [12]. In this retrospective study, the implantation rate and clinical pregnancy rate were significantly higher in the antagonist protocol (15.82 % and 30.26 %, respectively) than in the agonist protocol (5.26 % and 10.64 %, respectively). It was concluded that the GnRH antagonist protocol probably improved the outcome of pregnancy of older patients with a history of multiple failure of IVF-ET [12].

Recent meta-analysis summarized the study findings of RCTs [7, 8]. Based on these study

results, the implantation rate, clinical pregnancy rate, and miscarriage rates were similar in the GnRH antagonist as well the GnRH agonist long protocol. However, a significantly higher number of oocytes and a higher proportion of mature MII oocytes were retrieved per patient randomized, in the GnRH agonist group compared to the GnRH antagonist group. Moreover, a significant relationship was observed between the patient's age and the number of oocytes retrieved in the antagonist group, meaning that the GnRH antagonist allows a more natural recruitment of follicles in the follicular phase in an ovary that has not been suppressed, whereas a better synchronization of the follicular cohort is observed with the agonist treatment [7, 8].

GnRH Antagonist versus GnRH Agonist Protocol in Poor Responders

Mohamed et al. [13] compared the agonist flare-up and antagonist protocols in the management of poor responders to the standard long down-regulation protocol in a retrospective study [13]. They found both the flare-up and the antagonist protocols significantly improved the ovarian response of known poor responders. However, a significantly higher cycle cancellation rate and less patients having embryo transfer in the antagonist group tipped the balance in favor of the flare-up protocol [13]. Another recent retrospective study compared the efficacy of four different protocols including GnRH agonist (long, short and mini-flare), and GnRH antagonist on pregnancy outcomes in poor responders. They suggested that the application of four different protocols in poor responder patients seem to have similar efficacy in improving clinical outcomes such as implantation, pregnancy and cancellation rates [14].

The first published report of a prospective, RCT, comparing a fixed, multi-dose GnRH antagonist protocol with a long GnRH agonist protocol in poor responders undergoing IVF concluded that a protocol including a GnRH antagonist appears at least as effective as one using a GnRH agonist in patients who are poor responders to a long agonist protocol, and may be easier or more convenient to administer. Both the protocols have similar implantation and pregnancy rates [15].

Another randomized prospective study focused on the advantageous affect of antagonist protocols on embryologic data. In this randomized prospective study, the authors found that the flare-up protocol appears to be more effective than the GnRH antagonist protocol in terms of mature oocytes retrieved, fertilization rate, and top quality embryos transferred in poor-responder patients. However, they also reported similar findings in both protocols in terms of implantation and pregnancy rates [16]. We also published similar study results in 2009 [17]. We clearly reported in our randomized prospective study that the microdose flare-up protocol seems to have a better outcome in poor responder patients, with a significantly higher mean number of mature oocytes retrieved and higher implantation rate [17].

Kahraman et al. [18] also compared the efficacy of the microdose GnRH agonist flare-up and multiple dose GnRH antagonist protocols in patients who had a poor response to a long luteal GnRH agonist protocol in a prospective randomized study. The authors concluded that the microdose GnRH agonist flare-up protocol and multiple dose GnRH antagonist protocol seem to have similar efficacy in improving treatment outcomes in poor responder patients [18].

A recent randomized prospective study compared the efficacy of GnRH antagonist protocol with GnRH agonist protocol in poor responders in 364 women. They concluded that long GnRH agonist and fixed GnRH antagonist protocols have comparable pregnancy rates per transfer (42 % vs. 33 %, respectively). The higher cancellation rate (22 % vs. 15 %, respectively) observed in the antagonist group suggests the long GnRH agonist protocol as the first choice for ovarian stimulation in these patients [19].

Recently, in view of the discrepancies about the potential advantages of GnRH antagonist ovarian stimulation protocols compared with the GnRH agonist protocols in poor ovarian respond-

ers undergoing IVF/ICSI, a meta-analysis of the published data was performed to compare the efficacy of GnRH antagonist versus GnRH agonist protocols for ovarian stimulation in IVF poor responders. A meta-analysis involving 566 IVF patients in the GnRH antagonist protocol group and 561 patients in the GnRH agonist protocol group was performed. The main conclusion of this study was a clear advantage was gained in the duration of stimulation with GnRH antagonist in poor ovarian responders undergoing IVF, although there were no statistical differences in the number of oocytes retrieved, the number of mature oocytes retrieved, the cycle cancellation rate, and clinical pregnancy rates between the GnRH antagonist and GnRH agonist protocols. However, the authors also addressed the fact that further controlled randomized prospective studies with larger sample sizes are needed [20].

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Long-acting Gonadotropins and Route of Administration

6

Mausumi Das and Hananel E.G. Holzer

Abstract

Advances in recombinant DNA technologies have led to the development of longer-acting preparations with prolonged follicle-stimulating bioactivity. Corifollitropin alfa is a synthetic recombinant follicle-stimulating hormone (r-FSH) molecule containing a hybrid beta subunit, which provides prolonged follicle-stimulating activity while maintaining its pharmacodynamic activity. In controlled ovarian stimulation, long-acting gonadotropins have the ability to initiate and sustain multifollicular growth for 7 days. Current evidence suggests that the use of a medium dose of long-acting FSH is a safe treatment option and equally effective compared to daily FSH. This simplified treatment approach may provide a more patient-friendly approach to controlled ovarian stimulation. Further research is needed to determine whether long-acting FSH is safe and efficacious in patients at risk of ovarian hyperstimulation or poor responders. Studies are also needed to assess patient satisfaction and overall patient experience with the long-acting FSH preparations. Novel drug delivery systems developments will ultimately lead to greater ease of administration, more simplified and convenient dosing regimens and superior safety and efficacy, ultimately leading to greater patient satisfaction and improved patient experience.

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Introduction

Since the advent of in vitro fertilization (IVF), gonadotropins have been used to stimulate multiple follicle development [1]. This overcomes the physiologic selection of a single dominant follicle by increasing the duration during which serum follicle-stimulating hormone (FSH) levels remain above the threshold required for follicular recruitment and maturation [2, 3]. The presence of several mature oocytes for IVF and intracytoplasmic sperm injection (ICSI) procedures improves the chances of obtaining good-quality embryos and thereby, a successful pregnancy [4].

Several advances have taken place in the use of gonadotropins, beginning from the initial attempts at their extraction from animals, human cadavers and human urine to the production of recombinant products from Chinese hamster ovary (CHO) cells. These developments, especially the introduction of recombinant gonadotropins, have led to improved ovarian stimulation protocols by improving their efficacy and ease of administration.

At present, treatment regimens for IVF usually involve daily injections of FSH, either urinary FSH or recombinant FSH (r-FSH) with or without luteinizing hormone (LH) injections. Premature ovulation due to an LH surge is usually prevented by gonadotropin-releasing hormone (GnRH) agonists or GnRH antagonists.

Due to the relatively short half-life and rapid metabolic clearance of current FSH preparations, daily injections are required to maintain steady levels of FSH above the threshold during ovarian stimulation for follicular development [5]. Daily injections may increase discomfort and stress. It has been shown that as many as 40 % of non-pregnant couples withdraw after just one cycle of IVF due to emotional distress [6]. This has led to the search for more patient-friendly treatment protocols, which have the advantage of fewer errors during administration and improved patient compliance. Fewer injections may decrease the emotional stress associated with IVF. Simpler and more convenient treatment options may therefore, improve the overall patient experience.

Several studies have explored whether intermittent administration of r-FSH injections, by increasing the loading dose, could produce outcomes similar to that of daily FSH injections [7]. Alternatively, the development of FSH preparations with a longer half-life and a slower absorption to peak serum levels may be more helpful in obtaining a longer injection-free period than increasing the loading dose of current FSH preparations [8]. Besides improved patient compliance, long-acting compounds may result in more stable serum levels compared with repeated dosing using short-acting preparations.

In this chapter, we have discussed the latest advances in recombinant DNA technologies that have led to the development of longer-acting preparations with FSH bioactivity, including the production of Corifollitropin alfa, a new hybrid molecule with prolonged follicle-stimulating activity.

Gonadotropin Structure and Function

Follicle-stimulating hormone belongs to the glycoprotein hormone family, which also includes LH, human chorionic gonadotropin (hCG) and thyroid-stimulating hormone (TSH). The glycoprotein hormones are cysteine-rich dimeric proteins made up of two non-identical, non-covalently linked α- and β-subunits. The α-subunit is common to all family members, whereas the β-subunit is unique to each hormone and confers its bio-

logical and immunological specificity. Each of the two subunits of FSH has oligosaccharides that contain sites for the addition of terminal sialic acid residues [9]. FSH heterogeneity is due to the content of these sialic acid residues. Individual FSH isoforms differ in their extent of post-translational modification. Post-translational modification of the primary protein structure results in differential glycosylation, which in turn produces molecules with different isoelectric properties and bioactivity [9]. An increased sialic acid content produces more acidic isoforms with longer half-lives in vivo. Heavily sialylated FSH therefore, circulates for longer periods of time compared with more basic forms. FSH heterogeneity influences the amount of time that FSH is able to circulate, thereby regulating its in vivo bioactivity. The less acidic isoform was found to have a faster clearance from the circulation in rats as compared with the acidic isoform. The carbohydrate moieties on the FSH molecule play a role in regulating correct protein assembly and secretion of the gonadotropins and signal transduction [10].

Apart from hCG, the human glycoprotein hormones have relatively short terminal half-lives in vivo [11]. Although there is substantial amino acid sequence homology between hCG and LH, hCG has a much longer plasma half-life compared with LH [12]. The main difference between them is the presence of an additional 31 amino acids that form the C-terminal peptide (CTP) of the hCG β-subunit. Deletion of the CTP resulted in decreased in vivo activity of the hCG molecule compared with the wild type in a rat ovulation assay [13].

Development of Long-Acting FSH Compounds

Several techniques of developing longer-acting FSH molecules with increased half-life have been described. It has been suggested that altering the structure of the FSH molecule by additional glycosylation would increase the plasma half-life of FSH by reducing the glomerular filtration. However, there is a maximum plasma half-life beyond which further increases cannot be achieved by additional glycosylation [14].

Longer-acting FSH molecules have also been developed by introducing additional sequences containing glycosylation sites at the N-terminus of the FSH α-subunit [15] or by creating a contiguous, single-chain, covalently bound fusion protein containing the common α- and FSH β-subunits separated by the hCG β-CTP [16].

Using gene transfer techniques, Boime and co-workers [17] constructed a chimeric gene containing the sequence encoding the CTP of the hCG β-subunit fused to the translated sequence of the human FSH β-subunit [17]. The FSH β-CTP chimera was then transfected with the common glycoprotein α-subunit and expressed in Chinese hamster ovary (CHO) cells. This chimeric recombinant molecule was found to have similar in vitro receptor binding and steroidogenic activity compared with wild-type FSH but significantly increased in vivo activity and plasma half-life [17]. A single injection of this chimeric molecule could stimulate follicular maturation in rats enough to induce ovulation induction 52 h later. In contrast, a single injection of the same dose of wild-type FSH was not able to achieve the same effect. The production of a new CHO cell line, expressing the FSH hybrid molecule, has led to the development of Corifollitropin alfa, which has increased in vivo FSH bioactivity.

Other approaches to sustained-release drug delivery systems for long-acting recombinant human FSH that are currently being developed include encapsulation of the drug into small polymeric microspheres, which degrade slowly, releasing the drug at a controlled rate [18].

Corifollitropin Alfa: A Long-acting Recombinant FSH Compound

Recombinant DNA technologies have led to the development of a new recombinant molecule, which consists of the α-subunit of human FSH and a hybrid subunit consisting of the carboxyl-terminal peptide of the β-subunit of hCG, coupled with the FSH β-subunit. This design was a result of the observation that CTP is the main

distinguishing feature between LH and hCG and is most likely, responsible for the extended plasma half-life of hCG as compared to LH [18]. This recombinant molecule is a long-acting FSH compound, named Corifollitropin alfa or FSH-CTP [8]. Similar to the wild-type FSH, Corifollitropin alfa interacts only with the FSH receptor and lacks LH activity [19]. However, Corifollitropin alfa has a longer plasma half-life and an extended time interval to reach peak serum levels [20]. In controlled ovarian stimulation, a single subcutaneous dose of Corifollitropin alfa has been shown to have the ability to initiate and sustain multifollicular growth for 7 days. Subsequently, controlled ovarian stimulation for follicular development may be continued with daily FSH injections until the criteria for final oocyte maturation have been reached. In order to improve treatment simplicity, Corifollitropin alfa has been developed in combination with GnRH antagonist co-treatment.

Pharmacokinetics

Exposure after injection of Corifollitropin alfa can be measured most reliably with the help of a specific enzyme immunoassay, which does not cross-react with native or recombinant FSH [21]. Studies have demonstrated that the mean plasma half-life of Corifollitropin alfa is ~65 h for all doses tested between 60 and 240 μg, compared with ~35 h for r-FSH [21]. A single-dose of Corifollitropin alfa is slowly absorbed resulting in peak levels within 2 days after injection. Subsequently, serum Corifollitropin alfa levels decrease steadily, though the FSH activity may remain above the FSH threshold for an entire week in order to initiate and sustain multifollicular growth. The dose of the long-acting FSH compound should be as low as possible to avoid ovarian hyperstimulation syndrome (OHSS) but high enough to support controlled ovarian stimulation and follicular development over the 7-day period.

The efficacy of the long-acting FSH formulation, Corifollitropin alfa, has been tested in multicentre trials. In phase I clinical trials, the recombinant FSH molecule was administered to male hypogonadotrophic hypogonadal volunteers who received four subcutaneous injections of 15 μg Corifollitropin alfa to examine its safety and possible immunogenicity [22]. The plasma *t½* of Corifollitropin alfa in humans was found to be 94.7 ± 26.2 h, approximately two- to threefold longer than the *t½* of r-FSH.

Subsequently, the pharmacokinetics and ovarian response to a single dose of 30–120 μg Corifollitropin alfa were investigated in pituitary-suppressed female volunteers [20]. Twenty-four participants were treated with a high-dose oral contraceptives to suppress pituitary function. Participants were given a single dose of 15, 30, 60, or 120 μg of Corifollitropin alfa. The median time to reach maximal serum concentrations (t_{max}) ranged from 36 h in the 15, 60 and 120 μg groups to 48 h after administration of 30 μg. The calculated elimination half-lives ($t_{1/2}$) ranged from 60 h in the 30 μg group to 75 h in the 120 μg group. Other studies have also suggested that the serum concentration of Corifollitropin alfa is proportional to the dose within the 15–60 μg dose range [23]. Corifollitropin alfa administration also showed an inverse relationship with body-weight, which was found to be a significant covariate of clearance and volume of distribution [24].

Several studies have examined the efficacy of Corifollitropin alfa, in doses ranging 60–240 μg in women undergoing IVF. All these studies used GnRH antagonist to prevent premature LH surges. These studies suggest that the pharmacokinetic parameters of Corifollitropin alfa in IVF patients are similar to those noted in previous studies, with Cmax being reached on day 2 after injection (t_{max} 25–46 h) and plasma $t_{½}$ being approximately 65 h [20, 22].

The long-acting FSH preparations have an approximately two-fold longer elimination half-life and an almost four-fold extended time to peak serum levels as compared with the r-FSH compounds currently used [25]. Due to this pharmacokinetic profile of sustained FSH bioactivity, a single dose of long-acting FSH can maintain the circulating FSH level above the threshold necessary to support multifollicular growth over a 7-day

period [25]. A single injection of long-acting FSH on the first day of the stimulation can replace the first seven daily injections of r-FSH, thereby improving patient compliance.

Safety and Efficacy of the Long-acting FSH Formulation: Corifollitropin alfa

Corifollitropin appears to have a favourable safety profile similar to daily r-FSH injections. Moreover, the Corifollitropin molecule does not seem to be immunogenic. The safety and efficacy of Corifollitropin alfa have been evaluated in several randomized controlled trials (RCTs). In all these RCTs, GnRH antagonist was used to prevent premature LH surges. All the studies included women who were younger than 40 years of age and had regular menstrual cycles. Women with polycystic ovary syndrome (PCOS) and those with a history of over or poor response to gonadotropin stimulation or recurrent implantation failure were excluded.

In the first feasibility study, the efficacy and safety of a single dose of Corifollitropin alfa were investigated in IVF patients undergoing controlled ovarian stimulation with a flexible GnRH antagonist protocol. Participants were randomized to receive a single dose of 120 μg ($n=25$), 180 μg ($n=24$) or 240 μg ($n=25$) Corifollitropin alfa or to start daily fixed doses of 150 IU r-FSH ($n=24$). Subjects who received a single dose of Corifollitropin alfa continued 1 week after injection with fixed daily doses of 150 IU r-FSH until the day of triggering final oocyte maturation. The terminal half-life of Corifollitropin alfa was found to be, on average, 65 h and dose-independent. Headache and nausea were the commonest reported adverse effects. In this study, the authors reported that 12 subjects (17.6 %) in the Corifollitropin alfa groups and two subjects (8.3 %) in the r-FSH group experienced a premature LH rise (defined as LH ≥10 IU/L) before the start of the GnRH antagonist though this did not reach statistical significance. The authors suggested that this relatively high incidence of women demonstrating an early LH rise in the Corifollitropin alfa groups may be related to the higher initial rises of serum estradiol and the use of a flexible GnRH antagonist protocol. The mean number of oocytes recovered per started cycle was higher in the Corifollitropin alfa group compared with r-FSH-treated patients, but no difference could be noted between the number of good quality embryos and equal numbers of embryos were available for embryo transfer.

The second RCT was a dose-finding study, evaluating three different doses of Corifollitropin alfa [24]. A total of 315 women were randomized and received a single injection of 60 μg ($n=78$), 120 μg ($n=77$), or 180 μg Corifollitropin alfa ($n=79$) or daily injections of 150 IU r-FSH ($n=81$) from cycle days 2–3. If patients allocated to the Corifollitropin alfa group needed further stimulation to meet the hCG trigger criteria, they received a fixed dose of 150 IU/day r-FSH from stimulation day 8 onwards. Patients received a GnRH antagonist (Ganirelix 0.25 mg/day) from stimulation day 5 until the day of hCG. The authors reported that the number of cumulus-oocyte complexes retrieved showed a clear dose-response relationship ($P<0.0001$), being 5.2 (5.5), 10.3 (6.3) and 12.5 (8.0) in the three dose groups, respectively. The authors concluded that the optimal dose for a 1-week interval is higher than 60 μg and lower than 180 μg [24].

In a large, double-blind, randomized, non-inferiority trial, involving 1506 patients (ENGAGE 2009), the ongoing pregnancy rates were assessed after a single subcutaneous injection of 150 μg Corifollitropin alfa during the first week of stimulation and compared with daily injections of 200 IU r-FSH using a standard GnRH antagonist protocol. In both treatment groups, the median duration of stimulation was 9 days, implying that patients treated with Corifollitropin alfa needed, on average, 2 days of r-FSH to complete their treatment cycle prior to the hCG trigger. This study reported ongoing pregnancy rates of 38.9 % for the Corifollitropin alfa group and 38.1 % for r-FSH, with an estimated non-significant difference of 0.9 % [95 % confidence interval (CI): −3.9; 5.7] in favour of Corifollitropin alfa. The incidence of (moderate/

severe) ovarian hyperstimulation syndrome was comparable (4.1 and 2.7 %, respectively; $P=0.15$) [25]. It is noteworthy that the incidence of premature LH rise was 7 % versus 2.1 % ($P<0.01$) in the Corifollitropin alfa and r-FSH arms of the ENGAGE trial. However, the pregnancy rates for women with premature LH rises were not significantly different between Corifollitropin alfa (45.3 %) and r-FSH (31.3 %) groups [25].

In another double-blind randomized trial (ENSURE 2010), 396 women weighing 60 kg or less, who underwent controlled ovarian stimulation prior to IVF or ICSI, were randomized in a 2:1 ratio to a single dose of 100 μg Corifollitropin alfa or daily 150 IU r-FSH for the first 7 days of stimulation in a GnRH antagonist protocol. The mean ± SD number of oocytes retrieved per started cycle was 13.3 ± 7.3 for Corifollitropin alfa versus 10.6 ± 5.9 for r-FSH. The incidence of moderate and severe ovarian hyperstimulation syndrome was 3.4 % for Corifollitropin alfa and 1.6 % for r-FSH [26].

In a recent meta-analysis of four randomized controlled multicentre trials, involving 2335 participants, Pouwer and colleagues [27] evaluated the effectiveness of long-acting FSH versus daily FSH on pregnancy and safety outcomes in women undergoing IVF or ICSI treatment cycles. They compared subgroups by the dose of long-acting FSH administered, mainly low dose (60–120 μg), medium dose (150–180 μg) and high dose (240 μg) [27].

The age of the included participants in the four included trials ranged from 18 to 39 years, and the range of body mass index (BMI) was 17–32 kg/m^2. All the studies excluded poor responders, patients with a history of OHSS or PCOS and patients with explained fertility. None of the studies evaluated patient satisfaction. All included studies compared long-acting FSH with daily FSH in combination with a GnRH antagonist protocol. The studies varied in initial dose of long-acting FSH administered: 454 women received a low dose (60–120 μg), 869 women received a medium dose (150–180 μg) and 25 women received a high dose (240 μg). All studies used r-FSH for the control group: three studies used 150 IU r-FSH while the ENGAGE 2009 study used 200 IU r-FSH. ENSURE 2010 and ENGAGE 2009 used a body weight-adjusted dose of long-acting and daily FSH [27].

In this meta-analysis, there was evidence of a reduced live birth rate in women who received lower doses (60–120 μg) of long-acting FSH compared to daily FSH (OR 0.60; 95 % CI 0.40–0.91, 3 RCTs, 645 women, $I^2=0$ %). There was no evidence of effect on live births in the medium-dose subgroup (OR 1.03; 95 % CI 0.84–1.27). There was no evidence of effect on clinical pregnancy rate or ongoing pregnancy rates [27].

Likewise, there was no evidence of a difference in adverse events for rates of OHSS, multiple pregnancy, miscarriage and ectopic pregnancy between long-acting and daily FSH preparations. Additionally, treatment with Corifollitropin alfa did not induce hypersensitivity reactions. The authors concluded that the use of a medium dose of long-acting FSH is a safe treatment option and equally effective compared to daily FSH [27].

The effect of repeated ovarian stimulation with Corifollitropin alfa was assessed in the TRUST trial [28]. Most frequent adverse events reported included procedural pain, headache and pelvic pain. The cumulative ongoing pregnancy rate after three cycles, including frozen-thawed embryo transfer cycles and spontaneous pregnancies, was 61 % (95 % CI: 56–65 %) after censoring for patients who discontinued treatment. No clinically relevant immunogenicity or drug-related hypersensitivity was observed [28].

Conclusion

In controlled ovarian stimulation, long-acting gonadotropins have the ability to initiate and sustain multifollicular growth for 7 days. Current evidence suggests that the use of a medium dose of long-acting FSH is a safe treatment option and equally effective compared to daily FSH. This simplified treatment approach may provide a more patient-friendly approach to controlled ovarian stimulation. Studies seem to suggest that Corifollitropin alfa is an effective treatment option for potential normal responder patients undergoing ovarian stimulation with the GnRH antagonist protocol for

IVF, resulting in an ongoing pregnancy rate comparable to that achieved with daily r-FSH. However, it is noteworthy that there are still no patient satisfaction studies or studies seeking input from healthcare providers, which could help in evaluating whether the long-acting FSH preparations truly decrease stress and improve patient compliance and satisfaction.

Further research is needed to determine whether long-acting FSH is safe and efficacious in patients at risk of ovarian hyperstimulation or in poor responders. There is currently one ongoing trial relating to long-acting FSH in combination with a GnRH agonist protocol. Future trials, involving Corifollitropin alfa, are required to compare the clinical efficacy and safety outcomes using GnRH antagonist co-treatment with those achieved using long GnRH agonist protocols. Studies are also needed to assess patient satisfaction and overall patient experience with the long-acting FSH preparations. Novel drug delivery systems could lead to the development of less invasive methods, more long-acting compounds and various routes of administration that may include transdermal, inhaled or orally active gonadotropins. These developments would ultimately lead to greater ease of administration, more simplified and convenient dosing regimens and superior safety and efficacy, ultimately leading to greater patient satisfaction and improved patient experience.

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Mild Stimulation Cycles versus Controlled Stimulation Cycles: A Japanese Perspective

7

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Abstract

The aim of assisted reproductive technology (ART) is to achieve the best clinical results in a single treatment. Historically, controlled stimulation has been routinely carried out under the concept that the more oocytes utilized, the higher is the success rate. Recently, mild stimulation protocol has gathered attention because of its simplicity and easiness. It consists of 100 mg of Clomiphene plus 1–2 shots of follicle-stimulating hormone (FSH) or human menopausal gonadotropin (hMG), so no gonadotropin-releasing hormone (GnRH) agonist nor GnRH antagonist with 6–8 shots of FSH or hMG are necessary. If the clinical outcome following a mild stimulation is not significantly different from that of the controlled stimulation method, this mild stimulation method might be the first choice for ART patients. Now, the optimal number of collected oocytes can be controlled by choosing an appropriate stimulation method. Using the latest available techniques, we can now develop 10–15 oocytes without significant stress for patients with an individualized stimulation method. Controlled stimulation showed higher success rates compared to those of mild stimulation regardless of the number of ampules of hMG used or the number of oocytes collected in mild stimulation. These differences became more prominent in the group of older patients (over 34 years old and less than 40 years old), which is the age group where ART is most relevant. The main goal for Reproductive Medicine specialists should therefore be to find the best stimulation protocol through individualization to match the particular needs of the patient.

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Introduction

The history of ART is closely related to that of ovarian stimulation.

In vitro fertiilization and embryo transfer (IVF-ET) is now an established method for treating female infertility. The first successful pregnancy resulting from IVF-ET occurred following an unstimulated normal menstrual cycle [1]. However, following the extensive use of ovarian stimulation by exogenous follicle-stimulating hormone (FSH) to obtain more oocytes [2–4], treatment options using the natural cycle have been almost completely abandoned. This has resulted in fewer cancelled cycles and improved pregnancy rates, especially when downregulation with gonadotropin-releasing hormone (GnRH) analogs prior to ovarian stimulation is employed [5].

A long GnRH agonist pituitary suppression regimen, combined with relatively high doses of exogenous FSH, remains the most frequently used stimulation protocol [4, 6]. This method, referred to as the controlled stimulation (CS), promotes better clinical outcomes but is also associated with a high risk of ovarian hyperstimulation syndrome (OHSS) [7–9] and multiple births. On the other hand, the mild stimulation method (MS) is based on the administration of Clomiphene [2, 3, 10] and reduces the risks associated with CS. These two methods are the leading protocols of ovarian stimulation at present, although the mild stimulation method aims to be a safer, more patient-friendly protocol in which the risks of stimulation are minimized [7, 11–16].

The aim of this chapter is to compare the conventional controlled ovarian hyperstimulation (COH) method with the mild stimulation method, from a Japanese perspective. In 2011, Japan reported the largest number of ART cycles performed (161,980) [17] and the largest number of ART institutions (650) [17] in the world. Consequently, the present article has significant global relevance.

We conducted a randomized prospective study at 18 member institutions of JISART (Japanese Institution for Standardizing Assisted Reproductive Technology). A large number of papers have been published in the last 10 years, addressing natural and mild approaches to IVF [18, 19]. Recent studies have addressed the potential advantages of modified natural cycle and mild IVF in the light of current attempts to reduce patient distress, multiple births, and the cost of IVF cycles [18, 19]. It is not so easy to compare these two protocols across different countries due to differences in governmental financial support, in terms of the technical level of embryo cryopreservation [20, 21] and in terms of supporting neonatal intensive care unit systems and ethical issues [22]. This study, therefore, was conducted based only on Japanese data.

Is Mild Stimulation ART Patient-Friendly?

The ISMAAR (International Society for Mild Approaches in Assisted Reproduction) defines mild IVF cycle as the method used when FSH or hMG is administered at lower doses, and/or for a shorter duration in a GnRH antagonist co-treated cycle, or when oral compounds (anti-estrogens, or aromatase inhibitors) are used [23], either alone or in combination with gonadotropins to reduce the number of collected oocytes to between 2 and 7 [24]. In this definition, the kind of stimulation medication used does not matter, whether it is a GnRH agonist or GnRH antagonist [25–27], and the hMG units remains irrelevant as long as the number of collected oocytes is between 2 and 7 [19, 24]. From the patient's

point of view, the term "mild" is commonly associated not with the number of collected oocytes, but rather, with how hard the process of ovarian stimulation will be upon them. Patient stress is derived from the number of hMG injections, the number of developed oocytes, and the method of luteal support. In order to reduce the burden upon patients, it is imperative that we take these factors into consideration. Mild stimulation, performed in Japan, is defined as a low-dose stimulation that is based only on the administration of Clomiphene with 1–2 injections of hMG or FSH. However, in practice, more than 3 injections of hMG/FSH are sometimes used, and a GnRH antagonist [28] is occasionally, also used to control the LH surge. In these cases, there is little observed difference with regular stimulation. We therefore, believe that a more detailed definition of the term "mild stimulation" should be established.

The results of an unpublished questionnaire, collected at Saint Mother Hospital, showed that the main reason for patients dropping out from infertility treatment was the financial burden it causes. The second cause was psychological stress caused by frequent unsuccessful trials, which then resulted in serious financial burden [29, 30]. Judging from these results, what is most needed for ART patients is to reduce the financial burden [31]. Patients want to become pregnant in the least possible number of trials [22, 32–34]. Stimulation medicine, or anesthesia during oocyte pick-up, is not a significant problem for them.

Recently, the burden of ART caused by controlled stimulation, has been reduced thanks to self-injection, the development of techniques to count the number of antral follicles, and measurements of anti-Mullerian hormone (AMH) in advance, which make the prediction of OHSS easier. These advances make the selection of optimal methods of ovarian stimulation, based on these predictive findings, possible [35]. Nowadays, the physical and psychological burden following controlled ovarian stimulation is not likely to be as severe as it was before. Such advances in CS techniques reduce and, in some cases, eliminate the advantages that MS offers [36].

Comparison of Mild and Controlled Ovarian Stimulation Methods in Japan

Eighteen ART institutions conducted a randomized prospective study to compare the clinical results of mild and controlled stimulation in Japan. Patients were divided by age into two groups, younger than 35 years old (referred to as the "younger group") and between 35 and 39 years old (referred to as the "older group"). We analyzed clinical outcomes of mild and controlled stimulation methods from three points of view, as detailed below:

1. A comparison of results between the two groups according to fresh embryo transfer or frozen-thawed embryo transfer (FET).
2. A comparison of clinical results in the mild stimulation group according to the units of hMG used (two or less 150 IU ampules versus 3 or more 150 IU ampules)
3. A comparison of pregnancy rates per cycles versus per transfers.

The main findings of these comparisons are presented in the following tables (Tables 7.1 and 7.2).

In Table 7.1, there were statistically significant differences in the results obtained from each method, and they indicate that CS was more effective than MS in the older group following both of the fresh embryo transfer and the FET. There were no significant differences between CS and MS in the younger group following both the fresh embryo transfer and the FET.

In Table 7.1, the results show that statistically significant differences were found between mild stimulation when two or less ampules were used (MS $\leq$2A) and CS in both age groups after fresh embryo transfer. Statistically significant differences were found between CS and MS in the older group when three ampules or more of hMG (3A hMG) were used after frozen-thawed embryo transfer.

When looking at a pregnancy rate, we need to assess whether it is calculated per cycle or per transfer. Our data showed no significant

Table 7.1 Clinical results in mild stimulation or controlled stimulation

		Method			
		Fresh embryo transfer		Frozen-thawed embryo transfer	
Age		MS	CS	MS	CS
≤34	Pregnancy rates (per cycle)	22.7 % (17/75)	34.9 % (37/106)	55.8 % (24/43)	53.2 % (50/94)
	Miscarriage rates	23.5 % (4/17)	24.3 % (9/37)	25.0 % (6/24)	14.3 % (7/49)
≥35 ~ ≤39	Pregnancy rates (per cycle)	10.3 % (10/97)*	34.2 % (52/152)*	20.4 % (11/54)*	42.7 % (35/82)*
	Miscarriage rates	10.0 % (1/10)	28.8 % (15/52)	27.3 % (3/11)	22.9 % (8/35)

*$P<0.05$

Table 7.2 Clinical results in mild stimulation group according to units of hMG (150 IU × ≤ 2A vs. 150 IU × ≥ 3A) or controlled stimulation

		Method					
		Fresh embryo transfer			Frozen-thawed embryo transfer		
Age		≤2A	≥3A	CS	≤2A	≥3A	CS
≤34	Pregnancy rates (per cycle)	10.0 % (3/30)*	31.1 % (14/45)	34.9 % (37/106)*	45.5 % (5/11)	59.4 % (19/32)	53.2 % (50/94)
	Miscarriage rates	33.3 % (1/3)	21.4 % (3/14)	24.3 % (9/37)	20.0 % (1/5)	26.3 % (5/19)	14.3 % (7/49)
≥35 ~ ≤39	Pregnancy rates (per cycle)	6.3 % (3/48)*	14.3 % (7/49)	34.2 % (52/152)*	20.0 % (2/10)	20.5 % (9/44)*	42.7 % (35/82)*
	Miscarriage rates	0.0 % (0/3)	14.3 % (1/7)	28.8 % (15/52)	0.0 % (0/2)	33.3 % (3/9)	22.9 % (8/35)

*$P<0.05$

differences in per cycle pregnancy rate when compared between mild stimulation and controlled stimulation. However, for ART patients, what is most relevant is not the number of transfers but the number of cycles needed to acquire a successful pregnancy. The pregnancy rate should be presented not per transfer, but per cycle, including the number of cancelled cycles [18, 37]. Data at the clinic, which reported the largest number of cycles in Japan showed that a pregnancy rate of 32.3 % [37, 38], when calculated per transfer, decreased to 16.6 % when calculated per cycle.

Decision Tree Analysis as a Tool to Identify an Optimal Ovarian Stimulation Method

The skill that is most requested from Reproductive Medicine experts is the ability to choose the optimal ovarian stimulation protocol that will lead to the development of an appropriate number of high quality oocytes [10–15]. Each patient has a different background, which strongly influences the clinical outcome. Factors, such as age, height, body weight, AMH level, past chronic diseases, the number of antral follicles, and the measurement of serum estradiol (E2), LH, and FSH levels on the third day of the menstrual period all affect the outcome. Essentially, this means that the ovarian stimulation regimen should be individualized after taking these factors into consideration. The novel application of decision tree analysis [39] to ART treatments can help us identify the optimal stimulation method. Decision tree analysis is commonly used in statistics, data mining, and machine learning and uses a decision tree as a predictive model, which maps observations about an item to conclusions about the item's target value. Such an analysis can be successfully applied to ART treatment decisions (Fig. 7.1).

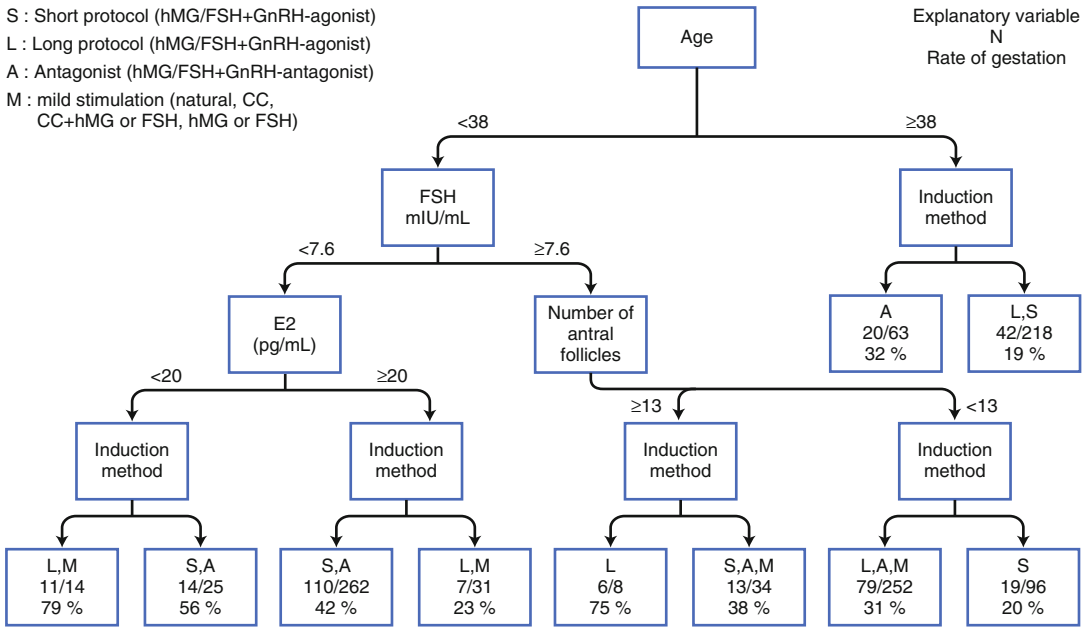

Fig. 7.1 Decision tree analysis for the selection of ART treatment

Discussion

We have investigated mild stimulation and controlled stimulation protocols in Japan. Clinical results for controlled stimulation showed a statistically significant difference for fresh embryo transfers, particularly in the 35–39 years age group, which represents the group with the most treatment cycles. This difference was more prominent when a dose of two or more injections (150 units) of FSH or hMG was used. Data suggests that the difference between the two methods is even greater when cumulative pregnancy rates are compared [40, 41].

Birth rate in Japan has sharply declined and currently sits at a figure of 1.4. This low birth rate has become a major social problem. It is considered that 2 % of all births are now achieved through infertility treatment, and this rate is expected to increase further. Therefore, the performance of ART treatments not only represents a personal issue but is also an important issue for Japan in general.

The selection of the specific ovulation induction method that leads to the best results is the most important task for clinicians. We used the same protocol in 18 facilities and conducted a comparative study between mild stimulation and controlled stimulation protocols. No significant difference was observed in the age group younger than 34 years old. We then divided mild stimulation protocol data into two groups (two or less, and three or more FSH or hMG ampule injections). It was found that mild stimulation, when defined as having two or less injections, was less effective than controlled stimulation.

In order to compare CS and MS objectively, we need to consider pregnancy rates not only per transfer but also per cycle as well as their relative cost performance. This is because patients wish to achieve a pregnancy in the lowest possible number of cycles [33, 42]. The cost performance of MS has been overlooked in Japan due to the fact that government subsidies are the same, regardless of the method used. In countries with more limited government support, the cost-effectiveness of the method is much more relevant.

An early pregnancy reduces the physical, psychological, and financial burden upon the patients [30]. The pregnancy rates for fresh embryo transfer and FETs in Japan (Saito H, 2010) are 21.9 %

and 32.7 %, respectively. FET exhibits a significantly higher pregnancy rate than the other two methods and is expected to become the standard method for ART in the future. As advanced techniques for freezing embryos have been developed, single embryo transfers have been established and the risk of multiple pregnancies has almost disappeared. In addition, the growing follicle number can be predicted more accurately, and the antral follicle number [43], E2 [44], LH, FSH [45], and AMH [46, 47] can be measured before treatment, making individualization of the ovulation induction method to be used (tailor-made treatments) possible. Furthermore, it is not an exaggeration to say that when using the whole embryo freezing method, the possibility of OHSS has almost disappeared. Therefore, instead of limiting our choices and uniformly applying mild stimulation protocols in an attempt to reduce the growing follicle number, it seems clear that thorough analysis of patient background, and choosing the best individualized ovulation induction method, must be the main goal of all Reproductive Medicine specialists.

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Ovarian Stimulation for PCO Patients and Management of OHSS

8

Yoshiharu Morimoto

Abstract

Polycystic ovary syndrome (PCOS) has been implicated as a main endocrine disorder that can cause oligo- or anovulation. PCOS has been studied for a long period and was first described by Stein and Leventhal in 1935.

A consensus between the European Society for Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) defined the diagnosis criteria for PCOS as oligo- and/or anovulation, hyperandrogenism (clinical and/or biochemical), and the appearance of polycystic ovaries on ultrasound. Most PCOS cases can be diagnosed using these criteria; however, there are some variant phenotypes, and the diagnosis is often difficult. Although PCOS has been well studied, an optimal treatment to achieve pregnancy remains unclear. In this chapter, the current status of PCOS and management methods of the disorder are discussed. Furthermore, how to control ovarian hyperstimulation syndrome (OHSS), the most troublesome side effect of PCOS during ovarian stimulation, is discussed.

Keywords

Polycystic ovary • Ovarian stimulation • Ovarian hyperstimulation syndrome • In vitro maturation • Hyperandrogenism • Cabergoline

Polycystic ovary syndrome (PCOS) has been implicated as a main endocrine disorder that can cause oligo- or anovulation. PCOS has been studied for a long period and was first described by Stein and Leventhal in 1935 [1].

A consensus between the European Society for Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) (Table 8.1) defined the

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Table 8.1 The ESHRE/ASRM consensus criteria for PCOS

2 out of 3
1. Oligo- or anovulation
2. Clinical and/or biochemical signs of hyperandrogenism
3. Polycystic ovaries
And exclusion of other etiologies (congenital adrenal hyperplasia, androgen-secreting tumors, Cushing's syndrome)

Revised diagnostic criteria, 2003

diagnosis criteria for PCOS as oligo- and/or anovulation, hyperandrogenism (clinical and/or biochemical), and the appearance of polycystic ovaries on ultrasound. Most PCOS cases can be diagnosed using these criteria; however, there are some variant phenotypes, and the diagnosis is often difficult. Although PCOS has been well studied, an optimal treatment to achieve pregnancy remains unclear. In this chapter, the current status of PCOS and management methods of the disorder are discussed. Furthermore, how to control ovarian hyperstimulation syndrome (OHSS), the most troublesome side effect of PCOS during ovarian stimulation, is discussed.

Physiology of PCOS

The hyperandrogenic status may affect folliculogenesis and oogenesis in PCOS patients. The sources of androgen during the fetal period are the fetal ovary itself and the hyperandrogenic adrenal cortex [2]. Fetal androgen excess in females may cause a heterogeneous PCOS phenotype later in life, because PCOS has been ascribed to genetic origins. Gharani et al. [3] reported a pentanucleotide repeat polymorphism in the *CYP11a* promoter region related to hyperandrogenism in PCOS patients. Insulin resistance frequently occurs in PCOS patients. Androgen exposure in the uterus and later in puberty has been implicated in impaired insulin action and may cause insulin resistance. The development of insulin resistance has been linked to fat distribution and thus, can be improved by weight loss.

Anti-Mullerian Hormone (AMH)

Anti-Mullerian hormone is a member of the transforming growth factor-β (TGF-β) superfamily and is recognized as an excellent parameter for predicting the ovarian reserve. To determine whether AMH could be used as a predictor of ovarian response in PCOS patients. Lie et al. [4] measured AMH and inhibin B concentrations during ovulation induction treatment with recombinant follicle-stimulating hormone (r-FSH) stimulation. However, they concluded that neither of these parameters were suitable to predict the outcome of ovulation induction.

Serum AMH levels are usually elevated in PCOS patients, and it is widely believed that AMH elevation is in response to robust follicle growth. However, AMH overproduction by granulosa cells may also be another cause [5]. Furthermore, AMH production was suppressed by FSH addition to the culture media. Lie et al. [5] indicated that enhanced promoter activity can cause excessive AMH production in PCOS granulosa cells and that, FSH may inhibit excessive AMH secretion by suppressing the luciferase activity of the AMH promoter. Collectively, these findings indicated that AMH elevation was not caused by the increased number of antral follicles but rather by the abnormal secretion of granulosa cells in PCOS patients [5].

Antral Follicle Count (AFC)

Antral follicle count is used to measure small (4–5 mm) follicles with transvaginal sonography and has been recognized as a predictor of ovarian reserve. Recent developments in ultrasonography have enabled the use of ovarian volume as a predictor. However, this methodology is complicated and thus, is not suitable for a routine testing [6].

The effectiveness of AFC is dependent on the measurement technique thus, results may vary during different menstruation phases. Holte et al. [7] indicated that an AFC up to 30 is an indicator of pregnancy and live birth rates in PCOS patients. Interestingly, this report indicated that

AFC predicts not the quantity, but rather the quality of oocytes. It is different from AMH that reflects the number of growing follicles.

Ovarian Stimulation for PCOS (Table 8.2)

The main symptoms of infertility in PCOS patients are anovulation and oligo-ovulation. For ovulatory PCOS patients, the prediction of the day of ovulation is difficult. Therefore, ovulation induction is routinely performed in these patients. Clomiphene citrate (CC) is commonly the first choice of induction; however, it may pose a risk of reduced endometrial receptivity and ovarian hyperstimulation syndrome (OHSS). To avoid this risk, Cyclofenil or aromatization inhibitors, such as Letrozole, can be used as alternative drugs. In medication-resistant cases, gonadotropin administration is sometimes effective, but it may increase the risk of OHSS, that is reportedly, the greatest risk factor in PCOS.

Clomiphene Citrate (CC)

Clomiphene citrate is commonly used for ovarian stimulation in PCOS patients and effectively yields mature follicles. It works by the feedback mechanisms in the hypothalamic and pituitary ovarian axis by occupying estrogen receptors. CC is a first-line stimulator, and the most effective dosage is 100–150 mg/day for 5 days from the third or fifth day of the menstrual cycle. Imani et al. [8] reported that over 75 % of ovulations occur within these dosages, and CC induces ovulation in almost 75–80 % of selected women with PCOS-related infertility [9]. However, CC administration is not recommend for more than 12 months, as the National Institute for Clinical Excellence (NICE) guidelines [10] contraindicate the extended use of CC because of the increased risk of ovarian cancer and decreased possibility of conception.

CC versus Low-Dose FSH

Clomiphene citrate is frequently administered to PCOS patients for ovulation induction because it is inexpensive, easy to give, and simple to administer for patients. However, the pregnancy outcome was better in a group who received FSH stimulation [11]. Thus, CC seems to be disadvantageous in terms of decreased cervical mucus production and has a deleterious effect on endometrial receptivity. Moreover, Homburg [12] reported that CC administration increased the rate of miscarriage and concluded that these detrimental effects occur via physiological features that work by its antiestrogenic actions and by the negative feedback mechanism on FSH secretion. In this study, CC was administered at a starting dosage of 50 mg/day and increased up to 150 mg/day, and 50 IU of recombinant FSH was added with 25 IU increments weekly. The results showed better clinical and cumulative pregnancy rates in the FSH administration group. However, multiple pregnancy rates increased slightly [12].

Table 8.2 Methods of ovarian stimulation and their feature

Method of ovarian stimulation	Feature
Clomiphene citrate	Easy to administer. Thin endometrium. Low implantation
Letrozole	It has an identical effect to Clomiphene citrate in ovarian stimulation but does not make endometrium thin
Selective estrogen receptor modulator (SERM)	Good for failed CC cases. Not well studied
Low-dose FSH	Decrease OHSS. Higher duration of stimulation. Costs a lot
Gonadotropins + GnRH agonist	Possible OHSS. Good clinical outcome
Gonadotropins + GnRH antagonist	GnRH is available as a trigger instead of human chorionic gonadotropin (hCG), which may exacerbate OHSS

Letrozole

Letrozole is an oral non-steroidal aromatase inhibitor and is used as an ovulation induction agent for PCOS patients. It has been used as an alternative to CC. Letrozole produces fewer follicles and reduces the incidence of multiple pregnancies and ovarian hyperstimulation compared with CC. Several different dosages of Letrozole have been used clinically. Rahmani et al. [13] showed that the yield of follicles is dose-dependent from 2.5 mg to 7.5 mg. In addition, they showed that large amounts of the agent increased not only follicle production but also the risk of OHSS. There are meta-analyses comparing efficacy of the induction potential between CC and Letrozole. He and Jiang [14] performed a meta analysis to compare the efficacy of the induction potential between CC and Letrozole and showed that the efficacy of Letrozole was not superior to that of CC but rather, identical as a report of Cochrane data base suggested.

Selective Estrogen Receptor Modulators (SERMs)

In addition to CC, SERMs, such as Tamoxifen, that can reduce the estrogen receptivity have been recently used for ovulation induction. The mechanism of Tamoxifen in improving folliculogenesis may involve direct action on the ovary without intervention of the hypothalamic-pituitary-adrenal axis. Dhaliwal et al. [15] showed that 38.8 % of PCOS patients in a group that failed to achieve pregnancy by CC administration conceived following Tamoxifen administration and achieved a pregnancy rate of 28.5 %. The pregnancy rate was higher in a group administered 80 mg/day than in a group administered 40 mg/day. It was elucidated that Tamoxifen administration did not increase the incidence of ovarian cancer [16]. Raloxifene (marketed as Evista by Eli Lilly and Company, Indianapolis, IN, USA) is an oral SERM that induces ovulation in a manner similar to CC [17].

Gonadotropins Combined with Gonadotropin-releasing Hormone (GnRH) Agonists and Antagonists

Many years have passed since the application of gonadotropins for ovulation induction in PCO patients. However, there is no consensus on an optimal protocol for ovulation induction that can also avoid side effects (mainly OHSS) and enable production of good quality oocytes/embryos. Gonadotropins are used in combination with GnRH agonists or antagonists. Commonly, an ovulation stimulation method for PCOS patients, using a GnRH antagonist is preferable to that using a GnRH agonist. The reason is because if OHSS is expected to occur, the human chorionic gonadotropin (hCG) injection used for triggering ovulation that may induce OHSS can be switched to GnRH agonist administration. Instead of hCG injection, we use 900 μg of a nasal spray of GnRH agonist twice daily.

Abuzeid et al. [18] reported a method to start the GnRH antagonist in the early stage of menstrual cycle for PCO patients. With the use of GnRH antagonists, implantation rates, pregnancy rates, and delivery rates were improved. Thereafter, they tried to start GnRH antagonist from day 1 and day 5, and the early starting group showed better implantation rates. This report suggested these improvements occurred via the suppression of luteinizing hormone (LH) level, which may have deleterious effects on follicular growth and oocyte quality.

Addition of hCG

Human chorionic gonadotropin administration for the final maturation of oocyte is essential; however, hCG may be a strong risk factor for OHSS. On the other hand, trials were conducted to enhance ovarian stimulation with the addition of low-dose hCG during stimulation protocols in PCOS patients [19, 20]. Ashrafi et al. [19] indicated that low-dose hCG combined with recombinant FSH reduced the use of FSH and yielded more mature oocytes. Furthermore, no severe cases of OHSS were reported in the study.

In Vitro Maturation (IVM)

The IVM procedure has been performed in many centers worldwide and mainly applied to PCO patients. The first study of IVM of mammalian oocytes was performed back in 1935 by Pincus and Enzmann [21]. Thereafter, Edwards et al. [22, 23] suggested its clinical application in humans, by obtaining immature oocytes from patients following ovarian stimulation. Veeck et al. [24] achieved the first successful birth from immature oocytes produced during an in vitro fertilization (IVF) program. Thereafter, Cha et al. [25] first reported the use of immature oocytes from unstimulated ovaries of patients in an oocyte donation program. In IVM, the size of aspirated follicle is 5–7 mm, but it is not easy to puncture follicles intravaginally. As the difficulty of the ovum pick-up (OPU) technique is one reason why this procedure cannot be mainstream in assisted reproductive technology, we developed a new needle for OPU in IVM and found that it was effective in easily acquiring many small follicles, even for beginners. The needle was composed of two segments; an inner fine needle to puncture the small follicles and an outer sheath to grasp the ovary because is liable to embed deep into the abdominal cavity during the puncture procedure.

The medium for culturing immature oocytes commonly contains FSH and hCG, based in a balanced salt solution. Nowadays, several media, specially designed for IVM, are commercially available. The issues to be discussed commonly are FSH and hCG priming. hCG priming has been performed in many centers since Chian et al. [26] proposed its significance, but the effectiveness of FSH priming in IVM is not yet recognized. Our group has applied IVM for PCOS patients since 1999. Up to 2010, we have performed the procedure in 1143 cycles and found that although pregnancy and implantation rates were acceptable, IVM was yet not superior to conventional IVF (Table 8.3).

In vitro maturation is the ultimate and only method for preventing OHSS in PCO treatment. Gremeau et al. [27] reported that IVM is a preferable alternative to IVF with ovarian stimulation, and effectively eliminates OHSS, but the live birth rate was significantly lower than with the conventional ovarian stimulation method.

Table 8.3 Clinical outcome of IVM procedure

	Fresh cycle	Frozen cycle	Thawed cycle	Total
No. of period	670	473		1143
No. of oocyte	5770	3532		9302
Ave. no. of oocyte	8.6	7.5		
% Maturation	50.7	52.6		51.7
% Fertilization	81.2	84.3		82.4
% Possible transfer	31.3		30.6	
% ET	60.9		66.1	62.8
No. of pregnancy	111		65	176
% Pregnancy/ET	27.2		24.3	26.1
% Implantation	13.2		11.3	12.5

1999–2010 IVF Namba Clinic and IVF Osaka Clinic

OHSS Management

It is well known and experienced that OHSS is often induced by ovarian stimulation for PCO patients; therefore, prevention of OHSS is the main consideration when performing this procedure. Once OHSS occurs, appropriate measures must be undertaken to save the patient's life.

Ovarian hyperstimulation syndrome is characterized by several symptoms, such as ovarian swelling and accumulation of abdominal, pleural, and pericardiac fluids. Hyperstimulation of the ovaries induces histamine secretion that may enhance vascular endothelial growth factor (VEGF) production by the granulosa cells. VEGF increases the permeability of blood vessels that causes plasma leakage into third spaces. Those symptoms induce secondary severe clinical symptoms such as respiratory disorders and kidney and cardiac failures. If appropriate measures, as shown at Table 8.4, are not taken, patients may face life-threatening conditions such as multiple organ failure (Fig. 8.1).

The incidence of OHSS is reportedly 0.6–1.9 % in severe cases [28]. Severe OHSS cases require hospitalization, and patients suffer from

critical conditions that are sometimes fatal. They have to endure pain caused by the pressure from enormously enlarged ovaries and ascites. Occasionally, patients may be incapable of assuming a supine position because of respiratory distress caused by the accumulation of pleural fluid.

The first choice for OHSS prevention is cryopreservation of all embryos, avoiding embryo transfer. This inhibits the effect of hCG from the placenta. Recently, pregnancy outcomes from cryopreserved and thawed embryos have been remarkably improved by the application of a vitrification procedure, and cryopreservation of all embryos is advantageous for PCOS patients.

The use of GnRH agonist as a trigger instead of hCG is an acceptable option to prevent OHSS. However, it is important to evaluate the impact of GnRH on oocytes and embryos. Acevedo et al. [29] reported no difference in maturation, fertilization, pregnancy, or implantation rates between triggering by hCG and a GnRH agonist.

Martinez et al. [30] reported a unique strategy for using a GnRH antagonist in a long protocol by a GnRH agonist. They withdrew the agonist during stimulation and replaced it with an antag-

Table 8.4 New strategies for prevention severe OHSS

1. Coasting: withholding gonadotropins and deferring the administration of hCG until E_2 levels start dropping
2. Continue to use GnRH agonist after OPU for a week at GnRH agonist protocol
3. Cabergoline administration: Start at the day of hCG administration at the dose of 0.5 mg for 7–8 days

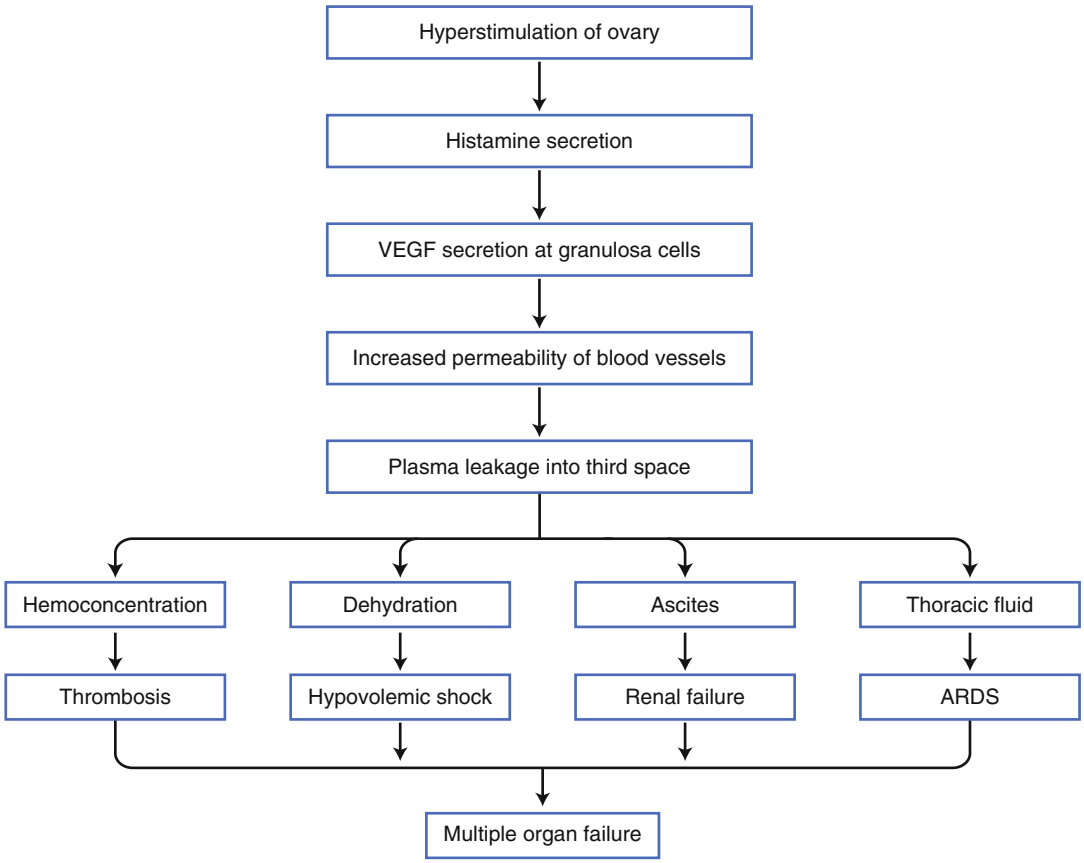

Fig. 8.1 Pathogenesis of OHSS

onist. Consequently, they could use, the agonist for triggering ovulation and thus, prevented OHSS in three cases.

The use of recombinant hCG instead of urinary hCG is one of options to reduce OHSS. However, a review of the Cochrane database [31] found no significant difference in the incidence of OHSS between the two drugs.

Coasting is also an effective method for preventing OHSS during ovarian hyperstimulation. It is a method to withdraw exogenous gonadotropins and withhold hCG until the estrogen titer decreases to a safe level. Ohata et al. [32] described that coasting for 3–6 days remarkably decreased the ovary size, ascites volume, and recovery time. The effectiveness of this procedure has encouraged further clinical application of this method because it is simpler to perform than other methods used for preventing OHSS.

Recently, the administration of Cabergoline (a dopamine receptor-2 agonist) has been employed to prevent OHSS. Cabergoline is commonly used for hyperprolactinemia treatment and can be initiated either from the day of hCG administration or the day of oocyte retrieval [33].

The growth factors and hormones such as insulin- like growth factor (IGF), AMH, inhibin B, and hepatocyte growth factor in follicular fluid decreased when Cabergoline was administered for patients at high risk for OHSS [34]. This is physiological evidence of the effectiveness of Cabergoline in OHSS patients. Esinler et al. [35] compared the effectiveness of Cabergoline with coasting and found no occurrence of OHSS in patients receiving Cabergoline. Thus, they concluded that Cabergoline was more beneficial than coasting.

It is well known that hCG is an exacerbating factor. To avoid hCG usage, GnRH agonist is alternatively used as a trigger for ovulation. Physiologically, OHSS dynamism starts when the FSH-primed ovaries are exposed to hCG, which increases vascular permeability. Thereafter, the vascular VEGF and VEGF receptor 2 (VEGFR-2) express dominantly. The mechanism of effective OHSS prevention can be explained by the fact that dopamine agonists, such as Cabergoline, prevent VEGF overexpression [36].

Conclusions

It is difficult to effectively and safely stimulate the ovaries of PCOS patients while avoiding side effects, especially such as the development of OHSS. It is important to carefully access the ovarian reserve of patients and choose an appropriate stimulation protocol. For the assessment of ovarian function, the use of AMH, FSH, and AFC is advantageous. CC is the first-line stimulation drug for ovaries in PCOS patients. However, newer medications such as Letrozole and SERMs are potential advantageous candidates. In patients at high risk of OHSS development, IVM is an excellent option that can be employed. It is necessary to keep it in mind that OHSS is sometimes fatal. It is essential to avoid the onset of this disorder by any means.

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Ovarian Stimulation for Poor Responders

9

Aisaku Fukuda

Abstract

Infertility specialists have been struggling to deal with poor responders, reported to range from 9 to 24 %. However, the incidence is abruptly increasing lately due to the increasing population of aged infertile women. The long gonadotropin-releasing hormone (GnRH) protocol is the first line of treatment if more than plural numbers of oocytes are expected, which is followed by the short protocol, and the GnRH antagonist stimulation is used as the last treatment option. Among the GnRH agonist protocols, the microdose agonist flare with oral contraceptive (OC) pretreatment appears more effective. Natural cycle or mild stimulation is used when the stimulation protocols fail. IVM is an alternative choice when none of the above-mentioned protocols succeed. Growth hormone (GH) appears to have a beneficial effect, but indications for using GH as well as growth hormone-releasing hormone (GH-RH) are not well defined. In addition, it is too early to determine if androgen pretreatment [dehydrepiandrosterone sulfate (DHEA), testosterone] is beneficial in the treatment of poor responders. It is most important for ART physicians to evaluate the patient response before starting the stimulation and choose the best protocol for the poor responder.

Keywords

DOR (diminished ovarian reserve) • AMH • AFC • Poor responder • Aged patient • IVM-IVF (in vitro maturation in vitro fertilization and embryo transfer)

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Background

The incidence of poor responders to ovarian stimulation has been reported to range from 9 to 24 % [1–3]. The poor response to ovarian stimulation is

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attributed to patients with advanced age and iatrogenic reasons, such as ovarian surgery, pelvic adhesions, and obesity, indicated by body mass index (BMI) [4–8]. The incidence of sporadic poor response to stimulation and primary ovarian insufficiency has been well known for a long time [9, 10]. Recently, there has been increased interest in improving the reproductive capacity of older women because of the changing social structure and the worldwide trend of delaying marriage and childbirth. In the United States, the number of births in women aged between 40 and 44 years has nearly doubled between 1990 and 2002 [11]. The birth rate in women aged 45–49 years is 0.5 births per 1000 women, indicating that it has increased by more than two-folds. However, majority of these births can be attributed to the use of donor oocytes [12]. In the United States, 19 % of all women using assisted reproductive technologies (ART) are ≥40 years in age [12]. In Europe, during 2005, the percentage of women aged ≥40 years undergoing ART, such as conventional in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) cycles, was 15.4 % and 13.0 %, respectively. The number of women delaying childbearing to the fifth decade of their life has markedly increased, and, consequently, 50 % of them will experience some difficulty in their attempt to have children [13]. The age-related decline in fecundity in spontaneous conception and ART success rates has long been known [14–17]. The decline in fertility is mainly due to a decrease in oocyte quality, which is linked to a single chromatid abnormality [18]. There is little evidence that uterine factors have a significant impact on age-related infertility [19]. Although ART with donor oocytes has helped woman in the fifth and sixth decades of their life to achieve a high pregnancy and childbirth rates [20], the procedure is associated with legal, ethical, religious, and cultural problems that have limited its universal accessibility in societies around the world [21, 22]. Extensive efforts to improve pregnancy rates in poor responders have been made using several stimulation protocols, but despite these efforts, pregnancy rates after IVF remain disappointingly low [23, 24]. Currently, the evidence available from both, retrospective and prospective studies is based on the variable definitions of poor ovarian response. The European Society for Human Reproduction and Embryology (ESHRE) consensus group has developed a new definition that may help in selecting a more uniform group of patients for future clinical trials [25]. A systematic appraisal of the available evidence, aiming to draw reliable conclusions is currently lacking. In this chapter, the current situation of stimulation protocols, interventions, and strategy at our facility is outlined.

Bologna Criteria as Defined by ESHRE

At least 2 of the following 3 features must be present to fulfill the Bologna criteria: advanced maternal age (≥40) or any other risk factor for poor ovarian response, previous poor response (≤3 oocytes with a conventional stimulation protocol), and abnormal ovarian reserve test (AFC < 5–7 follicles or AMH < 0.5–1.1 ng/mL). However, the definition of poor responder has not been standardized till date. Several groups have defined poor responders on the basis of variable numbers of mature follicles noted on ultrasound, ranging from <2 to <5. Others base their definition on elevated serum follicle-stimulating hormone (FSH) levels in the early follicular phase, with values ranging from 6.5 to 15 mIU/mL; the use of various maximal estradiol (E2) levels compared with the prior standard controlled ovarian hyperstimulation (COH); a minimal cumulative dose or the number of days of gonadotropin stimulation required in a prior cycle; or on the basis of differing numbers of mature oocytes obtained (≤4 or ≤6).

Which Types of Gonadotropins Are Effective?

Ovarian stimulation using recombinant FSH (r-FSH) is possibly associated with the retrieval of significantly higher numbers of oocytes, greater numbers of embryos, and higher pregnancy rates compared with ovarian stimulation

using urinary FSH (u-FSH) [26]. However, the potential benefit of ovarian stimulation using r-FSH with respect to pregnancy rates in poor responders is unclear [27]. Perhaps, the most logical approach to the management of patients, who fail to respond to a standard gonadotropin stimulation protocol, is to consider increasing the dose of gonadotropins. Although no single maximally effective gonadotropin dose has been defined, there would be little benefit in raising the initial daily dose of FSH to >450 IU/day.

Short or Long GnRH Agonist Protocol

Preference, not protocol efficiency, dictates the selection of a short- or long-agonist protocol for the suppression of a premature luteinizing hormone (LH) surge in women undergoing IVF treatment. The flare-up effect of gonadotropin-releasing hormone (GnRH) agonist on pituitary gonadotropin release is used in the short protocol to enhance initial follicular growth. In contrast, the long protocol results in a more co-ordinated follicular growth. The improvement in clinical pregnancy rate does not appear to be dependent on the type of GnRH agonist protocol applied [28].

GnRH Antagonist or GnRH Agonist Protocol

The use of GnRH antagonists to improve pregnancy rate in poor responders is based on the fact that endogenous gonadotropin secretion is not suppressed during follicular recruitment [29]. A meta-analysis suggests that the type of GnRH analog used to inhibit the LH surge does not appear to be associated with ongoing pregnancy [30]. In contrast, significantly better results were demonstrated with the use of GnRH antagonists with regard to the duration of stimulation, the total dose of gonadotropins required, and the number of cumulus-oocyte complexes retrieved, but further comparative studies may be required to substantiate these results.

Microdose GnRH Agonist Flare Regimen

Several studies have supported the use of a microdose GnRH agonist flare protocol in poor responders, which has demonstrated an improvement in the ovarian responses and clinical outcomes in these cases [31]. This approach takes advantage of the initial release of endogenous gonadotropins, induced by a low-dose GnRH agonist administration during the early follicular phase, and is aimed at enhancing the response to the subsequent administration of exogenous gonadotropins. Moreover, the blastocysts generated from a microdose GnRH agonist flare regimen showed a significantly lower incidence of blastocysts with chromosome aneuploidy [32].

Stimulation Protocols or Natural/ Mild Stimulation Cycles

Natural cycle IVF in poor responders has been proposed as an alternative to standard stimulation protocols. This approach appears to be less invasive and less expensive for poor responders who do not show an increase in oocyte production, with standard ovarian stimulation. However, there is a study suggesting that such a strategy is not beneficial for clinical pregnancy rates [33]. In contrast, Clomiphene citrate (CC) administration in the early follicular phase with r-FSH may improve the outcome of stimulation in poor responders [34]. The use of Letrozole with FSH does not appear to improve the pregnancy rates [35]. Moreover, safety concerns regarding Letrozole administration in assisted reproduction have been noted [36].

Transdermal Testosterone Priming

The addition of androgens during the early follicular phase may have a beneficial effect on the increase in number of small antral follicles and improve the ovarian sensitivity to FSH. Pretreatment with transdermal testosterone

(TT) may improve ovarian sensitivity to FSH and follicular response to gonadotropin treatment in previous IVF patients who were poor responders. This approach leads to an increased follicular response compared with a high-dose gonadotropin and minidose GnRH agonist protocols [37]. Moreover, the numbers of oocytes retrieved, mature oocytes, fertilized oocytes, and good quality embryos were significantly higher in the TT pretreatment group. Embryo implantation rate and clinical pregnancy rate per cycle initiated were also significantly higher in the TT group [38].

Dehydroepiandrosterone (DHEA) Supplementation

Supplementing poor responders with 75 mg of micronized DHEA daily for up to 4 months before the initiation of IVF resulted in significantly higher pregnancy rates. The beneficial effect of DHEA supplementation was suggested [39]; however, the definitive effect of this supplementation is still under discussion.

Growth Hormone (GH) and GH-Releasing Hormone (GH-RH)

The concept of potentiating the effect of exogenous gonadotropins with GH or GH-RH can be used as an alternative approach to improve pregnancy rates instead of changing the type or dose of gonadotropin administration. GH plays an important role in ovarian steroidogenesis and follicular development. Treatment with GH appears to modulate the action of FSH on granulosa cells by upregulating the local synthesis of insulin-like growth factor-I (IGF-I) [40, 41]. Live birth rates are improved when GH is co-administered to poor responders during ovarian stimulation for IVF; however, the clinical significance of this difference may be small. Interestingly, the inclusion of GH resulted in a significant decrease in the total dose of gonadotropins required for ovarian stimulation [42]. GH co-treatment and the optimal dose required in the ovarian stimulation of poor responders need to be evaluated further. An enhanced ovarian response, however, with little impact on conception rates was reported following treatment of poor responders with adjunctive GnRH [43].

Our Strategy for Poor Responders Based on the Treatment Protocols Used in Patients with Advanced Age

We established a new protocol for poor responders on the basis of the concept followed for treating patients >40 years old. Fecundity in female patients declines with age because of a decrease in the number of oocytes in the ovarian reserve and also because of impaired oocyte quality. These findings are similar to those observed in a poor responder. The pregnancy rate following ART is closely related to the number of oocytes/embryos obtained and the quality of the embryos prior to transfer. It is not easy to retrieve multiple oocytes from poor responders. At the same time, to achieve pregnancy, it is imperative to transfer >2 embryos. Therefore, our new strategy for the poor responder is to transfer ≥2 good frozen-thawed embryos, which are accumulated and chosen from several oocyte retrievals (Fig. 9.1). In addition to various stimulation protocols or natural/mild stimulation cycles, we also use in vitro maturation (IVM) to produce embryos [44].

Conclusions

Numerous papers have discussed the strategy for the treatment of poor responders during IVF, but the lack of a uniform definition of the poor responder makes an accurate comparison among results difficult. Conventional stimulation protocols should be applied if >2 follicles are present in each ovary on day 3 of ultrasound assessment. Recombinant FSH is usually more effective than u-FSH; however, in some instances, the addition of u-FSH to r-FSH can encourage follicular development

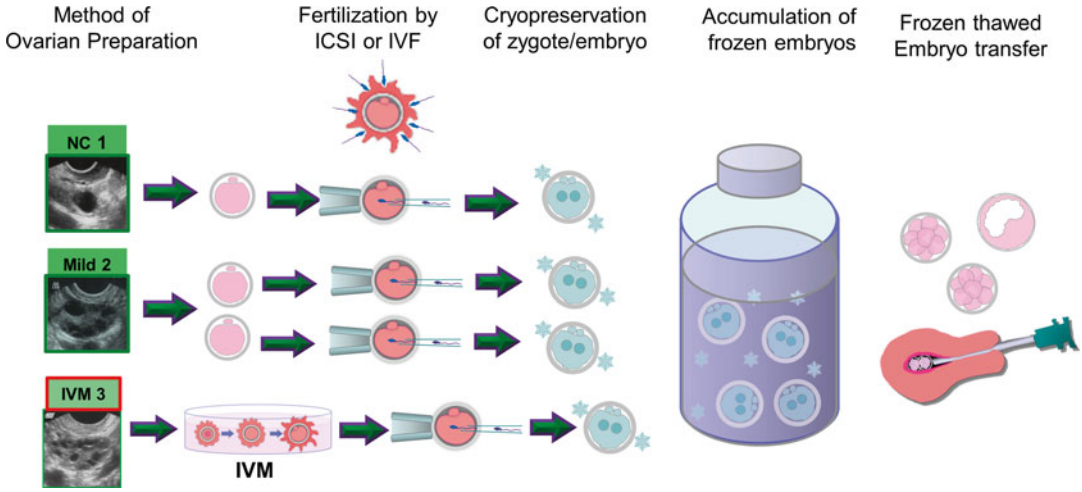

Fig. 9.1 New strategy to improve pregnancy rates for poor responders to IVF in the IVF Japan Group. *NC*: pure natural cycle IVF without any stimulation, *Mild*: mild stimulation IVF with CC and/or hMG, *IVM-IVF:* IVF using in vitro matured oocytes

in poor responders. The long protocol is the first line of treatment, which is followed by the short protocol, and the GnRH antagonist stimulation is used as the last treatment option. Among GnRH agonist protocols, the microdose agonist flare with OC pretreatment appears more effective. However, whether microdose flare protocols are more effective than GnRH antagonist protocols has not been determined till date. Natural cycle or mild stimulation is used when the stimulation protocols fail. IVM is an alternative choice when none of the above-mentioned protocols succeed. GH appears to have a beneficial effect, but indications for using GH as well as GH-RH are not well defined. In addition, it is too early to determine if androgen pretreatment (DHEA, testosterone) is beneficial in the treatment of poor responder cases in ART.

The available number of good quality embryos is the most important factor in achieving a pregnancy using ART. It is very difficult for poor responders to produce more than a few embryos in a single retrieval. Therefore, the accumulation of multiple cryopreserved embryos is key to achieve pregnancy in these patients. A Clomiphene regimen can be considered as an alternative choice for mild ovarian stimulation, as the endometrial condition is not considered if all embryos are frozen. Ovarian stimulation for poor responders is challenging; however, increasing the number of protocol choices may increase the prospects of achieving a pregnancy.

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Depot GnRH Agonists in COH for ART

10

Gautam N. Allahbadia and Rubina Merchant

Abstract

The ability of gonadotropin-releasing hormone (GnRH) agonists (GnRH-a), particularly when used in a long protocol, to fine-tune and conveniently program ovarian stimulation cycles with significant advantages in the prevention of premature luteinization and satisfactory clinical outcomes, has made them a preferred inclusion in stimulation protocols for assisted reproduction. GnRH-a may be administered as short-acting daily low-dose injections or as a single long-acting high-dose injection (depot). A remarkable improvement in clinical pregnancy rates has been reported following downregulation with GnRH-a depot formulation and gonadotropin stimulation compared to gonadotropins alone, particularly in hyperandrogenic patients. The higher duration of gonadotropin stimulation and gonadotropin requirement with the long-acting depot, owing to the suggested profound pituitary suppression, is controversial, and no significant differences in clinical outcomes, the levels of endogenous hormones, or time to pituitary desensitization have been reported in a majority of the studies compared with the short-acting daily preparations. Despite comparable pregnancy outcomes, the use of depot GnRH-a in controlled ovarian hyperstimulation (COH) protocols for assisted reproductive technology (ART) is controversial, with some favoring its use in terms of patient compliance and ease of administration, and results, especially with the reduced dose, while others favoring the short-acting daily GnRH-a in terms of cost-effectiveness, and ovarian response in poor responders. However, the

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GnRH antagonist single-dose protocol has proved to be superior to the depot GnRH-a for COH with advantages of a significantly reduced duration of gonadotropin stimulation and requirement, higher flexibility in treatment, economy, convenience, and safety despite comparable pregnancy outcomes. Poor responders, in contrast, are reported to benefit from a flare-up GnRH-a protocol with a depot formulation compared to the GnRH antagonist, with higher total pregnancy and implantation rates, possibly due to improved oocyte/embryo competence.

Keywords

Depot GnRH agonists • Stimulation protocols • Long-acting depot • Short-acting daily low-dose injections • GnRH antagonists • Controlled ovarian hyperstimulation • Poor responders • Assisted reproductive techniques

Introduction

The introduction of GnRH agonists (GnRH-a) combined with gonadotropins is considered to be one of the most significant events in the development of in vitro fertilization and embryo transfer (IVF-ET) programs [1], with advantages such as prevention of premature ovulation or premature senescence of the oocyte and increased oocyte yield in poor responders [2], increased oocyte quality [3], more supernumerary embryos for cryopreservation [1], convenient programming of oocyte recovery [1, 2], decreased cycle cancellation rates [2], and improved clinical pregnancy rates [1, 2, 4]. The problems, often associated with premature luteinizing hormone (LH) surges and premature luteinization, and thus, cycle cancellations, have efficiently been overcome by "reversible medical hypophysectomy" with GnRH-a, introduced in 1982 [5]. Hence, GnRH agonists are widely used in controlled ovarian hyperstimulation (COH) for assisted reproductive techniques (ART) [6].

The rationale behind the use of GnRH agonists is to downregulate the pituitary, suppressing the release of endogenous gonadotropins and hence, ovarian activity, to facilitate COH with gonadotropins. Following GnRH-a administration, an immediate flare response of pituitary gonadotropin secretion is followed by downregulation and pituitary desensitization akin to medical hypophysectomy. GnRH antagonists, on the other hand, achieve immediate downregulation by blocking the GnRH receptors. Both GnRH agonists and antagonists are used to block the secretion of endogenous gonadotropins in ovarian stimulation programs for assisted reproduction [7].

According to its initiation and duration, GnRH analog use has been divided into three protocols: the long protocol, the short protocol and the and ultra-short protocol. The long protocol is the most widely used protocol, as it has proved to be the best for suppression of high tonic endogenous LH levels, especially in polycystic ovary syndrome (PCOS) and normogonadotropic patients. The short and ultra-short protocols, have mainly been used in poor responders to ovarian stimulation treatment, older or hypergonadotropic patients with ovarian failure, because of the well-known "flare-up phenomenon" [5]. Attempts to use the GnRH-a to stimulate follicle maturation in a "short protocol" have resulted in variable and sometimes, poor results, leading to the development of the long GnRH-a gonadotropin protocol for ovarian stimulation [2]. The long protocol requires GnRHa administration until suppression of ovarian activity occurs, within approximately 14 days [8]. The superiority of the long protocols over the short and ultra-short protocols has been demonstrated in terms of increased clinical and patient compliance, improved efficacy of pituitary downregulation [1], and clinical pregnancy rates [8] with no evidence of an increased risk of pregnancy wastages or teratogenicity in human pregnancies [1].

GnRH-a can be used either as daily low-dose injections or through a single injection containing higher long-acting doses of the drug (depot) [8]. Traditionally, short-acting analogs have been employed because of concerns over long-acting depot preparations causing profound suppression, a longer period of stimulation, and higher doses of gonadotropins compared with short-acting agonists and luteal phase defects, adversely affecting pregnancy and miscarriage rates [1, 3, 4]. Although nasal administration may be suitable for short-term suppression (up to 28 days), it seems likely that long-acting depot preparations will be useful for more prolonged suppression [9]. However, according to a recent meta-analysis, it is unclear which of these two forms of administration is best, and whether single depot administration may require higher doses of gonadotropins [8].

Apart from their use in ART, GnRH analog depots have also been widely used to treat a variety of diseases including prostate cancer, breast cancer, endometriosis, uterine leiomyomas, and central precocious puberty [10]. GnRH analogs are now a well-established means of treating sex-steroid-dependent, benign and malignant disorders [7]. Monthly depot GnRH agonists are the preferred choice of medical treatment for endometriomas before IVF, with an average duration of treatment of 3 months [11]. In this chapter, we shall focus on the use of depot GnRH agonists for COH in ART.

Clinical Discussion

Endocrine Changes following GnRH-a Administration

Long-term pituitary suppression following high-dose GnRH agonist administration [DTrp6GnRH in microcapsules (Decapeptyl CR)], administered on day 3 of the cycle results in peak Triptorelin levels within 48 h and a gradual decline towards pretreatment values in 8 weeks. Administration of Triptorelin depot in the early follicular phase, results in an initial rapid rise in LH and follicle-stimulating hormone (FSH) levels to peak values after 4 h, followed by desensitization of the pituitary within 24 h and a subsequent decline in FSH levels to nearly normal levels. E2 levels are observed to peak at 12 h, returning to the follicular range thereafter. From the fourth till the seventh week after agonist administration, the LH pulse patterns showed a markedly increased pulse interval, decreased pulse amplitude, and a severely decreased mean LH level during which LH responses to GnRH were severely blunted or absent with the restoration of the pre-injection LH pulse pattern and the LH response to GnRH observed during the eighth and ninth week. Estradiol benzoate challenges showed an E2 rise to preovulatory levels in response to the injections. However, no changes were observed in LH and FSH concentrations. Pituitary responsiveness is completely absent in the second week and continues to exist until the eighth week after injection, when the agonist has disappeared from the circulation. These findings suggest profound alterations in GnRH receptor availability and post-receptor pathways that prevent the pituitary from responding to physiological stimuli [12].

Impact of GnRH-a on the Luteal Phase

The effect of long-acting GnRH-a, in the luteal phase during ART cycles varies from one patient to another. Geber et al. [13] evaluated the effect of long-acting GnRH-a, used for pituitary suppression, in the luteal phase of 367 patients undergoing ovulation induction for IVF/ICSI. Patients were stratified according to the period of action of the agonist in the luteal phase: group 1: ≤6 days; group 2: 7–12 days; and group 3: >12 days. Reporting pregnancy rates of 45.2 %, 38.9 %, and 47.4 % in groups 1, 2, and 3, respectively, they concluded no significant association between the duration of depot GnRH-a action in the luteal phase and pregnancy rates [13].

GnRH Agonist Depot versus Gonadotropins Alone

Schmutzler et al. [14] evaluated the role of GnRH-a in hyperandrogenic patients with elevated LH

levels and the consecutive development of polycystic ovaries in women undergoing IVF. A single depot injection of 3.6 mg Goserelin on cycle day 22, followed by individualized human menopausal gonadotropin (hMG) stimulation 14 days later ($n=33$) was compared to stimulation with hMG alone on cycle day 3 ($n=29$). They reported a significantly higher pregnancy rate per transfer (36.4 % vs. 20 %, respectively) and a strikingly lower abortion rate in the GnRH-a/hMG group compared to the hMG-only group, suggesting the benefit of the combined GnRH-a/hMG stimulation as a first-line therapy for hyperandrogenic IVF patients [14].

Influence of the Type of GnRH Agonist

Orvieto et al. [15] compared the use of two depot GnRH-a, Leuprolide and Triptorelin (3.75 mg depot formulations), administered on days 21–23 of the menstrual cycle in long-suppression GnRH-a protocols in 52 women undergoing COH-IVF. Stimulation with gonadotropins was initiated after pituitary desensitization was achieved. No significant differences were observed in the patient age, estrogen and progesterone levels on day of hCG administration, gonadotropin dosage, number of oocytes retrieved, fertilization rate, percentage of high-quality embryos, and number of embryos transferred. However, significantly higher clinical implantation and pregnancy rates were found in the Leuprolide group compared with the Triptorelin group when used in the mid-luteal phase [15].

Depot versus Daily GnRH Agonist Administration

Two different formulations of GnRH-a are now available: short formulations and depot formulations. Some authors have suggested that depot GnRH-a induce a too high pituitary suppression and reduced GnRH-a doses are enough for pituitary suppression during ovarian stimulation [6]. Partial pituitary desensitization, using GnRH agonists, may be sufficient in women undergoing controlled ovarian hyperstimulation for assisted reproduction; however, the minimal effective agonist dose remains to be determined [16]. Several studies [16–26] have attempted to compare the stimulation and clinical outcomes following pituitary desensitization with daily and depot administration of GnRH agonists and ovarian stimulation with gonadotropins in patients undergoing assisted reproduction with IVF/ICSI over the past two decades, with the aim to determine the adequate GnRH agonist dose and mode of administration for effective pituitary desensitization (Table 10.1).

With the view that traditional doses of depot GnRH agonist may be excessive for ovarian stimulation, Safdarian et al. [20] compared half-dose (1.87 mg) depot Triptorelin i.m. with reduced-dose daily Buserelin s.c. (0.5 mg reduced to 0.25 mg at the start of hMG stimulation) in a long protocol intracytoplasmic sperm injection (ICSI) embryo transfer initiated with oral contraceptives pretreatment for 21 days. The depot was followed by Baily s.c. injections of saline, while daily buserelin was administered after bolus injection of i.m. saline [20]. Some authors [18, 21, 23] compared the efficacy of a single reduced half dose of GnRH-a depot (1.88 mg) with a daily low dose (0.5 mg/day; s.c.) of Leuprolide acetate for pituitary desensitization followed by gonadotropin stimulation in a long protocol but without the OC pretreatment. Others [16] compared a single 3.75 mg depot injection (i.m.) of Triptorelin [(D-Trp-6-luteinizing hormone-releasing hormone (LHRH)] to 100 μg Triptorelin (D-Trp-6-LHRH) form of triptorelin daily, which was then reduced to 50 μg at the start of FSH stimulation [26]. Yet other compared a single 3.75 mg i.m. Leuprorelin depot injection versus Buserelin (0.3 mg sc twice daily) [25] for ovarian stimulation for IVF [25].

Clinical Outcomes

Despite the varied GnRH-a doses, stimulation protocols, gonadotropins used, and times and routes of administration of GnRH, no significant differences have been reported in the estradiol

Table 10.1 Daily versus depot GnRH administration

	GnRH-a (daily)					GnRH-a (depot)					P
Study	*n*	GnRH-a	Dose/mode of administration	Gn	Stimulation and clinical outcome	*n*	GnRH-a	Dose/mode of administration	Gn	Stimulation and clinical outcome	
Safdarian et al. (2007) [20]	91	Buserelin	0.5 mg s.c. reduced to 0.25 mg at the start of hMG	hMG	CPR OPR EPL Gn days: 10.6	91	Triptorelin	1.87 mg i.m.	hMG	CPR OPR EPL Gn days: 11.2	NS NS NS <0.03
Isikoglu et al. (2007) [21]	52	Leuprolide acetate	0.5 mg/d		CPR IR AR Gn units Gn days	51	Leuprolide acetate	1.88 mg		CPR IR AR Gn units Gn days	NS NS NS NS <0.01
Lorusso et al. (2004) [28]	46	Triptorelin acetate	0.1 mg/d s.c.			45	Triptorelin acetate	3.75 mg s.c.			
Geber et al. (2002) [22]	167	Leuprolide acetate		hMG	Gn units Gn days	292	Goserelin		hMG	Gn units Gn days	S: >40 year NS
Dal Prato et al. (2001) [16]	66	Triptorelin	0.1 mg i.m. reduced to 0.05 mg at the start of FSH	FSH	CPR: 34.9 % IR: 18.0 % AR: 9.1 % Gn units: 41.0 ± 26 Gn days: 11 ± 1.3	66	Triptorelin	3.75 mg s.c.	FSH	CPR: 38 % IR: 20.2 % AR: 8.3 % Gn units: 46.0 ± 25.3 Gn days: 11.8 ± 1.5	NS NS NS <0.03 <0.002
Vlaisavljević et al. (2000) [17]	260			FSH-HP	CPR: 20.2 % DR: 22.1 %	454			FSH-HP	CPR: 30.2 % DR: 23.4 %	NS NS
Hsieh et al. (2000) [23]	158	Leuprolide acetate	0.5 mg/d s.c.		CPR Gn days	289	Leuprolide acetate	1.88 mg s.c.		CPR Gn days	NS NS
Fábregues et al. (1998) [24]	30	Leuprolide acetate			CPR IR	30	Leuprolide acetate			CPR IR	NS NS

(continued)

Table 10.1 (continued)

	GnRH-a (daily)					GnRH-a (depot)					P
Study	*n*	GnRH-a	Dose/mode of administration	Gn	Stimulation and clinical outcome	*n*	GnRH-a	Dose/mode of administration	Gn	Stimulation and clinical outcome	
Tsai et al. (1995) [18]	52	Leuprolide acetate	0.5 mg/d s.c.	hMG 225 IU/d	CPR: 21.2 %	48	Leuprolide acetate	1.88 mg s.c.	hMG 225 IU/d	CPR: 25.0 %	NS
Porcu et al. (1995) [25]	57	Leuprorelin	0.3 mg twice daily/s.c.		CPR: 25.9 % IR: 12.3 % MR: 28.5 %	60	Leuprorelin	3.75 mg		CPR: 29.4 % IR: 11.9 % MR: 26.6 %	NS NS NS
Porcu et al. (1994) [26]	94	Triptorelin	0.1 mg/d s.c.	FSH	CPR: 25.6 % E2 Gn days	102	Triptorelin	3.75 mg	FSH	CPR: 28.7 % E2 levels Gn days	NS NS NS
Gonen et al. (1991) [19]	66	Buserelin	Intranasal	hMG	CPR: 27.1 % PL: 26.3 %	57	Decapeptyl depot	–	hMG	CPR: 12.3 % PL: 71.4 %	<0.05 <0.05

CPR clinical pregnancy rate, *OPR* ongoing pregnancy rate, *EPL* early pregnancy loss, *IR* implantation rate, *DR* delivery rate, *AR* abortion rate, *MR* miscarriage rate, *E2* estradiol, *Gn* gonadotropin, *FSH* follicle-stimulating hormone, *hMG* human menopausal gonadotropin, *NS* non-significant

concentrations, follicle number, the quantity of oocytes retrieved and fertilized, the number of embryos transferred [16, 18, 23, 24, 26], clinical pregnancy rates per transfer [8, 16–18, 21, 23–26], implantation rates [16, 21, 24, 25], ongoing pregnancy rates [8, 17, 20, 21], rates of early pregnancy loss [16, 20, 21], miscarriage rates [25, 26], or in the rate of severe OHSS [8] between the depot and daily GnRH agonist groups. No differences have been reported in follicular recruitment and growth during gonadotropin treatment, and the endometrial thickness on the day of hCG between patients randomized to a standard long protocol of s.c. Leuprolide acetate or a monthly injection of Leuprolide acetate depot for 4 months before gonadotropin stimulation [24]. A recent meta-analysis of 12 randomized controlled trials (RCTs) that compared depot and daily administration of GnRH-a in long protocols for IVF treatment cycles in couples with any cause of infertility, using various methods of ovarian stimulation concluded that the chance of achieving a clinical pregnancy, live birth or ongoing pregnancy, and severe ovarian hyperstimulation syndrome (OHSS) using daily GnRH-a injections was 30 %, 24 %, and 3 %, respectively, compared to a corresponding chance between 25 % and 35 %, 18 % and 29 %, and 1 % and 6 %, respectively, using a GnRH-a depot [8].

In contrast, Gonen et al. [19] observed significantly higher ($P<0.05$) clinical pregnancy rates per ovum pick-up (OPU) (27.1 % vs. 12.3 %, respectively) and significantly lower rates of pregnancy loss (26.3 % vs. 71.4 %; $P<0.05$) in patients who received short-acting GnRH-a (Buserelin) + hMG compared to those who received long-acting GnRH-a D-Trp6 (Decapeptyl Depot) + hMG showing the superiority of short-acting GnRH-a over the long-acting agents in achievement of pregnancy and its outcome, though neither was significantly different from the hMG-only protocol [19]. Significantly lower numbers retrieved oocytes, oocytes fertilized, cleaved embryos, embryos transferred, and estradiol levels have been reported in some studies following depot GnRH-a administration compared to the daily GnRH-a patients despite higher gonadotropin doses [24, 27, 28], suggesting that pituitary over suppression, induced by GnRH-a due to greater bioavailability, hence elevated circulating levels of the GnRH-a peptide, causes an increase in the gonadotropin requirement for ART and a reduction in the number of oocytes retrieved and fertilized [27, 28].

Stimulation Characteristics

While few studies have reported a significantly higher gonadotropin requirement [8, 16, 24, 28] and a significantly longer stimulation period [8, 16, 20, 21], others have, however, observed no significant difference in the gonadotropin requirement [21, 23, 26] or the duration of stimulation [22, 24, 26] between the depot and the daily GnRH agonist groups. On the other hand, though Geber et al. [22] observed a higher requirement for gonadotropin ampules in the depot group, this difference was only evident in patients >40 years that started GnRH-a in the follicular phase. Moreover, while the number of follicles aspirated and the number of oocytes retrieved was similar, the incidence of ovarian cysts in patients with >40 years was higher in patients administered GnRH-a daily [22].

Hormone Levels

Some authors [18, 23, 26] reported no statistical differences in baseline estradiol and FSH concentrations, and concentrations of estradiol, LH, and FSH on the day of human chorionic gonadotropin (hCG) administration between depot and daily GnRH-a administration, while others [2] observed significantly lower serum LH and FSH levels after downregulation with 3.75 mg of the GnRH agonist Triptorelin acetate depot compared to a daily dose of 0.1 mg Triptorelin acetate, necessitating significantly higher gonadotropin doses during subsequent ovarian stimulation to achieve comparable levels of serum estradiol and preovulatory follicles [27]. Profound endogenous LH suppression by depot GnRH agonists indicates a need for minimal LH activity in folliculogenesis and oocyte development [27]. There was no evidence of a premature LH surge in either group [18]. Porcu and Dal Prato reported [26] a high incidence of multiple pregnancy in both the groups [26].

Dada et al. [4] reported a significant difference in the suppression of estradiol from initial concentrations on day 15 of analog administration between patients on the short-acting Buserelin, short-acting Nafarelin and the depot formulation Leuprorelin (54 % vs. 72 % and 65 %, respectively; $p<0.05$), all commenced in the early follicular phase. They also reported a significant difference in the number of patients satisfactorily suppressed (80 %, 90 % and 90 %, respectively$p<0.05$), though there were no differences between the analogs by day 21. Similarly there was no difference in hormonal suppression during the stimulation phase or in the implantation, pregnancy, or miscarriage rates among the three agonists. They concluded that with Nafarelin and Leuprorelin, stimulation with gonadotropins may begin after 2 weeks of suppression and that, long-acting GnRH-a is as effective as short-acting analogs, with no detrimental effects on the luteal phase [4].

Time for Pituitary Desensitization

A series of GnRH tests during the late follicular and mid-luteal phases [26] and estradiol levels < 30 pg/mL have been used as an indication of pituitary desensitization and initiation of gonadotropin administration [28]. Lindner et al. [29] evaluated the efficacy of intranasal administration of the short-acting daily GnRH-a (Buserelin acetate; 1.2 mg/day) during the follicular phase (days 1–3; $n=84$),GnRH-a (Buserelin acetate; 1.2 mg/day) + 10 mg Medroxyprogesterone acetate (MDA) for 10 days during the early luteal phase ($n=41$), and intramuscular administration of the long-acting depot GnRH-a (Triptorelin acetate) + 10 mg MDA for 10 days during the early luteal phase ($n=42$). Pharmacological hypogonadotropism was assessed by the evaluation of serum LH, FSH, estradiol (E2), prolactin, and testosterone levels. Pituitary function was assessed by (1) measurement of fluctuations in endogenous LH levels, (2) response to LHRH (GnRH-a) administration, and (3) response to estradiol benzoate (E2 test). Complete pituitary desensitization was only assumed, if all three tests were negative. The LHRH test and the E2 test were shown to be the most reliable indicators of pituitary function. E2 administration led to further reduction of gonadotropin secretion after pituitary desensitization. They observed a significantly reduced desensitization time in the BA + MDA group compared to the BA only group (20.7 ± 10.5 days vs. 41.1 ± 11.7 days; $p<0.01$) and a further, non-significant shortening to 15.1 ± 3.0 days in the TA group. Changes in endocrine parameters demonstrated hypogonadotropic hypoestrogenism after initial pituitary stimulation [29].

However, later studies reported no significant differences in the time taken to achieve downregulation between the daily and depot GnRH-a dose [22, 25, 26], suggesting that both routes of GnRH-a have similar effects on pituitary suppression and ovulation induction in ART [22]. Resumption of pituitary activity occurred 7 days after the discontinuation of the daily form and in about 2 months after discontinuation of the depot form [25].

Interpretations

However, despite comparable clinical outcomes among majority of the studies, interpretations with regard to the choice of GnRH agonist for effective pituitary desensitization differ. Some authors concluded that a reduced GnRH dose is enough for pituitary suppression during ovarian stimulation and offers the possibility of a shorter GnRH-a treatment protocol, requiring lower amounts of gonadotropins that should be considered in view of its economic advantage, though it provides no significant improvement in IVF cycle outcome when compared with the depot formulation [16]. Long-term downregulation does not improve pregnancy rates in a general IVF program over the daily dose GnRH agonist [24], but depot GnRH-a may increase the overall costs of IVF treatment owing to a higher gonadotropin requirement and a longer duration of use [8]. Dal Prato et al. [6] suggested that thouth a reduced daily dose of Triptorelin provides no significant improvement in IVF cycle outcome when compared with depot formulation in normally responding women, it seems to improve ovarian response and overall results in poor responding patients [6].

However, others concluded that a single reduced depot dose (1.88 mg) of Leuprolide is as effective as the classical long multi-dose protocol for pituitary desensitization in COH [21] that may offer a

useful alternative for pituitary suppression in ovarian stimulation for IVF [18]. The long-acting GnRH-a is an excellent option, as only a single subcutaneous dose is necessary, decreasing the risk of the patient to forget its use and, most important, it does not interfere with the patient's quality of life [22]. Considering improved patient compliance and preference, depot forms are advantageous [25].

Dose of Depot GnRH-a

Appropriate dosage of the long-acting depot GnRH agonist has not been determined in long protocol for IVF. Envisaging excessive pituitary suppression by depot GnRH agonist for ovarian stimulation, Dal Prato et al. [30] equally randomized 180 patients to a standard full-dose (3.75 mg) and half-dose (1.87 mg) depot Triptorelin, in a long protocol to compare the efficacy of the two doses. They reported no premature LH surge, higher LH levels (1.04 ± 0.05 vs. 0.7 ± 0.06 IU/L on the day of hCG), lower number of FSH ampules (42 ± 2 vs. 59 ± 3), and significantly higher numbers of mature oocytes (10.1 ± 0.54 vs. 7.4 ± 0.55), fertilized oocytes (8.24 ± 0.35 vs. 6.34 ± 0.37) and of embryos (7.8 ± 0.36 vs. 5.9 ± 0.37) in the half-dose group compared to the full-dose group. No significant differences were found in pregnancy (38.8 % vs. 25.3 %), implantation (22.6 % vs. 13.8 %), or abortion (6.1 vs. 5.0 %) rates. Cumulative pregnancy (fresh plus frozen embryo transfers: 56.8 vs. 35.4 %) rate was significantly higher in the half-dose group. Hence, a half-dose of depot Triptorelin can be successfully used in ovarian stimulation for IVF and produces a higher number of good quality embryos with a good chance of implantation [30]. When these doses were compared in a smaller study ($n = 120$), Yim et al. [3] also reported significantly lower LH levels at 2 and 3 weeks (2.2 ± 1.0 and 1.1 ± 0.6 IU/L vs. 3.5 ± 5.5 and 2.7 ± 1.9 IU/l, respectively) in the conventional dose (3.75 mg) group compared to the half-dose (1.87 mg) group, respectively, but no significant differences between the doses of gonadotropins used, the number of oocytes and embryos available and the time to resumption of menses, nor in the pregnancy rates. Suppression was measured by evaluating serum LH levels at 2 and 3 weeks after the administration of the GnRH analogs, the dose of gonadotropin used, and the time to resumption of menses. The authors concluded that although the degree of suppression, as measured biochemically, was more profound with the conventional dose, this did not affect the IVF outcome; hence, the use of a lower dose would be equally effective and could contribute to a reduction in the cost of treatment [3].

In a recent study, Li et al. [31] compared further reduced doses: a one-third-dose (1.25 mg) depot Triptorelin with half-dose (1.87 mg) in a luteal long protocol in 100 patients undergoing IVF/ICSI. While no LH surge was observed in both the groups on day 3–5 of the menstrual cycle after downregulation, fewer patients showed low-level LH (<1.0 IU/L) and estradiol (<30 pg/mL) in the one-third-dose group ($p < 0.05$). They reported fewer retrieved oocytes ($p = 0.086$), significantly fewer total embryos and available embryos for cryopreservation ($p < 0.05$), a significantly higher good quality embryo rate ($p < 0.05$), non-significantly lower length and dose of ovarian stimulation and no significant differences in the clinical pregnancy (52 % vs. 40 %), implantation (48 % vs. 37.5 %), delivery (46 % vs. 32 %), or live birth (42 % vs. 32 %), and the abortion rates (8 % vs. 20 %) rates between the one-third and half-dose groups, respectively. The authors concluded that a one-third-dose depot Triptorelin (1.25 mg) can be successfully used with reduced pituitary suppression and lower cost in a long protocol for IVF [31].

Olivennes et al. [32] compared the ovarian response following a low-dose GnRH agonist protocol and a GnRH agonist long protocol depot formula in patients with high day 3 FSH (>6.5 IU/L). They reported a better ovarian response with the low-dose GnRH agonist with fewer ampules (37.1 vs. 46.6), a shorter duration of stimulation (10.5 vs. 12.4 days), a higher number of mature oocytes (5.9 vs. 4.5), a higher number of good quality embryos (3.2 vs. 2.3), higher E2 levels on day 8 (1065 vs. 460 pg/mL), and lower cancellation rates (14 % vs. 26 %) compared to the depot formula. However, randomized studies are needed to confirm these data [32].

Choice of Gonadotropins

With regard to the choice of gonadotropin stimulation (225 IU/day pure FSH or 225 IU/day hMG) following downregulation with an luteinizing hormone-releasing hormone (LHRH) agonist (Goserelin) depot, Gerli and Villani [33] reported no significant difference in the number of days and ampules required for follicular maturation, number of follicles developed, or in the pregnancy rates between the groups. However, estradiol values at the end of stimulation were significantly lower for the FSH group, suggesting that the contemporary administration of LH with FSH does not exert any effect on follicular development, but it seems to facilitate E2 synthesis, probably by providing more substrate for the aromatization process [33].

Balasch et al. [34] compared ovarian responses after ovarian stimulation with depot GnRH-a protocol combined with recombinant human FSH (rh-FSH) or hMG in normo-ovulatory patients undergoing ICSI. A fixed regimen of 150 IU rh-FSH or hMG was administered in the first 14 days of treatment. Although the dynamics of ovarian follicle development and serum estradiol concentrations on the day of hCG injection during gonadotropin treatment were similar in both the groups, the duration of treatment and the per cycle gonadotropin dose were lower in the hMG group. The number of leading follicles (>17 mm in diameter) on the day of hCG injection was higher and the number of oocytes, mature oocytes, and good quality zygotes and embryos obtained was significantly increased in the rh-FSH group. Hence, though supplemental LH may be required in terms of treatment duration and gonadotropin consumption, both oocyte, embryo yield and quality were significantly higher with the use of rh-FSH in IVF patients undergoing pituitary desensitization with a depot GnRH agonist preparation [34]. However, the choice of gonadotropins that may be used largely depends on the GnRH protocol used and the indication for infertility.

GnRH Agonist Depot versus GnRH Antagonist

Several studies have attempted to compare the outcomes of stimulation with the GnRH agonist long protocol and the GnRH antagonist protocol, followed by gonadotropin stimulation. Olivennes et al. [35] reported a shorter duration of stimulation, lower number of hMG ampules administered, lower occurrence of OHSS, and excellent patient tolerance with a single 3 mg dose of Cetrorelix (administered in the late follicular phase) but a lower number of oocytes and embryos compared to a depot preparation of Triptorelin (Decapeptyl) followed by ovarian stimulation with hMG (Menogon) in patients undergoing IVF-ET. No premature LH surge (LH level >10 IU/L, progesterone level >1 ng/L) was demonstrated after Cetrorelix administration. There was no difference in the percentage of mature oocytes and fertilization rates between the groups, and the pregnancy rates were not statistically different, suggesting that the Cetrorelix single-dose protocol compares favorably with the long protocol and could be a protocol of choice in IVF-ET [35]. Del Gadillo et al. [36] compared a flexible GnRH antagonist (GnRH-ant) protocol (Cetrorelix, 0.25 mg/day, administered when follicles reached a diameter of ≥14 mm) with a GnRH-a Triptorelin long protocol, which was continued during the gonadotropin hMG and/or r-FSH treatment until the induction of ovulation. The authors observed no difference in the mean length of stimulation and the dose of FSH required per patient but a significantly higher mean E2 level on the day of hCG administration (2076 ± 1430 vs. 1145 ± 605 pg/mL), a higher number of oocytes (6.34 vs. 5.38), higher fertilization rate (63.6 % vs. 59.3 %), and a higher pregnancy rate (15 % vs. 5 %) in the GnRH agonist compared to the GnRH antagonist protocol. The authors concluded that GnRH-ant and GnRH-a provide comparable results in unselected patients; however, GnRH-ant allows a higher flexibility in the treatment [36].

Roulier et al. [37] reported a significantly reduced ($p<0.01$) duration of FSH therapy (9.95 vs. 11.25 days), cumulative dose of rh-FSH (1604 vs. 1980 IU) and number of oocytes retrieved (8.5 vs. 11.2) following a GnRH-ant flexible protocol [Cetrorelix (Cetrotide) 3 mg, administered when the largest follicle reached 14 mm; $n=307$] compared to the administration of a GnRH agonist [Decapeptyl Retard 3.75 mg; $n=364$]. On the first day of menses, ovarian stimulation was carried out with rh-FSH, 150–225 IU/day, in both the protocols. Human chorionic gonadotropin, 10,000 IU, was administered when at least two follicles reached a mean diameter ≥18 mm. However, there was no difference in the number of embryos transferred or in the pregnancy rates per oocyte retrieval (24.5 %) between the antagonist and agonist protocols. The authors concluded that although fewer oocytes are recovered, the GnRH antagonist is simpler and more convenient for patients and yields similar pregnancy rates compared to the GnRH agonist protocol, with the added advantage of preventing both a premature LH surge and detrimental rises in LH during ovarian stimulation prior to assisted reproduction treatment [37]. A contemporary study also reported statistically significantly lower ($p<0.01$) mean number of ampules of FSH (25.9 vs. 34.5, respectively) and the duration of stimulation (9.6 vs. 12.2 days, respectively) in IVF/ICSI patients administered a flexible single-dose GnRH antagonist (Cetrorelix, 3 mg; $n=224$) in the late follicular phase, when the mean follicle diameter exceeded 12 mm compared to a single-dose depot GnRH agonist (Goserelin) long protocol ($n=236$) for ovarian stimulation for IVF/ICSI. There was no significant difference in the mean number of oocytes retrieved, fertilization, blastulation and blastocyst transfer rates, or in the clinical pregnancy (34.3 vs. 30.1 %) and delivery rates (31.9 vs. 28.3 %) per cycle between the Goserelin and Cetrorelix groups, respectively. The authors concluded that the flexible single-dose GnRH antagonist protocol is an advantageous alternative to the long GnRH agonist protocol, with similar efficacy, shorter duration, a significant reduction in the number of FSH ampules used, and without the menopause-like effects of the GnRH agonist [38].

A more recent study has also reported similar clinical outcomes with the fixed GnRH-ant and low-dose depot GnRH-a long protocols in infertile women with normal ovarian reserve function undergoing IVF or ICSI cycles, with advantages of economy, convenience, and safety with the GnRH-ant protocol [39]. However, a flare-up GnRH-a protocol with a depot formulation is reported to yield a higher total pregnancy and implantation rate in poor responders than a GnRH antagonist, possibly by improving oocyte/embryo competence [40].

Eldar-Geva et al. [41] compared the outcomes of frozen-thawed embryo transfer, using the long GnRH protocol with Triptorelin depot 3.75 mg ($n=215$) or 0.1 mg/day ($n=83$), or GnRH-ant protocol with either hCG ($n=69$) or GnRH agonist ($n=25$) for final oocyte maturation. They reported no differences in the implantation rate, clinical pregnancy rate, ongoing pregnancy rate, and embryo survival rate and concluded that the potential for frozen-thawed embryos to implant and develop following transfer is independent of the GnRH analog and the final oocyte maturation protocol used in the collection cycle [41].

Side Effects

Clinical studies with a number of agonists have demonstrated their efficacy in producing a hypogonadal state safely with rapid recovery following cessation of therapy [9]. Ovarian hyperstimulation following the sole administration of GnRH-a is exceedingly rare [42], with a few cases reported in the literature. Weissman et al. [42] reported massive ovarian multifollicular enlargement concomitant with high serum estradiol concentrations following mid-luteal depot administration of Triptorelin using the long protocol and early follicular administration of Triptorelin as daily subcutaneous injections,

which resolved spontaneously following expectant management, and Leuprolide acetate starting at the mid-luteal phase [42]. The authors suggested that ovarian hyperstimulation can occur following the sole administration of GnRH-a irrespective of the preparation used and the administration protocol. This rare entity probably represents an exaggerated form of ovarian cyst formation following GnRH-a administration, the underlying pathophysiology of which remains unresolved [42]. Park et al. [43] reported ovarian multifollicular enlargement with high estradiol level following administration of the GnRH depot preparation, Triptorelin (3.75 mg) without gonadotropins in a patient undergoing IVF for oocyte donation. However, a subsequent cycle in the same patient with a low dose of Triptorelin (0.05 mg) did not induce ovarian hyperstimulation and resulted in clinical pregnancy. Since only few such cases have been published, it is unclear what course to follow in subsequent cycles after ovarian hyperstimulation in the first cycle using only GnRH-a [43].

Conclusion

The GnRH-a depot formulation is a good option for pituitary downregulation in COH cycles for ART with advantages of patient compliance and ease of use and comparable clinical outcomes with the short-acting daily GnRH-a preparation. The feared profound pituitary suppression with the conventional dose (3.75 mg) depot GnRH-a preparation may be overcome with the use of reduced doses (1.87 mg/1.25 mg), which have proven to be equally effective. However, the choice of use of the GnRH-a depot formulation over the daily GnRH-a preparation, or the GnRH antagonist, for COH may be individualized according to cause of infertility and rests solely with the clinician.

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Luteal Phase Support in Controlled Ovarian Hyperstimulation

11

Budi Wiweko

Abstract

Luteal phase is the period between ovulation and either the establishment of a pregnancy or the onset of menses 2 weeks later [1]. Being the latter phase of the ovarian cycle, the luteal phase coincides with the secretory phase of the endometrium.

Keywords

Luteal phase • Ovarian cycle • Ovulation • Pregnancy • Luteal phase defect • Secretory phase • Endometrium

Introduction

Luteal phase is the period between ovulation and either the establishment of a pregnancy or the onset of menses 2 weeks later [1]. Being the latter phase of the ovarian cycle, the luteal phase coincides with the secretory phase of the endometrium.

Luteal phase defect (LPD) is described as a condition in which endogenous progesterone is not sufficient to maintain a functional secretory endometrium and to allow normal embryo implantation and growth [2]. It may be caused by inadequate progesterone secretion by the corpus luteum or inadequate response between the endometrium towards progesterone as a result of inadequate priming by estrogen. By consensus, LPD has been defined as a lag of more than 2 days in endometrial histological development compared with the expected day of the cycle [3, 4]. In LPD, there is an incompatibility between the endometrial cycle with the ovarian cycle, hence the term out-of-phase.

Luteal phase defect has been shown to be associated with ovarian stimulation alone, ovulation induction with or without gonadotropin-releasing hormone (GnRH) agonists, and assisted reproductive techniques (ARTs) [2, 5, 6]. It is also associated with other medical conditions such as anorexia, starvation, and other eating disorders [7], excessive exercise [8], stress [9], obesity and polycystic ovary syndrome (PCOS) [10], endometriosis [11], ovarian aging [12], thyroid dysfunction, and hyperprolactinemia [13]. These

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conditions should be ruled out, or treated adequately if present, before initiating other forms of therapy for infertility.

In ARTs, luteal phase defect results in higher failure rates. To manage the defect, luteal phase support is needed. Luteal phase support refers to the administration of medication to support the process of implantation [14]. It is commonly used in IVF cycles and has been well accepted. Nevertheless, there have been numerous researches and publications regarding the regimen, timing, dosage, and route of administration for luteal phase support [15]. These topics will be discussed in this chapter, along with the role of low molecular weight heparin (LMWH) during the luteal phase.

Rationale for Luteal Phase Support

There have been several hypotheses regarding the etiology of LPD in stimulated IVF cycles, most of which have been disproved (Table 11.1) [16]. These include removal of granulosa cells, prolonged pituitary recovery after administration of GnRH agonists, and suppression of LH by the administered human chorionic gonadotropin (hCG) [17–19]. Cycles co-treated with GnRH antagonists were also thought to enhance the recovery of pituitary function, but studies have shown that premature luteolysis also occurs in these cycles [20–22].

The most plausible explanation of LPD in stimulated IVF cycles is due to the multifollicular development during ovarian stimulation, resulting in supraphysiological level of steroids secreted by the abundant amount of corpora lutea during the early luteal phase. This condition causes a negative feedback at the hypothalamic-pituitary axis that directly inhibits LH release [23]. Inhibitions of LH will the cause the corpora lutea to degenerate and undergo premature luteolysis.

Considering the abnormal luteal phase in stimulated IVF cycles, exogenous support is crucial to achieve pregnancy [24]. There are two forms of exogenous support: administration of exogenous LH or hCG to save the corpus luteum and administration of products synthesized by the corpus luteum (progesterone and estrogen).

Table 11.1 Proposed etiologies of LPD and reason(s) for disproval

Etiology	Reason(s) for disproval
Removal of granulosa cells during oocyte retrieval	Aspiration of a preovulatory oocyte in a natural cycle did not diminish the luteal phase
Prolonged pituitary recovery after treatment with GnRH agonist	Luteolysis is also initiated prematurely in cycles co-treated with GnRH antagonists
Administration of hCG could suppress LH production	hCG did not downregulate LH secretion in the luteal phase of normal, unstimulated cycles in normo-ovulatory women

Choosing Regimens

The role of progesterone (P) in the luteal phase has been widely recognized and accepted. Progesterone-receptor blockers such as Mifepristone are known for their abortive properties. In stimulated IVF cycles, luteolysis of the corpus luteum results in reduced production of progesterone; therefore, exogenous supplementation of progesterone is needed for luteal phase support.

Different progestogen preparations have different characteristics, mainly in terms of the chemical structure, metabolism, pharmacokinetics, and potency [25]. The potency of progestogens to induce normal secretory transformation of the endometrium in luteal phase support is known as the transformational dose. The transformational dose of various progestogens are shown in Table 11.2.

Phases of Endometrial Transformation

A recent study by Wiweko et al. [27] revealed that serum progesterone level on the hCG day in pregnant women is significantly lower compared to women who failed to achieve pregnancy ($p = 0.024$) (Table 11.3). However, serum progesterone and hCG levels are higher in women with ongoing pregnancy compared to women only achieving clinical pregnancy ($p < 0.001$) [27].

Table 11.2 Transformational dose of various progestogens [26]

Progestin	Transformational dose (mg per cycle)	Transformational dose (mg per day)
Progesterone	4200	200–300
Dydrogesterone	140	10–20
Medrogestone	60	10
Medroxyprogesterone acetate	80	5–10
Chlormadinone acetate	20–30	10
Cyproterone acetate	20	1.0
Norethisterone	100–150	/
Norethisterone acetate	30–60	/
Lynestrenol	70.0	/
Ethynodiol	15.0	/
Levonorgestrel	6.0	0.15
Desogestrel	2.0	0.15
Gestodene	3.0	/
Norgestimate	7.0	/
Dienogest	6.0	/
Drospirenone	50	/
Promegestone	10	0.5
Nomegestrol acetate	100	5.0
Trimegestone	/	/

Reproduced with permission from Schindler et al. [26]

Table 11.3 Serum progesterone on hCG day and pregnancy rate

	Non-pregnant ($n=118$)		Pregnant ($n=37$)		
Variable	Mean	± SD	Mean	± SD	p
LH (mIU/mL)	1.9	1.6	1.4	1	0.164
P4 (ng/mL)	1.2	0.6	0.9	0.4	0.024*
P4/E2 ratio	0.7	0.5	0.5	0.3	0.01*
E2 (pg/mL)	2318	1472	2268	1132	0.829
Number of mature oocytes	6.2	3.7	7.3	2.7	0.023*
Number of 8 cells embryos	1.5	1.8	2.8	1.5	<0.05*

Variable	Clinical pregnancy ($n=73$)	Ongoing pregnancy ($n=57$)	p
Progesterone (ng/mL)	40 (7.6–955)	60 (15–955)	0.000
hCG (mIU/mL)	377 (16–1868)	413 (47–1868)	0.000

No	Variable	AUC	Cut-off point	p	Sensitivity (%)	Specificity (%)
1	Progesterone ($n=73$)	0.982	58.8 ng/mL	0.000	82.2	81
2	hCG ($n=228$)	0.860	74.05 mIU/mL	0.000	93.2	93.3

Data from Wiweko et al. [27]
*$p<0.05$

Regimen for Luteal Phase Support

Combined Estrogen + Progesterone

Var et al. [28] compared three different luteal phase support protocols among 280 samples: daily P + 4 mg of estradiol (E2), daily P + 1500 IU of hCG, and daily P-only. Pregnancy rates were similar in the first and second group but were significantly lower in women who only received daily P. However, Fatemi et al. [14] concluded that the addition of estradiol to progesterone did

Table 11.4 Comparison of pregnancy rates between E2 + P group and P-only group

	E2 + P		P-only	
Study	Regimen	Pregnancy rate	Regimen	Pregnancy rate
Fatemi et al. [14]	4 mg oral E2 valerate + 600 mg vaginal micronized progesterone	30/101 (29.7 %)	600 mg vaginal micronized progesterone	26/100 (26 %)
Tonguc et al. [29]	2 mg E2 + 90 mg/day vaginal P 4 mg E2 + 90 mg/day vaginal P 6 mg E2 + 90 mg/day vaginal P	30/95 (31.6 %) 38/95 (40 %) 31/95 (32 %)	–	–
Lin et al. [30]	6 mg E2 + 60 mg intramuscular P	103/202 (50.9 %)	60 mg intramuscular P	116/200 (58 %)
Jee et al. [31]	7 studies with GnRH agonist cycles Clinical pregnancy rate per patient: RR 1.32 (95 % CI 0.79–2.19) Ongoing pregnancy rate per patient: RR 1.34 (95 % CI 0.37–4.82) 3 studies with GnRH antagonist cycles Clinical pregnancy rate per patient: RR 0.94 (95 % CI 0.62–1.42) Ongoing pregnancy rate per patient: RR 1.09 (95 % CI 0.79–1.50)			
Kolibianakis et al. [32]	4 studies with GnRH analogs Clinical pregnancy rate: RR 0.94 (95 % CI 0.78–1.13) Live birth rate: RR 0.96 (95 % CI 0.77–1.21)			

not significantly increase pregnancy rates in patients stimulated with GnRH antagonist/r-FSH. Similar findings were reported by Tonguc et al. [29] and Lin et al. [30] with long GnRH agonist protocols. Meta-analyses by Jee et al. [31] and Kolibianakis et al. [32] concluded that the clinical pregnancy rate and live birth rate did not differ significantly between women who received a combination of progesterone and estrogen and women who only received progesterone (Table 11.4).

Progesterone-Only

In the most recent and large-scale systematic review, van der Linden et al. [33] concluded that progesterone is the best regimen as luteal phase support, favoring synthetic progesterone over micronized progesterone. The addition of estrogen or hCG to progesterone did not significantly increase the pregnancy rates, while addition of GnRH agonists significantly increased the odds of live birth, clinical, and ongoing pregnancy (Table 11.5). These findings are in contrast with the past systematic review by Fatemi et al. [1], which concluded that both hCG and progesterone increased the pregnancy rate. In both the reviews, hCG was associated with a significantly higher risk of ovarian hyperstimulation syndrome (OHSS).

Route of Progesterone Administration

The route of progesterone administration has been a debatable issue. There have been many theories and studies trying to prove which route is the best: oral, vaginal, or intramuscular progesterone, each with their own merits and weaknesses (Table 11.6).

Van der Linden et al. [33] found that any route of progesterone administration provides comparable results. This means that progesterone, as luteal phase support, may be administered orally, vaginally, or intramuscularly. Compared to micronized progesterone, synthetic progesterone significantly increases the clinical pregnancy rate (OR 0.79, 95 % CI 0.65–0.96) but not the live birth rate (OR 1.11, 95 % CI 0.64–1.91).

Patient preference and doctor's experience must be taken into account when choosing the

Table 11.5 Comparison of regimens for luteal phase support

Regimen	Live birth rate (OR, 95 % CI)	Clinical pregnancy rate (OR, 95 % CI)	Ongoing pregnancy rate (OR, 95 % CI)	Miscarriage rate (OR, 95 % CI)	OHSS (OR, 95 % CI)
hCG vs. placebo/no treatment	2.25, 0.37–13.80	1.30, 0.90–1.88	1.75, 1.09–2.81[a]	0.67, 0.15–3.09	0.28, 0.14–0.54[b]
P vs. placebo/no treatment	2.95, 1.02–8.56[a]	1.83, 1.29–2.61[a]	1.87, 1.19–2.94[a]	0.84, 0.33–2.11	0.06, 0.00–3.55
P vs. hCG	2.43, 0.84–6.97	1.14, 0.90–1.45	1.09, 0.66–1.80	0.75, 0.39–1.44	0.63, 0.38–1.03
P vs. hCG + P	1.93, 0.46–8.05	0.96, 0.74–1.25	1.04, 0.65–1.68	1.14, 0.27–4.74	0.45, 0.26–0.79[a]
P vs. estrogen + P	1.13, 0.43–2.94	1.25, 0.99–1.59	1.00, 0.77–1.31	0.99, 1.69–1.43	0.14, 0.01–2.21
P vs. GnRH agonist + P	2.44, 1.62–3.67[b]	1.36, 1.11–1.66[b]	1.31, 1.03–1.67[b]	0.59, 0.14–2.45	n/a

Adapted and modified from van der Linden et al. [33]
[a]Statistically significant favoring the first regimen
[b]Statistically significant favoring the second regimen

Table 11.6 Facts regarding route of progesterone administration

No	Route	Fact	Note
1	Oral	Very low level in blood Bioavailability < 10 % Very high transformational dose (600 mg/day)	Inactivated by hepatic metabolism
2	Vaginal	Low level in blood but still causing endometrium transformation	Directly distributed from vagina to uterus (first uterine pass effect)
3	Intra Muscular	Very high level in blood (2 h) but low in endometrium	Uncomfortable because of pain

Table 11.7 Trends of progesterone administration in various continents

Center	Cycles	Vaginal	im	Vaginal + im
Asia	*8095*	*4285 (52.9 %)*	*810 (10.0 %)*	*2250 (27.8 %)*
Europe	19,620	14,770 (75.3 %)	1250 (6.4 %)	1200 (6.1 %)
North America	14,600	6020 (41.2 %)	441 (30.2 %)	3960 (27.1 %)
Africa	1420	700 (49.3 %)	0	120 (8.5 %)
South America	2620	2620 (100 %)	0	0
Australia	4800	4800 (100 %)	0	0

regimen and route. Levine and Watson [34] reported that over 90 % of patients preferred vaginal over intramuscular progesterone, as it is less painful, easier to administer, and takes less time. A survey among various reproductive centers in different continents also showed different trends of route of progesterone administration (Table 11.7).

Timing of Luteal Phase Support

In controlled ovarian hyperstimulation (COH), GnRH agonist is used in order to suppress LH secretion. However, LH still declines for at least 10 days after cessation of GnRH agonists and causes negative effects on progesterone or hCG secretion [35]. As discussed before, pro-

gesterone is used as luteal phase support in order to maintain the LH level during declining levels of exogenous hCG in the luteal phase and the rise in endogenous hCG after IVF [36].

Another debatable issue regarding luteal phase support is when to initiate treatment. Time to start luteal phase support administration is diverse, ranging from the day before oocyte retrieval to 4 days after embryo transfer [35]. In a worldwide survey regarding progesterone usage in 81 countries during May to June 2012, progesterone supplementation was mostly initiated on the day of egg collection (80.1 %), followed by on the day of embryo transfer (15.4 %), on hCG administration (3.2 %), and a few days after ET (1.3 %) [37]. However, ongoing pregnancy rates and live birth rates, as the outcome of IVF, are not different clinically. The ongoing pregnancy rates after progesterone supplementation, given on day of oocyte retrieval (OR) compared to hCG administration on the day of ET were 20.8 % and 23.6 %, respectively [35].

In general practice, progesterone supplementation is frequently continued even after the patient has conceived; 44 % continue treatment until a gestational age of 8–10 weeks, 28 % continue until more than 12 weeks, 15 % continue until the pregnancy is present, and 13 % continue until fetal heart beats can be detected [37]. However, prolonged progesterone supplementation is not necessary and does not significantly impact the miscarriage and delivery rate. Progesterone supplementation can safely be withdrawn at the time of positive hCG test, 2 weeks after embryo transfer [36].

Schmidt et al. [36] also showed that there is no correlation between prolonged progesterone supplementation and delivery rates. In patients who received progesterone until 3 weeks of pregnancy after positive hCG, 4.6 % (95 % CI: 1.9–9.4) miscarried; the results are comparable to the miscarriage rate in the group of patients who withdrew progesterone (3.3 %, 95 % CI: 1.1–7.5). Moreover, delivery of babies reached 78.7 % in the group with continued progesterone administration and 82.4 % in the group who withdrew progesterone [36].

Role of Heparin to Prevent Recurrent Implantation Failure During the Luteal Phase

Implantation failure is defined as the failure to reach a stage in which there is no intrauterine gestational sac, confirmed by ultrasonography examination. A patients' failure to conceive after 2–6 IVF cycles in which more than 10 high-grade embryos were transferred to the uterus is called recurrent implantation failure [38].

Recurrent implantation failure is associated with thrombophilia patients, and low molecular weight heparin (LMWH) is used to prevent such condition [39]. LMWH can also increase the success rate for unknown etiology of recurrent spontaneous miscarriage patients [40]. Studies show that LMWH has an important role in increasing the endometrial receptivity towards embryo implantation due to its ability to interact with a wide variety of substances in the physiological process of implantation and trophoblastic development, a process that may be adversely influenced by assisted conception [41].

Heparin Plays a Role in Trophoblast Invasion During Implantation Mechanism and Endometrial Receptivity

There are three stages of embryo implantation in the human body: apposition, adhesion, and invasion. The first event is apposition, which is characterized by the attachment of microvilli on the apical surface of the syncytiotrophoblast with pinopodes on the apical surface of uterine epithelium. Furthermore, the blastocyst adheres to uterine epithelium and progresses into syncytiotrophoblast penetration. After the blastocyst is completely bonded in the uterine stromal tissue, the site of implantation (usually in the upper posterior area of uterus) is covered by uterine epithelium, and the trophoblast layer is developed. Cytotrophoblasts then invade the entire endometrium, and the uterine vasculature gets organized to arrange uteroplacental circulation [42]. These steps involve complex molecular signaling mechanisms that are not discussed in detail here.

Heparin has been known to influence the implantation of embryo in humans. There are some mechanisms in trophoblastic apposition, adhesion, and invasion that are regulated by heparin, such as [43]:

- LMWH reduces L-selectin on the entire embryo surface.
- LMWH interferes directly with the binding of APAs to the trophoblast and maintain normal trophoblast invasion.
- Heparin binds and activates epidermal growth factor (EGF) receptors. Heparin-binding epidermal growth factors (HB-EGF)-like growth factors induce trophoblast invasion and inhibit the apoptosis process. Furthermore, heparin also enables the improvement in matrix metalloproteinase (MMP) activation, which leads to the prevention of trophoblast apoptosis.
- LMWH also increases insulin-like growth factor binding protein (IGF-BP) synthesis, which modulates IGF-I and IGF-II effects in improving implantation.
- LMWH also improves selectins, which induce leukocytes for implantation.
- LMWH works as an E-cadherin downregulator. E-cadherin is already known to limit trophoblast invasion.
- LMWH improves trophoblast invasion by reducing transforming growth factor beta (TGF-β).
- LMWH increases interleukin-1 (IL-1) which increases integrin in the epithelial surface that facilitate adhesion and possibly implantation.

The use of heparin to facilitate implantation is still controversial and still needs to be studied furthermore (Fig. 11.1). Patients undergoing IVF cycles under LMWH treatment have higher pregnancy rates compared to the control group. However, the results are not significantly different. Some studies suggest the administration of heparin at a dose of 1 mg/kg/day after egg collection

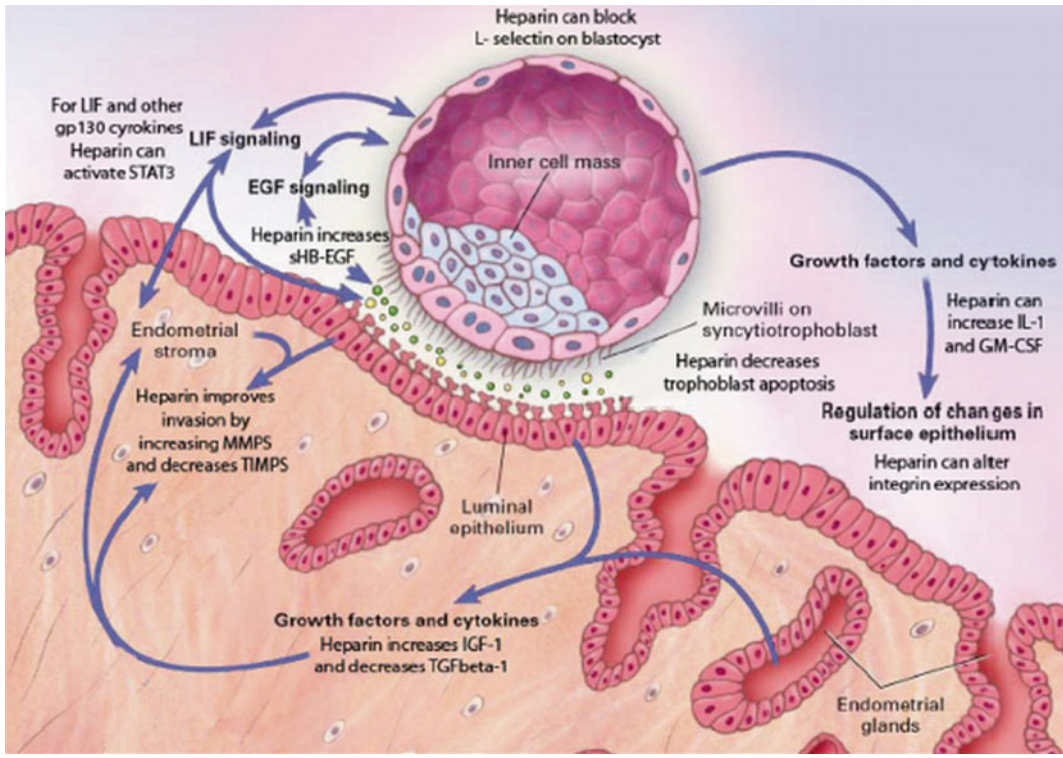

Fig. 11.1 Potential actions of heparin on implantation

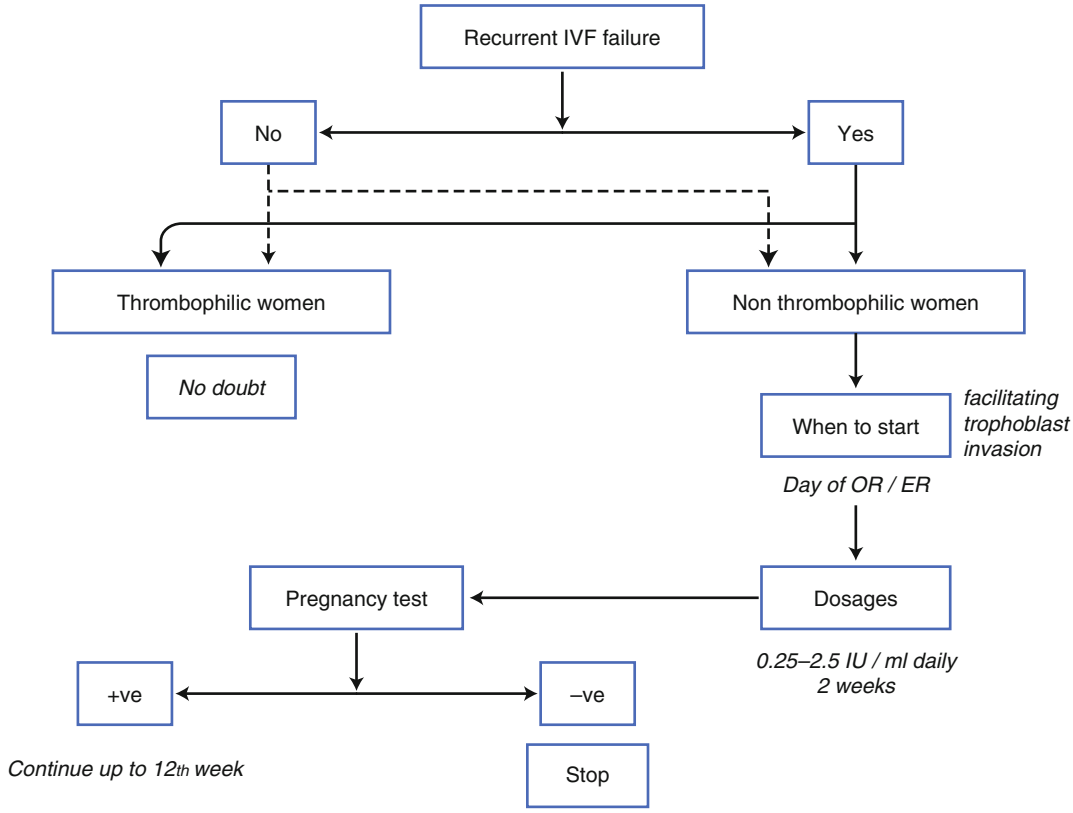

Fig. 11.2 Algorithm of LMWH usage for recurrent IVF failure

in women without laboratory findings of thrombophilia due to the beneficial effect of LMWH on the clinical outcome of pregnancy [44, 45].

In patients with thrombophilia, there is no doubt about using LMWH as it has been reported to significantly increase the success of implantation and prevent recurrent miscarriage. However, 0.25–2.5 IU/mL daily for 2 weeks can be given as a treatment during luteal phase support for patients without thrombophilias. Within the doses of 0.25–2.5 IU/mL, LMWH enhances trophoblast proliferation and invasion significantly, while high concentration of LMWH (25–250 IU/mL) will suppress its benefits in preventing miscarriage (Fig. 11.2).

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