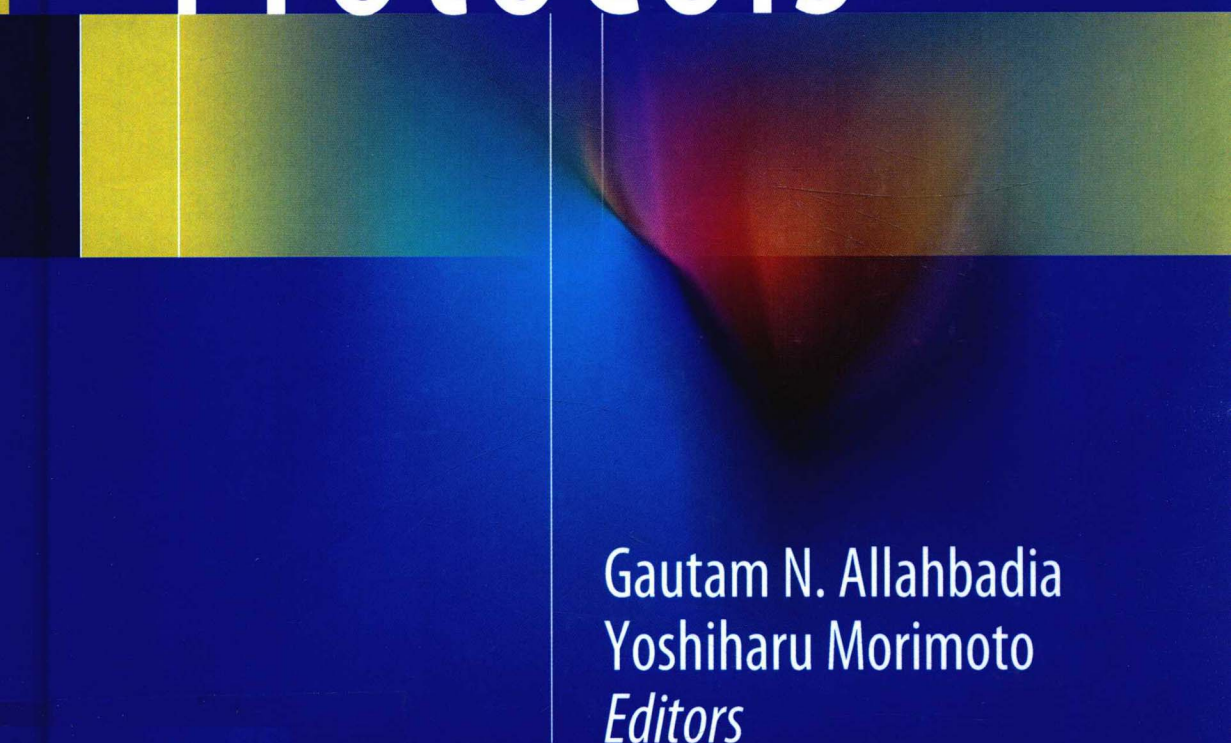
Ovarian Stimulation Protocols



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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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Contents

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

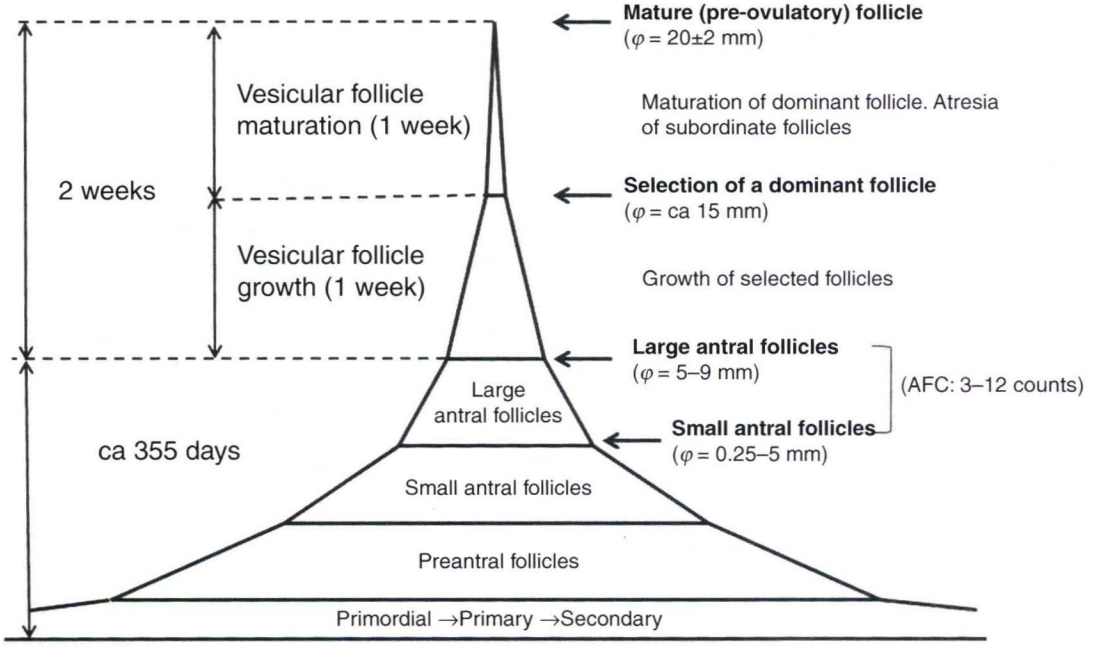

Fig. 1.1 Pyramid-imitative shape of dominant follicle selection in the human ovary. Since terminology and size of each stage of follicles are not uniformly standardized among authors, these are arbitrarily defined by the author (Reproduced with permission from Mori T et al. *Horm Front Gynec.* 2009)

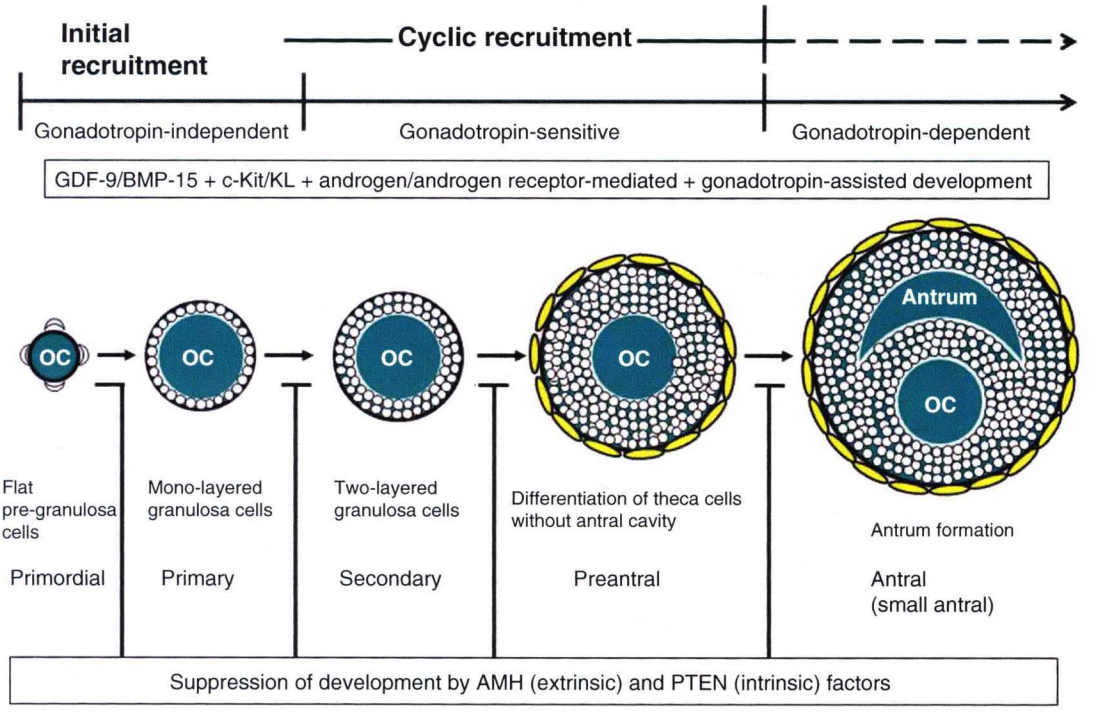

Fig. 1.2 Regulation of preantral follicle growth by stimulatory and inhibitory factors working at different stages of the preantral follicular development (Reproduced with permission from Mori T et al. *Horm Front Gynec*. 2009)

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

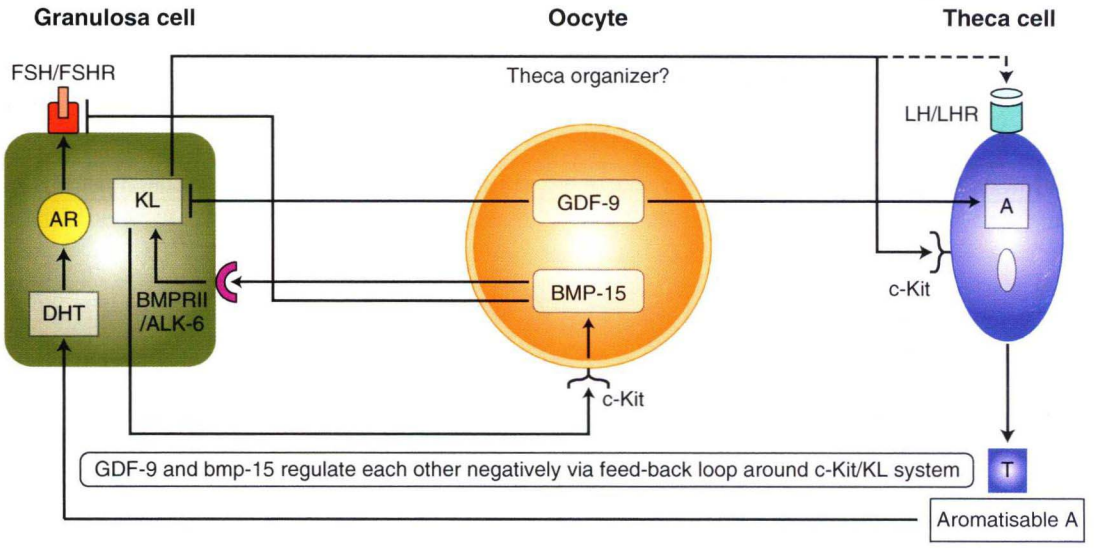

Fig. 1.3 Regulatory interplay of oocyte-originated GDF-9 and BMP-15 among oocytes and granulosa and theca cells for preantral follicle development. Testosterone converts to non-aromatizable androgen, 5α-dihydrotestosterone (5α-DHT) by 5α-reductase for acquisition of binding capacity to receptors for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

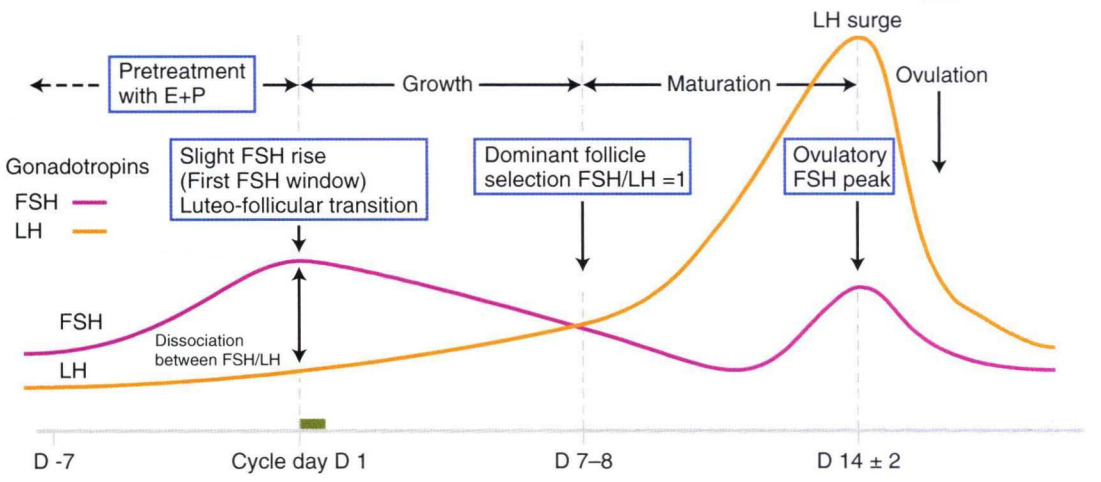

Fig. 1.4 Changes in serum follicle-stimulating hormone (*FSH*) and luteinizing hormone (*LH*) throughout the human menstrual cycle: Pretreatment with estrogen (*E*) plus progesterone (*P*) ensures dissociation between basal FSH and LH levels, resulting in perimenstrual rise of FSH

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

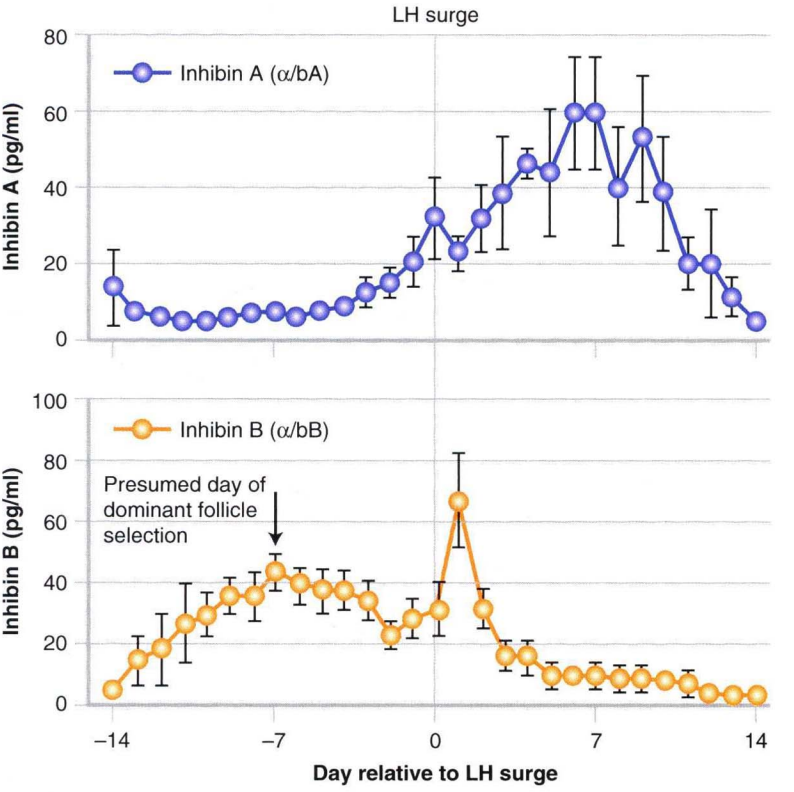

Fig. 1.5 Peripheral blood concentrations of inhibin A (Inh-A) and inhibin B (Inh-B) during human menstrual cycle: Inh-B increases to reach the highest level around on D-7 in the mid-follicular phase, the day when descending FSH curve just crosses with ascending LH curve, signifying the day for dominant 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