Regulatory Principles of Follicular Development

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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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G.N. Allahbadia, Y. Morimoto (eds.), *Ovarian Stimulation Protocols*,
DOI 10.1007/978-81-322-1121-1_1

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

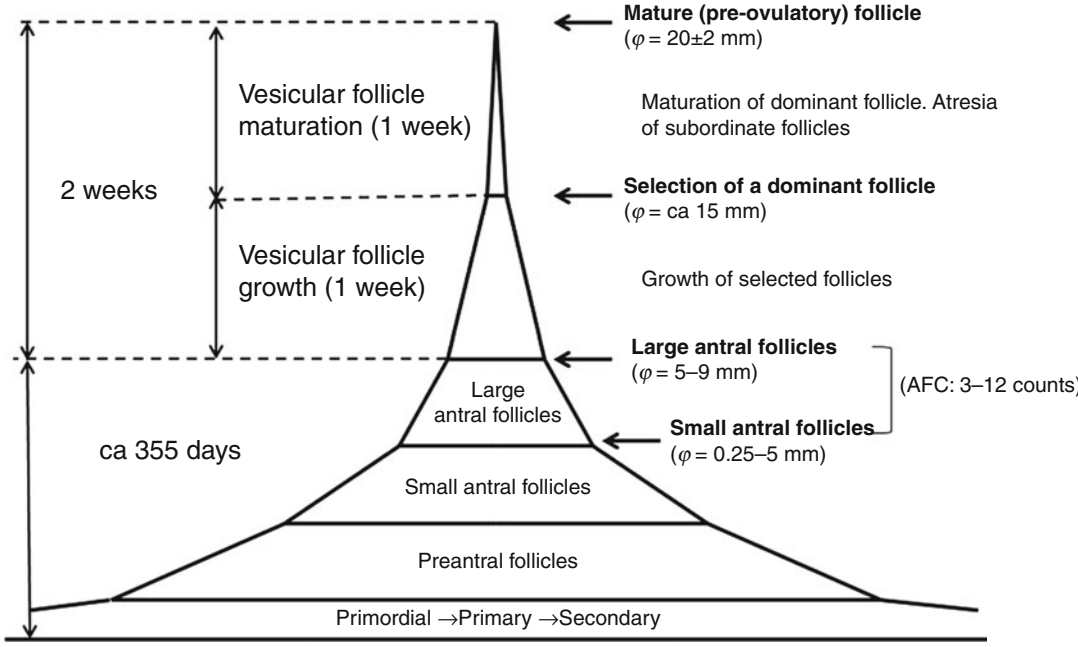

Fig. 1.1 Pyramid-imitative shape of dominant follicle selection in the human ovary. Since terminology and size of each stage of follicles are not uniformly standardized among authors, these are arbitrarily defined by the author (Reproduced with permission from Mori T et al. *Horm Front Gynec*. 2009)

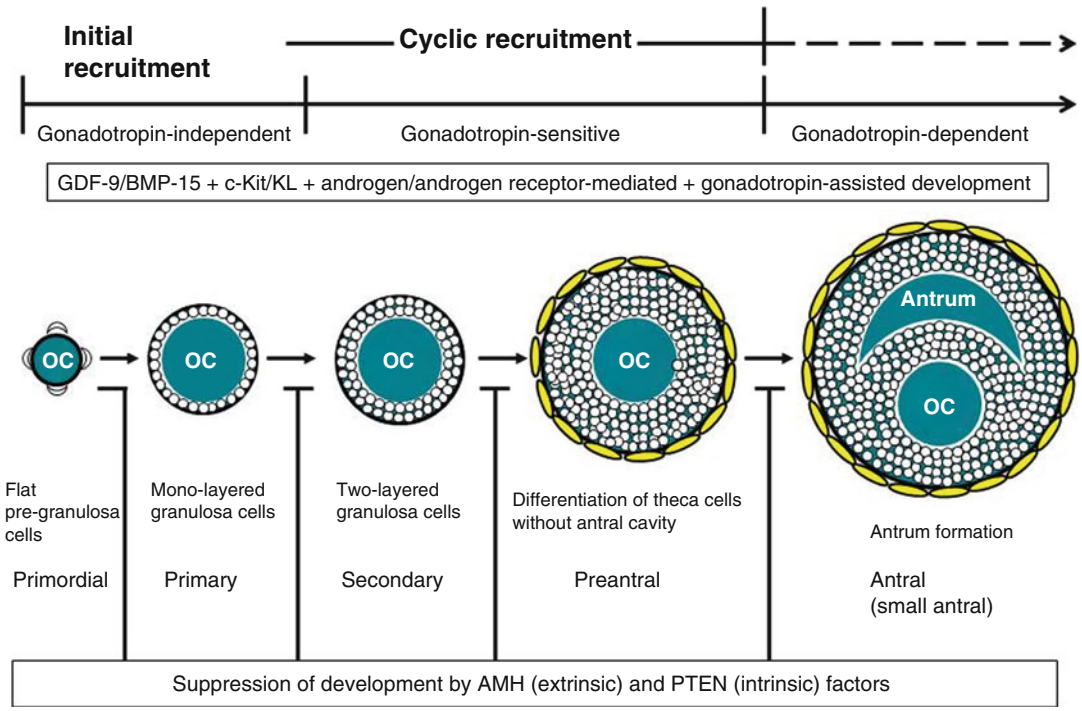

Fig. 1.2 Regulation of preantral follicle growth by stimulatory and inhibitory factors working at different stages of the preantral follicular development (Reproduced with permission from Mori T et al. *Horm Front Gynec*. 2009)

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

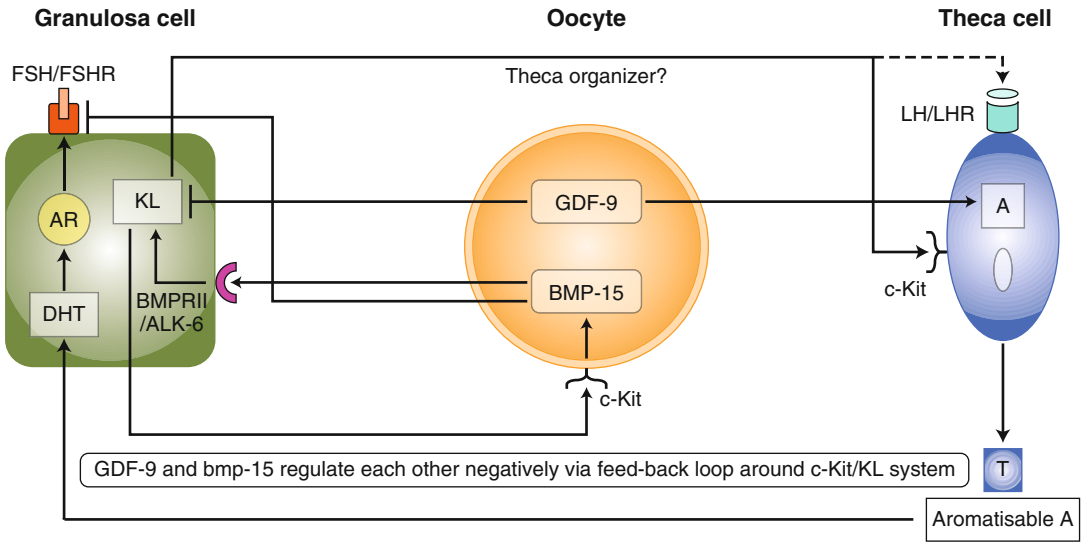

Fig. 1.3 Regulatory interplay of oocyte-originated GDF-9 and BMP-15 among oocytes and granulosa and theca cells for preantral follicle development. Testosterone converts to non-aromatizable androgen, 5α-dihydrotestosterone (5α-DHT) by 5α-reductase for acquisition of binding capacity to receptors for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

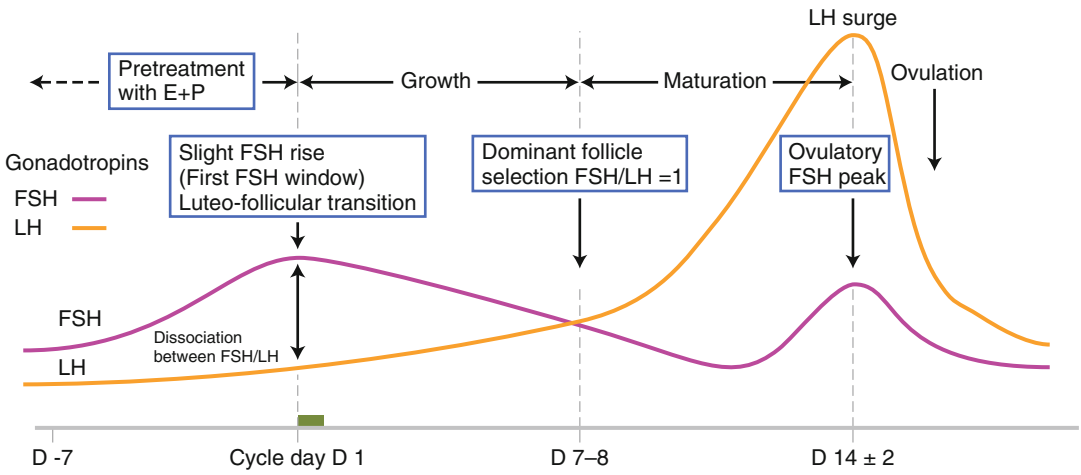

Fig. 1.4 Changes in serum follicle-stimulating hormone (*FSH*) and luteinizing hormone (*LH*) throughout the human menstrual cycle: Pretreatment with estrogen (*E*) plus progesterone (*P*) ensures dissociation between basal FSH and LH levels, resulting in perimenstrual rise of FSH

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

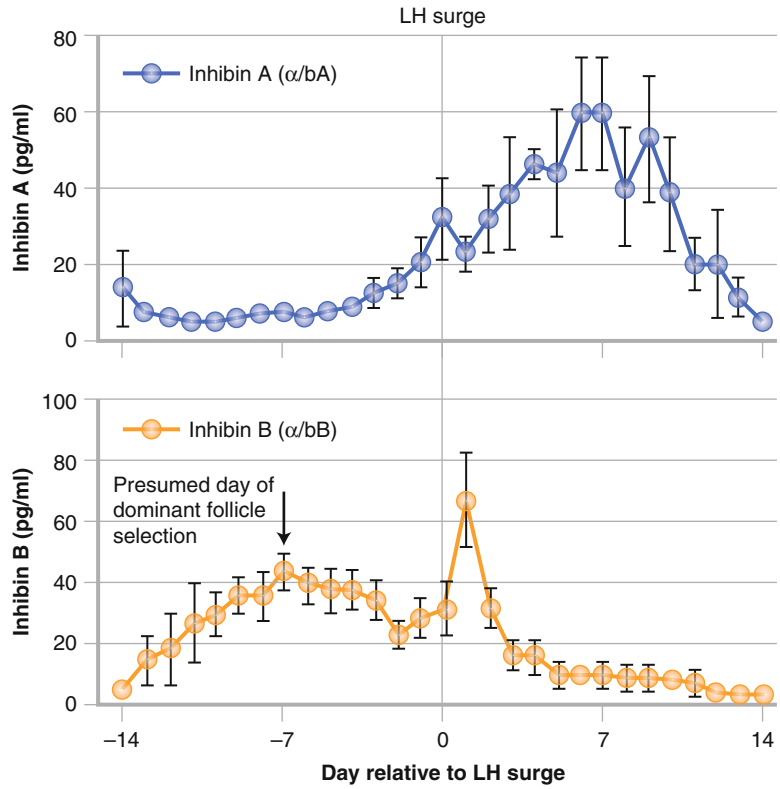

Fig. 1.5 Peripheral blood concentrations of inhibin A (Inh-A) and inhibin B (Inh-B) during human menstrual cycle: Inh-B increases to reach the highest level around on D-7 in the mid-follicular phase, the day when descending FSH curve just crosses with ascending LH curve, signifying the day for dominant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

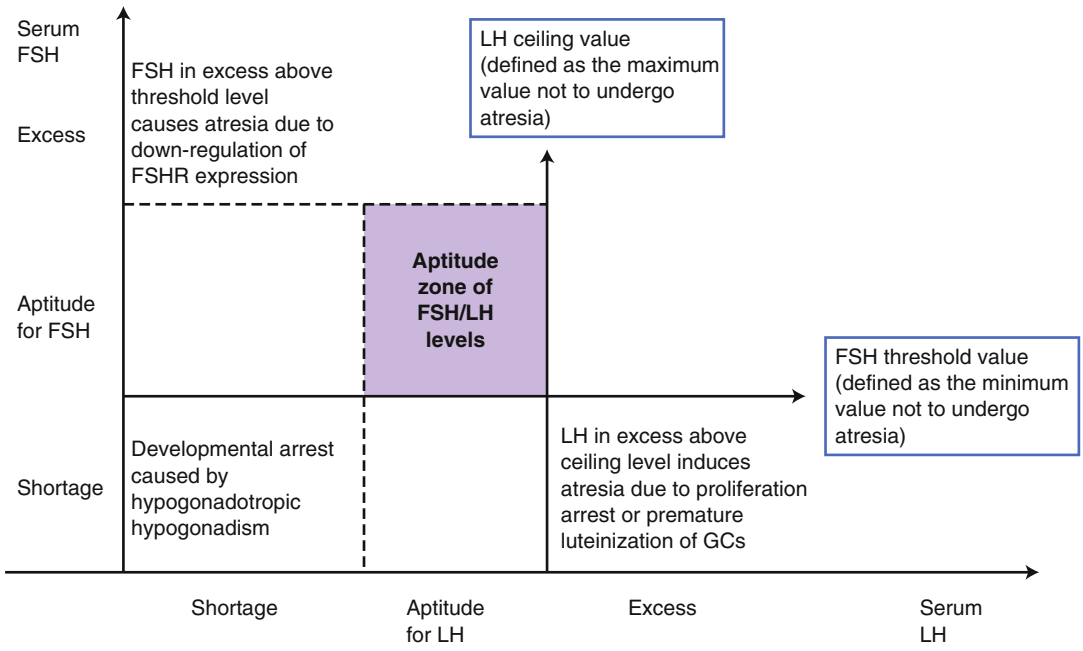

Fig. 1.6 Aptitude zone of FSH and LH levels for dominant follicle selection and subsequent maturation until preovulatory stage: Depicted by the author based on FSH threshold theory (Brown [18]) and LH ceiling hypothesis (Hillier SG 1992)

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.

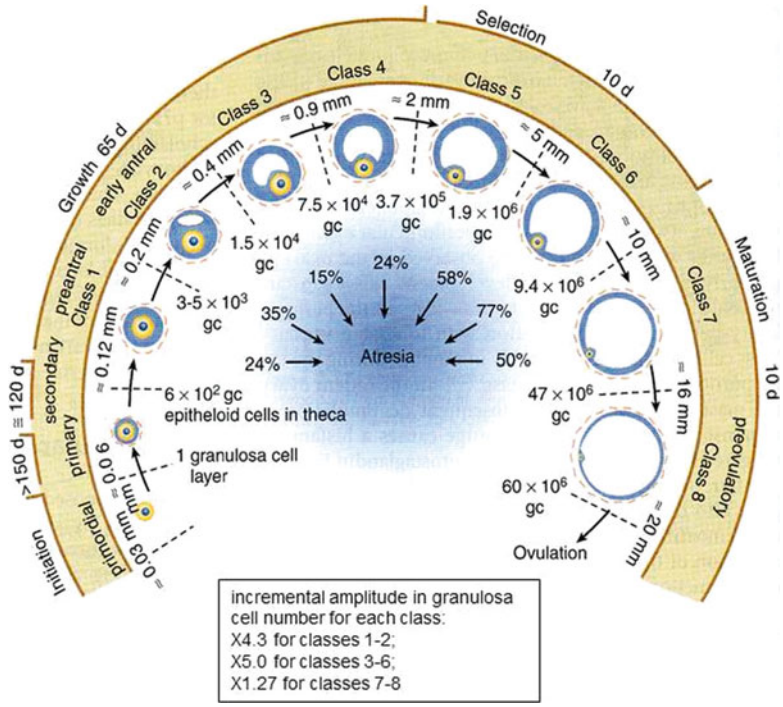

Fig. 1.7 Stages of follicular development in the adult human ovary and extent of atresia in the eight classes of growing follicles (Reproduced with permission from Gougeon [30]). Chapter author (T. Mori) notifies that incremental amplitude in granulosa cell number for each class is ×4.3 for classes 1–2, ×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.

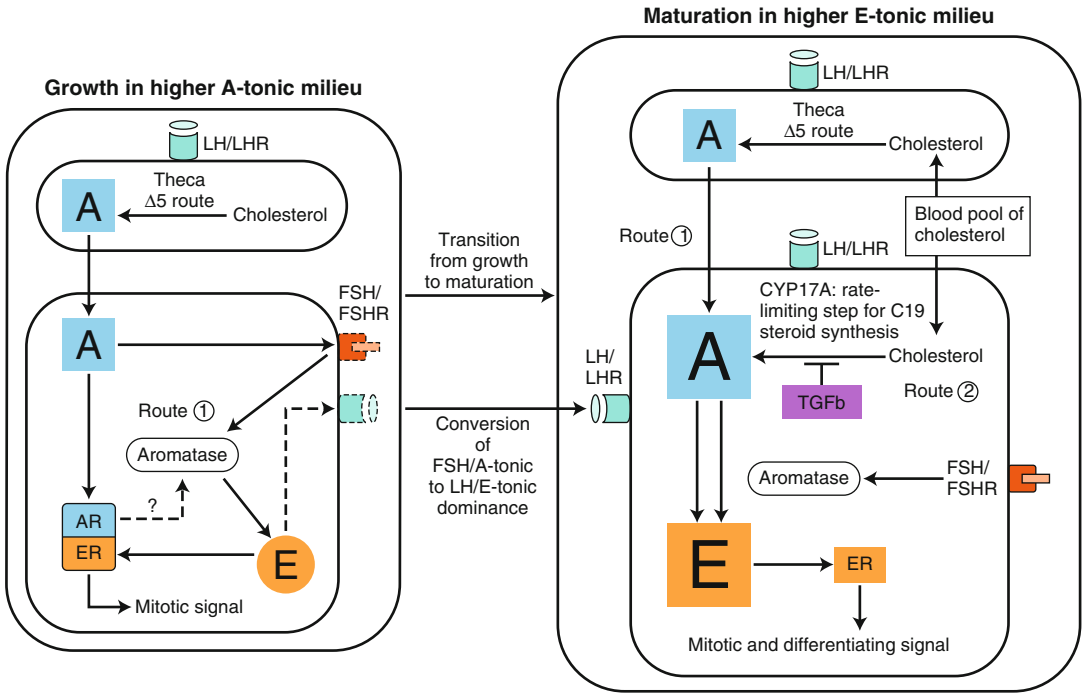

Fig. 1.8 Functional shift in regulatory system of Gn-gonadal steroids during transition from growing to maturing follicle development. While FSH/androgen system controls predominantly via single route of E synthesis in the stage of follicle growth, FSH + LH/estrogen govern follicle maturation via double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

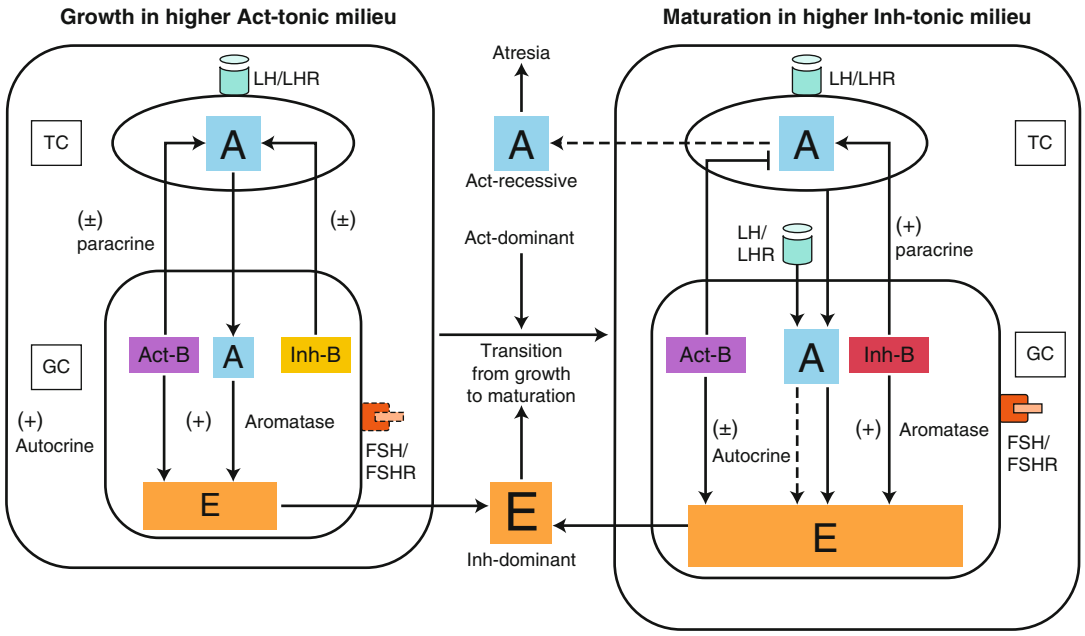

Fig. 1.9 Functional shift in Act-Inh regulatory system during transition from growing to maturing follicle development. Act tonus is relatively higher in antral to small vesicular follicles than in large vesicular follicles. In addition to suppressive action on FSH secretion at the pituitary level, Act-B 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

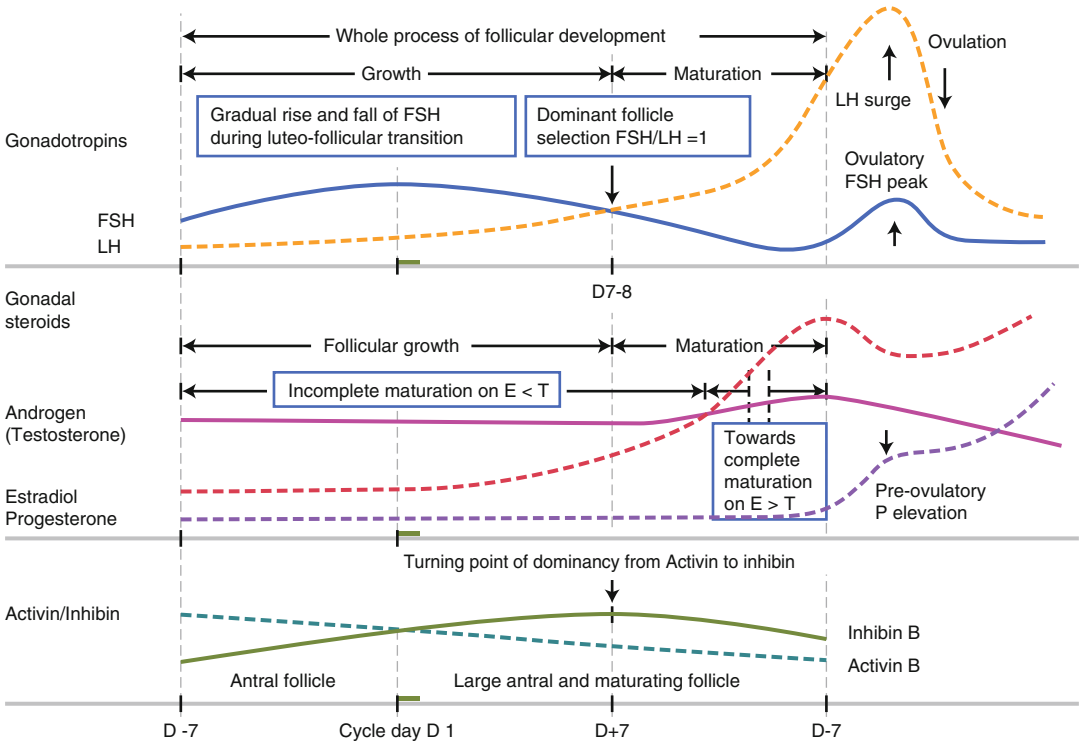

Fig. 1.10 Putative three-dimensional regulations with gonadotropins, gonadal steroids, and activin-inhibin systems of follicle development during the natural menstrual cycle. It is important to notice that α-subunit formation is the rate-limiting step to determine the direction of producing Act and/or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

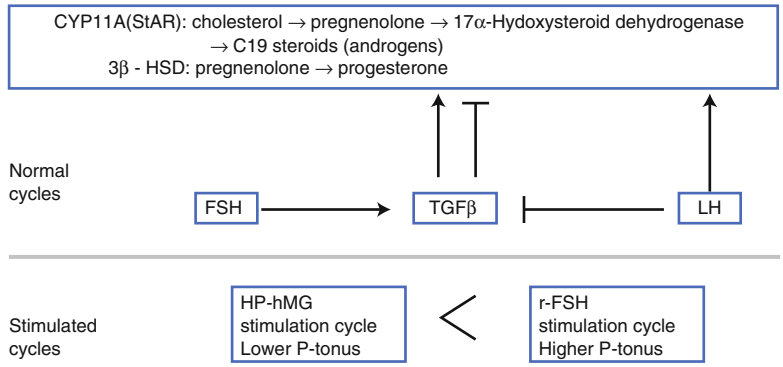

Fig. 1.11 Regulatory mechanism for appearance of preterm P elevation. TGF-β is upregulated by FSH but downregulated by LH in normal cycles, As proven, a limited amount of human chorionic gonadotropin (hCG) is reported to contain as an intrinsic constituent in terms of biological activity in highly purified 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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