CHAPTER 9

Ovarian endocrinology

Frank J Broekmans and Bart CJM Fauser

INTRODUCTION

The ovary in the female adult has a cyclic function that is both autonomous as well as directed by the hypothalamic–pituitary axis. The development of primordial follicles towards the antral stages and the elimination of the vast majority of these developing follicles along the way are fully under control of local factors. It is from the small antral stage of follicular development onwards that pituitary gonadotropin hormones facilitate the menstrual cycle. Dominant follicle growth, ovulation of the oocyte, and corpus luteum formation represent the key processes of the ovarian cycle and much is dependent on the interplay between pituitary gonadotropins, ovarian steroids, and peptides. The ultimate goal of the menstrual cycle is the implantation of a vital embryo in the endometrium that has been prepared under the influence of ovarian steroids.

FUNCTIONAL OVARIAN ANATOMY

The human ovary is an ellipsoid organ with a clear white appearance. On full through-cut the medulla part should be distinguished from the cortex (Figure 9.1). The medulla is continuous with the mesovarium and contains mainly blood vessels and connective tissue. The cortical tissue comprises the largest part of the ovary and contains the stroma in which the follicles are embedded. At the periphery of the ovary the stroma tissue is very dense, giving the ovary its white appearance.

Histologically, the outer lining consists of a flat or cuboidal epithelial cell layer, often erroneously referred to as 'germinal epithelium'. It is capable of expressing the mucus gene MUC1 and the surface cells have cilia and apical microvilli. The surface epithelium plays a role in the transport of substances to and from the peritoneal cavity and in the repair of surface defects, for instance after ovulation. The ovarian stroma contains fibroblastic cells with limited steroidogenic properties and the capability of producing growth factors and growth factor binding proteins. As such, the stroma is involved in the functional separation of follicles and corpora lutea through both physical and biochemical measures[1]. Also, various inflammation cell types are found in the interstitial tissue that are involved in tissue repair after ovulation and the elimination of atretic follicles. The medullary part of the ovary contains the hilar vascular plexus and extrinsic innervation of both sympathetic as well as parasympathetic fibers, responsible for regulation of ovarian blood flow and steroid

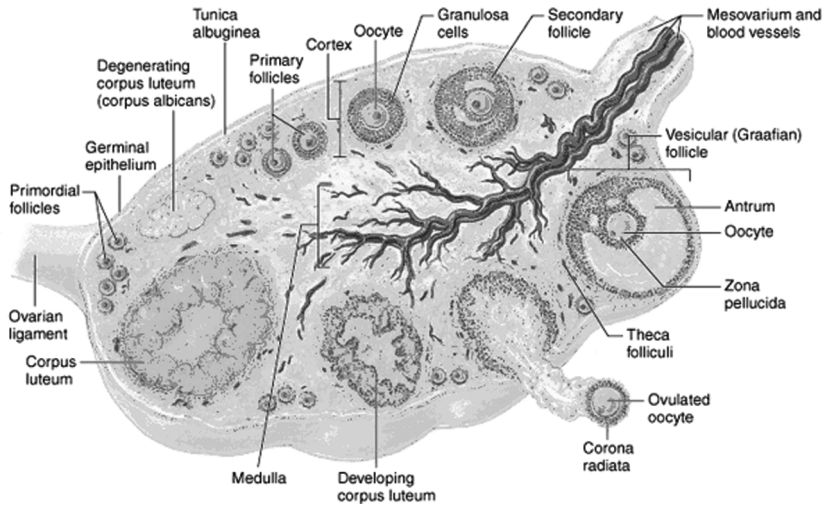

Figure 9.1 Section of the human ovary, showing the anatomic parts and the sequence of developmental stages of the follicles. Reprinted from Animal Reproduction Science, 78, Knight PG and Glister G: Local roles of TGF-β superfamily members in the control of ovarian follicle development, 165–183, 2003, with permission from Elsevier

synthesis of theca-interstitial cells. The cortex of the ovary contains large quantities of primordial follicles. This type of follicle consists of an oocyte surrounded by a single layer of flattened granulosa cells. Once the primordial follicle starts its development towards antral stages (primary follicle), the granulosa cells become cuboidal and an internal theca cell layer is acquired. These cells are elongated and separated from the granulosa cells by a basal membrane. With further expansion in follicle and oocyte size the outer thecal layer is developed by compressing the surrounding stroma, while the granulosa cells form several layers around the oocyte (secondary follicle, Figure 9.2), which by then becomes surrounded by the zona pellucida. The internal thecal layer is cell rich and highly vascularized and arterioles terminate in a network of capillaries at the basal membrane between the theca and granulosa layers.

As the secondary follicle is formed, FSH, androgen, and estrogen receptors develop in the granulosa and LH receptors in the thecal cells. Granulosa cells have no direct blood supply and are dependent on the passage of nutritional and regulatory substances through the basal membrane between the granulosa cell layer and the blood supply of the internal theca. Granulosa cells are interconnected by gap junctions that ensure exchange and transport of small molecules and have cytoplasmic processes through the zona pellucida that contact the plasma mem-

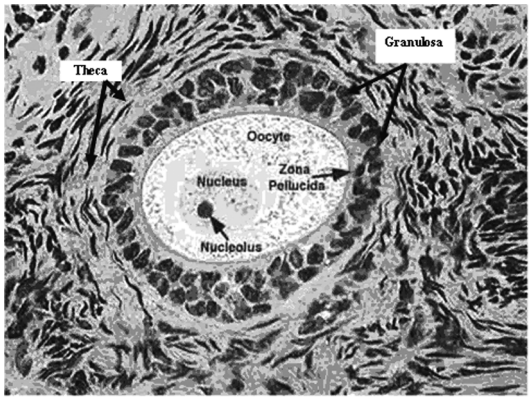

Figure 9.2 Histologic image of a primary human follicle

brane of the oocyte for exchange functions[2–4]. The structural composition of the gap junctions is determined by proteins called connexins, that are encoded for by both the oocyte and granulosa cells.

In its development during the primary and secondary follicle stage the oocyte attains meiotic and developmental competence[5,6]. Meiotic competence refers to the ability to carry out the meiotic divisions with proper chromosome segregation and re-establishment of genomic imprinting. Developmental competence is the ability to support pre-embryo development by remodeling sperm DNA. Once fluid collection takes place between the multiple granulosa cell layers of the secondary follicle it eventually leads to the formation of an antrum. This process requires rapid influx of water, enabled by active ion transport by granulosa cells into the developing antrum, thereby increasing osmolarity. The antrum and its fluid are thought to enable the release of the cumulus–oocyte complex from the ruptured follicle at ovulation and to play a role in nutrient and waste exchange for the avascular granulosa layer. Granulosa cells from the antral wall are called mural cells and express the greatest steroidogenic activity and the highest level of LH receptors. The cells surrounding the oocyte are named cumulus cells and have low LH responding properties[7], while being active in producing the extracellular matrix that is necessary for the preovulatory expansion of the cumulus–oocyte complex (Figure 9.3).

The final stage of antral follicle development is the Graafian follicle, with a diameter of 15–25 mm, which rapidly increases its size through increased accumulation of fluid and granulosa cell proliferation. At ovulation the ovum is released from this follicle after having resumed meiosis and the granulosa and theca cells will differentiate into luteinized cells under the influence of the LH surge. Thereby follicular remnants remain endocrinally active as the corpus luteum, a highly vascularized structure which will regress into a corpus albicans some

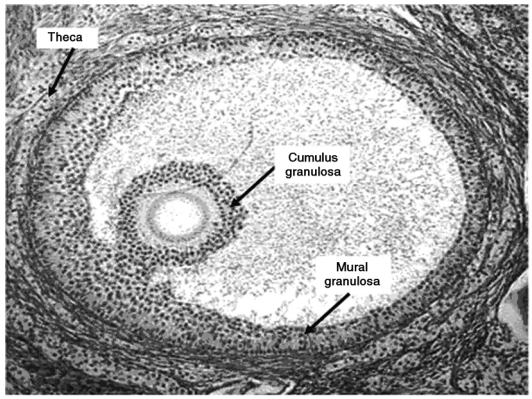

Figure 9.3 Histologic structure of the large antral follicle

9–11 days after ovulation, unless the exposure to human chorionic gonadotropin (hCG) released from an implanted blastocyst prevents this.

FOLLICLE DEVELOPMENT

Initial recruitment

In the third week of fetal development germ cells arise under the influence of transforming growth factor beta (TGF-β) superfamily members (bone morphogenetic proteins, BMPs)[8] in the yolk sac. From there the primordial germ cells migrate along the hindgut into the genital ridges, where they arrive at 6 weeks postfertilization[9] and start to proliferate. Migration and proliferation are under the control of kit ligand, integrin β, TIAR-1 and the Pog (proliferation of germ cells) gene, and leukemia inhibiting factor (LIF)[10–13]. At the fourth month of fetal development the ovaries contain some 6–7 million oogonia that develop into oocytes by entering the first meiotic division, after which they become arrested at the diplotene stage of the prophase[14,15]. Failure to enter meiosis will inevitably lead to apoptosis of the oogonium. Oocytes are then surrounded by a layer of flat granulosa cells to form primordial follicles. In humans, oocytes remain in this resting phase

for many decades until resumption of meiosis is effected by exposure to the mid cycle LH peak. Through a steady flow of primordial follicles and non-meiotic oogonia into apoptosis or atresia, mediated by a deficiency in survival factors like kit ligand and LIF or cell death inducers like TGF-β and activin, at birth 1–2 million primordial follicles are left[16,17]. After birth the rate of loss of follicles slows down so that at menarche some 3 to 4 hundred thousands are left[18]. During the reproductive years the loss of primordial follicles remains steady at some thousand follicles per month and is likely to accelerate after the age of 37 until the ovaries have become devoid of any follicles around the menopause.

Of the 1–2 million follicles present at birth, only some 400 will eventually develop into an ovulating dominant follicle (Figure 9.4). The remaining follicles will undergo atresia in the course of postnatal life due to a process referred to as apoptosis (programmed cell death[20]). Atresia occurs at almost every stage of follicle development, but not in postnatal primordial and dominant follicles. The development from the primordial follicle stage up till the moment of ovulation may take at least 6–8 months[21]. However, as in the fetal period, the vast majority of primordial follicles will never reach the stage of dominance and ovulation, but will undergo apoptosis or cell necrosis (Figure 9.5). It is proposed that follicle development before the antral stages is independent of FSH exposure and is regulated by intraovarian factors. As the normal fate of primordial follicles is programmed cell death, the 400 or so follicles that will reach full maturation and ovulation are rescued by processes that are principally dependent on gonadotropins.

Cyclic recruitment

From the size of 2 mm onwards, antral follicles gain FSH sensitivity as a result of increasing numbers of membrane receptors on their granulosa cells. Up to a follicle diameter of 5 mm only very

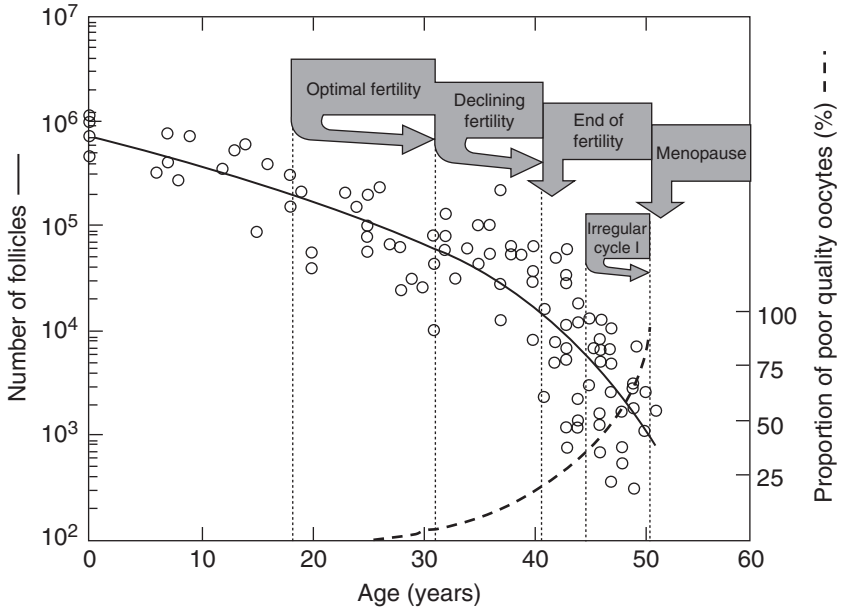

Figure 9.4 The decline in follicle number in relation to reproductive events with increasing female age. Reprinted with permission from Klinkert[19]

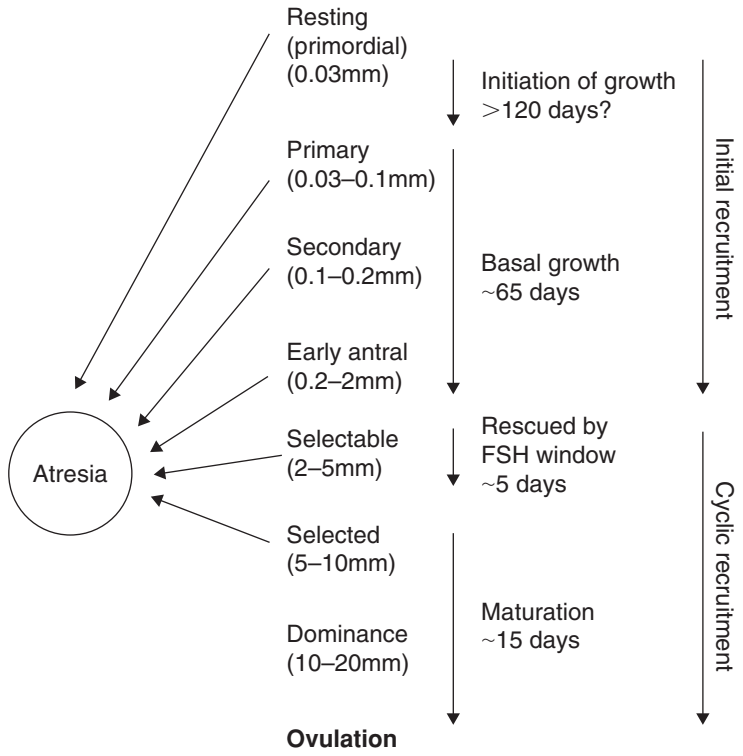

Figure 9.5 Classification and development of follicles in the human ovary. Reprinted and adapted with permission from te Velde and Pearson[22]

small amounts of gonadotropins are sufficient for follicle development[23,24]. For development into a dominant preovulatory follicle, exposure to higher levels of FSH is necessary. During that development, which will take about 2 weeks (Figure 9.6), the follicle will increase in size from 5 to about 20 to 25 mm just before ovulation[25]. Although the number of follicles that are present in the ovary in the small antral stage (2 to 5 mm) can amount to 20 to 25, only one follicle is selected to become the dominant follicle that will subsequently ovulate. The granulosa cells of this follicle have a high mitotic index and the follicular fluid contains FSH and estradiol. The mechanism underlying this single dominant follicle selection has become known as the threshold/window concept.

The demise of the corpus luteum at the end of the previous menstrual cycle and the resulting decrease in estradiol and inhibin A levels[26,27] cause FSH levels to rise at the end of the menstrual cycle[28]. By exceeding a certain threshold level[29,30], the cohort of FSH-sensitive antral follicles present at that time will start to grow and are thereby initially rescued from atresia. Rising FSH levels will, however, soon become depressed by negative feedback from estradiol[31] and inhibin B[32] produced by the cohort of developing antral follicles (Figure 9.7). Decreasing FSH levels provide the occurrence of a window or time period in which the FSH threshold of the individual follicles of the FSH sensitive cohort is exceeded[33,34]. The length of the time window and the hierarchy of FSH sensitivity of the various

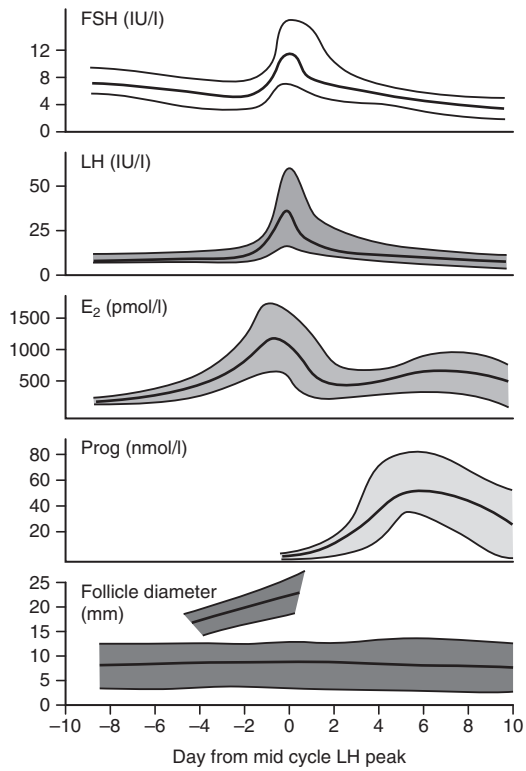

Figure 9.6 Endocrine fluctuations and follicle growth in the menstrual cycle. Reprinted with permission from Macklon and Fauser[39]

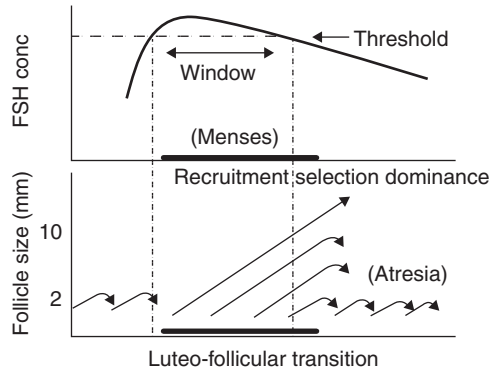

Figure 9.7 The FSH window/threshold concept for dominant follicle selection. Reprinted with permission from Macklon and Fauser[35]

follicles in the cohort will determine the number of follicles that are allowed to begin preovulatory development (dominant follicle growth). The single dominant follicle will gradually diminish its dependency on FSH and will start to produce rapidly increasing amounts of estradiol. The rising estradiol levels will subsequently further suppress the FSH plasma concentration, making dominant growth impossible for the other participants in the stimulated cohort[35,36]. Yet another mechanism whereby the dominant follicle escapes from becoming atretic is that the granulosa cells acquire LH receptors. Consequently, in addition to FSH, LH can support growth and differentiation of the dominant follicle and recent studies have shown that growth of the dominant follicle can be completed under the influence of LH alone[37,38].

The exponential rise in estradiol levels triggers the LH/FSH surge. As a result, resumption of meiosis in the oocyte, ovulation, and luteinization of the granulosa are established. The LH-induced synthesis of progesterone is believed to play an important role in the mechanism of follicle rupture. Enzymes that degrade the follicle wall, like plasminogen activators, the matrix metalloproteinase family members, and cadhepsin L, are expressed in granulosa cells under the influence of progesterone. Progesterone receptors induced in the granulosa cells by the LH surge are part of this autocrine loop phenomenon[40,41]. The end result of follicle wall degradation is the protrusion of the follicle through the ovarian capsule and the rupture and gentle release of the egg and follicular fluid. At this stage the expansion of the cumulus oophorus is a crucial process, enabling oocyte release from the follicle. LH-induced prostaglandin biosynthesis by COX/2 enzyme activity in granulosa cells is believed to play a role in the cumulus expansion[42]. Moreover, cumulus expansion results from LH-induced synthesis of hyaluronic acid[43].

Corpus luteum formation implies that a wide vascular network is formed which facilitates the delivery of precursors for steroid production and

release of secretory products into the circulation. Vascularization is enabled by angiogenic factors like VEGF and FGF, produced by granulosa cells in response to the LH stimulus of the mid cycle surge. The luteinization of granulosa cells includes a loss of their mitotic potential and expression of genes that encode for enzymes involved in progesterone synthesis, like StAR, P450scc, and 3β-hydroxysteroid dehydrogenase. Granulosa-lutein cells therefore mainly deliver progesterone, but through continued expression of aromatase they also remain the source of estradiol production. Luteinized theca cells, through the 17α-hydroxylase/17–20 lyase enzyme, continue to produce androgen precursors, but also 17α-hydroxyprogesterone. Luteal function is principally maintained by exposure to LH pulses that coincide with fluctuations in progesterone levels[44], while the increasing presence of LH/hCG receptors in the course of the luteal phase enables a steady rise in progesterone levels. Progesterone itself is believed to support its own production in an autocrine fashion[45]. After the mid luteal progesterone peak the intrinsic life cycle of the corpus luteum inevitably results in its physical and functional demise. The mechanism of luteolysis, leading to the formation of a scarring tissue zone known as the corpus albicans, is poorly understood[46]. Reduced LH signaling efficiency, possibly related to the presence of prostaglandin F2α, results in a fall in the presence of steroidogenic enzymes with a drop in progesterone release. Also, apoptosis and autophagia triggered by cytokines of the TNF-α superfamily and interferon-γ will imply structural destruction of the steroidogenic cells. Luteolytic substances like TNF-α, endothelin-1, and MCP-1 are believed to alter endothelial function, with vascular damage and reduced perfusion of the corpus luteum as the consequence.

The emergence of hCG from an implanted embryo will stimulate steroidogenesis and prevent the programmed structural and functional demise of the corpus luteum. In contrast, under the influence of rising hCG, the corpus luteum will show hypertrophy of the luteinized granulosa and theca cells and further expansion of the vascular network. Rescue of the corpus luteum by hCG will lead to increased levels of progesterone, inhibin A, and relaxin at the end of the luteal phase and into the first weeks of pregnancy.

GONADOTROPIN CONTROL OF CYCLIC FOLLICULAR RECRUITMENT

FSH and LH are heterodimer glycoproteins composed of a common α subunit and a specific β subunit. Release of FSH and LH from the anterior pituitary cells is primarily directed by the secretion of gonadotropin-releasing hormone (GnRH) from autonomic nuclei located in the medial basal and ventral hypothalamus. The pulsatile release of GnRH into the pituitary stalk portal vein system ensures the synthesis and pulsatile release of LH and FSH from the pituitary. FSH release seems to have an additional autonomic component and has a relatively high plasma half life, resulting in the pulsatile pattern of FSH in the plasma being far less clear. Typically, the frequency of gonadotropin pulses is rather high in the follicular phase with relatively low amplitudes, while in the luteal phase pulse frequency slows down to every few hours with a clear increase in the amplitude.

Specificity of the interaction between hormone and receptor is regulated due to the presence of the β subunit. The human LH and FSH receptors are glycoproteins that belong to the family of G-protein-coupled transmembrane receptors and are encoded for by genes located on chromosome 2p21. Through the linkage to G proteins located in the inner part of the cell membrane, coupling to the intracellular effector system is ensured. In addition to the cyclic AMP pathway, calcium channels, protein kinase B and C, and mitogen activated kinase are believed to play a role in transforming the gonadotropin signal into specific cell function[47]. According to the two-cell two-gonadotropin theory, LH

receptors are primarily present at the membranes of the internal theca cells, while the FSH receptor is expressed by granulosa cells (Figure 9.8). FSH receptor presence is attained in the early stage of antral follicle formation and FSH exposure in growing antral follicles will increase the number of FSH receptors, resulting in a feed forward system. In the course of an antral follicle obtaining dominance, FSH-induced LH receptors also come to expression at the granulosa cell membrane, allowing LH to partially take over FSH actions and ensuring responsiveness of the preovulatory follicle to the mid cycle LH surge[48].

The action of FSH at the level of the granulosa cell comprises enhancement of granulosa cell proliferation and differentiation, stimulation of the aromatase enzyme system that is responsible for the conversion of thecal

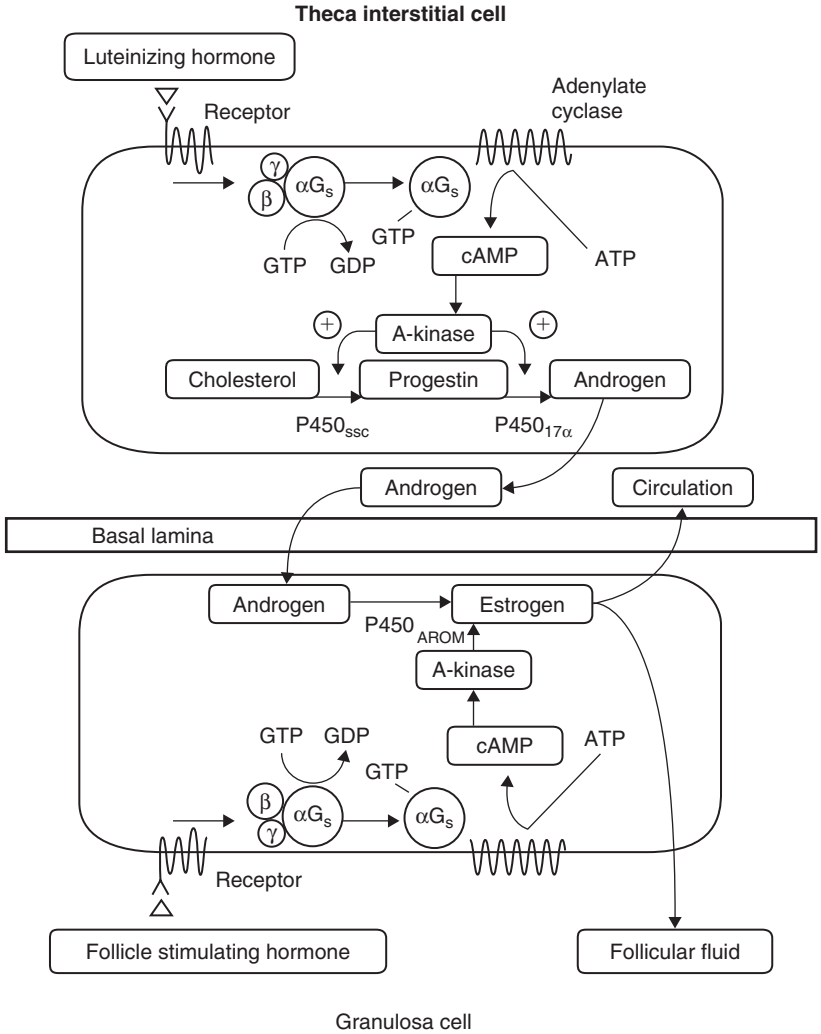

Figure 9.8 The two-cell two-gonadotropin concept [www.endotext.org/female/female1/figures1/figure19-gif]

androgens into estrogens, formation and development of the follicle antrum, maturation of the oocyte, presumably mediated through the function of the granulosa cells in the cumulus oophorus, as well as completion of the first meiotic division by the oocyte up to meiosis II metaphase stage.

The role of LH in the later stages of antral follicle development is mainly related to its effect on estradiol biosynthesis by regulating the production of androgens in the internal theca cells. LH also plays a role in supporting FSH in selection and regulation of the final growth of the dominant follicle[37,38]. In the course of dominant follicle growth only small amounts of LH are necessary for proper follicle function and high LH levels are believed to induce premature luteinization and atresia[49,50]. Finally, LH is uniquely essential for the process of ovulation of the dominant follicle and the resumption of meiotic division of the oocyte. Parallel to the process of ovulation, stimulation of the LH receptors at the granulosa level forces the granulosa cells to convert from an estradiol-producing unit into luteinized cells that produce both estradiol and progesterone. Corpus luteum function survival is dependent on the release of gonadotropins. However, in the absence of hCG release from an implanted blastocyst the corpus luteum will eventually regress in the late luteal phase. The subsequent fall in progesterone, estradiol, and inhibin A levels allows the levels of FSH to rise at the luteo-follicular transition, which induces the subsequent selection of a new cohort that delivers the dominant follicle for the upcoming cycle.

OVARIAN STEROID AND PROTEIN SYNTHESIS

Steroid hormone synthesis starts with the acquisition and storage of cholesterol by the steroid-producing theca cells. The enzyme complex cytochrome P450 side chain cleavage (P450-SSC) is responsible for the transformation of cholesterol into pregnenolone (Figure 9.9). For the rapid response to tropic hormones (especially LH) another enzyme, the steroid acute regulatory protein (StAR), is held responsible. Pregnenolone is further metabolized under the influence of two enzyme systems: the cytochrome P450-C17 and the 3β-hydroxy steroid dehydrogenase enzyme (3β-HSD). P450-C17 enables the transition of progestagens into androgens, first by allowing pregnenolone to change into 17OH-pregnenolone by hydroxylation (P450-17α-hydroxylase) and then by changing 17OH-pregnenolone into dehydroepiandrosterone (DHEA) through P450-17,20-lyase activity. The 3β-HSD enzyme converts the delta-5 steroids pregnenolone, 17OH-pregnenolone, and DHEA into the delta-4 steroids progesterone, 17OH-progesterone, and androstenedione, respectively. The 17β variant of HSD transforms androstenedione into testosterone. Finally, it is the CYP19 aromatase enzyme in the granulosa cells that converts C19 androgenic steroids into estradiol. This implies that granulosa cells are not capable of estradiol synthesis de novo, but fully rely upon androgen substrate production from the theca cells. The activity of the aromatase system is dependent on FSH exposure and, in the late proliferative phase, also on LH. Estradiol levels within the follicular fluid are extremely high. Through diffusion, estradiol reaches the blood circulation. Transport of steroids towards the effector organs is mainly through the blood system. In plasma they are bound to sex hormone binding globulin (SHBG) and albumin. Only in minor quantities do they circulate freely. Synthesis and release of SHBG from the liver is enhanced by estrogens and insulin, but decreases through the action of androgens. Steroid hormones will pass the cell membrane of the effector cell and bind to steroid receptors located at the cell nucleus. Steroid receptors are part of the superfamily of ligand-modified transcription factors that are responsible for growth, differentiation, and homeostasis.

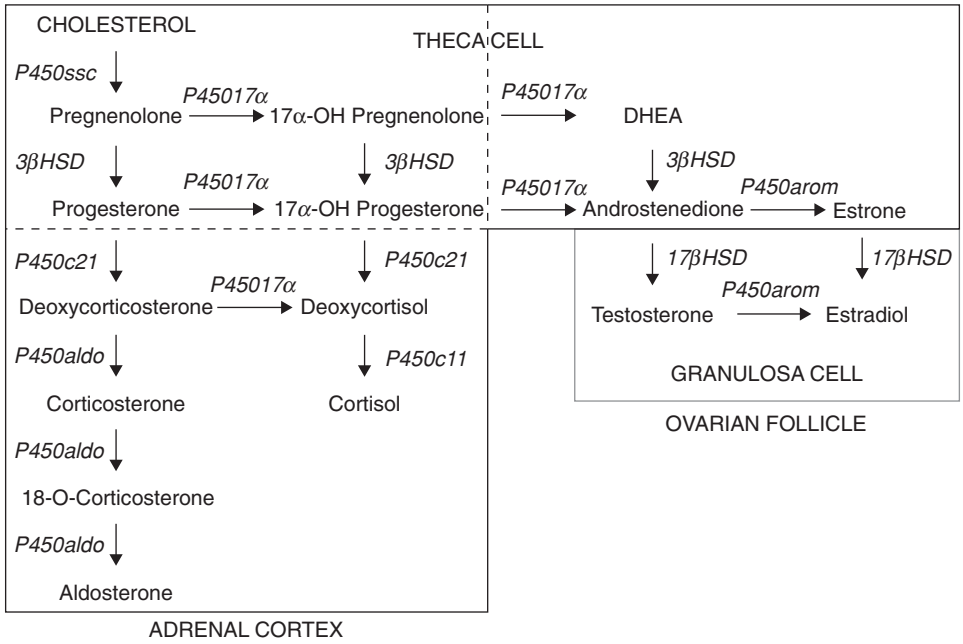

Figure 9.9 Steroid synthesis in the adrenal and the ovarian cortex

Apart from the classic target organs for steroid hormones like the endometrial tissue, cervix, fallopian tube, breast, bone, and brain, steroids also elicit effects in the ovary itself.

Progesterone synthesis from granulosa-lutein cells after ovulation is ensured by expression of the enzymes StAR, P450scc, and 3β-HSD (see Figure 9.9). Progesterone enhances corpus luteum function and induces development of the endometrium into the secretory stage.

Inhibins (A and B) are members of the TGF-β protein superfamily, with a common α-subunit but different β-subunits. The source of inhibin production is the granulosa cell. Inhibin B is secreted mainly from small antral follicles in the early follicular phase and levels fall to undetectable after the mid cycle gonadotropin surge. Inhibin A is low at the start of the cycle, but rises with dominant follicle growth into the preovulatory phase and will remain high until the mid luteal phase.

INTRAOVARIAN MODULATORS OF FOLLICLE DEVELOPMENT

The development of the primordial follicle up to the stage of ovulation and corpus luteum formation is, apart from extraovarian hormones like FSH and LH, regulated and controlled by a large number of para- and autocrine factors produced by granulosa cells, theca cells, and the oocyte itself. Most of these modulators are members of the superfamily of the TGF-β system[51], the insulin-like growth factor (IGF) system[52], and the epidermal growth factor (EGF) system[53,54], but ovarian steroid and protein hormones also play a role[55,56]. Intraovarian regulatory systems have not been elucidated to such an extent that there is full understanding of how primordial follicles eventually develop into the preovulatory follicle. Therefore in this section the role of intraovarian regulators will be discussed only briefly (Figure 9.10).

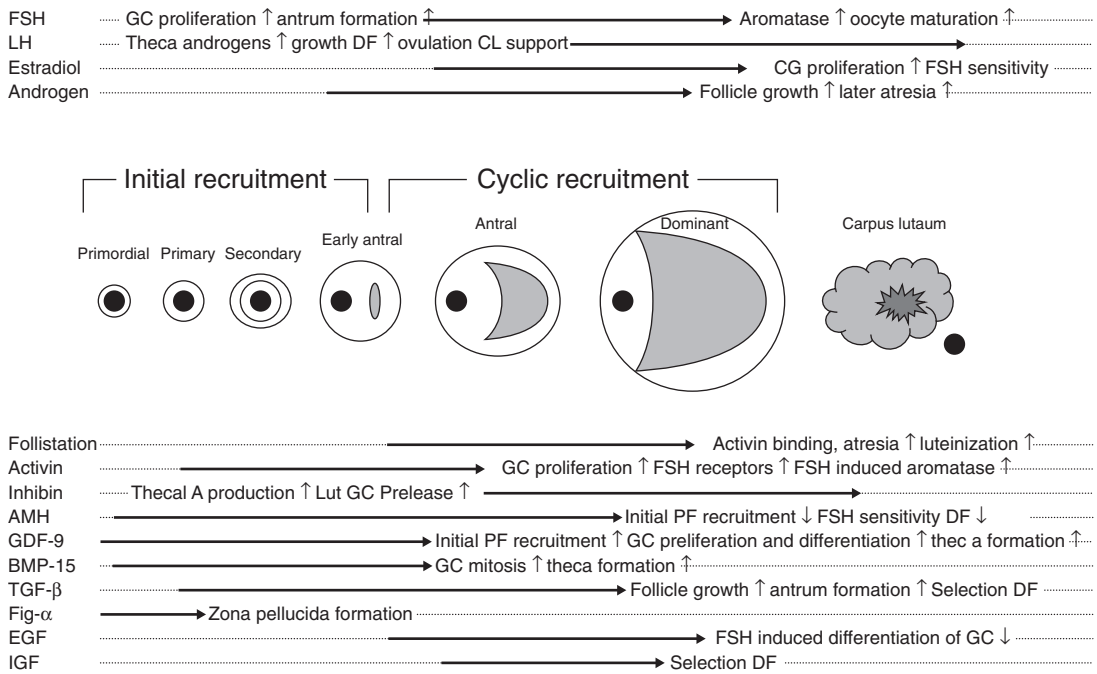

Figure 9.10 The role of intraovarian regulators in initial and cyclic recruitment of follicles. Bold arrows indicate the phase of development in which the modulator has (putative) action. Modified with permission from Knight and Glister[60] GC, Granulosa cell; DF, Dominant follicle; PF, Primordial follicle; Lut GC, Luteinized granulosa cells; CL, Corpus luteum

FSH is not necessary for the transition of primordial follicles into growing follicles as these follicles do not express FSH and LH receptors, and in FSH knockout mice the ovaries still contain growing follicles[57–59]. Still, LH and FSH regulation of early and late antral follicles may have indirect effects on the behavior of the primordial follicle pool, possibly through production by the antral follicles of one or more factors that affect the primordial pool. The transition of primordial follicles into growing follicles is a largely autonomous process in which growth differentiation factor-9 (GDF-9) and the bone morphogenetic protein 15 (BMP-15) are involved. GDF-9 is produced by the oocyte and considered an obligatory signal for further growth beyond the primordial stages[60,61]. It acts by promoting granulosa cell proliferation and differentiation and by enabling the formation of a thecal cell layer in primary follicles[62,63]. The thecal cell layer develops from primary interstitial cells present in the fetal ovary under the influence of oocyte-derived GDF-9 and kit ligand produced by granulosa cells[64,65]. Recombinant GDF-9 has also been shown to stimulate initial follicle recruitment in vivo[66]. BMP-15 plays a comparably essential role in early follicular growth by stimulating granulosa cell mitosis and initiation of theca cell layer formation[67–70]. Zona pellucida (ZP) formation is regulated by oocyte-specific genes that encode for a number of ZP proteins. These genes are expressed under the control of Fig(factor in the germ line)-α. The zona protects the oocyte and is essential for normal fertilization[71,72]. During further stages of follicle development, oocytes continue to express GDF-9

and BMP-15. Anti-müllerian hormone (AMH), produced by the granulosa cells of the primary follicles, has been shown to have an inhibitory effect on the transition from primordial into developing follicles and as such has a functional counteraction to GDF-9. In the absence of AMH, as shown in the knockout model, the pool of primordial follicles is reduced at a much higher rate than in the normal situation[73].

After the follicle has developed into a primary and secondary growing follicle, granulosa cells initiate synthesis and release of inhibins, activin, and follistatin. In the early growing phase activins are predominantly produced that enhance granulosa cell proliferation and protect the follicle from becoming atretic[74–76]. In small antral follicles, activins promote the expression of FSH receptors, further assisting the growth of follicles in response to FSH, and support FSH-dependent aromatase activity, as well as reduce the LH-dependent androgen production by theca cells[74]. They also enhance oocyte maturation and as such activins seem to protect the growing follicle from demise and prepare it for its steroid producing functions, while stimulating FSH release from the pituitary. Much of their action becomes counteracted by follistatin, produced from small antral follicles, that selectively binds to activins and neutralizes the follicle development promoting actions of activins. Inhibins are involved in growing follicles from the antral stages onwards. They selectively suppress FSH release from the pituitary, thereby indirectly influencing FSH action on the follicle, and exert a paracrine action on theca cells where they enhance LH-induced androgen secretion[77–79]. As such inhibins play a role in both facilitating steroid synthesis and enabling dominant follicle selection. Activins and inhibins thus have opposing actions, where activins are dominant in the early stages of the growing follicle, while inhibins come into play much more when the follicle has become antral and attained sensitivity to gonadotropins.

TGF-β, produced by both theca and granulosa cells, is believed to play a role that is comparable to activin in promoting follicular growth and may also be involved in antrum formation by interfering with the role of connective tissue growth factor (CTGF) in extracellular matrix modeling and angiogenesis[80,81]. Epidermal growth factor and TGF-α have been shown to be potent inhibitors of FSH-dependent differentiation of granulosa cells.

When follicles have reached the antral stage of development the crucial factor for ongoing development is FSH. FSH action may, in part, become expressed through intermediary factors like steroids and proteins released from granulosa cells. Inhibins are capable of suppressing FSH release from the pituitary, but also enhance LH- and IGF-mediated androgen synthesis from theca cells. Follistatin is another factor that comes to expression in antral follicle stages and is known for its FSH-suppressing ability and binding of activins. The neutralization of activin action by follistatin is important to reduce the suppression of estrogen and progesterone production from granulosa cells in antral and dominant follicles. Estrogens produced from granulosa cells promote their proliferation and have anti-atretic effects, while augmenting intercellular gap junctions and formation of the follicular antrum. The role of androgens in antral follicles is believed to be mainly folliculotropic, as evidenced by the high quantities of androgen receptors in granulosa cells of preantral and antral follicles. In later stages of follicular growth androgens may exert atretogenic effects by interference with the aromatase system. AMH is capable of mitigating the FSH-induced follicular growth of antral follicles. Once the dominant follicle has been selected AMH expression becomes severely reduced, enabling an increase in the FSH effects on this dominant follicle. As such AMH may contribute to dominant follicle selection[82].

The process of dominant follicle selection and growth from the cohort of small antral follicles is believed to be regulated according to

the threshold window concept, as explained earlier. Fine tuning of FSH levels seems to be the most important effector in this regulation. Still, the supposed changes in individual sensitivity for FSH within the cohort of follicles may be exerted by intraovarian paracrine and autocrine factors. Estradiol is believed to enhance the FSH response of the follicle and AMH exerts the opposite effect. In dominant follicles it is the production of these two substances that alters in such a way that the efficiency of the FSH stimulus is upgraded. Insulin-like growth factors and their binding proteins are believed to be the first factors that mark the attainment of dominance[83]. Members of the TGF-β family are known for their effects on steroid synthesis and induction of LH receptors in granulosa cells and growth inhibition of smaller subordinate antral follicles[61]. As such, the TGF and IGF systems may well be part of the integration of extraovarian signals and intrafollicular factors that determine whether a follicle will continue to develop into dominance or be diverted into atretic pathways.

REFERENCES

1. Jabara S, Christenson LK, Wang CY et al. Stromal cells of the human postmenopausal ovary display a distinctive biochemical and molecular phenotype. J Clin Endocrinol Metab 2003; 88: 484–92.
2. Matzuk MM, Burns KH, Viveiros MM, Eppig JJ. Intercellular communication in the mammalian ovary: oocytes carry the conversation. Science 2002; 296: 2178–80.
3. Albertini DF, Combelles CM, Benecchi E, Carabatsos MJ. Cellular basis for paracrine regulation of ovarian follicle development. Reproduction 2001; 121: 647–53.
4. Erickson GF, Shimasaki S. The physiology of folliculogenesis: the role of novel growth factors. Fertil Steril 2001; 76: 943–9.
5. Volarcik K, Sheean L, Goldfarb J et al. The meiotic competence of in-vitro matured human oocytes is influenced by donor age: evidence that folliculogenesis is compromised in the reproductively aged ovary. Hum Reprod 1998; 13: 154–60.
6. McLay DW, Carroll J, Clarke HJ. The ability to develop an activity that transfers histones onto sperm chromatin is acquired with meiotic competence during oocyte growth. Dev Biol 2002; 241: 195–206.
7. Lawrence TS, Dekel N, Beers WH. Binding of human chorionic gonadotropin by rat cumuli oophori and granulosa cells: a comparative study. Endocrinology 1980; 106: 1114–18.
8. Ying Y, Qi X, Zhao GQ. Induction of primordial germ cells from pluripotent epiblast. Sci World J 2002; 2: 801–10.
9. Motta PM, Makabe S, Nottola SA. The ultrastructure of human reproduction. I. The natural history of the female germ cell: origin, migration and differentiation inside the developing ovary. Hum Reprod Update 1997; 3: 281–95.
10. Anderson R, Fassler R, Georges-Labouesse E et al. Mouse primordial germ cells lacking beta1 integrins enter the germline but fail to migrate normally to the gonads. Development 1999; 126: 1655–64.
11. Cheng L, Gearing DP, White LS et al. Role of leukemia inhibitory factor and its receptor in mouse primordial germ cell growth. Development 1994; 120: 3145–53.
12. Beck AR, Miller IJ, Anderson P, Streuli M. RNA-binding protein TIAR is essential for primordial germ cell development. Proc Natl Acad Sci USA 1998; 95: 2331–6.
13. Agoulnik AI, Lu B, Zhu Q, Truong C, Ty MT, Arango N et al. A novel gene, Pog, is necessary for primordial germ cell proliferation in the mouse and underlies the germ cell deficient mutation, gcd. Hum Mol Genet 2002; 11: 3047–53.
14. Byskov AG. Differentiation of mammalian embryonic gonad. Physiol Rev 1986; 66: 71–117.
15. Baker TG. A quantitative and cytological study of germ cells in human ovaries. Proc R Soc Lond B Biol Sci 1963; 158: 417–33.
16. Peters H, Byskov AG, Grinsted J. Follicular growth in fetal and prepubertal ovaries of humans and other primates. Clin Endocrinol Metab 1978; 7: 469–85.

17. Peters H, Himelstein-Braw R, Faber M. The normal development of the ovary in childhood. Acta Endocrinol (Copenh) 1976; 82: 617–30.

18. Hillier SG. Regulation of follicular oestrogen biosynthesis: a survey of current concepts. J Endocrinol 1981; 89(Suppl): 3P–18P.

19. Klinkert ER. Clinical significance and management of poor response in IVF. Academic thesis, Utrecht, 2005.

20. Hsueh AJ, Billig H, Tsafriri A. Ovarian follicle atresia: a hormonally controlled apoptotic process. Endocr Rev 1994; 15: 707–24.

21. Gougeon A. Regulation of ovarian follicular development in primates: facts and hypotheses. Endocr Rev 1996; 17: 121–55.

22. te Velde ER, Pearson PL. The variability of female reproductive ageing. Hum Reprod Update 2002; 8: 141–54.

23. Hillier SG. Current concepts of the roles of follicle stimulating hormone and luteinizing hormone in folliculogenesis. Hum Reprod 1994; 9: 188–91.

24. Govan AD, Black WP. Ovarian morphology in oligomenorrhea. Eur J Obstet Gynecol Reprod Biol 1975; 5: 317–25.

25. Pache TD, Wladimiroff JW, de Jong FH, Hop WC, Fauser BCJM. Growth patterns of nondominant ovarian follicles during the normal menstrual cycle. Fertil Steril 1990; 54: 638–42.

26. Le Nestour E, Marraoui J, Lahlou N et al. Role of estradiol in the rise in follicle-stimulating hormone levels during the luteal–follicular transition. J Clin Endocrinol Metab 1993; 77: 439–42.

27. Roseff SJ, Bangah ML, Kettel LM et al. Dynamic changes in circulating inhibin levels during the luteal–follicular transition of the human menstrual cycle. J Clin Endocrinol Metab 1989; 69: 1033–9.

28. Hall JE, Schoenfeld DA, Martin KA, Crowley WFJ. Hypothalamic gonadotropin-releasing hormone secretion and follicle-stimulating hormone dynamics during the luteal–follicular transition. J Clin Endocrinol Metab 1992; 74: 600–7.

29. Brown JB. Pituitary control of ovarian function – concepts derived from gonadotrophin therapy. Aust NZJ Obstet Gynaecol 1978; 18: 47–54.

30. Schoemaker J, van Weissenbruch MM, Scheele F, van der Meer M. The FSH threshold concept in clinical ovulation induction. In: Evers JLH, ed. Ovulation Induction: The Difficult Patient. Bailliere Tindall, London, 1993: 297–308.

31. Zeleznik AJ, Hutchison JS, Schuler HM. Interference with the gonadotropin-suppressing actions of estradiol in macaques overrides the selection of a single preovulatory follicle. Endocrinology 1985; 117: 991–9.

32. Groome NP, Illingworth PJ, O'Brien M et al. Measurement of dimeric inhibin B throughout the human menstrual cycle. J Clin Endocrinol Metab 1996; 81: 1401–5.

33. Fauser BC, Van Heusden AM. Manipulation of human ovarian function: physiological concepts and clinical consequences. Endocr Rev 1997; 18: 71–106.

34. van Santbrink EJ, Hop WC, van Dessel TJ, de Jong FH, Fauser BC. Decremental follicle-stimulating hormone and dominant follicle development during the normal menstrual cycle. Fertil Steril 1995; 64: 37–43.

35. Macklon NS, Fauser BC. Follicle-stimulating hormone and advanced follicle development in the human. Arch Med Res 2001; 32: 595–600.

36. Schipper I, Hop WC, Fauser BC. The follicle-stimulating hormone (FSH) threshold/window concept examined by different interventions with exogenous FSH during the follicular phase of the normal menstrual cycle: duration, rather than magnitude, of FSH increase affects follicle development. J Clin Endocrinol Metab 1998; 83: 1292–8.

37. Zeleznik AJ. The physiology of follicle selection. Reprod Biol Endocrinol 2004; 2: 31.

38. Filicori M, Cognigni GE, Pocognoli P, Ciampaglia W, Bernardi S. Current concepts and novel applications of LH activity in ovarian stimulation. Trends Endocrinol Metab 2003; 14: 267–73.

39. Macklon N, Fauser BC. Regulation of follicle development and novel approaches to ovarian stimulation for IVF. Hum Reprod Update. 2000; 8: 141–154.

40. Borman SM, Chaffin CL, Schwinof KM, Stouffer RL, Zelinski-Wooten MB. Progesterone promotes

oocyte maturation, but not ovulation, in nonhuman primate follicles without a gonadotropin surge. Biol Reprod 2004; 71: 366–73.

41. Chaffin CL, Stouffer RL. Local role of progesterone in the ovary during the periovulatory interval. Rev Endocr Metab Disord 2002; 3: 65–72.

42. Norman RJ. Reproductive consequences of COX-2 inhibition. Lancet 2001; 358: 1287–88.

43. Zhuo L, Kimata K. Cumulus oophorus extracellular matrix: its construction and regulation. Cell Struct Funct 2001; 26: 189–96.

44. Filicori M, Butler JP, Crowley WF, Jr. Neuroendocrine regulation of the corpus luteum in the human. Evidence for pulsatile progesterone secretion. J Clin Invest 1984; 73: 1638–47.

45. Stouffer RL. Progesterone as a mediator of gonadotrophin action in the corpus luteum: beyond steroidogenesis. Hum Reprod Update 2003; 9: 99–117.

46. Davis JS, Rueda BR. The corpus luteum: an ovarian structure with maternal instincts and suicidal tendencies. Front Biosci 2002; 7: d1949–d1978.

47. Zeleznik AJ, Saxena D, Little-Ihrig L. Protein kinase B is obligatory for follicle-stimulating hormone-induced granulosa cell differentiation. Endocrinology 2003; 144: 3985–94.

48. Sullivan MW, Stewart-Akers A, Krasnow JS, Berga SL, Zeleznik AJ. Ovarian responses in women to recombinant follicle-stimulating hormone and luteinizing hormone (LH): a role for LH in the final stages of follicular maturation. J Clin Endocrinol Metab 1999; 84: 228–32.

49. Shoham Z, Jacobs HS, Insler V. Luteinizing hormone: its role, mechanism of action, and detrimental effects when hypersecreted during the follicular phase. Fertil Steril 1993; 59: 1153–61.

50. Shoham Z. The clinical therapeutic window for luteinizing hormone in controlled ovarian stimulation. Fertil Steril 2002; 77: 1170–7.

51. Findlay JK, Drummond AE, Dyson ML et al. Recruitment and development of the follicle; the roles of the transforming growth factor-beta superfamily. Mol Cell Endocrinol 2002; 191: 35–43.

52. Poretsky L, Cataldo NA, Rosenwaks Z, Giudice LC. The insulin-related ovarian regulatory system in health and disease. Endocr Rev 1999; 20: 535–82.

53. Ashkenazi H, Cao X, Motola S et al. Epidermal growth factor family members: endogenous mediators of the ovulatory response. Endocrinology 2005; 146: 77–84.

54. Park JY, Su YQ, Ariga M et al. EGF-like growth factors as mediators of LH action in the ovulatory follicle. Science 2004; 303: 682–4.

55. Findlay JK, Britt K, Kerr JB et al. The road to ovulation: the role of oestrogens. Reprod Fertil Dev 2001; 13: 543–7.

56. Findlay JK, Drummond AE, Britt KL et al. The roles of activins, inhibins and estrogen in early committed follicles. Mol Cell Endocrinol 2000; 163: 81–7.

57. Kumar TR, Wang Y, Lu N, Matzuk MM. Follicle stimulating hormone is required for ovarian follicle maturation but not male fertility. Nat Genet 1997; 15: 201–4.

58. Rannikki AS, Zhang FP, Huhtaniemi IT. Ontogeny of follicle-stimulating hormone receptor gene expression in the rat testis and ovary. Mol Cell Endocrinol 1995; 107: 199–208.

59. Oktay K, Briggs D, Gosden RG. Ontogeny of follicle-stimulating hormone receptor gene expression in isolated human ovarian follicles. J Clin Endocrinol Metab 1997; 82: 3748–51.

60. Aaltonen J, Laitinen MP, Vuojolainen K et al. Human growth differentiation factor 9 (GDF-9) and its novel homolog GDF-9B are expressed in oocytes during early folliculogenesis. J Clin Endocrinol Metab 1999; 84: 2744–50.

61. Knight PG, Glister C. Local roles of TGF-beta superfamily members in the control of ovarian follicle development. Anim Reprod Sci 2003; 78: 165–83.

62. Eppig JJ. Oocyte control of ovarian follicular development and function in mammals. Reproduction 2001; 122: 829–38.

63. Elvin JA, Yan C, Matzuk MM. Growth differentiation factor-9 stimulates progesterone synthesis in granulosa cells via a prostaglandin E2/EP2 receptor pathway. Proc Natl Acad Sci USA 2000; 97: 10288–93.

64. Erickson GF, Magoffin DA, Dyer CA, Hofeditz C. The ovarian androgen producing cells: a review of structure/function relationships. Endocr Rev 1985; 6: 371–99.

65. Nilsson EE, Skinner MK. Kit ligand and basic fibroblast growth factor interactions in the induction of ovarian primordial to primary follicle transition. Mol Cell Endocrinol 2004; 214: 19–25.

66. Vitt UA, McGee EA, Hayashi M, Hsueh AJ. In vivo treatment with GDF-9 stimulates primordial and primary follicle progression and theca cell marker CYP17 in ovaries of immature rats. Endocrinology 2000; 141: 3814–20.

67. Otsuka F, Yao Z, Lee T et al. Bone morphogenetic protein-15. Identification of target cells and biological functions. J Biol Chem 2000; 275: 39523–8.

68. Otsuka F, Shimasaki S. A novel function of bone morphogenetic protein-15 in the pituitary: selective synthesis and secretion of FSH by gonadotropes. Endocrinology 2002; 143: 4938–41.

69. Moore RK, Otsuka F, Shimasaki S. Molecular basis of bone morphogenetic protein-15 signaling in granulosa cells. J Biol Chem 2003; 278: 304–10.

70. Shimasaki S, Moore RK, Erickson GF, Otsuka F. The role of bone morphogenetic proteins in ovarian function. Reprod Suppl 2003; 61: 323–37.

71. Zhao M, Dean J. The zona pellucida in folliculogenesis, fertilization and early development. Rev Endocr Metab Disord 2002; 3: 19–26.

72. Soyal SM, Amleh A, Dean J. FIGalpha, a germ cell-specific transcription factor required for ovarian follicle formation. Development 2000; 127: 4645–54.

73. Durlinger AL, Kramer P, Karels B et al. Control of primordial follicle recruitment by anti-Mullerian hormone in the mouse ovary. Endocrinology 1999; 140: 5789–96.

74. Zhao J, Taverne MA, van der Weijden GC, Bevers MM, van den HR. Effect of activin A on in vitro development of rat preantral follicles and localization of activin A and activin receptor II. Biol Reprod 2001; 65: 967–77.

75. Phillips DJ. Activins, inhibins and follistatins in the large domestic species. Domest Anim Endocrinol 2005; 28: 1–16.

76. Muttukrishna S, Tannetta D, Groome N, Sargent I. Activin and follistatin in female reproduction. Mol Cell Endocrinol 2004; 225: 45–56.

77. Luisi S, Florio P, Reis FM, Petraglia F. Inhibins in female and male reproductive physiology: role in gametogenesis, conception, implantation and early pregnancy. Hum Reprod Update 2005; 11: 123–35.

78. Laven JS, Fauser BC. Inhibins and adult ovarian function. Mol Cell Endocrinol 2004; 225: 37–44.

79. Cook RW, Thompson TB, Jardetzky TS, Woodruff TK. Molecular biology of inhibin action. Semin Reprod Med 2004; 22: 269–76.

80. Harlow CR, Davidson L, Burns KH et al. FSH and TGF-beta superfamily members regulate granulosa cell connective tissue growth factor gene expression in-vitro and in vivo. Endocrinology 2002; 143: 3316–25.

81. Harlow CR, Hillier SG. Connective tissue growth factor in the ovarian paracrine system. Mol Cell Endocrinol 2002; 187: 23–7.

82. Weenen C, Laven JS, Von Bergh AR et al. Anti-Mullerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. Mol Hum Reprod 2004; 10: 77–83.

83. Fortune JE, Rivera GM, Yang MY. Follicular development: the role of the follicular microenvironment in selection of the dominant follicle. Anim Reprod Sci 2004; 82–83: 109–26.