

Review

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## The physiology of follicle selection

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### Abstract

During the follicular phase of the primate menstrual cycle, a single follicle usually matures to the preovulatory stage and releases its oocyte for fertilization and the potential establishment of pregnancy. In assisted reproductive technology procedures, it is desirable to override the natural process of follicle selection to produce many oocytes that are capable of being fertilized and undergoing normal embryo development. The goal of this chapter is to summarize the current views regarding the natural process of follicle selection in primates and to discuss how this process may be amplified to produce a greater number of oocytes.

### Review

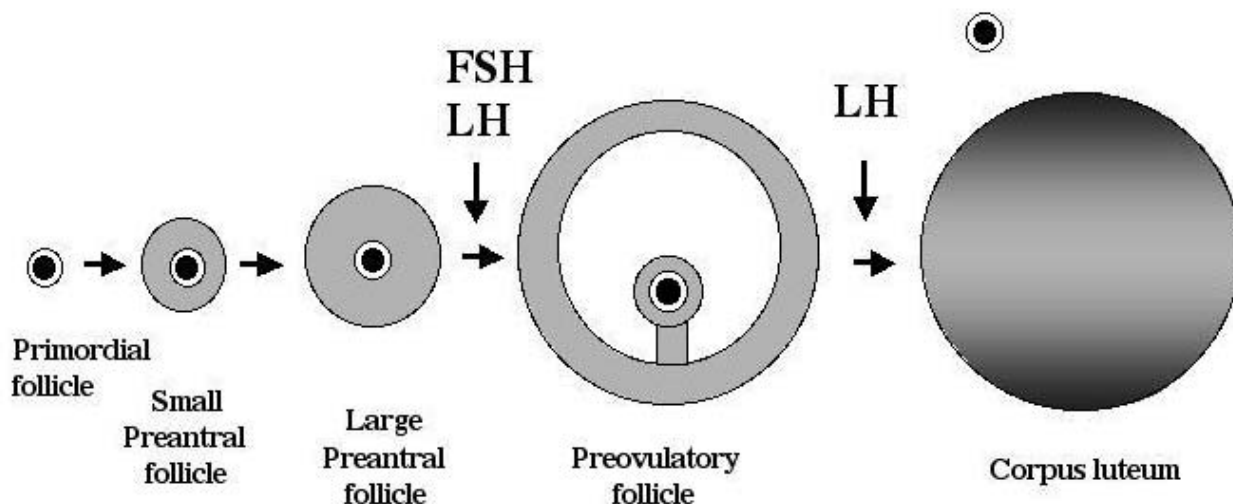
The goal of this review is to summarize the current views regarding the regulation of the primate menstrual cycle with particular reference to the physiological mechanisms involved in the selection of a single preovulatory follicle and to discuss the mechanisms by which this process may be overridden to achieve the production of multiple preovulatory follicles to obtain more oocytes for use in assisted reproductive technology studies. For this purpose, the review will focus on the following: i) the characteristics of preantral follicular growth, ii) the initiation of preovulatory follicular growth during the early through mid follicular phase of the menstrual cycle, iii) the selection of the preovulatory follicle during mid through late follicular phase of the menstrual cycle and iv) the mechanisms by which the normal process of follicular selection may be overridden to produce multiple oocytes. This review summarizes early work which has also been reviewed elsewhere [1-3].

### Characteristics of preantral follicular development

As summarized in Figure 1, follicular maturation to the preovulatory stage is the culmination of a lengthy process

in which the maturation of dormant primordial follicles is initiated as the granulosa cells begin to proliferate and form preantral follicles. Granulosa cell division continues and the number of granulosa cell layers increase as the preantral follicle grows. After the preantral follicle attains six-seven granulosa cell layers, the theca interna layer becomes pronounced and the formation of the antral cavity begins. In the absence of appropriate gonadotropic stimulation, follicles do not develop beyond the early antral stage and atresia occurs. It has been estimated that the duration of time required for the growth of a follicle from the primordial stage to the large preantral stage takes in excess of 150 days [4]. Thus, a follicle which ovulates in any given menstrual cycle will actually have begun to grow at least five menstrual cycles earlier.

In primates, early antral follicles are present in ovaries throughout the follicular as well as the luteal phase and even prior to the onset of puberty [5,6]. It is generally accepted that the stages of follicular development up to and including the early antral follicle are relatively independent of the pituitary gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH). In nonhuman primates, autoradiographical studies have

**Figure 1**

**Gonadotropic requirements for follicular development.** The early stages of follicular development do not appear to require cyclic fluctuations in the secretion of FSH and LH. The role of FSH is to stimulate the formation of a large preovulatory follicle which, because of its FSH-dependent maturation, is capable of ovulation and forming a corpus luteum in response to the midcycle surge of LH.

shown that preantral follicles possess FSH receptors, but not LH receptors, similar to that previously shown in rats [7,8]. Because early antral follicles are FSH responsive and are present throughout the menstrual cycle as a product of the continual supply of preantral follicles from the primordial pool, it is generally accepted that the process of preantral folliculogenesis serves to provide a constantly available source of maturing follicles for final maturation to the preovulatory stage when provided with the appropriate hormonal support, which, in primates, begins upon the onset of puberty.

As noted above, the growth of preantral follicles does not appear to require adult levels of FSH and LH as growing preantral follicles are present in ovaries of prepubertal monkeys and humans [6]. Likewise, in some humans with inactivations of the FSH receptor, follicular growth occurs to the large preantral stages [9]. In vitro, activin appears to stimulate granulosa cell division in preantral follicles [10,11] and preantral follicles are also responsive to other members of the TGF- $\beta$  family of growth factors including TGF- $\beta$ , bone morphogenetic proteins and growth and differentiation factor-9 [9]. In addition, granulosa cells of preantral follicles are responsive to estrogens, androgens, insulin and insulin-like growth factor-1. However, much of the information regarding the effects of

non-gonadotropic regulators of follicular growth has been limited to in vitro studies and the extent to which they may be active in vivo remains an uncertainty.

#### **The initiation of preovulatory follicular growth during the early through mid follicular phase of the menstrual cycle**

Primates are unique with respect to the long duration required for the maturation of the preovulatory follicle when compared with other species. Baird et al. [12] proposed that the lengthy follicular phase of primates is because the primate corpus luteum, unlike that of sheep, cows and horses, produces sufficient amounts of estrogen to suppress FSH secretion below the level necessary to advance the development of early antral follicles thus more time is required for a follicle to develop to the preovulatory stage. Upon the demise of the corpus luteum, serum levels of FSH and LH increase (the peri-menstrual rise) and the process of preovulatory follicular development is initiated. Although the occurrence of a peri-menstrual rise in serum FSH concentrations was recognized by Ross et al. [13] in their original descriptions of the hormonal profiles of the human menstrual cycle, the extent to which such a slight (30–50%) rise in FSH concentrations during the early follicular phase participated in follicular development was not fully appreciated until Brown [14]

demonstrated that changes in FSH concentrations on the order of 10–30% are sufficient to initiate follicular development in anovulatory women. Based on this finding, Brown introduced the concept of an "FSH threshold" to indicate that a critical concentration of FSH must be achieved to initiate the process of follicular development.

### **The mid-follicular phase through the late follicular phase**

It is during this period that follicular selection is accomplished. As mentioned in the previous section, a perimenstrual rise in FSH secretion occurs following the regression of the corpus luteum. Thereafter, there is a reciprocal relationship between the plasma concentrations of FSH and estradiol. During the early follicular phase prior to the emergence of a stimulated follicle, serum FSH concentrations are elevated while estradiol concentrations are low. Approximately five days before the midcycle gonadotropin surge, serum estrogen concentrations begin to rise as the result of the emergence of a maturing follicle. Associated with the gradual increase in estradiol concentrations is a progressive fall in FSH concentrations due to the feedback actions of estrogen (and possibly inhibin) on gonadotropin secretion [15,16]. This classical negative feedback relationship between estradiol and FSH is an essential component in the process of follicular selection. Owing to the steady exit of follicles from the primordial pool, there will always be a maturational distinct population of early antral follicles within the ovaries ready for development to the preovulatory stage under the influence of FSH. As a growing follicle acquires sufficient aromatase activity as a result of FSH stimulation, its production of estrogen suppresses FSH secretion below that necessary to sustain the development of less mature follicles which consequently undergo atresia.

The estrogen-feedback hypothesis has been tested directly in nonhuman primates by manipulating the pattern of estrogen concentrations during the follicular phase of the menstrual cycle. In rhesus monkeys, subcutaneous implants of estrogen-containing capsules on days 3–6 of the follicular phase during the menstrual cycle prematurely elevated estrogen concentrations by 50–80 pg/mL and resulted in a slight but significant fall in the plasma concentration of FSH and an interruption of spontaneous follicular development [17]. This negative feedback model for follicle selection would also predict that negating the gonadotropin suppressing effects of estrogen during the mid through late follicular phase of the menstrual cycle should prevent the fall in FSH concentrations and override the process of follicle selection. Indeed, passive immunization of rhesus monkeys with anti-estradiol antibodies during the mid through late follicular phase of the menstrual cycle prevented the fall in FSH concentrations and caused the maturation of more than one preovulatory

follicle [18]. In humans, it is well known that blockage of the biological actions of estrogen with the antiestrogen clomiphene results in an augmentation of gonadotropin secretion and maturation of more than one preovulatory follicle [19,20].

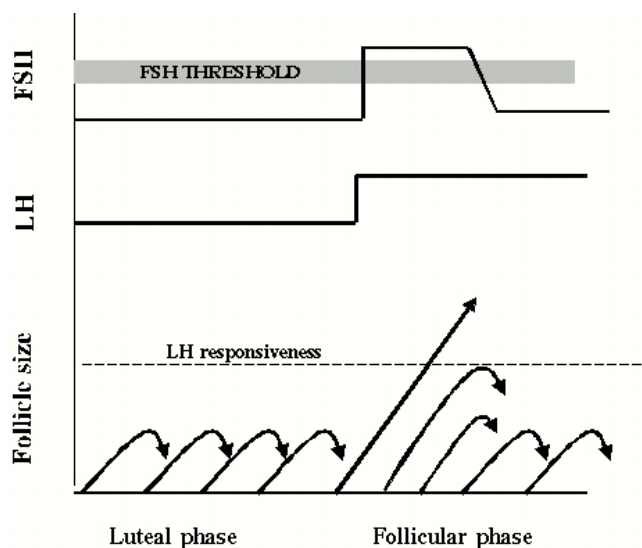
Given that FSH is essential for follicular development, how is it then that the maturing follicle continues to develop in the presence of FSH concentrations that are unable to maintain the development of less mature follicles? The only explanation for this paradox is that as the follicle matures, it must become less dependent upon FSH such that the concentration of FSH necessary to initiate preovulatory follicular development is greater than the concentration of FSH necessary to maintain preovulatory follicular growth. This hypothesis was tested directly by intravenous infusion of highly purified hFSH and hLH into cynomolgus monkeys whose endogenous gonadotropin secretion was blocked by a GnRH antagonist [21]. Results of this study demonstrated that when plasma FSH levels were maintained at approximately 10 mIU/ml, which is the concentration of FSH circulating during the luteal phase of the menstrual cycle, there was no evidence of estrogen secretion. When plasma FSH concentrations were elevated to approximately 20 mIU/mL, which is typical of FSH concentrations during the early follicular phase, preovulatory follicular development was initiated, as reflected by increasing concentrations of estrogen. Once preovulatory follicular growth was apparent, a reduction of plasma FSH concentrations to 10 mIU/ml over a five day period was associated with a continued rise in estrogen production. That estrogen secretion continued to rise despite the progressive fall in FSH concentrations demonstrates that the maturing follicle, as a consequence of FSH stimulation, acquires increased sensitivity to FSH such that it continues to mature in the presence of FSH concentrations that are unable to initiate the development of less mature follicles.

This finding indicates that there must be specific functional changes in the FSH-stimulated follicle that renders it less dependent on FSH than other lesser mature follicles. A hallmark action of FSH during preovulatory follicular development is the induction of LH receptors on granulosa cells [8]. Granulosa cells from early antral follicles possess FSH receptors and stimulation of the cells by FSH results in the activation of adenylyl cyclase and the production of cAMP. In response to FSH stimulation, granulosa cells acquire LH receptors and, like that the FSH receptor, occupancy of the LH receptor by LH also results in the activation of adenylyl cyclase and the production of cAMP [22]. As would be predicted by the common intracellular cAMP pathway, granulosa cells from FSH-stimulated follicles respond similarly to both FSH and LH; moreover, at non-saturating levels of FSH and LH, the

responses are additive [23]. The overall significance of these findings is that while granulosa cells from early antral follicles are *only* responsive to FSH, granulosa cells from FSH-stimulated follicles are responsive to *either* FSH or LH. Thus it is possible that the maturing follicle reduces its dependence on FSH by acquiring LH receptors, and hence LH responsiveness.

Recent studies conducted in humans using recombinant FSH and LH by Sullivan et al. [24] support the hypothesis that the acquisition of LH receptors on granulosa cells protects the follicle from the decline in FSH concentrations during the mid through late follicular phase of the menstrual cycle. Women were treated with recombinant FSH to stimulate follicular development to the antral stages (approx. 14 mm diameter) following which FSH treatment was terminated. In subjects who received no additional gonadotropin treatment, peripheral estrogen concentrations declined within 48 hr after the cessation of FSH treatment. However, subjects who received recombinant LH following the cessation of FSH treatment exhibited rising estrogen concentrations over the subsequent 48 hr, indicating that LH was able to substitute for FSH in supporting the growth of FSH-stimulated follicles. This observation is further supported by the studies in women by Willis et al. [25] in which estrogen and progesterone production in response to FSH and LH by granulosa cells from different sized follicles was assessed. They observed that LH responsiveness (estradiol and progesterone production) became apparent in follicles with diameters  $\geq 10$  mm, a size attained by the maturing follicle during the midfollicular phase of the menstrual cycle when estradiol levels just begin to increase [26]. A similar role for LH in follicle selection in sheep and humans has recently been described [27,28].

A working model for follicle selection is presented in Figure 2. During the luteal phase of the menstrual cycle, preovulatory follicular development is curtailed because the corpus luteum, via its secretions of estrogen, progesterone and possibly inhibin suppress FSH secretion below that necessary to stimulate the maturation of follicles beyond the early antral stage of development. Upon the regression of the corpus luteum, feedback inhibition of FSH secretion is relieved and FSH concentrations rise and stimulate the progression of follicles beyond the early antral stages. Two hallmark responses of granulosa cells to FSH are the induction of aromatase and the induction of LH receptors. The induction of aromatase results in the rise in peripheral levels of estrogen which, as noted earlier, suppress FSH secretion such that plasma concentrations of FSH fall below the threshold that is necessary to stimulate the maturation of other less mature follicles. The concurrent induction of LH receptors may provide the maturing follicle with an additional source of gonadotropic support



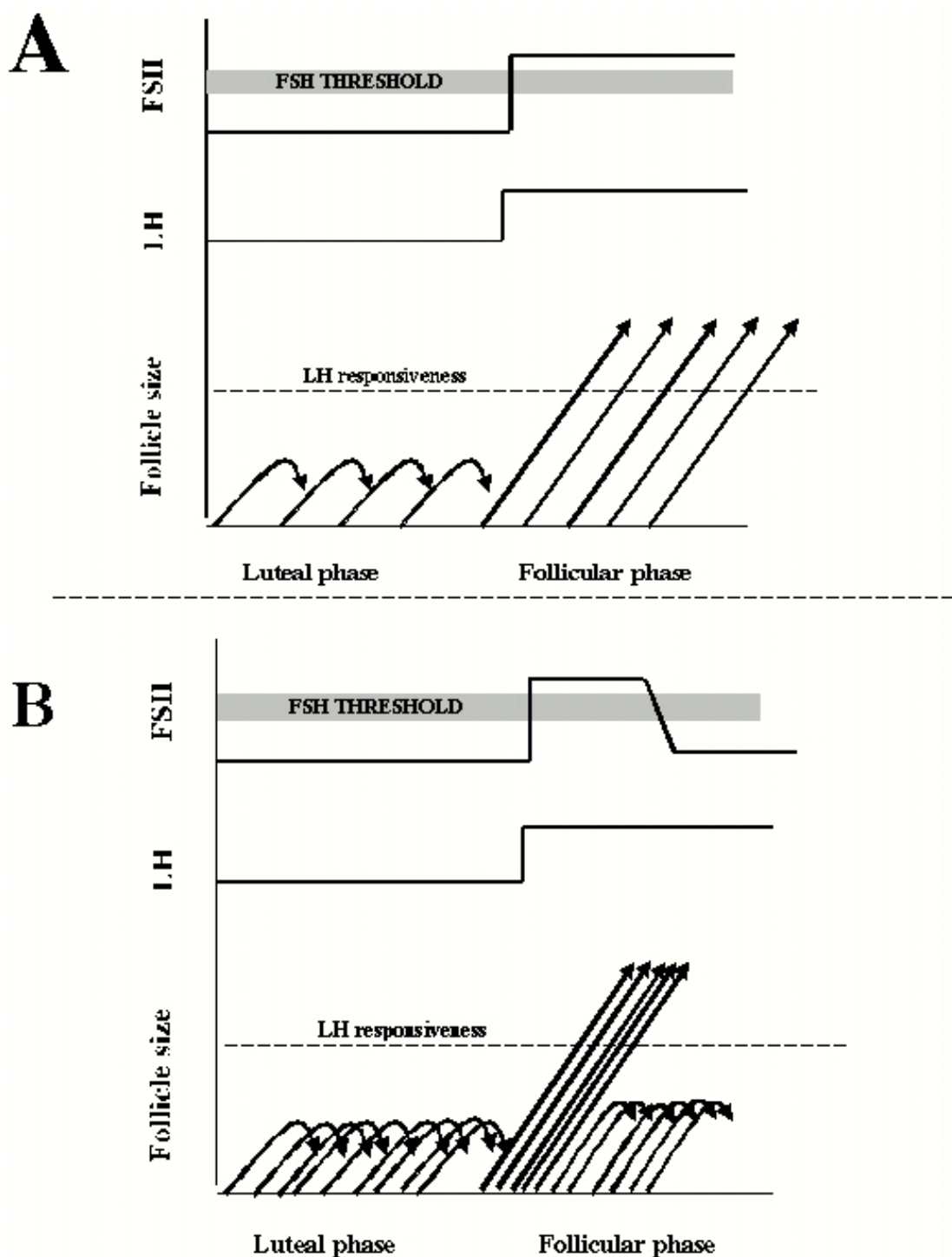
**Figure 2**  
**Schematic diagram of the "FSH threshold" model for the selection of the preovulatory follicle in primates.**

During the luteal phase of the menstrual cycle circulating FSH concentrations are held below the FSH threshold by secretions of the corpus luteum. As a result, growing follicles do not advance beyond the preantral stage and undergo atresia. Upon the regression of the corpus luteum at the end of the nonfertile menstrual cycle, the negative feedback suppression of FSH secretion is released and FSH concentrations rise above threshold levels. One (or occasionally more) of the maturing preantral follicles is stimulated in response to the elevation of FSH and develops both the aromatase enzyme and LH receptors. The acquisition of aromatase results in a rise in systemic levels of estradiol which result in the suppression of FSH secretion, which, in turn, prevents the maturation of less mature follicles. The FSH-stimulated induction of LH receptors and the acquisition of LH responsiveness of granulosa cells of the stimulated follicle permit it to mature in the presence of FSH concentrations which are insufficient to stimulate the maturation of other less mature follicles.

which enables it to continue to mature in the presence of FSH concentrations which are insufficient to support the development of other follicles.

**Physiological basis of controlled ovarian stimulation**

Knowledge of the normal process of follicular selection allows for the understanding of the physiological principles that underlie various strategies for increasing the number of preovulatory follicles that can be stimulated to mature. Figure 3A illustrates a description of the process of controlled ovarian stimulation achieved by increasing the duration that serum FSH concentrations are maintained above threshold levels. Prolonged elevation of FSH can be



**Figure 3**  
**Physiological basis of ovarian hyperstimulation.** Panel A illustrates the conventional mechanism of ovarian hyperstimulation in which concentrations of circulating FSH are elevated above threshold levels either by direct administration of exogenous FSH or by interfering with the negative feedback actions of estrogen on FSH secretion either by the administration of anti-estrogens or aromatase inhibitors. The number of preovulatory follicles increases but in an asynchronous manner owing to the asynchronous nature of preantral follicular development. Panel B illustrates a hypothetical way to provide a more synchronous population of preovulatory follicles by increasing the number of preantral follicles prior to elevating FSH concentrations and substituting FSH with LH when an appropriate number of follicles are selected.

achieved by direct administration of exogenous FSH. Alternately, administration of the anti-estrogens clomiphene and tamoxifen as well administration of an aromatase inhibitor, in the presence or absence of exogenous FSH, also can result in ovarian stimulation presumably by diminishing the negative feedback effects of estrogen on FSH secretion [19,20,29]. As can be seen in Figure 3A, one of the inherent difficulties in this approach to ovarian stimulation is that follicular maturation is likely to be asynchronous due to the asynchronous nature of the development of preantral follicles, and this asynchrony would become greater as the duration of elevation of FSH persists. Thus, oocytes collected from these follicles could differ in their maturational states as well. One possible way of reducing the variability of differing maturational states of follicles could be by providing a sequential FSH and LH treatment regimen to limit follicular recruitment to a group of follicles. Switching from FSH to LH would maintain the growth of follicles with LH receptors on granulosa cells but would prevent the additional maturation of less mature follicles. In addition, administration of LH in the absence of FSH may actually reduce the number of smaller follicles, possibly by elevating intrafollicular androgen levels [28].

A hypothetical mechanism for ovarian stimulation is shown in Fig 3B. In this model, the number of follicles that are "recruitable" by FSH is increased either by stimulating the growth or reducing the atresia of preantral follicles. This potentially could result in a greater number of follicles at a given maturational state which would lead to a more synchronous development of preovulatory follicles. To date, however, this not possible largely because, as described earlier, the factors which regulate the growth of preantral follicles and their atresia in the primate ovary have not been identified. A number of potential candidates have been suggested from in vitro studies which include estrogen, androgens and IGF-1. However, when tested in primate models *in vivo*, none of these have been shown to augment the ovarian responsiveness to FSH [30-32]. Whether other potential autocrine/paracrine agents such as activin or GDF-9 may be effective in increasing the number of recruitable preantral follicles awaits further study.

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