STEROID HORMONES AND REGULATION OF OVARIAN FUNCTION

Role for Cholesterol Precursors

A family of C29 4,4-dimethylsterol intermediates in the cholesterol biosynthetic pathway from lanosterol has been found to induce oocytes to resume meiosis.[248] One of these sterols, 4,4-dimethyl-5α-cholest-8,14,24-triene-3β-ol, was extracted from human follicular fluid and named follicular fluid meiosis–activating substance (FF-MAS). A related compound, 4,4-dimethyl-5α-cholest-8,24-diene-3β-ol, was isolated from bull testis and called T-MAS. These compounds are synthesized from lanosterol by P450 14α-demethylase, which is encoded by the CYP51 gene. FF-MAS and T-MAS are present in micromolar concentrations in preovulatory follicle follicular fluid: 1.6 μM for FF-MAS and about half that for T-MAS.

The accumulation of FF-MAS and T-MAS in mature follicles may be the result of increased synthesis as well as inhibition of cholesterol synthesis at steps beyond the formation of FF-MAS and T-MAS. Gonadotropins have been reported to cause a several-fold increase in Cyp51 gene expression in rodent ovaries, which could contribute to enhanced MAS formation.[249] Additionally, progestins at concentrations found in follicular fluid in the preovulatory period block cholesterol synthesis at late steps, which would result in an accumulation of FF-MAS and T-MAS.

When perfused into rodent ovaries, FF-MAS can induce maturation of cumulus cell–deprived oocytes or oocyte maturation. However, experiments using various inhibitors of sterol synthesis—including drugs that block 14α-demethylase and those that inhibit enzymes that metabolize MAS—have yielded conflicting results. Inhibitors of 14α-demethylase block gonadotropin-stimulated, but not spontaneous, meiosis in rodents, whereas drugs that block MAS metabolism generally result in germinal vesicle breakdown of cumulus-enclosed oocytes. Consequently, the physiologic roles of FF-MAS and T-MAS in oocyte maturation, if any, remain uncertain. The pharmacologic value of FF-MAS and T-MAS also is uncertain. Some, but not all, studies on in vitro oocyte maturation suggest effects of these compounds on maturation by stimulating progression to metaphase II or increasing the survival of oocytes without affecting maturation.

Role of Estrogens

In addition to their systemic effects on the reproductive tract, hypothalamus, and pituitary, estrogens have important actions on granulosa, theca, and luteal cells in the ovaries of laboratory animals and domestic species. There are conflicting reports in the literature regarding the expression of estrogen receptor α and β in the primate ovary.[250] The most convincing of these studies indicates that both estrogen receptor α and estrogen receptor β are expressed by the surface epithelium, granulosa cells (with estrogen receptor β predominating over estrogen receptor α in medium-sized and preovulatory follicles), theca cells, and luteinized granulosa cells.

Estrogen receptor α transcripts also have been detected by polymerase chain reaction (PCR) in human oocytes by some authors, but these findings have not been confirmed by others. Although there are differences among the various reports that probably reflect the sensitivity of the method of detection of estrogen receptor expression (i.e., reverse transcriptase PCR, Northern blotting, Western blotting, immunohistochemistry), and in the case of immunochemical methods, the specific antibodies employed, the existing data support the notion that the ovary is a site of estrogen action via the classic receptor-mediated signaling pathways.

The physiologic roles of estrogen within the primate ovary are matters of current debate, as are the mechanisms by which they might influence cellular function (i.e., genomic versus nongenomic actions), given the very high concentrations achieved during follicular maturation and corpus luteum function.[250] Indeed, the extremely high levels of estradiol reached in the antrum of the preovulatory follicle (approximately 1 μg/mL) raise serious questions as to the function of the classic estrogen receptor system, which would be fully saturated by ligand during the later stages of follicular maturation.

In animal granulosa cells, estrogens have pleiotropic actions. They promote proliferation and exert antiatretic effects. Estrogens augment intercellular gap junction and antrum formation, and they also increase the estrogen receptor content of granulosa cells. Estrogens synergize with gonadotropins at several levels, including the promotion of ovarian...
growth, LH and FSH receptor expression, and the augmentation of aromatase activity.

Insight into the importance of estrogens in ovarian function in women comes from the study of subjects in whom estrogen synthesis is impaired. Limited studies have been performed on women with 17α-hydroxylase/17-20 desmolase deficiency who are incapable of producing thecal androgens to support granulosa cell estradiol synthesis. Promotion of follicular growth to the preovulatory stage in an estrogen-impooverished environment is possible in these individuals with exogenous gonadotropins after pituitary desensitization. The same is true in severely hypogonadotropic women given exogenous FSH. Follicles grow, but in the absence of exogenous LH, estradiol synthesis is minimal. Moreover, the development of follicular cysts with low estrogen levels is common in women with StAR, 17α-hydroxylase/17-20 desmolase, and aromatase deficiency. Hence, one can conclude that the high levels of estrogen associated with normal follicular maturation are not required for the growth of follicles to the size equivalent to the preovulatory stage.

Whether the oocytes that are recovered from such follicles are endowed with the properties that will lead to successful embryonic development after fertilization is less certain. Successful in vitro fertilization of oocytes recovered from estrogen-poor follicles of a woman with 17α-hydroxylase-17/20 desmolase deficiency has been described, with the formation of cleavage-stage embryos, but a pregnancy was not achieved after embryo transfer.

There are pharmacologic data suggesting that estrogens are important for oocyte function. Monkeys treated during follicular maturation with doses of an aromatase inhibitor that substantially reduces circulating estradiol levels showed no effects on follicular growth. A greater proportion of the oocytes recovered from follicles of the aromatase-treated animals were in prophase I, however, and there was retarded completion of maturation to MII. Whether this is a direct reflection of estradiol deficiency, a consequence of the aromatase inhibitor (1,4,6-androstatrien-3,17-dione), or the result of compensatory changes in endocrine status due to the decline in estradiol is not known. The implication of these observations for ovulation induction in women using aromatase inhibitors is not clear.

In vitro studies on primate granulosa cells have yielded inconsistent findings with respect to the actions of estrogens. Estradiol inhibits progesterone secretion by Rhesus monkey granulosa cells, whereas in marmoset granulosa cells, it has no effect on progesterone production, but stimulates aromatase when added in the presence of IGF-I. As discussed previously, exogenous estrogen exerts a luteolytic effect in the primate corpus luteum, probably through actions on the central nervous system.

In summary, although the primate ovary expresses the receptors that allow a variety of cells to respond to estradiol, the physiologic significance of estrogen in follicular maturation and luteal function in the primate ovary is still unknown. Evidently, follicle growth per se does not require high levels of estradiol, but the orchestration of events that result in a mature oocyte capable of developing into a viable embryo after fertilization may require estrogen action on either the granulosa cells or the oocyte.

**Role of Androgens**

In addition to serving as substrates for estrogen production, androgens have a number of effects on the primate ovary. Administration of testosterone or 5α-dihydrotestosterone to Rhesus monkeys promotes accumulation of primary follicles as well as follicle survival, suggesting a folliculotropic action. In this model, androgen receptors are abundant in the granulosa cells of healthy preantral and antral follicles, with lesser expression in the theca and stroma. Moreover, androgen receptors were positively correlated with a marker of cell proliferation (Ki-67) and negatively correlated with apoptosis. These observations contrast with the view that androgens are atretogenic, a concept that emerged primarily from studies on the rodent ovary in which androgens block granulosa cell proliferation in vitro in some systems and promote follicular atresia. For example, in the absence of gonadotropins, androgens provoke follicular atresia and antagonize estrogen-associated ovarian weight increases in hypophysectomized immature rats. Similarly, treatment with 5α-dihydrotestosterone abolishes the ability of FSH to induce LH receptors in granulosa cells and inhibits granulosa cell proliferation.

Studies in the marmoset indicated stage-dependent effects of androgens on granulosa cell function in vitro. Androgen enhanced FSH-stimulated aromatase expression and progesterone production while inhibiting hCG-stimulated aromatase activity and progesterone synthesis in cells from large preovulatory follicles. Evidence that androgens have a detrimental effect on human follicular function includes the observation that follicular fluid enriched in 5α-dihydrotestosterone and poor in estradiol is characteristic of atresia. However, this steroid profile may be a consequence rather than a cause of atresia. Favoring a causal relationship are reports that high follicular concentrations of 5α-reduced androgens, such as 5α-dihydrotestosterone, act as competitive inhibitors of granulosa cell aromatase activity. In this regard, follicles from patients with PCOS have greater 5α-reductase activity than follicles from normal ovaries. Thus, androgens may exert both positive and negative effects on follicular growth and
function in a stage-dependent manner through androgen receptors as well as by non-receptor-mediated mechanisms.

Role of Progesterone

Progesterone production by the preovulatory follicle is required for ovulation, as discussed previously. It also may have a role in regulating corpus luteum function. Pharmacologic blockade of ovarian progesterone production with a 3β-hydroxysteroid dehydrogenase inhibitor indicated that progesterone exerts antiapoptotic and prodifferentiation effects on luteinizing cells and maintains luteal function. The progesterone receptor antagonists mifepristone and HRP2000 inhibit hCG-stimulated progesterone and relaxin secretion by human granulosa–lutein cells.[259] Progesterone receptors, both the A and B forms, are present in the Rhesus monkey and human corpus luteum, with progesterone receptor mRNA increasing from the early to the midluteal phase. The ratio of progesterone receptor B to progesterone receptor A increases from the early to the late luteal phase. The action of progesterone receptor antagonists on lutein cell steroidogenesis is presumably a reflection of altered transcription regulated by these nuclear receptors.