

Endocrine and Anatomical Correlations in Human Ovarian Pathology

by A. F. Haney*

Knowledge of normal gonadal hormone production and function provides the basis for understanding the ovarian pathologic effects resulting from perturbations in endocrine balance and feedback. A precisely timed, complex, and well-coordinated cascade of ovarian steroidogenesis accompanies normal cyclical follicular function. This cascade involves both estrogens and androgens. Alterations in the hormonal milieu are associated with specific morphological changes in the ovary. While predictable hormonal changes accompany commencement of menopause, several disease states are associated with ovarian dysfunction. These diseases include polycystic ovarian syndrome and hyperthecosis, both associated with androgenization. Ovarian tumors may also be associated with morphological and clinical alterations. While endocrinologically inert ovarian tumors are associated with morphologic evidence of stromal activation, endocrinologically active ovarian tumors may cause differentiation along either male or female lines as a consequence of differential productions of androgens and estrogens.

Introduction

Reproduction is a complex process involving interaction of the hypothalamus, pituitary, ovary, and the lower genital tract. The cyclic pattern of sex steroid production by the ovary is dramatically different from that of the testis. Testicular hormone production is independent of germ cells and remains relatively constant over the life-span of the individual, with only a slight decline beyond the sixth decade of life. By contrast, the reproductive cycle in women is critically dependent on the presence of ovarian follicles. As the number of oocytes in the ovary is fixed during embryonic life, a decline in ovarian function, termed the climacteric, is observed over a relatively short interval at the end of the reproductive life-span. While certainly not all ovarian pathology is associated with alterations in ovarian function, aberrations of the menstrual cycle and abnormal bleeding after menopause represent important clinical clues to the presence of ovarian pathology. Consequently, a thorough understanding of the physiology of gonadal hormone production is helpful in understanding ovarian pathology.

Gamete Physiology

Oocytes can only remain viable within the human ovary when they are enclosed by a complicated arrangement of supporting cells that are collectively termed the follicle. The follicle is composed of avascular granulosa

cell layers in direct contact with the oocyte, a basement membrane surrounding the granulosa cells, and a vascularized thecal compartment. A functional relationship between the follicle and the surrounding ovarian stroma has never been proven, but the theca is thought to differentiate from the stroma. Table 1 depicts the fate of the follicles throughout a woman's life.

Germ cells migrate from the yolk sac into the primitive gonad in the first several months of embryonic life.

Table 1. The complement of ovarian follicles throughout life.

Age	No. of oocytes	Physiologic state of the follicles	Theoretical rate of follicular atresia
Week 20 of gestation	$5-7 \times 10^6$	Primordial follicle organization	—
Weeks 20 to 40 of gestation	—	Inactive primordial follicles	29,000-43,000/day
Birth	$1-2 \times 10^6$	Inactive primordial follicles	—
Childhood	—	Inactive primordial follicles	20-125/day
Puberty	$3-4 \times 10^6$	Disorderly follicular maturation	—
Reproductive years	—	Preovulatory follicles and corpora lutea	8/day
Climacteric	1×10^4	Disorderly follicular maturation	—
Ovarian senescence	Negligible	Inactive follicles	—

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By 20 weeks gestation, they have actively undergone mitosis and now total 5 to 7 million oocytes. The oogonia are rapidly incorporated into primitive follicles and are arrested in the prophase of the first meiotic division. At birth, there remain approximately 1 to 2 million follicles, indicating a high rate of atresia in these early primordial follicles. The cause for these early follicular losses is unknown.

Rapid atresia continues throughout childhood, and at puberty only 300,000 to 400,000 follicles remain. The increasing gonadotropin levels at puberty result in orderly follicular maturation and ovulation. By the end of the sixth decade of life, an occasional follicle remains, and the decline in ovarian hormone production, termed the climacteric, heralds the loss of reproductive potential. As only 300 to 400 oocytes are lost by the process of ovulation, the overwhelming majority of human oocytes are lost prior to full differentiation. As the lowest rate of atresia occurs during the reproductive years when levels of sex steroids and gonadotropins are high, the hormonal balance likely has a significant influence on the maintenance of these follicular structures.

Throughout history, the mean life expectancy of women has dramatically increased from 25 years to 80 years. Thus, living into the climacteric and beyond is a relatively new phenomenon for our species. The impact of a longer life-span is reflected in what constitutes the most common causes of death. Several hundred years ago, the most common causes of death for women were infectious diseases and childbirth; cardiovascular disease and neoplasia are now the most common modes of death for women.

Gonadal Steroid Production

Ovarian hormone production to the levels observed during the reproductive years is dependent on the presence of oocytes. The follicular cells not only protect the oocyte from undergoing degeneration, but also possess gonadotropin receptors. These receptors couple follicular development to hypothalamic/pituitary function and actively synthesize steroids and other protein hormones. The oocyte itself does not possess gonadotropin receptors nor does it synthesize significant quantities of steroids.

Prior to puberty, the primordial oocytes are enclosed by a single layer of primitive granulosa cells without an identifiable thecal layer. During the onset of puberty, the hypothalamus is desensitized to the suppressive effect of circulating steroids, causing gonadotropin levels to rise, initiating follicular development. This in turn stimulates gonadal hormone production, resulting in the well-known physical and psychological changes attendant to sexual maturity.

Based on morphologic criteria, it takes approximately 65 days for primordial follicles to attain preovulatory status, with all but the last 14 to 16 days independent of gonadotropins. The general endocrinologic milieu is important for early follicular maturation, however, pro-

viding a favorable environment for granulosa cell mitosis and thecal differentiation (Fig. 1). This continuous gonadotropin-independent differentiation sets the stage so that at any point in time, a cohort of follicles reaches the critical stage where exposure to gonadotropins may continue preovulatory development. If sufficient gonadotropins are not present during this critical interval, the follicles uniformly undergo atresia (Plate 1). In clinical terms, excision of the dominant cyclic structure, i.e., either a preovulatory follicle or corpus luteum, initiates recruitment of a new cohort of follicles with ovulation occurring 14 days later. The follicular events under these circumstances are indistinguishable from those of the normal follicular phase.

With the functional decline of the corpus luteum at the end of the luteal phase, there is a reduction in the negative feedback effect of gonadal steroids, increasing the levels of the circulating gonadotropins, primarily follicle-stimulating hormone (FSH). As a consequence, another cohort of follicles reaching that critical gonadotropin-dependent stage is recruited for the next cycle. FSH appears to be the key gonadotropin at this stage by virtue of its ability to induce aromatase activity in granulosa cells. Attainment of the ability to respond to FSH, i.e., the presence of functional FSH receptors, is probably the key event in that process. With an enhanced aromatase system, the granulosa cells produce large quantities of estradiol from thecally derived precursors and undergo active mitosis, the two principal characteristics of healthy follicles.

As humans are a monotocous species, follicle selection is necessary so that only a single follicle reaches preovulatory status each cycle. The process by which the other competing follicles are retarded in their maturation is unclear, but likely involves inhibition or loss of FSH responsiveness. Little is known of the dynamics of follicular steroidogenesis prior to follicle selection, as the quantity of steroids produced by all the compartments at that stage is small. In healthy follicles, the estrogen/androgen ratio is high, whereas androgen predominates in the follicular fluid of atretic follicles. This is consistent with the concept of FSH-induced aromatase activity being the critical step in avoiding the otherwise inevitable atresia.

The selection of a single dominant follicle culminates in high preovulatory estradiol levels. Estrogen and FSH synergize to induce luteinizing hormone (LH) receptors in the granulosa cells, preparing the follicle to respond to the mid-cycle surge of gonadotropins. LH is released in the largest amounts during the mid-cycle surge, causing a decline in aromatization and an increase in progesterone synthesis. This is obviously a well-coordinated event, optimizing the hormonal milieu for early embryonic events.

In understanding follicular steroidogenesis, it is important to divide the follicle into its basic components, the avascular granulosa compartment, the theca, and the ovarian stroma. Both the theca and ovarian stroma possess all the steroidogenic enzymes necessary to pro-

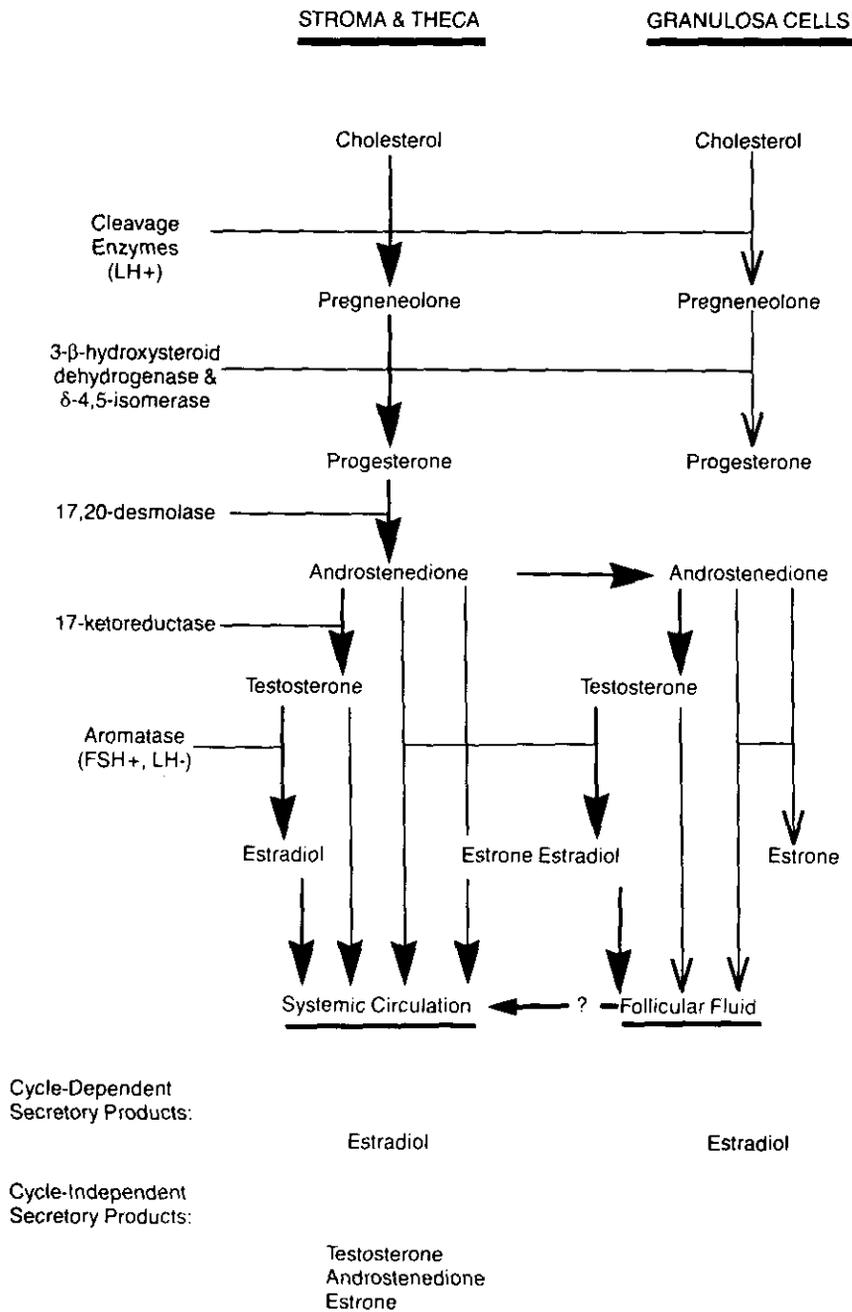


FIGURE 1. Compartmental follicular steroidogenesis.

duce the three classes of sex steroids in women: androgens, estrogens, and progesterone. Despite the highest FSH-inducible aromatase activity, the granulosa cells lack the 17-20 desmolase enzyme required to reduce C-21 progestogens to C-19 androgens (Fig. 1). As follicular fluid contains estrogen levels 1000-fold higher than those observed in peripheral serum, it is likely that androgens from the theca provide the substrate for granulosa cell aromatization. As the granulosa compartment is avascular, it is likely that granulosa cell estrogen production is predominantly responsible for

the high follicular fluid estrogen levels, whereas the vascularized stroma and theca produce estrogens and androgens primarily for the systemic circulation (Plate 2).

The steroidogenically active cells in the theca do not bind FSH but are rich in LH receptors. LH stimulation of the theca increases production of all classes of sex steroids, but androgens are by far the greatest quantitatively in the form of androstenedione and, to a lesser extent, testosterone. While the stromal compartment is also steroidogenically active, the quantity of hor-

mones produced is insignificant relative to the other follicular compartments.

With the onset of the LH surge, both the granulosa and thecal cells undergo a morphologic luteinization and form the corpus luteum (Plate 3). Simultaneously, aromatization of thecally derived androgens to estrogens by granulosa cells is dramatically reduced. While estrogens and androgens are still produced by thecal and stromal cells throughout the luteal phase, the quantity of progesterone produced by the granulosa cells is by far greater, dominating luteal steroidogenesis (Fig. 2).

In nonconceptive cycles, the estrogen and progesterone production begins to decline midway through the luteal phase. The mechanism of luteolysis in humans is unknown. In conceptive cycles, the fetal signal, human chorionic gonadotropin (hCG), maintains the corpus luteum and supports luteal progesterone production through the early part of pregnancy. The fetal hCG binds to the same receptor as LH, but its secretion is not subject to the same negative feedback by steroids as are the pituitary gonadotropins. Geometrically increasing quantities of hCG are required for luteal main-

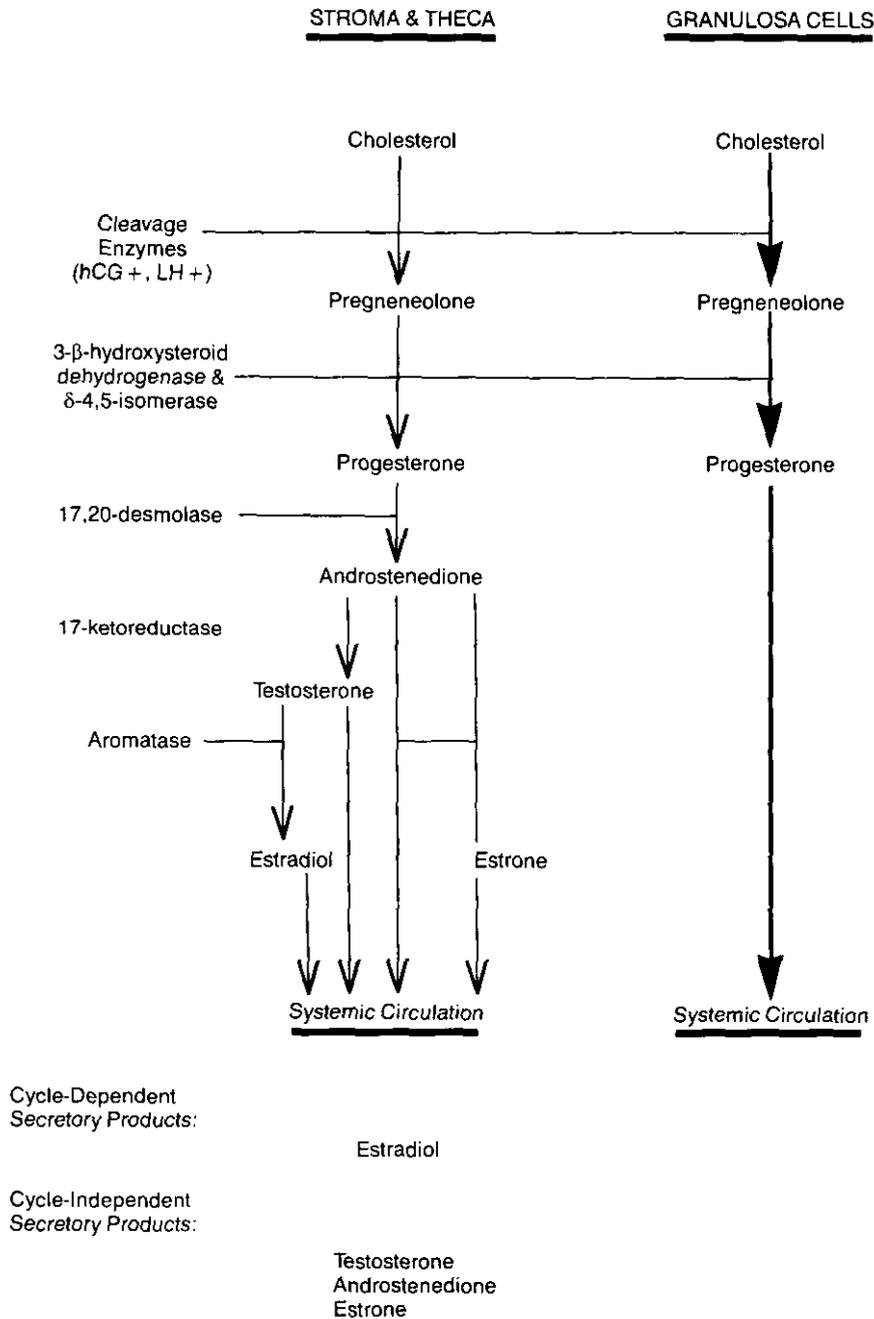


FIGURE 2. Compartmental luteal steroidogenesis.

tenance, consistent with the pattern of the rise in hCG observed in early pregnancy. The corpus luteum hypertrophies in early pregnancy, and clinical ovarian enlargement is common. By 8 to 10 weeks of gestation, the trophoblast has assumed the role as the primary producer of steroids, and the corpus luteum regresses. Throughout the remainder of pregnancy, the ovary is hormonally inactive, as the gonadotropins are suppressed by the high concentrations of circulating steroids.

Gonadotropin suppression, while preventing ovulation, does not prevent follicle loss through atresia. Follicles continue to mature independent of the gonadotropins, but without sufficient gonadotropins during the critical interval of gonadotropin sensitivity, atresia inevitably results. Consequently, the reproductive life-span is not lengthened by the use of agents such as oral contraceptives that act to suppress the pituitary's release of gonadotropins.

As women enter the latter stages of their reproductive years, the steroid production by morphologically normal follicles declines. Simultaneously, FSH levels gradually rise, most prominently in the early follicular phase. It is unclear whether this is due to intrinsically unhealthy follicles that have not matured throughout the reproductive years or a necessity for a large number of follicles to be present. Eventually, a point is reached at which anovulatory cycles predominate, and atresia prior to ovulation is the rule. Estrogen without the opposition of progesterone predominates, and peripheral target organs are constantly exposed to estrogen. This climacteric interval covers the approximately 8 to 10 year span of declining ovarian function. As the estrogen levels decrease, eventually a point is reached at which menstruation ceases. The date of the last menstruation is termed the menopause, but represents only a highly identifiable clinical marker of the climacteric. After the climacteric, only stromal estrogen production remains and this is insufficient to maintain the peripheral estrogen target organs, resulting in clinical symptoms of hypogonadism, i.e., atrophic vaginitis, vasomotor hot flashes, etc. (Plate 4). Follicles can still be observed occasionally after the menopause in the ovarian stroma, but their functional capabilities are obviously limited.

Clinical Syndromes of Ovarian Dysfunction

Premature Ovarian Failure

As women possess a fixed number of oocytes at birth, all women eventually undergo ovarian failure if their life-span exceeds the number of years during which follicles continue to be present. The mean age of menopause has been remarkably constant throughout recorded history regardless of the population studied, averaging approximately 50 years. Premature loss of oocytes results in morphologic and functional gonadal failure at a chronologically inappropriate time. Despite the high levels of gonadotropins circulating at this time,

the steroid production by the ovarian stroma is low, and no obvious hypertrophy of the ovarian stroma is observed. A variety of disease states have been associated with ovarian failure, as listed in Table 2.

The category of the "resistant ovary" is one in which histologically normal follicular structures are present, but apparently they are unresponsive to the circulating gonadotropins. This could be related to either defective gonadotropin synthesis or absent/nonfunctional ovarian gonadotropin receptors. In patients with an autoimmune basis for ovarian failure, an inflammatory process is usually present in the ovary. In all other circumstances of premature ovarian failure, the ovaries are morphologically indistinguishable from those of normal postmenopausal women (Plate 4).

Polycystic Ovary Syndrome

A unique syndrome of anovulation was identified in the 1930s and termed polycystic ovary syndrome. In this disease, multiple atretic follicles with active-appearing thecal compartments are present just under the ovarian capsule. The capsule itself is thickened and the ovarian stromal compartment is increased in size. The syndrome is clinically manifested by altered levels of circulating gonadotropins with an elevated LH to FSH ratio (greater than 3 versus a normal ratio of 1), hyperandrogenism manifested by elevated testosterone and androstenedione, and chronic unopposed estrogen effects on target organs. While this was originally thought to represent a single syndrome, it is now apparent that there are multiple subgroups, indistinguishable on clinical criteria. The morphology of the ovary in polycystic ovary syndrome appears to be a consequence of the anovulation with a large number of atretic follicles rather than any specific characteristic of the disease. Consequently, anything which alters gonadotropin levels and impedes follicular development results in this clinical syndrome.

The hypothalamic-pituitary axis in women with polycystic ovary syndrome is intact with operative negative and positive gonadotropin feedback function. The

Table 2. Causes of ovarian failure.

Cause	Description
Idiopathic	
Chromosomal abnormalities	
Iatrogenic	Surgery Chemotherapy Radiation therapy
Autoimmune oophoritis	Primary in ovary Associated with other autoimmune endocrine diseases
Viral oophoritis	
Resistant ovary syndrome	Primordial follicles present in the ovary
Congenital absence of the thymus	
17-Hydroxylase deficiency	
Galactosemia	

aberrant gonadotropin profiles can be mimicked by excess androgen, whatever the source. Women with androgen-secreting tumors have been identified to have a histologic pattern consistent with polycystic ovary syndrome in the contralateral ovary. When their tumors are removed and their circulating androgens decline, the ovary reverts to normal both morphologically and functionally. A primary ovarian defect in polycystic ovary syndrome has also been postulated based on altered gonadotropin responsiveness. Theoretically, this could be due to an alteration in gonadotropin binding or inappropriate production of gonadotropin receptor regulatory proteins.

Compartmentalized ovarian hormone secretion has recently been investigated. It appears that in polycystic ovary syndrome, the ovarian androgens are produced primarily from the theca, and the large amounts of androgens are attributable to the large number of atretic follicles. During the atretic process, estrogen production by the granulosa cell compartment declines, but androgen and estrogen production by the theca is maintained. This results in the seemingly paradoxical situation of simultaneous androgen excess and chronic unopposed estrogen.

Despite the inability to ovulate, a follicular response to gonadotropins is still present. Treatment with both clomiphene citrate, whose action is mediated through the hypothalamus, and human menopausal gonadotropins, which act directly on the ovary, result in follicular development and ovulation. With the use of clomiphene citrate, the positive feedback effect of estrogen triggers the midcycle gonadotropin surge, whereas with exogenous gonadotropins, the spontaneous surge will not occur and hCG is used as a surrogate LH surge.

Isolated luteinized cells are observed scattered throughout the ovarian stroma in polycystic ovary syndrome. The term "luteinized cells" simply describes lipid-containing cells consistent morphologically with active steroidogenesis. Whether these are stromal cells responding to the altered gonadotropins or are remnants of the theca of previously atretic follicles is unknown.

Hyperthecosis

Hyperthecosis is a syndrome similar in clinical presentation to polycystic ovary syndrome, but the androgenization is more severe. While the circulating gonadotropin levels are similar, the testosterone concentrations are significantly higher, often overlapping the range usually seen with androgen-secreting ovarian tumors.

The term "hyperthecosis" is used because of the large number of steroidogenically active cells present in the ovarian stroma (Plate 5). These cells are indistinguishable in appearance from the luteinized cells present in the stroma of polycystic ovarian patients, except the number of cells is greater constituting large areas of the ovarian stroma. Reminiscent of the confusion with polycystic ovary syndrome, it is unclear whether these

represent remnants of previous thecal compartments from follicles that have previously undergone atresia or are a unique thecal or stromal response. Similarly, their gonadotropin dependence is not known. The ovary is typically not as large as in polycystic ovary syndrome, as there is a smaller complement of atretic follicles. Consistent with that observation, it is much more difficult to stimulate ovulation in these women.

Stromal Activation by Ovarian Tumors

The association of endocrinologically inert ovarian tumors with clinical signs of sex steroid production has frequently been observed. Often, lipid-laden stromal cells are noted adjacent to the tumors in the remaining nontumorous ovarian stroma. These morphological changes have suggested activation of these surrounding stromal cells, with the observed clinical hormone production attributed to this presumed stromal steroidogenesis. Virtually any histologic tumor type can be present, including primary or metastatic ovarian cancer. Because of the difficulty in mechanically isolating these stromal cells, steroid production has never been experimentally verified but is likely on histologic criteria alone. The mechanism of this activation is unknown.

Endocrinologically Active Ovarian Tumors

When neoplastic, the gonadal stroma can differentiate along either male or female lines (Table 3). This includes Leydig and Sertoli cells that mimic male differentiation, and granulosa and thecal cells that yield female differentiation. It is not clear whether these represent analogous processes, as the granulosa and thecal cells are normally present within the ovary. Either one or both cell types in each category can be present, and rarely, both male and female cell types will be present in the same tumor (Plate 6). Although the majority of these tumors are benign, they can occasionally be malignant. Their major clinical importance is the peripheral consequences on the sex hormone target organs. Marked

Table 3. Classification of endocrinologically active ovarian tumors.

Result	Tumor type
Female differentiation	Granulosa cell Thecoma Granulosa/theca cell
Male differentiation	Leydig cell Sertoli cell Sertoli/Leydig cell (arrhenoblastoma)
Male and female differentiation	Gynandroblastoma Gonadoblastoma (germ cell tumor with stromal elements)
Other	Lipoid cell Hilus cell Clear cell (adrenal rest cell tumor) Luteoma of pregnancy

virilization can be observed with androgen-secreting tumors, while endometrial adenocarcinomas are common when estrogen-secreting tumors are present after menopause.

Any of these tumors can produce either androgens or estrogens, and the clinical presentation will reflect the particular steroid or steroids secreted. Those that differentiate along male lines tend to be androgen-secreting tumors, and those that differentiate along female lines tend to be estrogen-secreting tumors. Occasionally, both androgens and estrogens are secreted, and the women are simultaneously androgenized and estrogenized. A unique histologic marker for androgen production, regardless of the tumor cell type, is the intracellular crystalloid of Reinke.

The luteoma is an ovarian tumor, usually masculinizing, which is found only in pregnancy. It is typically yellow in color and histologically is composed of sheets of lipid-laden cells resembling a corpus luteum. It is not clear whether this is a true neoplasm or simply represents an exaggerated physiologic response of the ovarian stroma to hCG. Whether development of all these tumors is dependent on gonadotropins is unknown. As

they generally respond with increased hormone output when given hCG, a relationship to gonadotropin stimulation remains an intriguing possibility.

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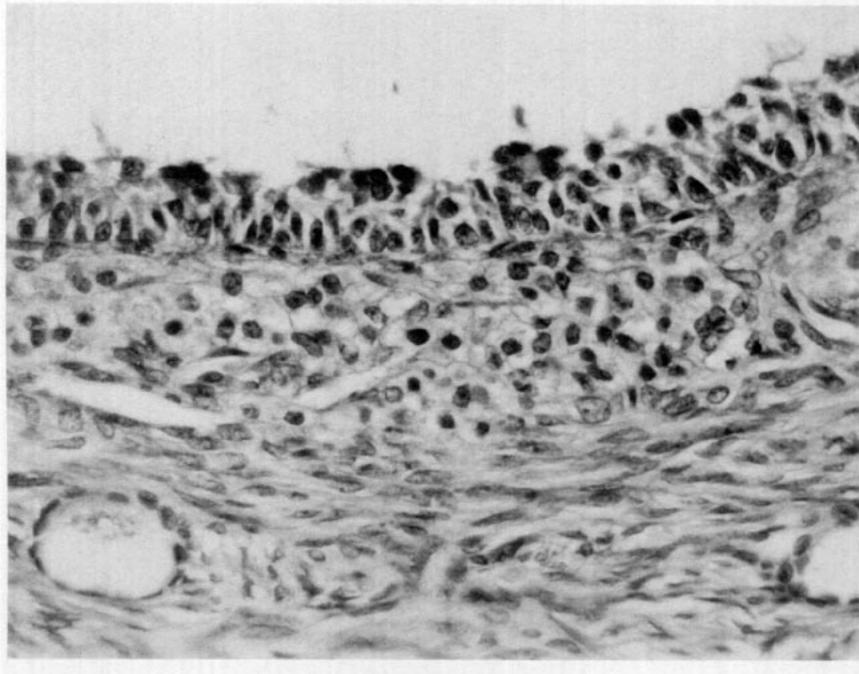


PLATE 1. The follicle wall of an atretic follicle. The pyknotic granulosa cell layers characteristic of an atretic follicle are present, overlying relatively active-appearing theca. The individual thecal cells still appear steroidogenically active, consistent with the maintenance of hormone production by the atretic theca. H&E, $\times 400$.

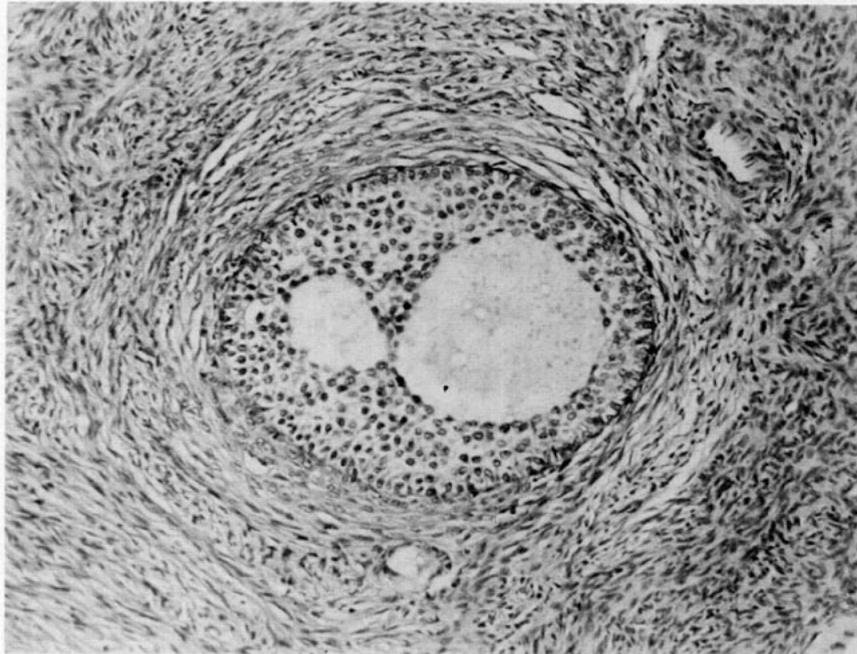


PLATE 2. An early antral follicle. A developing antral follicle is present with coalescing pools of follicular fluid. The oocyte is not in the plane of section and the number of granulosa cell layers is small. The theca is not yet prominent but is distinguishable from the surrounding ovarian stroma. H&E, $\times 400$.

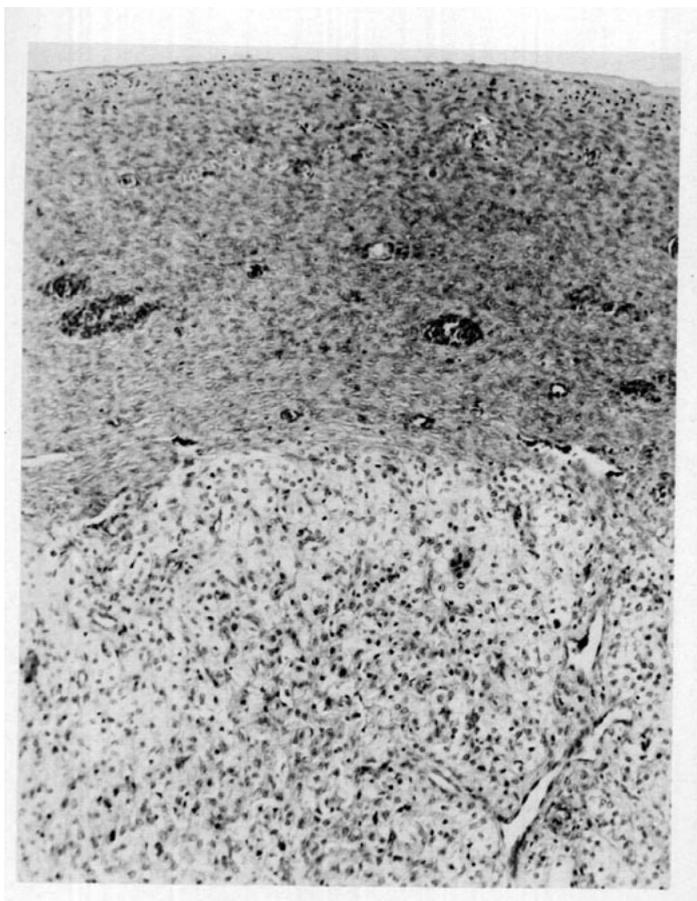


PLATE 3. The corpus luteum. A portion of a corpus luteum is present surrounded by typical ovarian stroma with the capsule of the ovary at the top of the picture. The distinction between the luteinized granulosa and theca layers is difficult, as the granulosa compartment becomes vascularized rapidly after ovulation. The individual luteal cells have the characteristics of steroidogenically active cells, with large, clear areas in the cytoplasm representing lipid accumulations. H&E, $\times 130$.

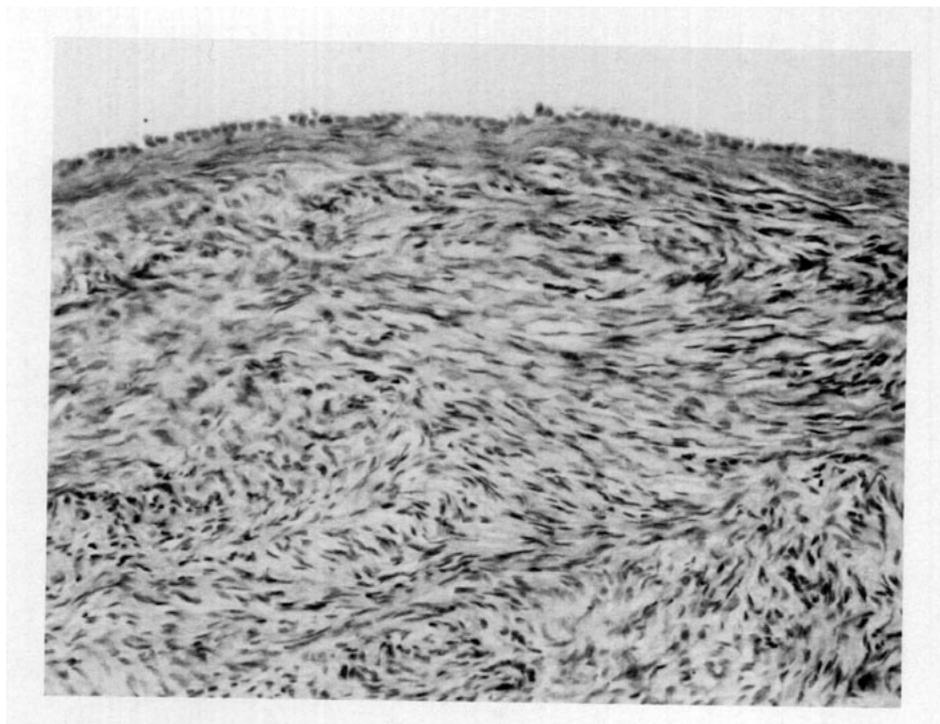


PLATE 4. The postmenopausal ovary. A single layer of germinal epithelium covers the ovarian capsule with typical ovarian stroma devoid of follicles. H&E, $\times 250$.

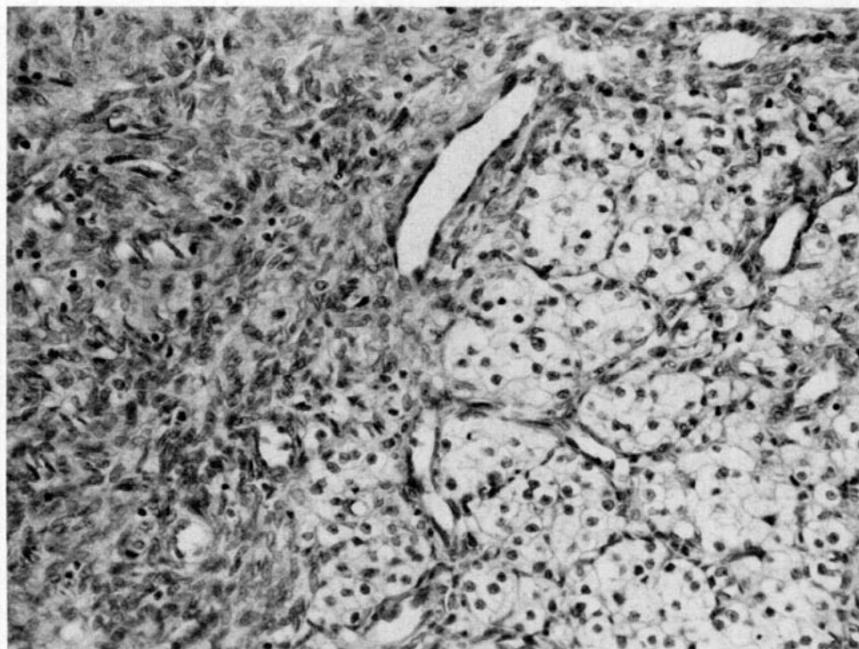


PLATE 5. Ovarian hyperthecosis. The ovarian stroma of an androgenized woman with chronic anovulation demonstrates an area of steroidogenically active-appearing cells consistent with excessive androgen production. These luteinized cells are scattered throughout the normal ovarian stroma. H&E, $\times 250$.

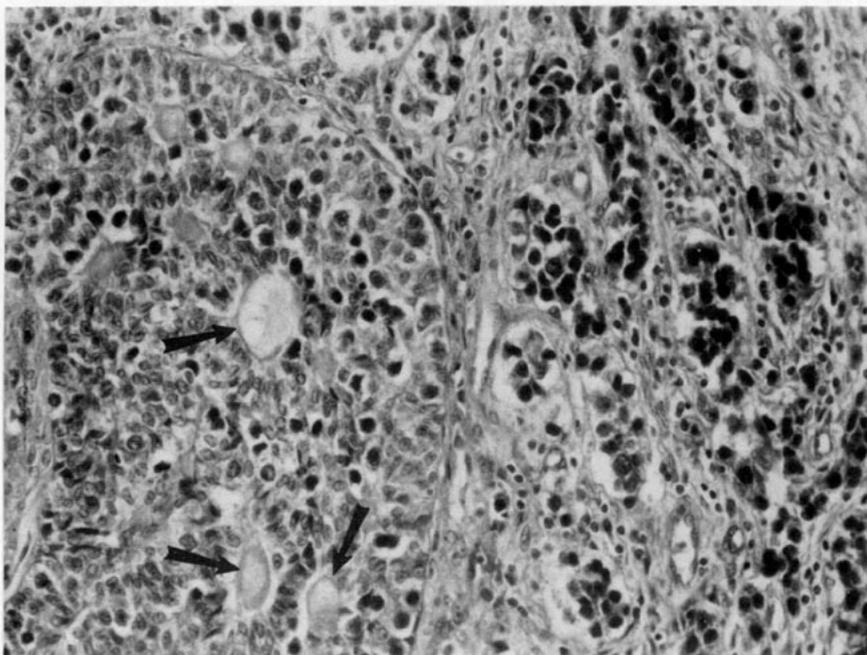


PLATE 6. Gonadoblastoma. A typical steroidogenically active gonadoblastoma is shown with both granulosa cell elements on the left side and attempts at seminiferous tubule formation on the right. Areas of apparent follicular fluid formation (Call-Exner bodies) are present in the granulosa cell elements (\rightarrow) typical of normal granulosa cells. H&E, $\times 250$.