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Gonadogenesis, Female

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GLOSSARY

female gametogenesis (oogenesis) A process that includes migration of primordial germ cells into the developing ovary, and continuous development of ovarian follicles in the adult as primordial follicles are recruited to develop.

granulosa cell A female somatic cell type in the ovarian follicle that surrounds the oocyte providing the required microenvironment for its maturation.

oocyte A female germ cell that develops within ovarian follicles until released from the ovary at ovulation.

ovary The female gonad; a structurally dynamic organ that supports oocyte maturation within developing follicles.

ovulation A process in which the mature Graafian ovarian follicle wall is ruptured and an oocyte is released.

primordial follicle A dormant ovarian follicle that consists of a single oocyte surrounded by a single or partial layer of squamous "pregranulosa cells" and will initiate follicular development and ovulate or degenerate. The pool of primordial follicles contains the available oocytes that a female will ever have.

theca cell A female somatic cell type that surrounds and provides structural integrity for the ovarian follicle. Theca cell–granulosa cell interactions are essential for ovarian follicular development.

Mammalian reproduction is dependent on the maturation of germ cells in the gonad. In the female, germ cells (i.e., oocytes) are contained in the ovary, which is a structurally dynamic organ that supports maturation of oocytes within developing follicles. Ovarian follicular development begins with the recruitment of primordial follicles to initiate development and continues through ovulation. After the oocyte is released at ovulation, the remaining theca cells and granulosa cells in the follicle develop into the corpus luteum. In humans, one dominant follicle ovulates from a single ovary during each menstrual cycle. Follicles that do not ovulate become atretic and degenerate. A great deal of research during the past 100 years has provided an understanding of some of the factors that control ovarian follicular

development. However, two important aspects of ovarian folliculogenesis are still largely unknown. These processes are (i) the initiation of primordial follicle development and (ii) the selection of the dominant follicle.

I. INTRODUCTION

During embryonic development, the process of ovarian development in the embryo establishes the tissue structures and cell populations that are necessary for follicular development in the adult. Most important, the germ cell population is established during early ovarian development. Ovarian organogenesis involves the coordinated movements and induction of several dynamic cell populations. The sequence of events at specific developmental stages is conserved from mice to humans (Table 1). Although the primary function of the mammalian ovary is to support germ cell (i.e., oocyte) development, all germ cells initially develop outside of the genital ridge. Interestingly, primordial germ cells follow a specific migratory pathway through the embryo into the gonad.

II. EARLY DEVELOPMENT OF THE OVARY

In the mammalian embryo, gonads develop from (i) the coelomic epithelium, (ii) the mesenchyme of the mesonephric ridge, and (iii) the primordial germ cells. The development of gonads is unique in that the gonadal rudiment can differentiate into an ovary or a testis. This developmental decision determines the subsequent sexual development of the organism. Before this decision is made the mammalian gonad first develops through an indifferent stage. During this stage the gonadal rudiment is first recognizable as a thickening of the coelomic epithelium and budding mesonephric mesenchyme on the medial side of the mesonephric ridge. As the thickening tissue buds out from the mesonephros, it forms the genital ridge (Fig. 1A) during Embryonic Days 31–35 in humans and on Embryonic Day 9 in mice. The coelomic epithelial cells of the genital ridge proliferate and migrate into the adjacent loose mesenchymal tissue in the budding indifferent gonad (Fig. 1B), recognizable during Embryonic Week 6 in humans and Day 11 in mice.

Two important paired ducts are also present in the mesonephros during early embryonic development.

TABLE 1
Early Development of Germ Cells and the Ovary

Age of embryo		
Human	*Mouse*	*Developmental process*
Day 28	Day 7.5	First primordial germ cells recognizable
Weeks 4–7	Days 8–12.5	Germ cells proliferate during migration
Week 4 (Days 25–30)	Day 9	Mesonephric tubules form
Weeks 4–5 (Days 28–30)	Day 9	Germ cells migrate to hindgut
Weeks 4–5 (Days 28–30)	Days 9–10	Wolffian ducts form
Week 5 (Days 31–35)	Day 9	Coelomic epithelium thickens to form genital ridge
Week 6 (Days 35–42)	Days 10–11	Germ cells migrate to dorsal mesentery
Week 6 (Days 38–42)	Day 11	Budding indifferent gonad is recognizable
Week 6 (Days 40–42)	Day 11	Müllerian duct forms
Week 7 (Days 42–48)	Days 11–12	Germ cells migrate to indifferent gonad
Week 7 (Day 49)	Day 12.5	Germ cell migration to gonads is complete
Weeks 7–8	Day 13	Gonad is recognizable as ovary
Months 2–7	Days 12–13	Oogonia proliferate within ovary
Months 2.5–7	Days 13–16	Oocytes initiate then arrest in meiosis (oogenesis)

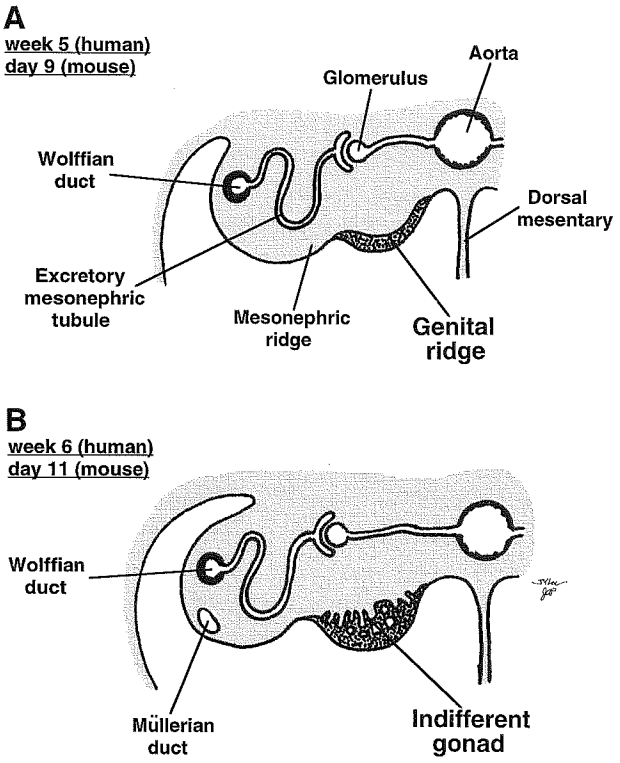

FIGURE 1 Early development of the indifferent gonad. The indifferent gonad can develop into an ovary or a testis. (A) During this stage the gonadal rudiment begins to bud out from the genital ridge to form the indifferent gonad. This is first observed as a thickening of the coelomic epithelium. (B) As the gonad grows, the coelomic epithelium of the genital ridge proliferates and migrates into the adjacent loose mesenchymal tissue. Germ cells are not present when the indifferent gonad buds out from the genital ridge. Germ cells migrate into the gonad during Embryonic Weeks 6 and 7 in humans and on Embryonic Days 11–12.5 in mice.

The Wolffian duct appears during Embryonic Week 4 in humans and on Day 10 in mice (Fig. 1A). The Müllerian duct is first seen during Embryonic Week 6 in humans and on Day 11 in mice (Fig. 1B). The Wolffian and Müllerian ducts will eventually develop into reproductive tracts of the male or female, respectively. Later in male development, the Müllerian duct will regress due to the production of mullerian-inhibiting substance by Sertoli cells in the testis. Androgen production by Leydig cells in the developing testis will support development of the Wolffian duct into the epididymus, vas deferens, and seminal vesicles. Later in female development, the Wolffian duct will regress due to the lack of high androgen production by the gonad (i.e., ovary). The Müllerian duct will develop into the oviduct, uterus, and upper part of the vagina. The development of the female reproductive tract from the Müllerian duct is not dependent on estrogen. For example, targeted disruption of the estrogen receptor gene in mice (i.e., the ERKO mouse) has no effect on prenatal development of the reproductive tract.

In essentially all mammals, germ cells are not present in the indifferent gonad when the genital ridge begins to bud out from the mesonephros. Primordial germ cells migrate along a characteristic pathway and enter the indifferent gonad when the coelomic epithelium is invading the loose mesenchyme. Primordial germ cells begin to enter the developing ovary during Embryonic Weeks 6 and 7 in humans and on Embryonic Day 11 in mice. This process of germ cell migration is highly conserved from flies to frogs to humans and is thought to be the result of a specific evolutionary process. In particular, primitive Metazoa had germ cells but no gonads to harbor them. As higher animals acquired gonads, biological strategies evolved to support germ cell migration to developing indifferent gonads. Migration of primordial germ cells is controlled by short-range cell-to-cell contacts and long-range effects of the genital ridge.

Analysis of germ cell migration during embryonic development has been an area of research for several decades. Most of the initial work was performed in mice. A significant advance in mapping mammalian germ cell migration was achieved with the observation that primordial germ cells contained extremely high levels of alkaline phosphatase that distinguished embryonic cells. Using this staining procedure, primordial germ cells can first be seen in the allantois on Embryonic Day 28 in humans and Embryonic Day 7.5 in mice (Table 1). The germ cells then begin to migrate into the yolk sac at the base of the allantois (Fig. 2A). By this time, the germ cells have split into two populations that will eventually migrate into the left or right gonad. Migration continues from the yolk sac through the newly formed hindgut and up the dorsal mesentery into the genital ridge (Fig. 2B). Germ cells migrate by ameboid movements and migration into the gonads is complete by the end of Embryonic Week 7 in humans and Embryonic Day 12.5 in mice (Table 1).

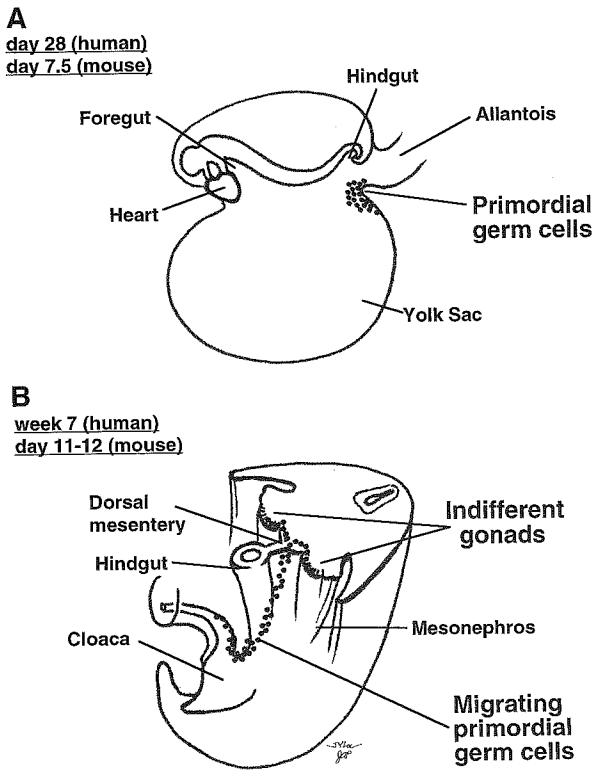

FIGURE 2 Germ cell migration in mammals. Primodrial germ cells are detected by staining for alkaline phosphatase. (A) Primordial germ cells can first be seen in the allantois on Embryonic Day 28 in humans and Embryonic Day 7.5 in mice. Germ cells then begin to migrate into the yolk sac at the base of the allantois. By this time, the germ cells have split into two populations that will migrate into the left or right gonad. (B) Migration continues caudally from the yolk sac through the newly formed hindgut and up the dorsal mesentery into the genital ridge. Primordial germ cell migration into the gonads is complete by the end of Embryonic Week 7 in humans and Embryonic Day 12.5 in mice. The overall process of germ cell migration is common from flies to frogs to humans.

The number of primordial germ cells is relatively small before germ cell migration takes place. In 70% of early vertebrate embryos, 20–100 primordial germ cells are present. The degree of consistency of early germ cell numbers in vertebrates suggests that some common processes are involved in the induction of the germ cell line. Primordial germ cells dramatically increase in number by proliferating. During germ cell migration in the human (Embryonic Weeks 4–7) and mouse (Embryonic Days 8–12.5), the number of primordial germ cells increases from about 100 to 2500–5000. After migration to the gonad is complete, primordial germ cells (i.e., oogonia) organize into clusters and continue to proliferate. Oogonia continue to proliferate until Embryonic Month 7 in humans and Day 13 in mice (Table 1). In humans the number of oogonia increases to 6 or 7 million.

Migration and proliferation of primordial germ cells is critical for fertility. White Spotting (W) mutations in mice caused sterility because the W locus encodes the c-*kit* gene that is necessary for germ cell migration in the embryo. The c-*kit* gene is a protooncogene receptor that interacts with the kit ligand (KL) (described in Section IV). Both KL and c-*kit* are essential for germ cell migration and proliferation during embryonic development. The somatic cells that line the migratory pathway express KL, whereas migrating primordial germ cells express c-*kit*. In the absence of KL or c-*kit*, embryonic germ cell migration and proliferation is inhibited. Subsequent studies have demonstrated that KL and c-*kit* are important for several stages of ovarian folliculogenesis in the adult. One exciting function of KL/c-*kit* during recruitment of primordial follicles is currently being investigated (described in Section IV).

Indifferent gonads are first recognizable as ovaries when primordial germ cells (i.e., oogonia) stop proliferating and enter meiotic prophase. This process occurs during Embryonic Weeks 7 and 8 in humans and on Day 13 in mice (Table 1). Oogonia that have entered meiosis are called oocytes. Initiation of oocyte meiosis in the ovary marks the initiation of oogenesis. However, not all oogonia enter meiosis at the same time. Therefore, proliferating oogonia and meiotic oocytes are present in young ovaries at the same time. As shown in Table 1, oogenesis occurs during Embryonic Months 2.5–7 in humans and on Embryonic Days 13–16 in mice. Meiosis continues through the first meiotic prophase until arresting at the diplotene stage. The ability of germ cells to develop from primordial germ cells to meiotic oocytes does not require the presence of the gonad. However, the gonad is necessary for oocytes to develop beyond this stage. A single layer of presumptive granulosa cells organizes around each oocyte. These oocyte–pregranulosa complexes are called primordial follicles. Oocytes in primordial follicles remain arrested in meiosis until later in life when they resume devel-

opment. In humans some oocytes are arrested in meiotic prophase in the form of primordial follicles for up to 50 years. The factors that control recruitment of primordial follicles to develop are not known.

Not all oocytes in the ovary survive. Proliferation of oogonia in the embryo establishes an abundance of germ cells in females. After this proliferative phase is complete, the number of germ cells in the ovary decreases progressively throughout life. Germ cells degenerate during embryonic ovarian development or during follicular development in the adult. The majority of germ cells are eliminated in females, perhaps to select germ cells with stable, viable genomes. In humans the number of germ cells is reduced from 6 or 7 million in the 7-month-old embryo to 2 million at birth and 400,000 at puberty.

III. PRIMORDIAL FOLLICLE DEVELOPMENT

A crucial event for ovarian development is the enclosure of oocytes by somatic cells into individual "compartments." These cellular structures are called primordial follicles. Survival and differentiation of germ cells depend on their organization into primordial follicles. Primordial follicles are formed when mesonephric-derived cells within the ovary contact and organize into a single layer of pregranulosa cells around the oocyte (Figs. 3A and 4A). These cell–cell

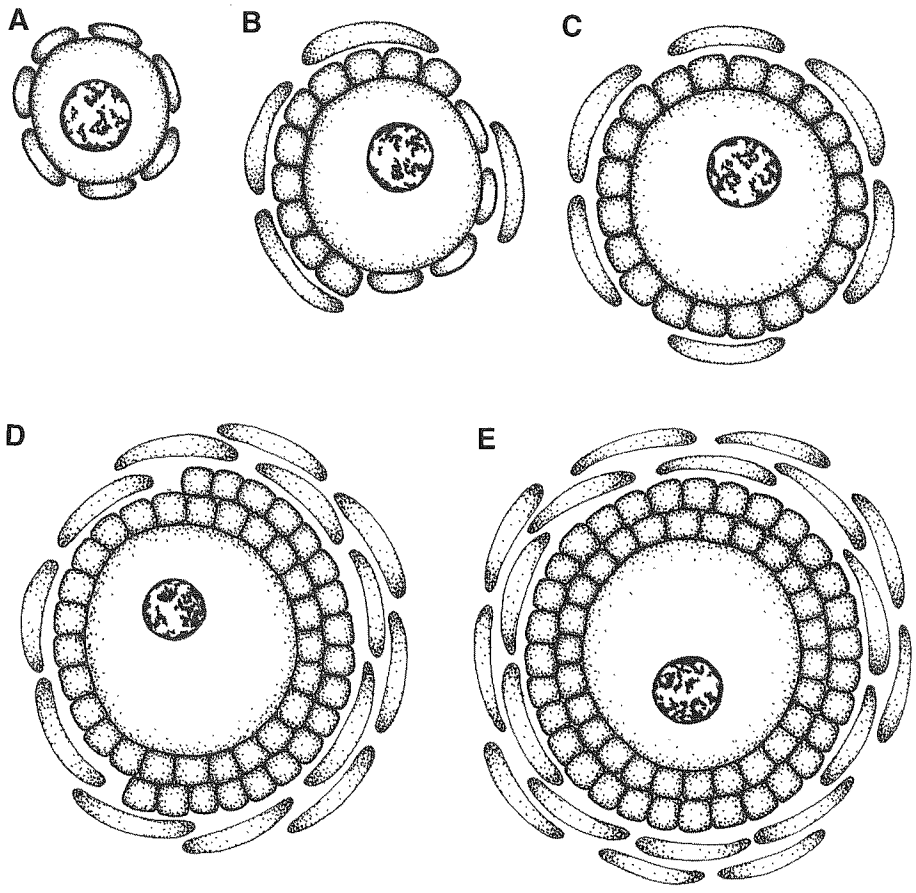

FIGURE 3 Schematic of primordial follicle development in the ovary. (A) Primordial follicles (stage 0) consist of an oocyte surrounded by a full or partial layer of flattened pregranulosa cells. (B) Early primary follicles (stage 1) have initiated development and contain some columnar (enlarged) granulosa cells. Theca cells are being recruited and are starting to organize around the follicle. (C) Primary follicles (stage 2) are surrounded by a single layer of cuboidal granulosa cells around the oocyte. (D) Transitional follicles (stage 3) contain 1 or 2 layers of columnar granulosa cells. Formation of theca cell layers continues. (E) Preantral follicles (stage 4) have two or more layers of columnar granulosa cells. Theca cells are well organized around the follicle.

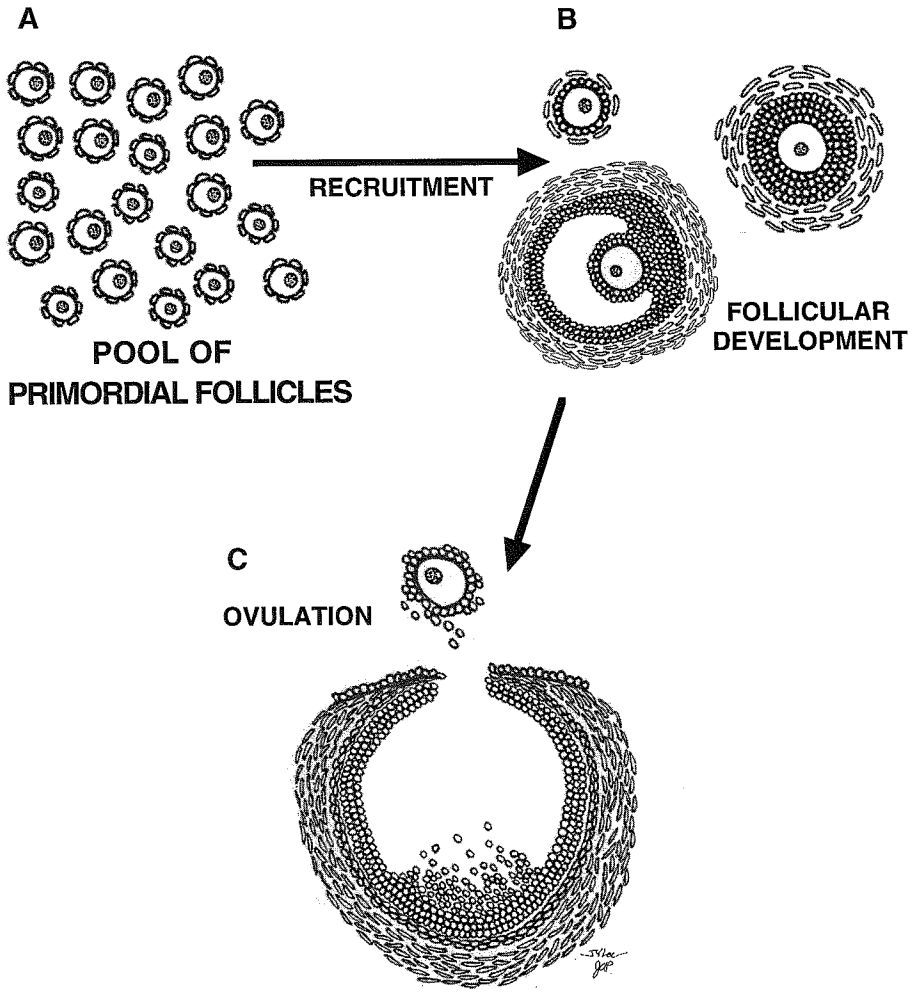

FIGURE 4 Primordial follicles are recruited to develop later in life. (A) A single layer of presumptive granulosa cells organizes around oocytes to form the pool of primordial follicles. Primordial follicles represent the complete pool from which all ovarian follicles can develop. Oocytes arrest at the diplotene stage of the first meiotic prophase. Oocytes remain dormant in the form of primordial follicles until later in life when they are recruited to develop. (B) Follicular development involves follicular expansion due to theca cell and granulosa cell proliferation as well as the formation of an antrum. Oocytes remain arrested in meiosis. (C) After the luteinizing hormone surge at ovulation, oocyte meiosis is resumed. In humans some oocytes are arrested in meiotic prophase in the form of primordial follicles for up to 50 years.

contacts may act as a trigger for the onset of meiosis in germ cells. After arrest in prophase of meiosis, oocytes remain dormant in the form of primordial follicles until recruited for follicular development later in life (Fig. 4).

Extensive research (i.e., histological, histochemical, and autoradiographic techniques) has established that primordial follicles represent the pool from which all developing follicles will emerge. Waldeyer correctly proposed in 1870 that the germ cells observed in the embryonic ovary developed sequentially into mature ova throughout life. In 1887 and 1903, Paladino claimed there was a continuous destruction of follicles with constant formation of new egg tubes and ova, with the process going on from birth to old age in mammals. Subsequent studies showed that *de novo* formation of oocytes does not occur in ovaries depleted of germ cells. Today it is

well accepted that the oocytes established in the pool of primordial follicles are the only oocytes a female animal will ever have.

The large pool of primordial follicles in mammalian ovaries is progressively reduced through follicular development. Throughout life, a subset of available primordial follicles is continuously recruited to develop (Figs. 4 and 5). In embryonic and prepubertal ovaries, all developing follicles degenerate. In pubertal and adult ovaries, some follicles will continue development and successfully ovulate (Fig. 4C). All primordial follicles that initiate follicular development are destined to ovulate or degenerate through atresia. Therefore, factors that control recruitment and initiation of primordial follicle development ultimately determine the number of available follicles in the ovary.

The factors that control initiation of primordial follicle development in the ovary are not known. Identification of such factors has been an extensive area of research for more that 100 years. During this time, no hormone (including gonadotropins and estrogen) has been identified that influences this process. However, there is evidence that the total number of primordial follicles (i.e., the pool) in the ovary may influence the number of developing follicles.

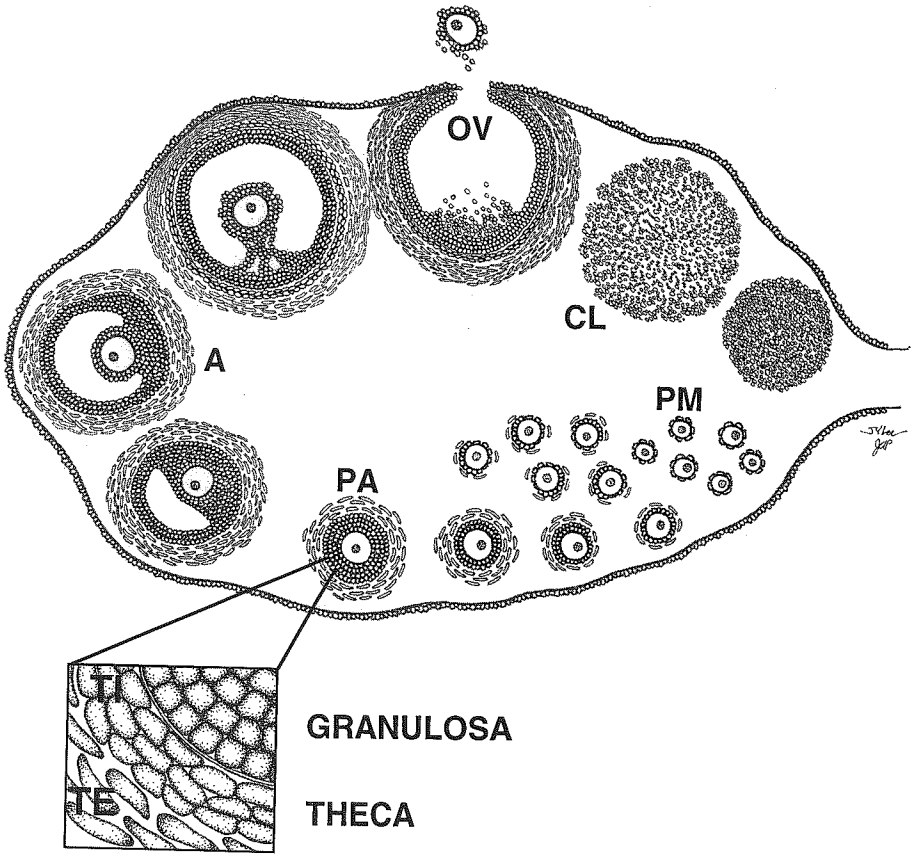

FIGURE 5 Schematic of cycling adult ovary. The basic functional unit is the ovarian follicle. Follicles are recruited to initiate development from the pool of primordial follicles (PM). Developing follicles consist of a single oocyte and two somatic cell types (enlarged box). Granulosa cells surround the oocyte and provide the critical microenvrionment for oocyte maturation. Surrounding the granulosa cells, theca cells provide structural integrity for the follicle. Theca cells differentiate into two distinct layers during folliculogenesis. Theca interna (TI) are adjacent to granulosa cells and theca externa (TE) are further outside. Theca cells and granulosa cells proliferate and differentiate to form preantral follicles (PA) and fluid-filled antral follicles (A). A small number of follicle continue development and eventually ovulate (OV). After ovulation the remaining theca cells and granulosa cells organize into the corpus luteum (CL).

Nevertheless, initiation of primordial follicle development is proposed to be controlled by a hormone or soluble factor. The mechanism of how only a subset of primordial follicles can be recruited to initiate development was being considered by Hargitt in 1930. Hargitt argues that

> If an assumption be made that many latent primary follicles are stored in the mammalian ovary, a serious difficulty is encountered in explaining the stimulation of a few to renewed development, while others remain quiescent. Likely enough, some hormone might stimulate renewed activity, but why of a few not all of the same age?

This question has led to the proposal that initiation of primordial follicle development is controlled locally by a factor(s) produced in the ovary.

Peters' discussion of follicular development in immature mouse ovaries suggested that initiation of primordial follicle development may be influenced by a factor(s) produced by developing follicles. Such factors may induce or inhibit recruitment of primordial follicles. The local presence of stimulatory and inhibitory factors for this process may determine how a subset of primordial follicles begins to develop. The influence of antral follicles on initiation of primordial follicle development was tested by injecting follicular fluid (bovine) into neonatal mice for 4 days. Within a few days, the number of primordial follicles that initiated development was reduced by 35%. These results suggest that developing antral follicles produce an unknown inhibitory factor(s) for primordial follicle development. It has also been suggested that granulosa cells in early developing follicles produce an unknown factor that acts on theca cells to promote early follicular development. The identity of these putative factors is not known despite their importance for control of primordial follicle development.

IV. RECENT PROGRESS: KIT-LIGAND INDUCES PRIMORDIAL FOLLICLE DEVELOPMENT

KL and its receptor c-*kit* are important for gametogenesis, melanogenesis, and hematopoiesis. During embryonic development, KL and c-*kit* are essential for germ cell migration. In the adult ovary, granulosa cells produce KL that may be important for oocyte function during follicular development (e.g., oocyte development and meiotic arrest). Most of our understanding about the function of KL in the ovary is based on observations of KL actions on oocytes. However, granulosa cell-derived KL also influences theca cell growth and functional differentiation (i.e., steroidogenesis). Expression of the receptor c-*kit* has been observed in stromal cells, theca cells, and oocytes in very early developing follicles. Based on the variety of KL actions and the expression patterns of KL and c-*kit* in the ovary, KL has been proposed to induce initiation of ovarian primordial follicle development.

Ovaries from 4-day-old rats contain large numbers of primordial follicles that can initiate development. After 5 days in ovary organ culture, some of these primordial follicles spontaneously initiated development and KL induced primordial follicles to initiate development. When control ovaries were cultured with ACK-2, a c-*kit* antibody that strongly inhibits KL actions, spontaneous primordial follicle development was completely inhibited. These results suggested that KL was necessary and sufficient to induce initiation of primordial follicle development in ovarian organ cultures.

Initiation of primordial follicle development is one of the most basic aspects of ovarian folliculogenesis. KL is the first identified factor that is involved in initiation of primordial follicle development. The mechanism of how KL induces primordial follicle development is not known. It is possible that KL has important actions on oocytes, theca cells, and adjacent stromal–interstitial cells during early follicle development.

Acknowledgment

The figures were provided by my friend and colleague, Janet Y. Lee. Thank you for your dedication and kindness.

See Also the Following Articles

CORPUS LUTEUM; FOLLICULAR DEVELOPMENT, CONTROL OF; OVARY, OVERVIEW; THECA CELLS

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