First Week of Development: Ovulation to Implantation

Ovarian Cycle

At puberty, the female begins to undergo regular monthly cycles. These **sexual cycles** are controlled by the hypothalamus. **Gonadotropin-releasing hormone (GnRH)** produced by the hypothalamus acts on cells of the anterior pituitary gland, which in turn secrete **gonadotropins**. These hormones, **follicle-stimulating hormone (FSH)** and **luteinizing hormone (LH)**, stimulate and control cyclic changes in the ovary.

At the beginning of each ovarian cycle, 15 to 20 primary (preantral) stage follicles are stimulated to grow under the influence of FSH. (The hormone is not necessary to promote development of primordial follicles to the primary follicle stage, but without it, these primary follicles die and become atretic.) Thus, FSH rescues 15 to 20 of these cells from a pool of continuously forming primary follicles (Fig. 2.1). Under normal conditions, only one of these follicles reaches full maturity, and only one oocyte is discharged; the others degenerate and become atretic. In the next cycle, another group of primary follicles is recruited, and again, only one follicle reaches maturity. Consequently, most follicles degenerate without ever reaching full maturity. When a follicle becomes atretic, the oocyte and surrounding follicular cells degenerate and are replaced by connective tissue, forming a **corpus atreticum.** FSH also stimulates maturation of **follicular (granulosa)** cells surrounding the oocyte. In turn, proliferation of these cells is mediated by growth differentiation factor-9 (GDF-9), a member of the transforming growth factor-β (TGF-β) family.

In cooperation, granulosa and thecal cells produce **estrogens** that (*a*) cause the uterine endometrium to enter the follicular or **proliferative phase**; (*b*) cause thinning of the cervical mucus to allow passage of sperm; (*c*) stimulate the pituitary gland to secrete LH. At mid-cycle, there is an **LH surge** that (*a*) elevates concentrations of maturation-promoting factor, causing oocytes to complete meiosis I and initiate meiosis II;
(*b*) stimulates production of progesterone by follicular stromal cells **(luteinization);**
(*c*) causes follicular rupture and ovulation.

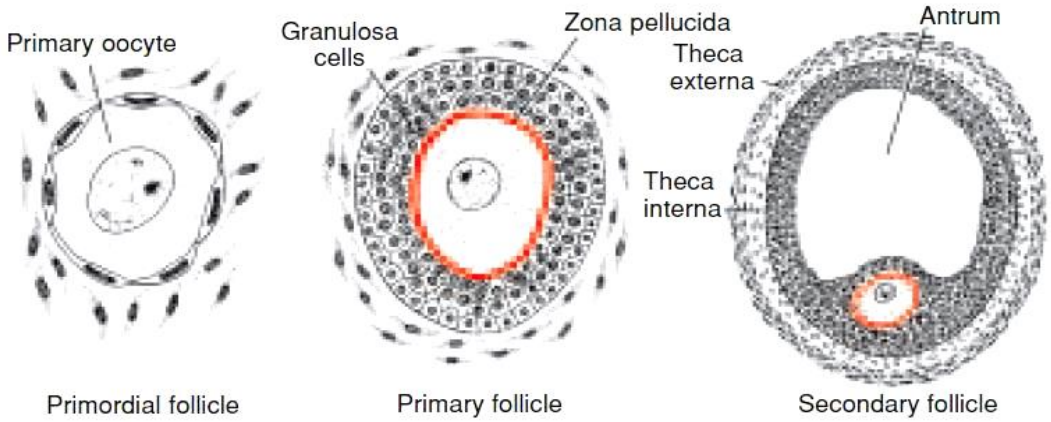

Figure 2.1 From the pool of primordial follicles, every day some begin to grow and develop into secondary (preantral) follicles, and this growth is independent of FSH. Then, as the cycle progresses, FSH secretion recruits primary follicles to begin development into secondary (antral, Graafian) follicles. During the last few days of maturation of secondary follicles, estrogens, produced by follicular and thecal cells, stimulate increased production of LH by the pituitary (Fig. 2.13), and this hormone causes the follicle to enter the preovulatory stage, to complete meiosis I, and to enter meiosis II where it arrests in metaphase approximately 3 hours before ovulation.

OVULATION

In the days immediately preceding ovulation, under the influence of FSH and LH, the secondary follicle grows rapidly to a diameter of 25 mm. Coincident with final development of the secondary follicle, there is an abrupt increase in LH that causes the primary oocyte to complete meiosis I and the follicle to enter the preovulatory stage. Meiosis II is also initiated, but the oocyte is arrested in metaphase approximately 3 hours before ovulation. In the meantime, the surface of the ovary begins to bulge locally, and at the apex, an avascular spot, the **stigma**, appears. The high concentration of LH increases collagenase activity, resulting in digestion of collagen fibers surrounding the follicle. Prostaglandin levels also increase in response to the LH surge and cause local muscular contractions in the ovarian wall.

Those contractions extrude the oocyte, which together with its surrounding granulosa cells from the region of the cumulus oophorus, breaks free **(ovulation)** and floats out of the ovary (Figs. 2.2 and 2.3) Some of the cumulus oophorus cells then rearrange themselves around the zona pellucida to form the **corona radiata** (Figs. 2.4–2.6).

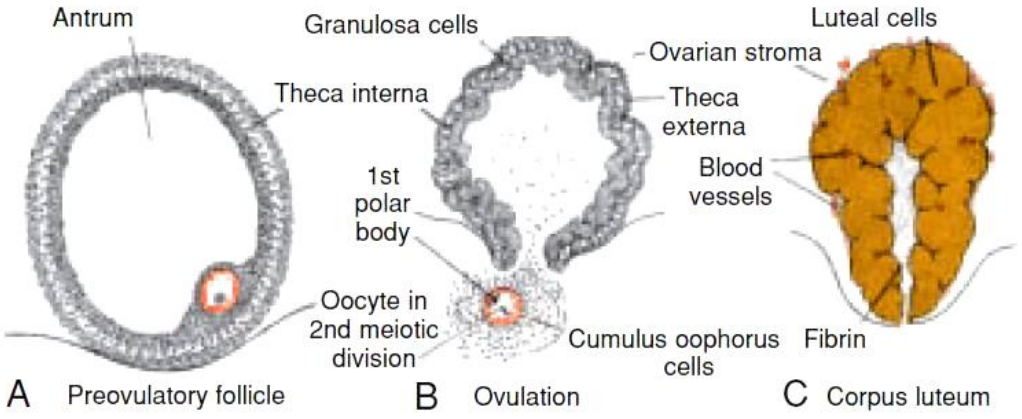

Figure 2.2 A. Preovulatory follicle bulging at the ovarian surface. **B.** Ovulation. The oocyte, in metaphase of meiosis II, is discharged from the ovary together with a large number of cumulus oophorus cells. Follicular cells remaining inside the collapsed follicle differentiate into lutean cells. **C.** Corpus luteum. Note the large size of the corpus luteum, caused by hypertrophy and accumulation of lipid in granulosa and theca interna cells. The remaining cavity of the follicle is filled with fibrin.

CORPUS LUTEUM

After ovulation, granulosa cells remaining in the wall of the ruptured follicle, together with cells from the theca interna, are vascularized by surrounding vessels. Under the influence of LH, these cells develop a yellowish pigment and change into **lutean cells**, which form the **corpus luteum** and secrete the hormone **progesterone** (Fig. 2.2*C*). Progesterone, together with estrogenic hormones, causes the uterine mucosa to enter the **progestational** or **secretory stage** in preparation for implantation of the embryo.

OOCYTE TRANSPORT

Shortly before ovulation, fimbriae of the oviduct begin to sweep over the surface of the ovary, and the tube itself begins to contract rhythmically. It is thought that the oocyte surrounded by some granulosa cells (Figs. 2.3 and 2.4) is carried into the tube by these sweeping movements of the fimbriae and by motion of cilia on the epithelial lining. Once in the tube, cumulus cells withdraw their cytoplasmic processes from the zona pellucida and lose contact with the oocyte.
Once the oocyte is in the uterine tube, it is propelled by cilia with the rate of transport regulated by the endocrine status during and after ovulation. In humans, the fertilized oocyte reaches the uterine lumen in approximately 3 to 4 days.

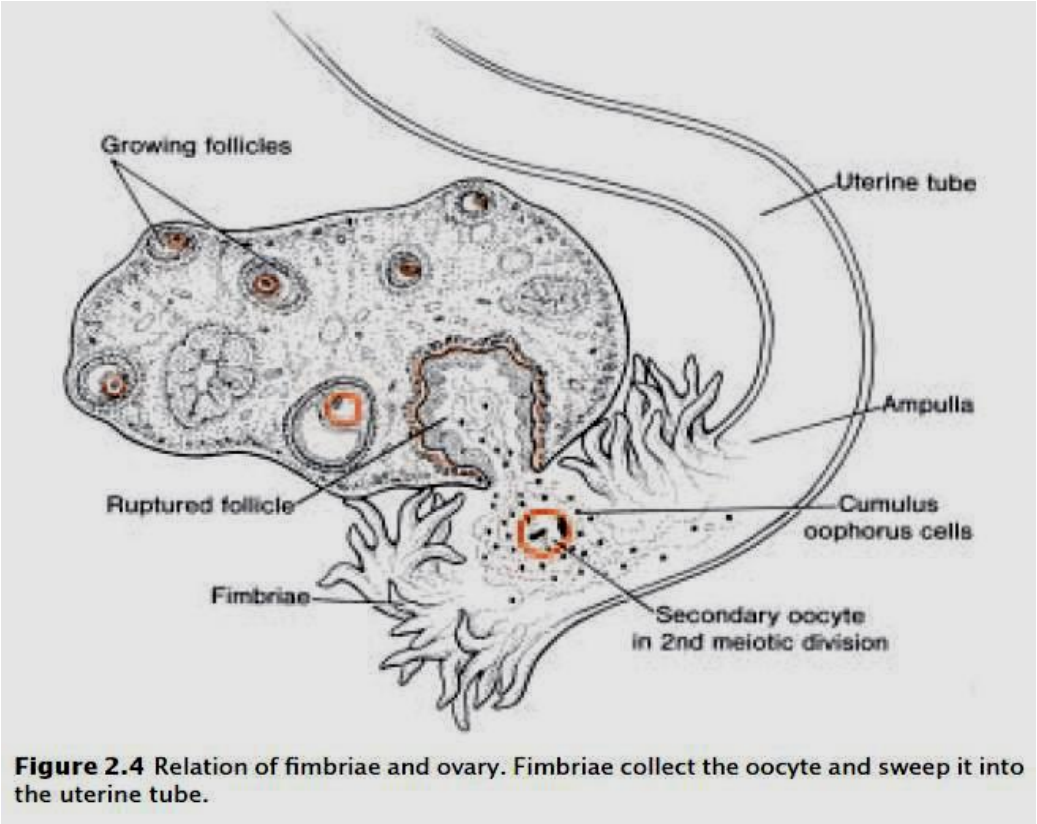

Figure 2.4 Relation of fimbriae and ovary. Fimbriae collect the oocyte and sweep it into the uterine tube.

CORPUS ALBICANS

If fertilization does not occur, the corpus luteum reaches maximum development approximately 9 days after ovulation. It can easily be recognized as a yellowish projection on the surface of the ovary. Subsequently, the corpus luteum shrinks because of degeneration of lutean cells and forms a mass of fibrotic scar tissue, the **corpus albicans**. Simultaneously, progesterone production decreases, precipitating menstrual bleeding. If the oocyte is fertilized, degeneration of the corpus luteum is prevented by **human chorionic gonadotropin)hCG),** a hormone secreted by the syncytiotrophoblast of the developing embryo.

The corpus luteum continues to grow and forms the **corpus luteum of pregnancy (corpus luteum graviditatis).** By the end of the third month, this structure may be one-third to one-half of the total size of the ovary. Yellowish luteal cells continue to secrete progesterone until the end of the fourth month; thereafter, they regress slowly as secretion of progesterone by the trophoblastic component of the placenta becomes adequate for maintenance of pregnancy. Removal of the corpus luteum of pregnancy before the fourth month usually leads to abortion.

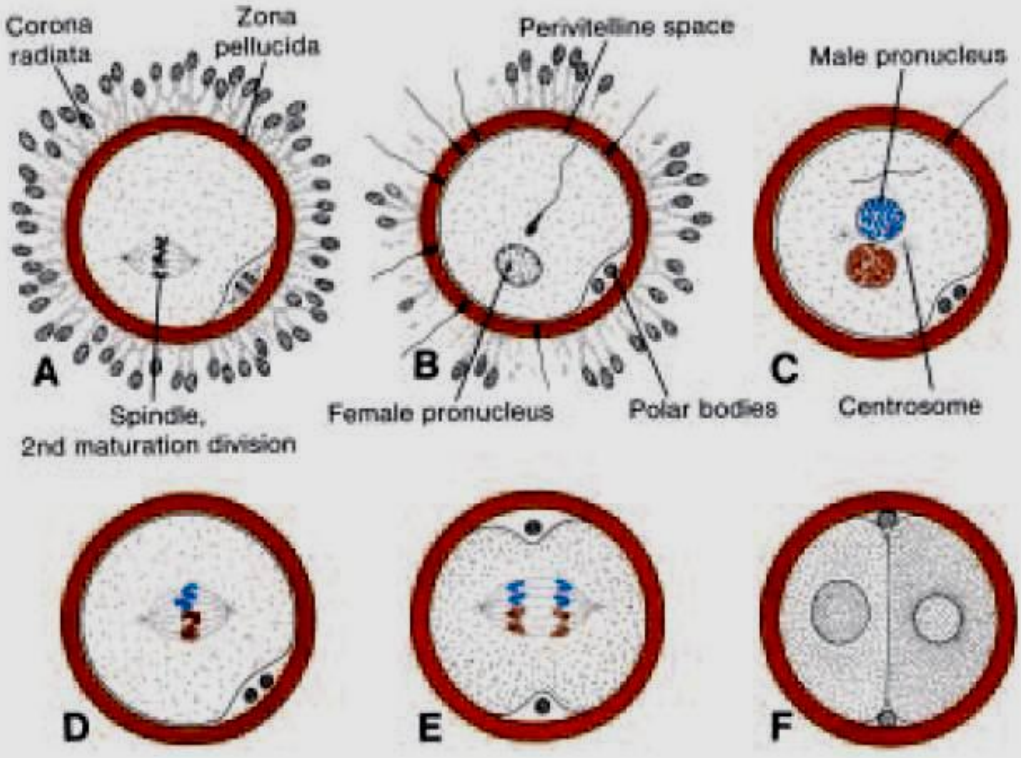

Figure 2.6 A. Oocyte immediately after ovulation, showing the spindle of the second meiotic division. **B.** A spermatozoon has penetrated the oocyte, which has finished its second meiotic division. Chromosomes of the oocyte are arranged in a vesicular nucleus, the female pronucleus. Heads of several sperm are stuck in the zona pellucida. **C.** Male and female pronuclei. **D** and **E.** Chromosomes become arranged on the spindle, split longitudinally, and move to opposite poles. **F.** Two-cell stage.

Fertilization

Fertilization, the process by which male and female gametes fuse, occurs in the **ampullary region of the uterine tube**. This is the widest part of the tube and is close to the ovary (Fig. 2.4). Spermatozoa may remain viable in the female reproductive tract for several days.

Only 1% of sperm deposited in the vagina enter the cervix, where they may survive for many hours. Movement of sperm from the cervix to the oviduct is accomplished primarily by their own propulsion, although they may be assisted by movements of fluids created by uterine cilia. The trip from cervix to oviduct requires a minimum of 2 to 7 hours, and after reaching the isthmus, sperm become less motile and cease their migration. At ovulation, sperm

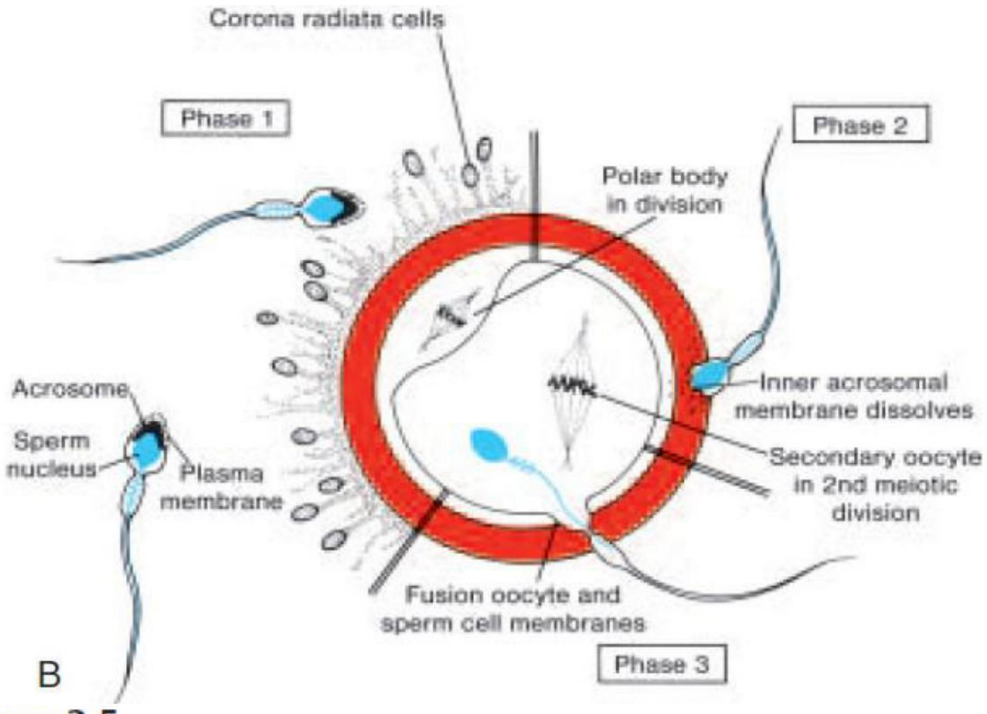

Figure 2.5
B. The three phases of oocyte penetration. In phase 1, spermatozoa pass through the corona radiata barrier; in phase 2, one or more spermatozoa penetrate the zona pellucida; in phase 3, one spermatozoon penetrates the oocyte membrane while losing its own plasma membrane. *Inset.* Normal spermatocyte with acrosomal head cap.

Again become motile, perhaps because of chemoattractants produced by cumulus cells surrounding the egg, and swim to the ampulla where fertilization usually occurs. Spermatozoa are not able to fertilize the oocyte immediately upon arrival in the female genital tract but must undergo: (*a*) **capacitation**. (*b*) the **acrosome reaction** to acquire this capability.

Capacitation is a period of conditioning in the female reproductive tract that in the human lasts approximately 7 hours. Much of this conditioning, which occurs in the uterine tube, entails epithelial interactions between the sperm and mucosal surface of the tube. During this time a glycoprotein coat and seminal plasma proteins are removed from the plasma membrane that overlies the acrosomal region of the spermatozoa. Only capacitated sperm can pass through the corona cells and undergo the acrosome reaction.

The **acrosome reaction**, which occurs after binding to the zona pellucida, is induced by zona proteins. This reaction culminates in the release of enzymes needed to penetrate the zona pellucida, including acrosin and trypsin-like substances (Fig. 2.5).

The phases of fertilization include phase 1, penetration of the corona radiata; phase 2, penetration of the zona pellucida; and phase 3, fusion of the oocyte and sperm cell membranes.

PHASE 1: PENETRATION OF THE CORONA RADIATA

Of the 200 to 300 million spermatozoa deposited in the female genital tract, only 300 to 500 reach the site of fertilization. Only one of these fertilizes the egg. It is thought that the others aid the fertilizing sperm in penetrating the barriers protecting the female gamete. Capacitated sperm pass freely through corona cells (Fig. 2.5).

PHASE 2: PENETRATION OF THE ZONA PELLUCIDA

The zona is a glycoprotein shell surrounding the egg that facilitates and maintains sperm binding and induces the acrosome reaction. Both binding and the acrosome reaction are mediated by the ligand ZP3, a zona protein. Release of acrosomal enzymes (acrosin) allows sperm to penetrate the zona, there by coming in contact with the plasma membrane of the oocyte (Fig.. 2.5) Permeability of the zona pellucida changes when the head of the sperm comes in contact with the oocyte surface. This contact results in release of lysosomal enzymes from cortical granules lining the plasma membrane of the oocyte .

In turn, these enzymes alter properties of the zona pellucida(zona reaction) to prevent sperm penetration and inactivate species-specific receptor sites for spermatozoa on the zona surface. Other spermatozoa have been found embedded in the zona pellucida, but only one seems to be able to penetrate the oocyte(Fig. 2.6).

PHASE 3: FUSION OF THE OOCYTE AND SPERM CELL MEMBRANES

The initial adhesion of sperm to the oocyte is mediated in part by the interaction of integrins on the oocyte and their ligands, disintegrins, on sperm. After adhesion, the plasma membranes of the sperm and egg fuse (Fig. 2.5). Because the plasma membrane covering the acrosomal head cap disappears during the acrosome reaction, actual fusion is accomplished between the oocyte membrane and the membrane that covers the posterior region of the sperm head (Fig. 2.5). In the human, both the head and tail of the spermatozoon enter the cytoplasm of the oocyte, but the plasma membrane is left behind on the oocyte surface. As son as the spermatozoon has entered the oocyte, the egg responds in three ways:

1. **Cortical and zona reactions**. As a result of the release of cortical oocyte granules, which contain lysosomal enzymes,

(a) the oocyte membrane becomes impenetrable to other spermatozoa,

(b) the zona pellucida alters its structure and composition to prevent sperm binding and penetration.

These reactions prevent polyspermy (penetration of morethan one spermatozoon into the oocyte).

2. **Resumption of the second meiotic division**. The oocyte finishes its second meiotic division immediately after entry of the spermatozoon. ofOne the daughter cells, which receives hardly any cytoplasm, is knowntheas second polar body; the other daughter cell is the definitive oocyte. female pronucleus (Figs. 2.6 and 2.7).

3. Metabolic activation of the egg. The activating factor is probably carried by the spermatozoon. Postfusion activation may be considered to encompass the initial cellular and molecular events associated with early embryogenesis.
The spermatozoon, meanwhile, moves forward until it lies close to the female pronucleus. Its nucleus becomes swollen and forms the **male pronucleus** (Fig. 2.6); the tail detaches and degenerates. Morphologically, the male and female pronuclei are indistinguishable, and eventually, they come into close contact and lose their nuclear envelopes (Fig. 2.7*A*). During growth of male and female pronuclei (both haploid), each pronucleus must replicate its DNA. If it does not, each cell of the two-cell zygote has only half of the normal amount of DNA. Immediately after DNA synthesis, chromosomes organize on the spindle in preparation for a normal mitotic division. The 23 maternal and
23 paternal (double) chromosomes split longitudinally at the centromere, and sister chromatids move to opposite poles, providing each cell of the zygote with the normal diploid number of chromosomes and DNA (Fig. 2.6, *D* and *E*). As sister chromatids move to opposite poles, a deep furrow appears on the surface of the cell, gradually dividing the cytoplasm into two parts (Figs. 2.6*F* and 2.7*B*).
The main results of fertilization are as follows:
- **Restoration of the diploid number of chromosomes,** half from the father and half from the mother. Hence, the zygote contains a new combination of chromosomes different from both parents.
- **Determination of the sex** of the new individual. An X-carrying sperm produces a female (XX) embryo, and a Y-carrying sperm produces a male (XY) embryo. Hence, the chromosomal sex of the embryo is determined at fertilization.
- **Initiation of cleavage**. Without fertilization, the oocyte usually degenerates
24 hours after ovulation.

Cleavage

Once the zygote has reached the two-cell stage, it undergoes a series of mitotic divisions, increasing the numbers of cells. These cells, which become smaller with each cleavage division, are known as **blastomeres** (Fig. 2.8). Until the eight-cell stage, they form a loosely arranged clump (Fig. 2.9*A*). However, after the third cleavage, blastomeres maximize their contact with each other, forming a compact ball of cells held together by tight junctions (Fig. 2.9*B*). This process, **compaction**, segregates inner cells, which communicate extensively by gap junctions, from outer cells. Approximately 3 days after fertilization, cells of the compacted embryo divide again to form a 16-cell **morula** (mulberry). Inner cells of the morula constitute the **inner cell mass**, and surrounding cells compose the **outer cell mass**. The inner cell mass gives rise to tissues of the **embryo proper**, and the outer cell mass forms the **trophoblast**, which later contributes to the **placenta**.

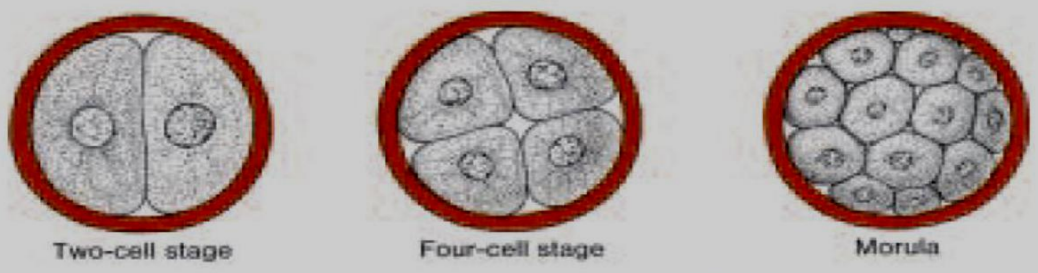

Figure 2.8 Development of the zygote from the two-cell stage to the late morula stage. The two-cell stage is reached approximately 30 hours after fertilization; the four-cell stage, at approximately 40 hours; the 12- to 16-cell stage, at approximately 3 days; and the late morula stage, at approximately 4 days. During this period, blastomeres are surrounded by the zona pellucida, which disappears at the end of the fourth day.

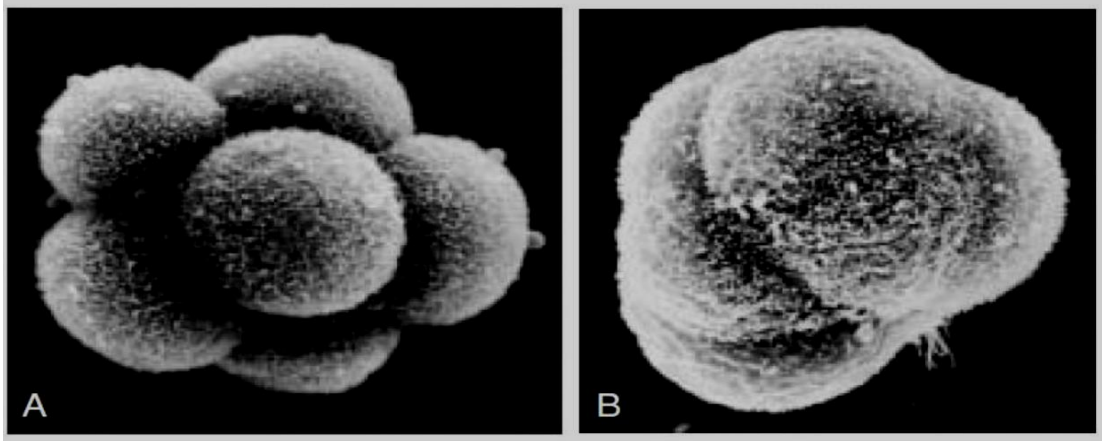

Figure 2.9 Scanning electron micrographs of uncompacted **(A)** and compacted **(B)** eight-cell mouse embryos. In the uncompacted state, outlines of each blastomere are distinct, whereas after compaction cell-cell contacts are maximized and cellular outlines are indistinct.

Blastocyst Formation

About the time the morula enters the uterine cavity, fluid begins to penetrate through the zona pellucida into the intercellular spaces of the inner cell mass. Gradually the intercellular spaces become confluent, and finally a single cavity, the **blastocele**, forms (Fig. 2.10, *A* and *B*). At this time, the embryo is a **blastocyst**. Cells of the inner cell mass, now called the **embryoblast**, are at one pole, and those of the outer cell mass, or **trophoblast,** flatten and form the epithelial wall of the blastocyst (Fig. 2.10, *A* and *B*). The zona pellucida has disappeared, allowing implantation to begin.

In the human, trophoblastic cells over the embryoblast pole begin to penetrate between the epithelial cells of the uterine mucosa about the sixth day (Fig. 2.10*C*). Attachment and invasion of the trophoblast involve integrins, expressed by the trophoblast, and the extracellular matrix molecules laminin and fibronectin. Integrin receptors for laminin promote attachment, while those for fibronectin stimulate migration. These molecules also interact along signal transduction pathways to regulate trophoblast differentiation so that implantation is the result of mutual trophoblastic and endometrial action. Hence, by the end of the first week of development, the human zygote has passed through the morula and blastocyst stages and has begun implantation in the uterine mucosa.

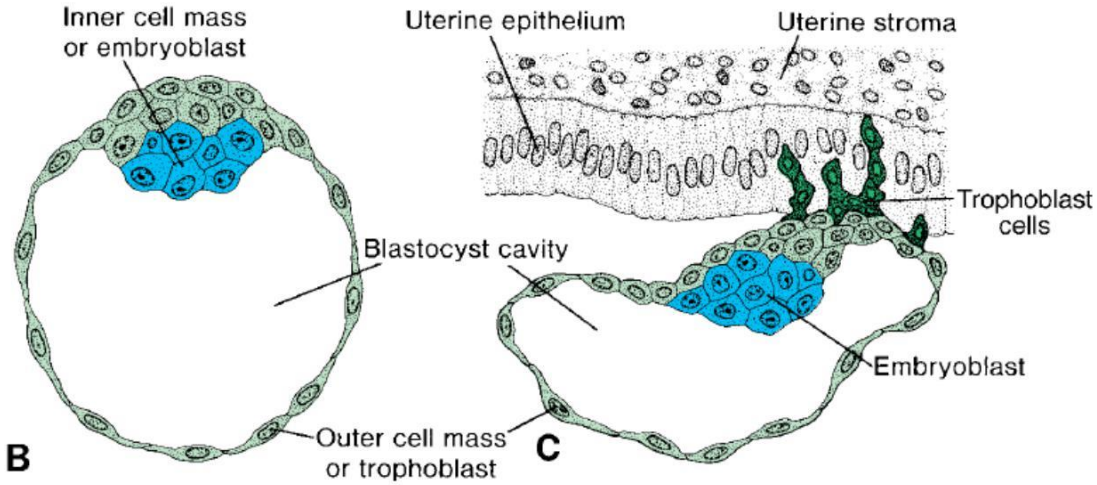

Figure 2.10

B. Schematic representation of a human blastocyst recovered from the uterine cavity at approximately 4.5 days. *Blue*, inner cell mass or embryoblast; *green*, trophoblast. **C.** Schematic representation of a blastocyst at the ninth day of development showing trophoblast cells at the embryonic pole of the blastocyst penetrating the uterine mucosa. The human blastocyst begins to penetrate the uterine mucosa by the sixth day of development.

Uterus at Time of Implantation

The wall of the uterus consists of three layers:
(*a*) **endometrium** or mucosa lining the inside wall;
(*b*) **myometrium**, a thick layer of smooth muscle;
(*c*) **perimetrium**, the peritoneal covering lining the outside wall (Fig. 2.11). From puberty (11–13 years) until menopause (45–50 years), the endometrium undergoes changes in a cycle of approximately 28 days under hormonal control by the ovary. During this menstrual cycle, the uterine endometrium passes through three stages, the **follicular** or **proliferative phase**, the **secretory** or **progestational phase**, and the **menstrual phase** (Figs. 2.11– 2.13).

The proliferative phase begins at the end of the menstrual phase, is under the influence of estrogen, and parallels growth of the ovarian follicles. The secretory phase begins approximately 2 to 3 days after ovulation in response to progesterone produced by the corpus luteum. If fertilization does not occur, shedding of the endometrium (compact and spongy layers) marks the beginning of the menstrual phase. If fertilization does occur, the endometrium assists in implantation and contributes to formation of the placenta.

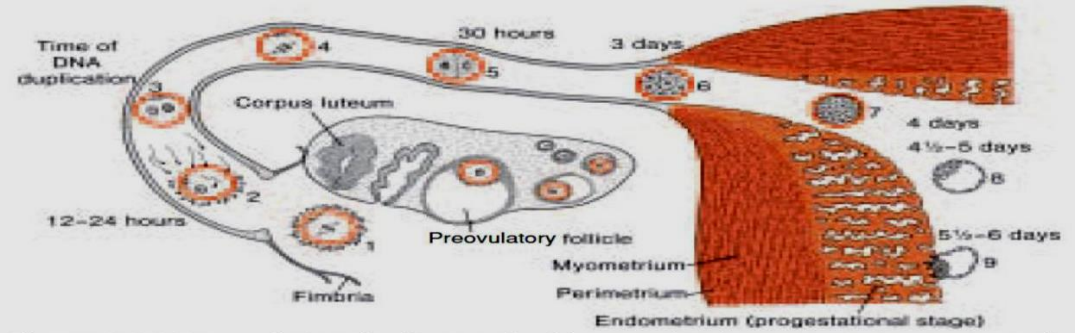

Figure 2.11 Events during the first week of human development. *1*, Oocyte immediately after ovulation. *2*, Fertilization, approximately 12 to 24 hours after ovulation. *3*, Stage of the male and female pronuclei. *4*, Spindle of the first mitotic division. *5*, Two-cell stage (approximately 30 hours of age). *6*, Morula containing 12 to 16 blastomeres (approximately 3 days of age). *7*, Advanced morula stage reaching the uterine lumen (approximately 4 days of age). *8*, Early blastocyst stage (approximately 4.5 days of age). The zona pellucida has disappeared. *9*, Early phase of implantation (blastocyst approximately 6 days of age). The ovary shows stages of transformation between a primary follicle and a preovulatory follicle as well as a corpus luteum. The uterine endometrium is shown in the progestational stage.

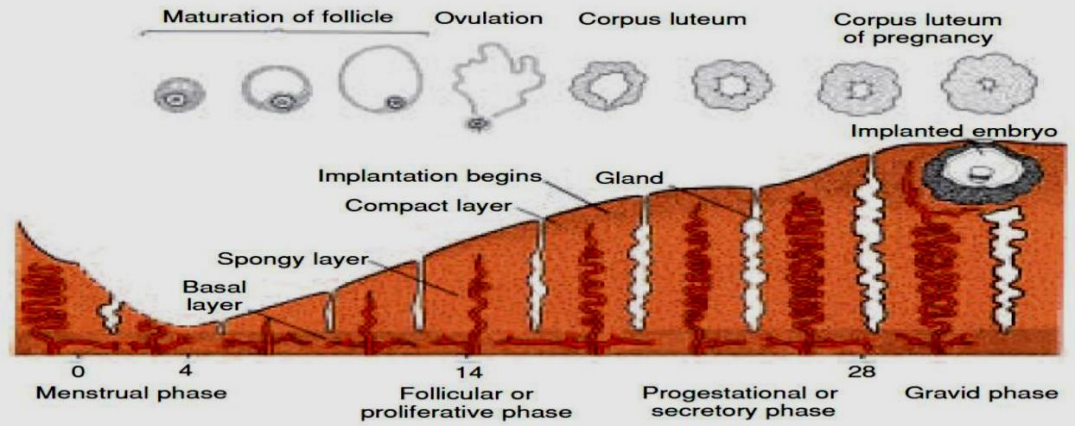

Figure 2.12 Changes in the uterine mucosa correlated with those in the ovary. Implantation of the blastocyst has caused development of a large corpus luteum of pregnancy. Secretory activity of the endometrium increases gradually as a result of large amounts of progesterone produced by the corpus luteum of pregnancy.

At the time of implantation, the mucosa of the uterus is in the secretory phase (Figs. 2.11 and 2.12), during which time uterine glands and arteries become coiled and the tissue becomes succulent. As a result, three distinct layers can be recognized in the endometrium: a superficial compact layer, an intermediate **spongy layer**, and a thin **basal layer** (Fig. 2.12). Normally, the human blastocyst implants in the endometrium along the anterior or posterior wall of the body of the uterus, where it becomes embedded between the openings of the glands (Fig. 2.12).

If the oocyte is not fertilized, venules and sinusoidal spaces gradually become packed with blood cells, and an extensive diapedesis of blood into the tissue is seen. When the **menstrual phase** begins, blood escapes from superficial arteries, and small pieces of stroma and glands break away. During the following 3 or 4 days, the compact and spongy layers are expelled from the uterus, and the basal layer is the only part of the endometrium that is retained (Fig. 2.13). This layer, which is supplied by its own arteries, the **basal arteries**, functions as the regenerative layer in the rebuilding of glands and arteries in the **proliferative phase** (Fig. 2.13).

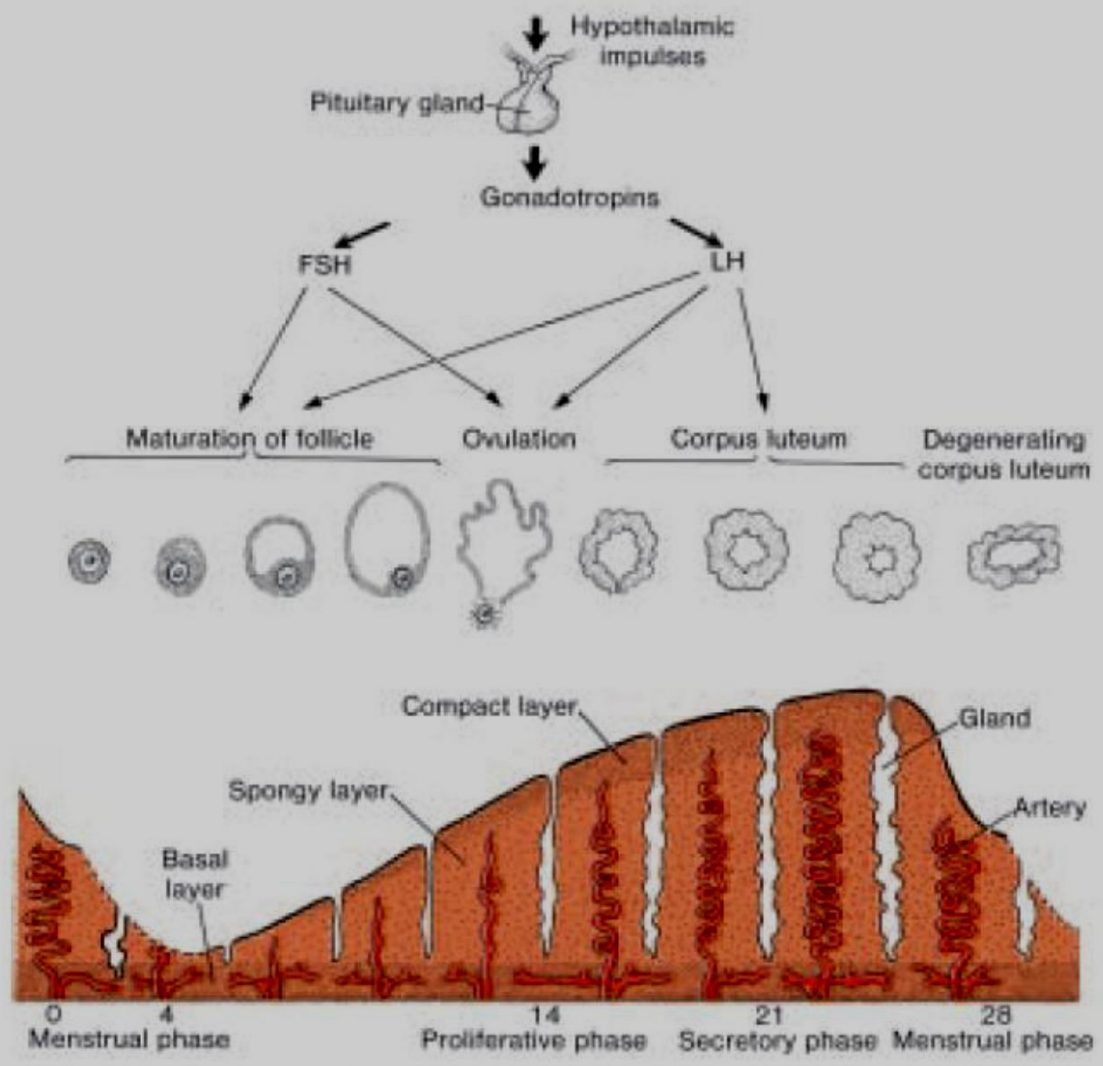

Figure 2.13 Changes in the uterine mucosa (endometrium) and corresponding changes in the ovary during a regular menstrual cycle without fertilization.