Ovum Maturation, Ovulation, Fertilisation, Implantation and Embryo Development.

Introduction

- During Intra Uterine Fetal Development the ovary passes through a series of clearly identifiable stages:
  - The genital ridge stage in which the sex cells can first be identified. They begin as hypertrophy of the coelomic epithelium (future peritoneum) overlying the developing mesonephroi. It is thought any further growth of the ridges is dependent upon the arrival of germ cells.
  - Indifferent stage where there is proliferation of germinal and somatic cells. The germ cell proliferates rapidly by mitosis.
  - Sexual differentiation in which fundamental histological differences between the ovary and the testis are established.
- To maintain the species-specific Chromosome compliment, the male and female gametes must go through the process of Meiosis. There are, however, considerable differences between male and female as to the timing of this process. In the human male meiosis occurs after puberty and continues throughout life owing to the persistent of mitotically active "Stem Cells", (spermatogonia). By contrast, meiosis in the female is initiated during fetal life and all stem cells are eliminated around the time of birth when meiosis is suspended in the middle of the first meiotic division. The resumption of meiosis occurs shortly before ovulation in response to LH surge.
- Increasing numbers of oogonia become transformed into primary oocytes in human ovaries from the end of the second month post-conception. During this period approximately 600,000 germ cells are present, the majority being oogonia either at interphase or in mitosis, and the remainder in the prophase of meiosis. The oogonia continue to proliferate so that the total number of germ cells reaches a maximum at mid-pregnancy. Baker's calculations show that at the 5th month of gestation an ovary contains approximately 2 million oogonia together with 4.8 million oocytes representing all the stages of meiotic prophase. During the second half of gestation, the total number of germ cells is markedly reduced as more and more oocytes are eliminated, although the rapidity of decline is accentuated by the cessation of mitosis among the remaining oogonia, and their entry into meiosis. Thus between 5 months post-conception and full term the numbers of oogonia and of oocytes at the Leptotene and Zygotene stages fall practically to zero. Of the oocytes, which have reached Pachytene, considerable number show degenerative changes, and the remainder, progress to Diplotene. The proportion of cells at Diplotene increases and at birth constitutes about 65% of the population, the remaining germ cells being at earlier stages.
- The ovaries of a newborn child contain only about 2,000,000 oocytes. At 12 to 14 years of age there are approximately 250,000 to 300,000 oocytes, while at 45-55 years of age, perhaps only a few hundreds survive.

OOCYTE (Ovum) MATURATION

In human embryos, primordial germ cells are first identified at about 4 weeks post conception when they appear to be embedded in the wall of the york sac near to the allantois region. In succeeding days the cells migrate around the allantois and the developing gut into the dorsal mesentery near to the transitory kidney. These primordial germ cells constitute the first population of mitotically active stem cells, the oogonia in the ovary, and spermatogonia in the testis.

The process of Oogenesis begins when the primordial germ cells migrate into the embryonic gonad and become oogonia. The oogonia proliferate by mitotic division, become invested with a single layer of granulosa cells, and differentiate into primary oocytes. The primary oocyte, duplicates its complement of DNA, reaches Prophase-1-Meiosis, and then enters a state of prolonged "hibernation". The primary oocyte remains arrested in this state until it is recruited into a pool of developing follicles during any one of the normal menstrual cycles. At around the 14th day to the commencement of menstruation and under the influence of the Luteinizing Hormone surge, the oocyte completes meiosis I, extrudes the first polar body and becomes a secondary oocyte. The secondary oocyte proceeds to metaphase II of meiosis and released from the ovary to await fertilization.

In summary, as the Graafian Follicle develops to maturity, the following well-coordinated changes are observed after the LH surge:

1. The germinal vesicle condenses into meiotic chromosomes in Diakinesis.

2. Then followed rapidly by Metaphase, Anaphase and Telophase of the first meiotic division. The extrusion of the first polar body (small, organelle-free cytoplasmic vesicle) results in haploid number of chromosomes.

3. Oocyte maturation also involves cytoplasmic changes resulting in a defence against polyspermy, established by the migration of granules from the cytoplasm to the cortex of the ooplasm. This peripheral position of the cortical granules and their association with microtubules, actin and myosin probably establishes the effective block against polyspermy.

4. Rapid synthesis of cytoplasmic protein macromolecules. Such proteins probably arise by translation from preformed mRNA, rather than by the modification of existing proteins. Cytoplasmic maturation is essential for normal fertilization and early embryonic development.

5. The second meiotic division, which results in formation of a second polar body (similar in size to the first), is completed only if fertilization occurs.

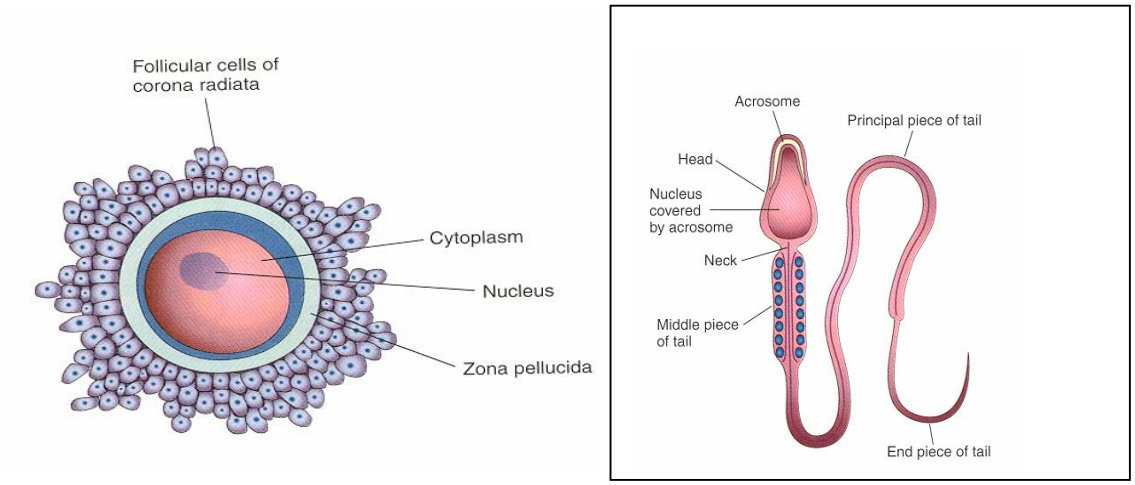
Note:

- Φ When removed from their follicles, human oocytes require 36-37 hours to complete their maturation. There is a strict diurnal rhythm in the timing of the LH surge in women, beginning in the great majority at 03.00 AM, hence ovulation must occur in most women at a prescribed time of day, perhaps in the early afternoon. The induction of the LH surge appears to be linked to the cortisol rhythm.

OVULATION & OVUM TRANSPORT

As indicated above the primary oocyte remains arrested at Prophase-1-Meiosis until it is recruited into a pool of developing follicles. Under the influence of the Luteinizing Hormone surge, the oocyte completes meiosis I, extrudes the first polar body, and becomes a secondary oocyte. The secondary oocyte then proceeds to metaphase II of meiosis. At the same time there is loosening up of the corona radiata cells, which eventually rupture on the outer surface leading to the oozing out of the oocyte. (Ovulation). At this time of ovulation, a protein covering, the Zona Pellucida, surrounds the ovum. Layers of follicular cells form what is called the cumulus oophorus about the egg. The follicular cells closest to the Zona Pellucida are arranged in a radiate pattern and therefore referred to as the corona radiata. These cells send projections in and through the Zona Pellucida, establishing cytoplasmic contact with the egg membrane (vitelline membrane).

After a short period of ovum retention in the ampulla, it progresses through the isthmic portion of the tube into the uterus. During this interval, the ovum if fertilized, cleaves to between the 8 and 16 cell stage. The ovum lives approximately 72 hours after it is extruded from the follicle but is probably fertilizable for less than half this time. Sperms apparently survive in the female genital tract for no more than 48 hours.



Indicators of Ovulation:

- Φ History: Regular periods; mid-cycle pain, (Mittelschmerz); cervical mucus changes, (thinnest with maximum elasticity at the time of ovulation).
- Φ Rise in Basal Body Temperature. The cause of this temperature rise at ovulation is unknown but is probably due to the increase in progesterone secretion, since progesterone is thermogenic.

- Φ Finding of thick, cellular cervical mucus that does not form fern pattern in the luteal phase of the cycle. (Provided there is no cervical/vaginal infection or Bleeding).
- Φ Rise in both urinary and serum LH levels.
- Φ Histology: secretory pattern of the endometrium.

FERTILIZATION

Human development begins at Fertilization, a complex sequence of 'coordinated molecular events' that begins with contact between the male gamete - (Sperm or Spermatozoon) and the female gamete - (Ovum or Oocyte), and ends with the intermingling of maternal and paternal chromosomes at metaphase of the first mitotic division of the zygote, a unicellular embryo. Carbohydrate binding molecules on the surface of gametes are possibly involved in the process of fertilization through gamete recognition and union of the cells. The fertilization process takes about 24 hours.

Approximately 20 – 250 million sperms/ml are deposited on the cervix and the posterior vaginal fornix during sexual intercourse. The fertilization process begins when the capacitated spermatozoon contacts the ovum, followed by penetration through the cellular coverings: the cumulus oophorus, the corona radiata and Zona Pellucida, and finally fusion with the oocyte. The cumulus cells are embedded in a sticky matrix, rich in hyaluronic acid. The acrosome, located on the head of the spermatozoon contains various enzymes, including hyaluronidase, which are capable of depolymerising hyaluronic acid, hence cumulus dispersion. The action of the cilia of the fallopian tube also aids the process of cumulus dispersion.

In summary the key steps in the process of fertilization are:

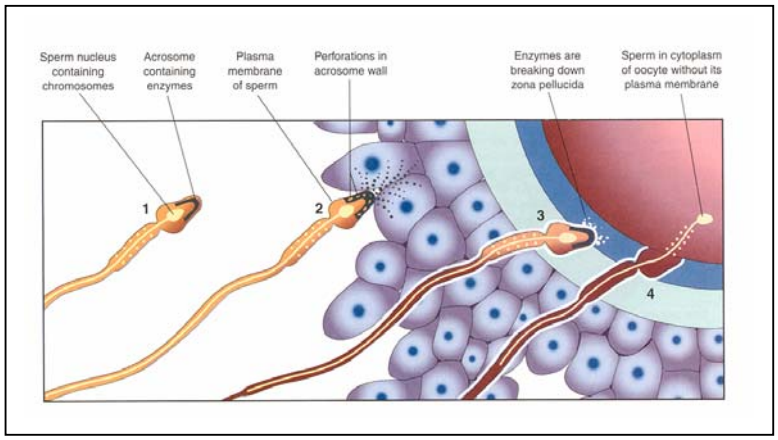
1. Capacitation: A series of enzymic changes involving proteinases, glycosidases and other enzymes in vivo to modify the plasma membrane of the spermatozoon, exposing specific glycoproteins or sugar residues which are then involved in the binding of spermatozoa to the Zona Pellucida or to the membrane of the oocyte. Sperms are usually capacitated in the uterus or uterine tubes by substances secreted by these parts of he female genital tract.
2. Binding of sperm to Zona Pellucida.
3. Acrosome reaction: This reaction is triggered by specific proteins contained in the zona pellucida and involves fusion and finally disruption of the sperm plasma membrane and outer acrosomal membrane so releasing Acrosomal enzymes, including acrosin, which assist in the penetration of spermatozoa between the cumulus cells and their attachment to and possibly movement through the Zona Pellucida.
4. Penetration of sperm through Zona Pellucida. The penetration of the zona pellucida occurs as a result of release from the inner acrosomal membrane of the penetrating spermatozoon of a trypsin-like enzyme, referred to as acrosin. Other enzymes involved include esterases and neuraminidase.
5. Once attachment of plasma membranes of sperm and oocyte has occurred, rapid electrical changes occur on the membrane surface that prevents penetration of additional spermatozoa (polyspermy). The spermatozoon (both head and the tail), is then incorporated into the cytoplasm of the oocyte, completing the penetration process.
6. Sperm nucleus enters egg cytoplasm. Within the cytoplasm of the oocyte, the nucleus of the sperm enlarges to form the male pronucleus and the tail of the sperm degenerates. After entry of the sperm, the oocyte, which has been arrested in metaphase of the second meiotic division, completes this division and forms a mature oocyte and a second polar body. Following decondensation of the maternal chromosomes, the nucleus of the mature oocyte becomes the female pronucleus. Morphologically the male and female pronuclei are indistinguishable.
7. Both pronuclei increase in size, migrate towards the centre of the ovum, and fuse as the second polar body is formed and extruded from the oocyte. With the union of the male and female pronuclei, the cell once again contains a diploid number of chromosomes. Membranes of pronuclei break down; the chromosomes condense and become arranged for a mitotic cell division – the first cleavage division. The fertilized oocyte or zygote is a unicellular embryo with 46 chromosomes. The first mitotic division occurs, with cleavage to the two-cell stage.

Abnormalities of fertilization include:

a. Triploidy: accounting for 20% of chromosomally imbalanced human fetuses.

b. Dispermy or fertilization with a diploid spermatozoon: leading to the formation of a hydatidiform mole. These embryos are androgenic in origin and possess a diploid set of paternal chromosomes because the female pronucleus either fails to form or is excluded at syngamy.

c. Nondisjunction (failure of a chromosome pair to separate) occurs during an early cleavage division of a zygote, an embryo with two or more cell lines with different chromosomes complements is produced.



IMPLANTATION

A successful implantation depends upon precise synchrony between the arrival of the blastocyst at the site of implantation and the sensitisation of the endometrium for implantation. In the days prior to ovulation, under hormonal influence, predominantly estrogen, the endometrial cells exhibit marked mitotic activity. Following ovulation, the combined influence of estrogen and progesterone, produced in increasing quantities by the corpus luteum, continue to increase the endometrial vascular and glandular activity.

Approximately 30 hours after fertilization, cleavage of the zygote begins. Cleavage consists of repeated mitotic divisions of the zygote, resulting in a rapid increase in the number of cells. These cells –blastomeres – become smaller with each cleavage division. Subsequent divisions follow one another, forming progressively smaller blastomeres. After the nine-cell stage, the blastomeres change their shape and tightly align themselves against each other to form a compact ball of cells. This phenomenon-known as compaction is probably mediated by cell surface adhesion glycoproteins. Compaction permits greater cell-to-cell interaction and is a prerequisite for segregation of the internal cells that form the inner cell mass (embryoblast) of the blastocyst. When there are 12 to 16 blastomeres, the developing human is called a morula and is ready to enter the uterus for further development. This latter takes place at around the 3-4 day of fertilization.

Shortly after the morula enters the uterus, a fluid filled space called the blastocyst cavity (blastocele) appears inside it. As fluid increases in the blastocyst cavity, it separates the blastomeres into two parts: A thin outer cell layer called the trophoblast, which gives rise to the embryonic part of the placenta, and a group of centrally located blastomeres known as the inner cell mass, the embryoblast, which gives rise to the embryo. At this stage of development, the conceptus is called a blastocyst.

After the blastocyst has floated in the uterine secretions for about 2 days, the zona pellucida gradually degenerates and disappears (Shedding process), followed by release of the blastocyst, (hatching process). Shedding of the zona pellucida permits the blastocyst to increase rapidly in size.

At around day 6 after fertilization (day 20 of a 28 day menstrual cycle), the blastocyst attaches to the endometrial epithelium, usually adjacent to the inner cell mass, the embryonic pole. As soon as it attaches to the endometrial epithelium, the trophoblast starts to proliferate rapidly and gradually differentiates into two layers: An inner layer of cytotrophoblast and an outer mass of syncytiotrophoblast consisting of a multinucleated protoplasmic mass in which no cell boundaries can be observed.

The initial process of implantation involves the adherence of the trophoblast tissue adjacent to the inner cell mass to the epithelium of the endometrium. Subsequently, the finger-like processes of syncytiotrophoblast begin to invade the endometrial tissue, and within 3-5 days the embryo is completely embedded under the uterine epithelium and into the endometrial stroma.

In summary, implantation is an interstitial process with three main stages:

- Φ Preattachment or apposition: Epithelia lining opposing endometrial surfaces move together and into close contact with the blastocyst, so that microvilli of the epithelium and trophoblast interdigitate.
- Φ Attachment: The embryonic microvilli become more numerous, the apposing trophoblast and epithelium flatten, and the contact becomes more intimate. The vascular permeability of the uterus increases, perhaps through the action of histamine, prostaglandins and cAMP from the embryo. Large amounts of glycosaminoglycans or high molecular weight on the outer surface of embryos and changes in cell surface may be involved in adhesion.
- Φ Invasion. The invasive stage involves contact between trophoblast and endometrial stroma. Syncitiotrophoblast proliferates from the cytotrophoblast overlying the embryonic disc and penetrates between the epithelial cells, disrupting them from the basement membrane and possibly phagocytosing them, perhaps through its secretion of Proteolytic enzymes.

Note:

The chances of an embryo implanting appear to be much lower in man than in animals, since 50-90% implant in animals, but only 25% in women. The proportion of women conceiving during any particular menstrual cycle rises from 28% if coitus occurs six times to 45% if it occurs twelve times in the cycle. Approximately one-half of oligomenorrhoeic women given Clomiphene conceive after three cycle of treatment, a ratio similar to that occurring in the general population, and similar observations have been made after AID.

THE DEVELOPMENT OF THE EMBRYO: (To be covered in another lecture)

Embryos undergo a series of cleavage divisions as they move down the oviduct. Cleavage involves a series of mitotic divisions, the cytoplasm of the oocyte being parcelled out into blastomeres without any increase in the diameter of the embryo. After the 4-cell stage, the synchronous cleavage of the blastomeres becomes less obvious, and the distinct shape of individual blastomeres becomes blurred from around 16-cell-stage. At this stage the cells become more adherent to each other. This change, called ' compaction' is associated with the formation of the blastocyst and involves: modifications in the ultra structures of the cytoplasm and cytoplasmic organelles, the formation of a layer of outer cells with junctional complexes between them and the formation of inner groups of cells which develop into the inner cell mass. Blastocyst differentiates in the uterus before implantation.

- Φ At around the second and third week of development the inner cell mass differentiates into two distinct masses, the outer or ectodermal germ disc and the inner or entodermal germ disc. Further differentiation produces a third layer between them, the mesoderm. Mesoderm grows outward eventually lining the blastocyst. The combination of the trophoblast and primitive mesoderm is termed the chorion.
- Φ Two cavities appear, one in the ectoderm the amniotic sac and the other in the entoderm the yolk sac. The trilaminar disc formed by the ectoderm mesoderm and entoderm is destined to form the actual embryo.
- Φ As the embryo differentiates, the amniotic cavity expands, enfolding onto the yolk sac.
- Φ Blood vessels develop in the embryonic mesoderm and in the mesoderm of the trophoblast. Extension of these vessels along the connecting stalk results in the formation of the umbilical arteries and vein.
- Φ Within the embryo, the vessels at the cephalic end differentiate to form the heart.
- Φ Fetal blood formation occurs within the primitive blood vessels of the trophoblast and embryo.
- Φ Formation and differentiation of the haemopoietic vascular system occurs between the 3rd and 4th week of pregnancy.

Note:

- ***DERIVATIVES OF THE GERM LAYERS***: See your lecture notes.
- ***DEVELOPMENT OF THE ORGANS-TIME TABLE***: See your lecture notes.
- ***OSSIFICATION CENTRES***: See your lecture notes.
- ***PLACENTAL DEVELOPMENT:*** See your lecture notes.

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