LECTURE 2

From Gametes to Parturition

A. Cell Division; There are two types of cell division, **Mitosis** and **Meiosis**. Mitosis is the cell division process that all types of somatic cells undergo while meiosis is limited to only two cell types, the male and female germ cells. After a brief description of the cell cycle and cell division we will look at the male and female germ cells and their resulting gametes

1. The **Cell Cycle** is a molecular and physiologic process that all somatic cells follow in order to accomplish cell division. The cell cycle is necessary for replication of DNA prior to cell division. The cell cycle is divided into 4 major phases and one secondary phase as follows:
   - A) **G1** (growth 1 or gap 1) is the phase that occurs immediately after cell division. During G1 the cell resumes DNA transcription, grows and resumes normal function.
   - B) **G0** is a secondary phase that some cells enter. G0 is a resting phase and follows the G1 phase. G0 implies that the cell will not be dividing and has lost the DNA replication factors on the chromosomes that allow the S phase to begin. G0 is induced by cell-cell contact inhibition (hepatocyte) or terminal differentiation of the cell (neuron for instance). G0 can be induced in cell culture by growth factor and serum starvation. In cloning by nuclear transfer the nucleus that is to be fused to the oocyte must be in G0 for success.
   - C) **S** (synthesis) is the DNA replication phase. During S the dispersed DNA inside of the nucleus duplicates itself and the DNA content of the cell increases from 2N to 4N. The chromosome number is still considered to be 2N though, as the DNA strands lie adjacent and are closely apposed to each other. The single **Centriole** that was inherited from the parent cell replicates itself during S phase. The centriole is a protein structure that resembles a short section of the 9x2 microtubular structure of the axoneme of the sperm tail or the microtubular structures in cilia and flagella. After replication the 2 centrioles separate to opposite poles of the nucleus. They will act as the **Microtubule Organizing Center** from which the **Mitotic Spindle apparatus**, necessary for cell division grows.
   - D) **G2** is the second growth phase that occurs in preparation for cell division. In some cells there is no G2 phase. In mammals the germ cells entering meiosis do not have a G2 phase as the S phase occurs immediately prior to the M phase. Rapidly dividing cells in the pre-implantation embryo may also skip G2 and move directly to M phase.
   - E) **M** is the actual cell division phase or; mitosis in somatic cells and meiosis in germ cells undergoing terminal differentiation.

2. **Mitosis**: Is the term that describes cell division in all somatic cells. Mitosis is divided into 4 phases that describe the main nuclear and chromosomal events

A) **Prophase** is the phase where the chromosomes reorganize and condense into chromatids. Each chromosome is divided into 2 sister chromatids, which contain the DNA that was duplicated during S phase. One chromatid contains the intact DNA strand from the previous cell division while the other chromatid contains identical, replicated DNA from the S phase of the current cell cycle. The sister chromatids are joined together by a centromere that lies at some point along the length of the chromosome. During prophase the nuclear membrane (nuclear envelope) breaks down, exposing the chromosomes to the cell's cytoplasm. The spindle apparatus grows from the centrioles and the chromosomes become attached to the spindle microtubules at an area on the chromosome called the Kinetochore. The chromosomes then begin migration towards the center of the spindle apparatus.

B) **Metaphase** is the phase where the chromosomes, attached to the developing spindle apparatus, become aligned and are referred to as the **Metaphase Plate**. The metaphase plate lies half way between the two centrioles.

C) **Anaphase** is the phase where the sister chromatids separate from each other and the microtubules of the spindle apparatus slide together and shorten, thus pulling the sister chromatids apart. The Chromatids migrate until they are adjacent to the centrioles.

D) **Telophase** is the phase where two new nuclear envelopes form around the separated groups of sister chromatids (now referred to as chromosomes) and the cell's cytoplasm and cell membrane begin to constrict and divide into two new cells (cytokinesis).

E) **Interphase** occurs between cell divisions. Once cell division is complete the cell enters the G1 phase of the next cell cycle. If the cell is to divide again it completes another complete cell cycle; G1, S, G2 and M. If the cell is to become quiescent it enters G0 after G1.

3. **Meiosis**: Is the term that describes cell division in terminally differentiating germ cells. Meiosis consists of two cell divisions that result in four spermatids or one secondary oocyte awaiting fertilization.

A) **Meiosis 1 (M I)** is the first meiotic division.

1) **M I Prophase**; As in mitosis the germ cell must first duplicate it's DNA. The main difference is that S phase of the cell cycle occurs immediately prior to prophase and there is no G2 phase in the oogonia or spermatogonia. Once prophase starts, the oogonium is then referred to as a primary oocyte. In prophase of meiosis 1 as in mitosis the DNA condenses and shortens into microscopically visible chromosomes. In meiosis the homologous chromosomes are closely apposed to each other so that the process of **Synapsis** and **Crossing Over** can occur. The processes of prophase are subdivided into five morphological stages:

a. **Leptotene**, condensation of the DNA (4N) into distinctly separated chromosomes

b. **Zygotene**, The homologous chromosomes form pairs and synapsis begins. Synaptonemal Complexes, which are the areas

on chromosome pairs where crossing over occurs, can be visualized with EM microscopy.

c. **Pachytene**, formation of distinct sister chromatids

d. **Diplotene** is the stage in which **Chiasmata** form between homologous chromosomes. Chiasma are visible even with light microscopy. The chiasma or chiasmata refers to the chromosomal morphology that occurs due to one or more crossings of chromatids on homologous chromosomes. Synaptonemal complexes occur at the points of crossing. This is the area where exchange of DNA occurs between homologous chromosomes and is the basic event in **Meiotic Genetic Recombination**.
In oocytes the cell becomes arrested at diplotene of meiosis 1. The oocyte may remain in the state of **Meiotic Arrest** for many months, years, or even decades, depending on the species. Spermatocytes do not have an arrested stage during meiosis.

e. **Diakinesis** is the stage where the recombined chromosomes separate and migrate toward the meiotic metaphase plate. In the oocyte and spermatocyte the nuclear membrane does not break down during most of prophase. The nuclear membrane finally does break down during diakinesis, exposing the chromosomes to the cytoplasm. Diakinesis is the event that occurs upon **Resumption of Meiosis 1** in the oocyte. The nuclear envelope of the primary oocyte if usually referred to as the **Germinal Vesicle** or the **GV**. **Germinal Vesicle Breakdown (GVB)** is a microscopically visible event that heralds the beginning of final maturation of the oocyte in preparation for fertilization.

2) **M I Metaphase** is the phase where the chromosomes, attached to the developing spindle apparatus, become aligned and are referred to as the **M I Metaphase Plate**. The metaphase plate lays half way between the two centrioles.

3) **M I Anaphase** differs from mitotic anaphase in that the sister chromatids do not separate from each other. Instead the homologous chromosomes separate and migrate to opposite poles of the spindle apparatus. The resulting daughter cells have a 1N chromosome content and a 2N DNA content. Since the resulting cells have only ½ of normal number of chromosomes M I is referred to as the **Reduction Division**.

4) **M I Telophase** is the phase where two groups of separated chromosomes remain clustered and condensed as chromatid pairs in preparation for Meiosis 2. The cell's cytoplasm and cell membrane begin to constrict and divide into two new cells (cytokinesis). Primary oocytes divide into two very unequal cells; one secondary oocyte which will enter Meiosis 2 and one polar body which is a vestigial cell that will degenerate. The polar body receives a very minimal amount of cytoplasm (<1%), preserving the cytoplasm for the oocyte. Primary spermatocytes divide into 2 equally viable secondary spermatocytes. In both oocytes and spermatocytes the cell proceeds immediately into Meiosis 2.

A) **Meiosis 2 (M II)** is the second meiotic division.

1) **M II Prophase** is a brief stage as the chromosomes are already condensed in preparation for the M II metaphase. The primary event of M II prophase is the duplication of the centriole in preparation for MII spindle apparatus formation.
2) **M II Metaphase;** the chromosomes become attached to the developing spindle apparatus and are aligned on the **M II Metaphase Plate**. In most species the oocyte is arrested in MII metaphase and awaits activation by a fertilizing sperm cell. Also it is at M II metaphase that the oocyte is ovulated from follicle. One notable exception is the canine where a primary oocyte that has not yet resumed meiosis is ovulated. Secondary spermatocytes spontaneously proceed through M II metaphase and enter anaphase.
3) **M II Anaphase**; Upon fertilization and activation of the oocyte, the sister chromatids separate from each other and migrate to opposite poles of the spindle apparatus. In the oocyte anaphase does not commence unless fertilization has occurred. In the spermatocyte anaphase proceeds spontaneously. The resulting daughter cells have a 1N chromosome content and a 1N DNA content and can be truly considered to be gametes at this point.
4) **M II Telophase** As with M I telophase, the cell's cytoplasm and cell membrane begin to constrict and divide into two new cells (cytokinesis). Primary oocytes divide into two very unequal cells; one fertilized ootid and the second polar body, which is also a vestigial cell that will degenerate. The polar body once again receives a very minimal amount of cytoplasm. Primary spermatocytes divide into 2 equally viable spermatids.

A. The male **gamete**, spermatozoon (sperm cell), are formed in the **seminiferous epithelium**, (the epithelium of the seminiferous tubules) during a process called **spermatogenesis**. As the spermatogonia develop into spermatocytes, then spermatids and finally spermatozoa, they migrate from the deep basal layer of the epithelium towards the lumen surface. When the nuclear (head) and cytoplasmic (acrosome and midpiece) development of the spermatozoon is complete the cell is released into the lumen of the seminiferous tubule and muscular contractions carry it to the epididymis for final maturation of the tail and plasma membrane structures. Each of these cell types undergoes dramatic changes in the molecular, nuclear and cytoplasmic function and morphology. Following is a brief description of the stages of spermatogenesis:

1. **Spermatogonia**: The type A spermatogonia are somatic stem cells with a 2N complement of chromosomes. The type A cells undergo mitotic cell division and are responsible for maintaining a constant supply of Type B spermatogonia. Type B cells have stopped dividing and are the precursors of spermatocyte I cells.
2. **Spermatocyte I** cells: the transition from spermatogonia to spermatocyte is heralded by the commencement of active manufacture of DNA and commencement of prophase of

the first meiotic division. Upon completion of the first meiotic division two Spermatocyte II cells are produced.

3. **Spermatocyte II** cells are short lived because they rapidly undergo the second meiotic division. After the second meiotic division there are 4 spermatids with a 1N chromosome content resulting from a single spermatogonia.
4. **Spermatid** cells are intermediary cells that begin maturation processes that will result in the formation of spermatozoa. This maturation process has been divided into many stages (8 to 19 depending on species). The early stage spermatid cells are round with fairly abundant cytoplasm and are the last cell stage that resembles a somatic cell. As development continues the acrosome and the flagella are produced, and the nucleus and cytoplasm are reorganized into the configuration typical of a spermatozoon. Basically, when the cell is released into the lumen of the seminiferous tubule the cell is considered to be a spermatozoon.
5. **Spermatozoa**; although released into the lumen of the tubule these cells still must undergo final maturation. At this stage the sperm cells are still immotile and incapable of fertilization. The spermatozoa are propelled through the efferent ducts to the head of the epididymis by ciliary action in the ducts. In the epididymis peristalsis is responsible for transport of the immotile sperm. Maturation of the sperm occurs in the head and the body of the epididymis. The sperm cells spend about 2 weeks in these structures. Sperm cells in the tail of the epididymis are mature and ready for ejaculation.

   Major structural features of the spermatozoa cell include:
   - A) Head of the sperm cell
     - 1) **Nucleus** with a 1N chromosome complement
     - 2) **Acrosome** with hydrolytic enzymes
   - B) Midpiece
     - 1) **Centriole** (root of tail)
     - 2) **Mitochondria** supply energy for motility
     - 3) **Axoneme,** the complex 9x2 + 1x2 microtubules structure responsible for motility. The Axoneme originates at the centriole.
   - C) Principle piece of tail
   - D) Terminal piece of tail

B. The female gamete or **oocytes (egg)**: The oocyte is derived from oogonia cells in the gonadal ridge (fetal structure) during fetal life. Following is a brief description of the stages of oogenesis:

1. **Oogonia**: The initial germ cell types that migrate to the fetal mesonephros (primitive urogenital system) along with other ovarian somatic cells. The oogonia undergo rapid mitotic division during the development of the fetal ovary.
2. **Oocytes**: oogonia cells lie in the cortex of the fetal ovary and become associated with follicular cells. These oogonia enter **prophase of meiosis 1** and are then referred to as **primary oocytes**. Many of the oogonia and primary oocytes degenerate but those that form a complete basement membrane and associate with follicular cells will survive and form the complete pool of oocytes that a female will possess. Proliferation of germ cells and development of the oogonia into primary oocytes is complete, in most species, by the time that the female is born. The primary oocyte will lie quiescent for many years, arrested in prophase of the first meiotic division (**meiotic arrest**).

Final pre-ovulatory maturation of the oocyte occurs in the **graafian follicle**. During final maturation, just prior to ovulation, resumption of the first meiotic division occurs. The first meiotic division results in a vestigial cell referred to as the first polar body and the **secondary oocyte, which** is finally a fertilizable egg in most species.

Development of the oocyte can also be described within the context of the follicle that contains it. Follicular development can be divided into several stages:

- Non-hormonally responsive follicles
  - A) **Primordial follicle**: .02 mm (20 micron) Dia., contains an oocyte with no zona pellucida, a few flattened granulosa precursor cells, called follicular cells, and no thecal cells
  - B) **Intermediary follicle**: .03 mm (30 micron) Dia., contains an oocyte with no zona, a mixed layer of granulosa cells and follicular cells,
  - C) **Primary follicle**: .04 mm (40 Micron) Dia., contains an oocyte with no zona and a single complete layer of granulosa cells,
  - D) **Secondary follicle**: .06 mm (60 micron) Dia., contains an oocyte which is beginning to deposit the glycoprotein which will form the zona pellucida, two complete layers of granulosa cells, and a primordial thecal cell layer,
- Hormonally responsive follicles
  - E) **Preantral follicle**: .11 to .15 mm Dia., these are follicles that have exited the reserve pool and are commencing development that will result in **ovulation or atresia.** These follicles have 2 or more layers of granulosa cells, a well developed thecal cell layer, a well developed basement membrane and the oocyte is enveloped by a zona pellucida. The granulosa and thecal cells membranes express FSH and LH receptors indicating that these follicles are responsive to endocrine signals from the pituitary gland.
  - F) **Small antral follicle**: These follicles have developed a fluid filled antrum and are available to be recruited into a **follicular wave**. The antrum forms when the follicle is about .2 mm in diameter and continues to enlarge. As cell numbers increase and the fluid volume increases these follicles will grow to 2 mm in diameter at which time they are recruited into a follicular wave and finish development.
  - G) **Large antral follicles**: These follicles have been recruited into a follicular wave and will continue development until ovulation or atresia occurs. The diameter of these follicles ranges from 2 to 15 mm in most species. The mare's dominant follicle (the one that will ovulate) can grow to 50 mm prior to ovulation.

3. **Ovulation**: when a large antral follicle obtains dominance and becomes the largest follicle it is sometimes referred to as a **graafian follicle**, the dominant follicle will usually only rupture and release the oocyte if there is no CL present. An exception is mares that may ovulate several follicles at about 40 days (+) of pregnancy and form accessory CLs.
   - A) **LH surge**: the rapid rise in the pituitary hormone **Luteinizing Hormone** (LH) starts the cascade of ovulatory events that result in:

B) **Resumption of meiosis** in the primary oocyte; the first meiotic division is completed, resulting in a **secondary oocyte** and the small **first polar body**, a cellular remnant containing half of the chromosomal DNA.

C) The follicle becomes hyperemic and the theca becomes edematous.

D) Granulosa cells and cumulus cells secrete hyaluronic acid, which result in a breakdown of the intercellular matrix.

E) Other factors, which assist in this process, include Prostaglandin I_2 (PGI_2), histamines, platelet activating factor, collagenase and plasmin. This process resembles (is) an inflammatory process.

F) Once the follicular surface ruptures, the secondary oocyte, which is enclosed in several layers of cumulus cells and referred to as the **Cumulus Oocyte Complex** or **COC**, is releases slowly from the surface of the ovulated follicle.

G) The fimbria encloses the ovary and ciliary action draws the COC into the infundibulum and subsequently into the ampulla of the oviduct where fertilization takes place.

NOTE: Oogenesis can be contrasted with spermatogenesis in the adult male. In males **spermatogonia** (which are analogous to oogonia) persist throughout adulthood and continue to undergo mitotic division prior to meiotic division and maturation. This situation results in an "unlimited" supply of spermatozoa.

C. Fertilization, Conception and the Zygote

Preparatory steps

1. **Oocyte Maturation,** nuclear and cytoplasmic events

A) **Germinal Vesicle Breakdown** (GVB): The germinal vesicle is the cell nucleus of the oocyte. In order to complete meiosis I and form the metaphase plate of the meiotic cell division the nuclear membrane must breaks down, as it would for any cell division.

B) Completion of **meiosis I**; The chromosomes of the primary oocyte exit their meiotic arrest in prophase of meiosis I, align on the metaphase plate, rapidly proceed into anaphase and completes the first meiotic cell division. Remember that the oocyte has been arrested at meiosis I since birth of the female, perhaps several years.

C) **Extrusion of the first polar body** and formation of the **Secondary Oocyte**; this event is telophase of the first meiotic division. At this division one half of the chromosomal DNA is incorporated into the small non-functional cell called the first polar body and half into the secondary oocyte. The distinguishing characteristic of the first polar body is that almost no cytoplasm is incorporated into this cell, preserving the cytoplasm for the use and development of the secondary oocyte and the fertilized zygote. After completion of the first meiotic division, the oocyte is ready for fertilization by a sperm cell. The chromosome number of the oocyte is 2N after the meiosis I division.

2. **Ovulation:** Almost all species ovulate a secondary oocyte, shortly after meiosis I. Once ovulated, secondary oocyte must be fertilized within 6 to 8 hours or irreversible degeneration will begin. In the canine, primary oocytes are ovulated and the first meiotic division does not occur until about 2 days after ovulation. In spite of this the bitch's immature oocyte can be fertilized prior to meiosis I and is viable for 3 or 4 days after ovulation.
3. **Sperm maturation:** Primarily events that affect the sperm cell membrane
   A) Sperm **Capacitation:** Fertile sperm cells can only fertilize an oocyte after the process of capacitation has occurred. Sperm undergo capacitation in the female reproductive tract only after spermatozoa swim out of seminal plasma residue, which inhibits capacitation. Seminal plasma proteins coat the sperm cell masking and stabilizing surface membrane proteins necessary for sperm attachment and completion of the acrosome reaction. Glycosaminoglycans, such as heparin, present in the female reproductive tract assist in removing the seminal plasma inhibiting substances. The sperm cell surface receptors are thus exposed and undergo rearrangement. Capacitation also results in hyperactivity of the sperm flagella, which will assist the cell in penetrating the cumulus oophorus and the zona pellucida
   B) **Sperm penetration of the cumulus oophorus**: The sperm head cell membrane has hyaluronidase like enzymes that may assist it in penetrating the cumulus cells surrounding the oocyte.
   C) **Sperm attachment to the zona pellucida:** Specific zona receptors on the sperm head attach to the oocyte zona pellucida.
   D) Sperm **Acrosome reaction**; After attachment to the zona, the cell membrane over the acrosome and the outer acrosomal membrane begin to fuse and form membrane vesicle. This results in exposure of the acrosomal enzymes: hyaluronidase and acrosin. The acrosome reaction is dependant on successful completion of capacitation including rearrangement of trans membrane and membrane surface proteins, thus forming protein particle free areas of the cell membrane. It is at these particle free areas where fusion with the outer acrosomal membrane occurs.
4. **Sperm - Zona penetration**: Once the sperm cell's acrosome is exposed and under the propulsion of flagellar hypermotility, the sperm cell may be able to penetrate the zona of a freshly ovulated oocyte. Only one (or very few) sperm cell is able to fully penetrate the zona and contribute to fertilization due to the natural block to polyspermy of the oocyte (see below).

Fertilization

5. **Sperm - Vitelline membrane fusion**: Once inside the perivitelline space a single sperm cell will fuse with the oocyte cell membrane. The remaining sperm cell plasma membrane will become part of the oocyte cell membrane while the remainder of the sperm cell, including the tail and inner acrosomal membrane, is incorporated into the oocyte's cytoplasm.
6. **Oocyte Activation** and the **Block to Polyspermy**,
   A) **Hyperpolarization**: As soon as the sperm cell has fused with the oocyte cell membrane an **intracellular C++ release** results in K+ efflux and a wave of negative membrane hyperpolarization. The intracellular C++ release and negative

hyperpolarization are the hallmarks of **oocyte activation**. Without oocyte activation, completion of oocyte maturation and the normal block to more than one sperm cell entering the oocyte (**block to polyspermy**) would not occur. Hyperpolarization of the cell membrane causes the cortical granule reaction (described below). Together, hyperpolarization and cortical granule enzymes probably serve as a direct deterrent to additional sperm cell fusion with the vitelline membrane.

B) **Cortical Granule Reaction**: The cortical granules are exocytotic vesicles formed by the golgi apparatus of pre-ovulatory follicles. These vesicles contain hydrolytic enzymes. At the time of resumption of meiosis in a dominant follicle these granules move to the periphery of the oocyte adjacent to the vitelline membrane. At the time of sperm cell fusion and as a result of the intracellular C++ release in the oocyte, the cortical granules fuse with the vitelline membrane and release their contents. The hydrolytic enzymes diffuse throughout the perivitelline space and into the zona pellucida. The enzymatic action on the zona proteins causes functional **hardening of the zona pellucida**, resulting in blockage to any additional sperm cells entering the perivitelline space.

C) **Failure of the block to polyspermy**: Obviously there are several places where the normal block to polyspermy can fail. Most important is the chance occurrence of more than one sperm cell fusing with the vitelline membrane at the same time resulting in a **polyploid** zygote (diploid is the normal condition). If **dispermy** (a case of polyspermy with 2 sperm cells) occurs and both sperm cells form pronuclei that contribute to **syngamy** (see below), the resulting zygote will have a 3N, or **triploid**, chromosome content. A 3N zygote may or may not develop beyond a few cell divisions but occasionally a triploid embryo will develop and result in early failure of a pregnancy (resorbtion). Rarely abortion of a late term fetus or birth of a fetus with assorted lethal defects may occur. Polyspermy occurs at a rate of 2% to 5% in most species and is a common cause of breeding failure.

7. Oocyte completes **meiosis II**; the **reduction division** that causes the oocyte to finally achieve the 1N (Haploid) chromosome content. At this division one half of the chromosomes are incorporated into the small non-functional cell called the **second polar body**. The completion of meiosis II is induced by fertilization and Ca++ dependant activation of the oocyte.
8. **Extrusion of second polar body:** This event is telophase of the second meiotic division. As in telophase of meiosis I, almost no cytoplasm is incorporated into the second polar body, preserving the cytoplasm for the use of the fertilized zygote. Both the first and the second polar bodies degenerate and serve no function beyond being repositories for the unnecessary chromosomes of oocyte meiosis. Triploidy can occur if the second polar body is not extruded at meiosis II (an occasional occurrence) and the chromosomes that should have been removed contribute to syngamy.
9. **Male and female pronucleus formation**: Both the female and male chromosomes are incorporated into separate nuclear membranes called pronuclei within 3 to 6 hours of sperm penetration. DNA replication occurs in the pronuclei in preparation for Syngamy and Cleavage

Conception

10. Finally: **Syngamy** and **zygote** formation
    A) Breakdown of the 2 pronuclear membranes
    B) Joining of the male and female chromosomes on the metaphase plate
    C) Anaphase and telophase of the first mitotic division
    D) The first mitotic division is referred to as **cleavage**.

D. Pregnancy and Developmental anatomy

The **embryonic phase** of pregnancy lasts from conception until the completion of organogenesis. It consists of two distinct periods; the **period of blastulation** and the **period of organogenesis.**

1. **Period of blastulation** lasts from conception until the advent of organogenesis (the period of organ development), at about 11 to 12 days. During this period the most noticeable changes that is occur are the development of the embryo from a 1 cell **zygote** to a 30+ cell organism called a **morula** at 3 to 7 days. The morula is encased in a non-calcified proteinaceous shell called the **zona pellucida**. At 4 to 7 days the embryo develops into a **blastocyst** associated with the development of a **blastocoele** or fluid filled cavity in the center of the embryo and concurrent differentiation of the cells into the outer **trophoblast** cells (primitive placenta) and the **inner cell mass** or **embryonic disc** cells. At 5 to 8 days the blastocyst "hatches" from the zona pellucida and marked growth of the trophoblast occurs prior to initiation of organogenesis. During these stages the entire **conceptus** is referred to as an embryo.

   Also of note is the fact that the first 3 to 5 days of development after conception occur in the oviduct. The embryo, still encased in the zona pellucida then enters the uterus. The ovaductal and uterine secretions provide all nutrients and oxygen for the developing morula and blastocyst respectively.
   If an embryo is defective (unfortunately a relatively common situation) due to chromosomal or genetic defects or if the uterine environment is not adequate for growth, the embryo will die during this stage. If the animal is **monotocous** (bearing only one fetus) the pregnancy will be lost and the dam will return to estrus in a normal interval. **Polytocous** species may experience reduced litter size or complete loss of the pregnancy.

   As a poor generalization **attachment** or **fixation** (but not implantation) of the trophoblast to the endometrium occurs at the end of the blastulation period or early in the organogenesis period. Equine embryos fixate in the uterus at 15 days but don't form placental villi and implant until after 30 days. Bovine embryos do not attach until after 20 days. Primate and rodent embryos on the other hand begin the actual implantation process as early as 5 days, when the zona pellucida is shed.

   Implantation is the process where trophoblast / chorion cells invade the epithelium of the uterus in animals with invasive placentation (man, dogs, cats, rodents) or extend villi into the endometrial glandular system in animals with non-invasive placentation. In general the more invasive the placentation type the earlier that implantation occurs.

2. The **period of organogenesis** lasts until all organs and major body parts are formed and placentation is complete. Until completion of organogenesis the pre-natal animal is still best described as an embryo (as opposed to a fetus). Typically the entire conceptus at this point is simply referred to as a pregnancy while the term embryo is used in describing the developing pre-natal animal.

   By the end of organogenesis several thing are evident. The embryo (or fetus) resembles the adult in most aspects and development of the trophoblast has progressed to the formation of the **chorio-allantoic** fetal placenta with invasion of placental villi into the endometrium (**implantation**). The end of the organogenesis stage is as follows:

   * Cow, and other large ruminant species; Approx. 50 days
   * Human and other large species this is Approx. 60 days
   * Horse; Approx. 60 days
   * Sheep & goats; Approx. 45 days
   * Swine; Approx. 45 days
   * Dog, Cat; Approx. 30 days
   * Rodents and other small species this is Approx. 10 days

   The period of organogenesis is the period where most **teratogenic** effects are initially noted. Aberrations of development at this stage can result in many lethal or deforming defects in the fetus and resulting neonate

3. The **period of fetal growth** is the 3rd and longest stage of gestation (over two trimesters). During this stage the pre-natal animal is referred to as a **fetus**. The overwhelming activity of the conceptus during this stage of gestation is growth. Many small developmental and maturational changes occur also. The fetus is grossly identifiable by it's species and sex as early as the beginning if the fetal period.

4. **Placental types**, function and passive transfer of antibodies
   A) Types of placentation:
      1) **Epithelio-Chorial**, in this type of placenta the intact endometrial epithelium is in direct contact with the superficial chorio-allantoic membrane of the placenta. As in all types of placentas the fetal vasculature in the areas of attachment and implantation of the chorio-allantoic membrane forms dense capillary beds. Gaseous and nutritional support of the fetus is via molecular diffusion and plasma transudation through all layers between maternal and fetal vascular systems. Passive transfer of antibodies does not occur in this type of placentation. This type of placenta is found in ruminants, the equine and swine.
      2) **Endothelio-Chorial**, in this type of placenta the endometrial epithelium is eroded in the process of implantation. The chorio-allantoic membrane of the placenta is thus in direct contact with the maternal vasculature. Gaseous and nutritional support of the fetus is via molecular diffusion and plasma transudation through maternal vascular wall, chorial layer of the fetal membranes and fetal vascular wall. Passive transfer of antibodies does occur in this type of placentation. This type of placenta is found in canine, feline and other carnivorous species.

3) **Hemo-Chorial**, in this type of placenta the endometrial epithelium is deeply eroded in the process of implantation. In addition the maternal capillary bed "breaks down" and forms a network of sinuses that join to and bathe the chorion directly with maternal blood. Gaseous and nutritional support of the fetus is via molecular diffusion and plasma transudation through only the chorial layer of the fetal membranes and fetal vascular wall. Passive transfer of antibodies does occur in this type of placentation. This type of placenta is found in primates and rodents.

B) Distribution of placental - maternal attachment; see illustration

1) **Diffuse**, equine and porcine; this type of placenta is epithelio-chorial
2) **Cotyledonary**, ruminants; this type of placenta is epithelio-chorial
3) **Zonary**, canine and feline; this type of placenta is endothelio-chorial
4) **Discoid**, primates and rodents; this type of placenta is hemo-chorial

C) Endocrine function of the placenta;

1) **Maternal Recognition of Pregnancy**: The dam must recognize that she is pregnant so that the cyclic corpus luteum is maintained and transformed into the CL of pregnancy. The first endocrine function of the embryonic trophoblast cell is to secrete a chemical signal that prevents normal cyclic regression of the corpus luteum. The signal in ruminants is a protein called **Trophoblast Protein-1 (Trophoblastin or TP-1)**. Similar proteins are probably produced by most polyestrus species except for primates (see HCG below). TP-1 belongs to the **Interferon** class of proteins and indeed has antiviral activity like alpha Interferon. TP-1's action is local in the uterus. It suppresses production of Prostaglandin F2 by the endometrium, which would otherwise result in regression of the CL and return to estrus. It also modifies local immune functions in the uterus, inducing immune tolerance and preventing rejection of the foreign tissues of the embryo. TP-1 is secreted from the embryo starting at about the time of hatching from the zona pellucida (days 7 to 10). Maternal recognition of pregnancy must occur prior to the time of prostaglandin release (days 12 to 17). It is noteworthy that canines and other **Monoestral** carnivourous species do not require an inhibitor of prostaglandin release since the CL is maintained for the normal length of gestation whether pregnant or not.
2) **Gonadotropins**;

a. **Equine Chorionic Gonadotropin**; ECG is produced by chorionic cells that have invaded the endometrium at about 35 to 40 days of pregnancy. These cells form structures that are called **Endometrial Cups.** ECG has primarily FSH activity with some LH activity. ECG is responsible for growth and ovulation of **Accessory Follicles** and the resulting **Accessory Corpus Lutea** that develop and persist from 40 to 120 days of pregnancy in the mare. There are usually 3 to 6 accessory CL present during this time causing a marked elevation in progesterone.

b. **Human Chorionic Gonadotropin**; HCG is produced in primates by syncytiotrophoblast cells of the placental disk. HCG has primarily LH activity and is responsible for luteal support during pregnancy. In actuality it serves two functions: inhibition of luteal regression and also direct luteal support.

3) **Progesterone**; During pregnancy the placenta produces progesterone, assisting in the maintenance of pregnancy. In some species the placenta completely replaces the corpus luteum in progesterone production.

4) **Estrone Sulphate,** During early and later pregnancy estrone sulphate is a unique estrogen that is produced by the placenta. The function of this hormone is not clear. An assay for this hormone can be used as an early pregnancy test in horses, swine and perhaps other species.

5. Birth; Initiation of **parturition** is a complex event involving maternal and fetal endocrine events:

A) Fetus exerts overriding control of gestation length.

B) Normal fetal endocrine maturation result in increased fetal hypothalamus and pituitary activity. Increased secretion of ACTH from the fetal anterior pituitary gland results in an increase in cortisol secretion from fetal adrenal glands. Fetal stress near the end of gestation may have a normal place in elevating cortisol secretion from the adrenal glands. Placental insufficiency, infection, or other fetal pathology late in gestation may also cause a premature release of fetal cortisone from the adrenal glands, causing premature birth, stillbirth or abortion. Fetal cortisol, whether of normal or pathological origin, crosses the placenta and enters the maternal circulation.

C) Placental maturation in conjunction with increased fetal cortisol levels results in a shift of placental steroidal hormone secretion from progesterone to estrogen. Cortisol is responsible for inducing the enzymatic machinery necessary for the conversion of placental progesterone to estrogen. Increased circulating estrogen levels induce PGF2 alpha production in the uterus and also induce oxytocin receptors in the uterus and cervix. Increased estrogen, PGF2 alpha and oxytocin results in regression of the corpus luteum. The decrease in ovarian and placental progesterone causes a rapid drop in circulating progesterone levels at the same time that circulating estrogen levels are increasing. The decreased circulating progesterone level in conjunction with an elevated circulating estrogen level is responsible for the physiological initiation of events leading to parturition in the pregnant female.

D) Other placental and uterine changes that occur as a result of decreased progesterone and placental maturation include: decreased placental circulation, atrophy of villous crypts.

E) Relaxin, a polypeptide hormone may also have a role in preparation of the uterus, cervix, vagina and pelvic ligaments for parturition. Relaxin is secreted by the corpus luteum, endometrium and placenta in several species throughout gestation. During most of gestation relaxin causes relaxation of uterine smooth musculature. The uterus looses receptors for relaxin as parturition approaches. Relaxin appears to induce the production of collagenase during late pregnancy.

This collagenase activity may be responsible for cervical softening and relaxation of pelvic ligaments. The physiological levels and actions of relaxin during pregnancy vary widely, depending on the species of animal.

F) Increased estrogen levels cause the cervical musculature to relax. In addition to relaxation there is an estrogen and perhaps relaxin induced increase in collagenase activity that breaks down the fibrous collagen support present in the cervix of pregnancy. Relaxation and the biochemical changes in the connective tissues allow for dilation of the cervix to occur.

G) Estrogen also induces changes in the vaginal mucosa similar to the changes evident at estrus. In addition to the estrogenic changes to the vaginal mucosa, there is also relaxation of the muscular layers. Estrogen and perhaps relaxin induce an increase in collagenase activity, allowing for softening and dilation of the fibro-elastic layers of the vagina, similar to the situation in the cervix.

H) Relaxin and estrogen are responsible for relaxation of the pelvic ligaments (sacro-sciatic ligament, pubic-symphyseal ligament), allowing for an overall increase in the cross-sectional area of the pelvic canal.

I) Once all of the above physical changes have occurred and dilation of the cervix and vagina are complete, the fetus enters the vagina.

J) The presence of the fetus in the vagina stimulates myometrial contraction (probably due to oxytocin release) as well as a straining reflex by the female, and the fetus is expelled.

K) The final stage of parturition is expulsion of the fetal membranes.

6. Lactation and passive transfer of antibodies

A) Elevated progesterone during pregnancy and increased estrogen at the end of pregnancy are responsible for development of the mammary gland alveoli and lactation ducts respectively.

B) In the last 20% of pregnancy, after progesterone has stimulated development of the mammary alveoli, there is a cell receptor mediated transfer of serum IgG antibodies into the mammary glands. These antibodies will form the basis of the colostrum (first milk) which is extremely high in antibodies. The active transfer of immunoglobulins from the serum into the mammary gland can cause a precipitous fall in serum immunoglobulin levels, lowering maternal immunity prior to parturition. This seems to be a serious problem in dairy cattle.

C) Prolactin and oxytocin are involved in initiation of lactation once the glandular and duct systems are in place. Prolactin may also be involved in colostrum formation.

D) Oxytocin is involved in immediate “milk let-down”; being secreted by the posterior pituitary gland as the efferent response to the suckle reflex.