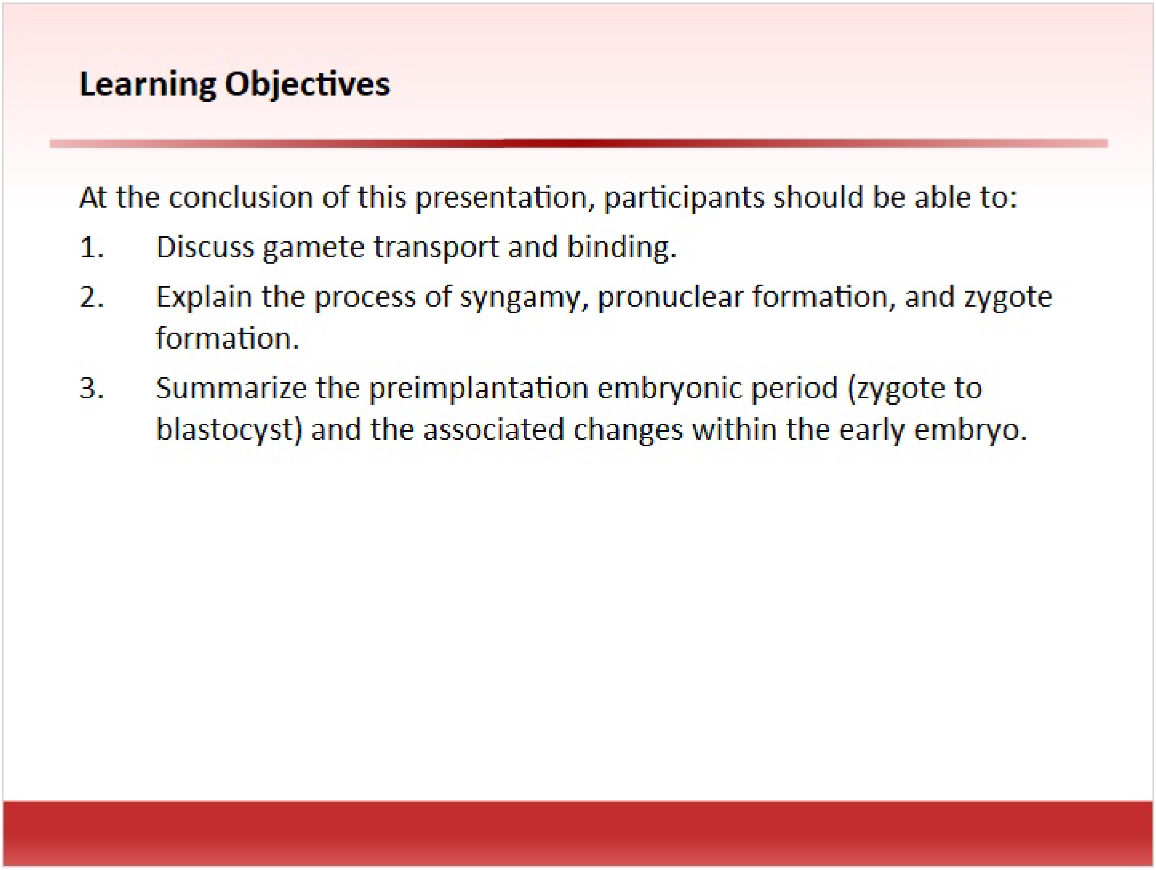
LABCC100 Lesson 4

1.1 Fertilization and Early Embryo Development

Notes:

Welcome to the American Society for Reproductive Medicine's eLearning modules. The subject of this presentation is Fertilization and Early Embryo Development.

1.2 Learning Objectives



Notes:

At the conclusion of this presentation, participants should be able to:

Discuss gamete transport and binding.

Explain the process of syngamy, pronuclear formation, and zygote formation.

Summarize the preimplantation embryonic period (zygote to blastocyst) and the associated changes within the early embryo.

1.3 Ovulation

Ovulation

- Occurs when egg is released into peritoneal cavity
- Under influence of **follicle-stimulating hormone (FSH) and luteinizing hormone (LH)**
- LH Surge
  - Immature follicle matures into vesicular (Graafian) follicle
  - Increase in collagenase activity
  - Increase in prostaglandin levels
- Oocyte discharged with granulosa cells into peritoneal cavity
- Remaining granulosa cells become **corpus luteum**

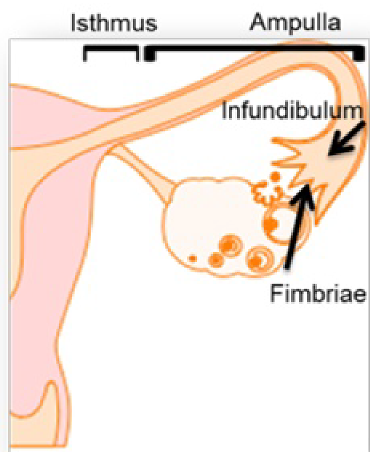
Notes:

First, a review of the physiologic process of ovulation:
In the days immediately preceding ovulation, under the influence of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), the immature follicle grows rapidly to become a mature vesicular (Graafian) follicle as shown by the black arrow. An abrupt increase in LH secretion stimulates the primary oocyte to complete Meiosis I, causing the follicle to enter the preovulatory mature vesicular stage. Meiosis II is also initiated at this time, but is then arrested in metaphase just 3 hours before ovulation. Collagenase activity and prostaglandin levels will also increase in response to the LH surge. These are responsible for digesting the collagen fibers around the follicle and inducing local muscular contractions in the ovarian wall. These contractions discharge the oocyte together with its surrounding granulosa cells, which then develop into what is known as the corona radiata. The granulosa cells that remain in the ruptured follicle (those that do not become the corona radiata), develop into the corpus luteum. These lutein cells secrete estrogens and progesterone, preparing the uterine mucosa (endometrium) for embryo implantation (progestational or secretory stage).

1.4 Gamete Transport

Gamete Transport

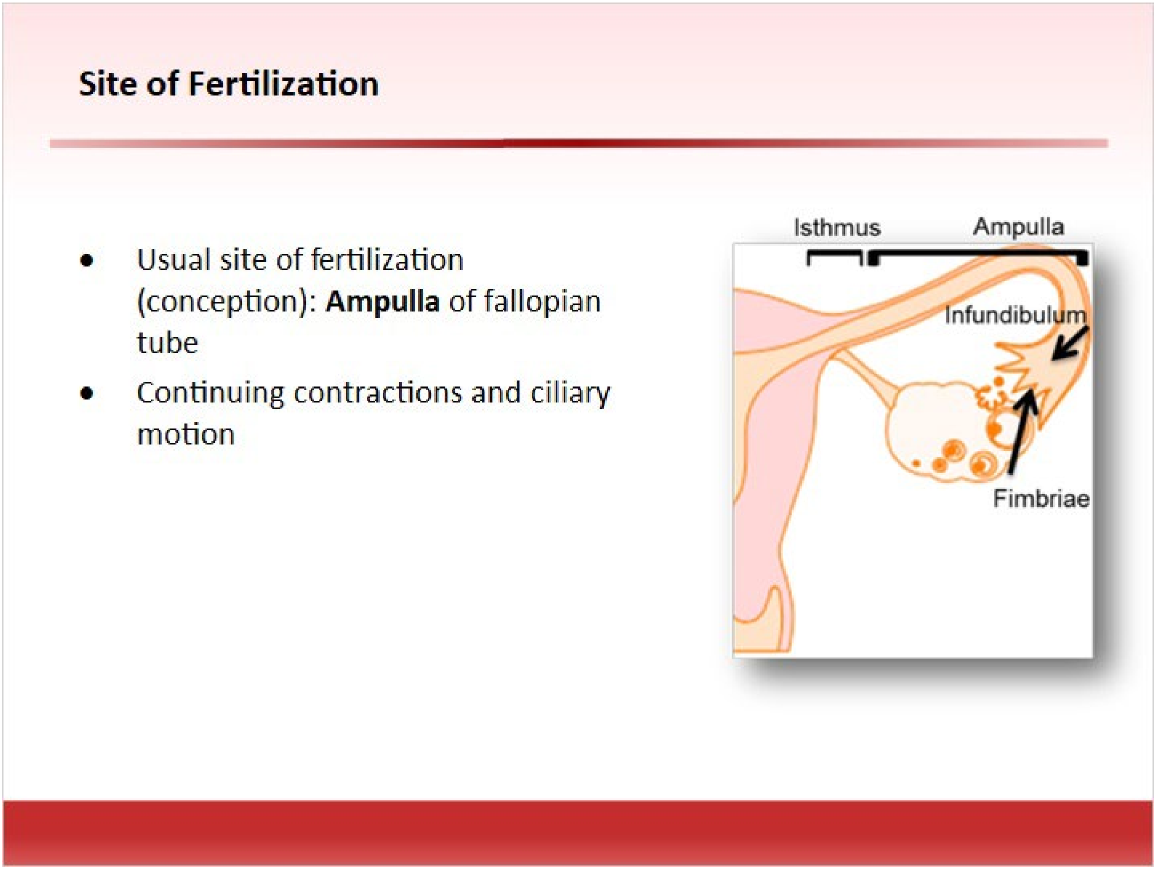
- Oocyte must migrate from fimbria to ampulla and isthmus
  - Mucosal cilia activity
  - Regulated by endocrine status
- Sperm must migrate from vagina to ampulla
  - Uterine contractions
  - Sperm motility and propulsion



Notes:

After ovulation, the oocyte is swept into the infundibulum and ampulla of the fallopian tube by the motion of the fimbriae. After ejaculation, sperm travel through the vaginal canal, cervix, and uterus primarily by the muscular contractions of the uterus as well as their own propulsion. The trip from the cervix to the fallopian tube can last anywhere from 30 minutes to 6 days.

1.5 Site of Fertilization



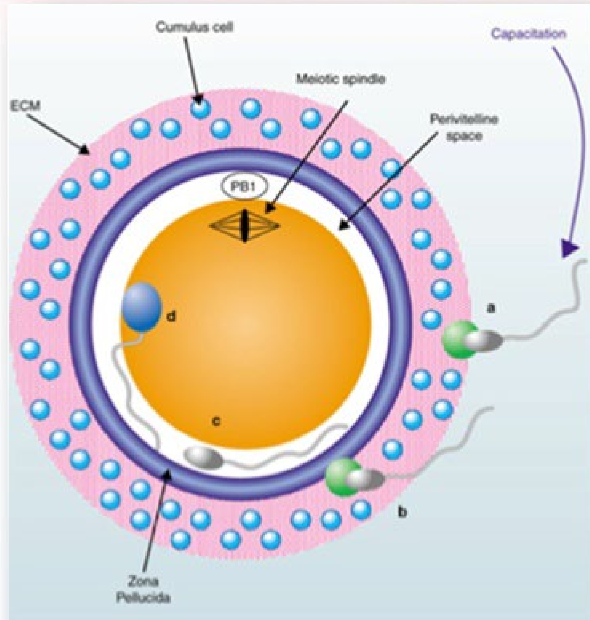
Notes:

Fertilization typically occurs in the ampulla, or more specifically the ampullary-isthmus junction as the fallopian tube narrows. Once fertilized, the oocyte is continually propelled through the ampulla and isthmus by the rhythmic contractions of the tube and beating motions of the mucosal cilia. The rate of transport is regulated by the endocrine status during and after ovulation. In humans, the fertilized oocyte reaches the uterine lumen in 3-4 days.

1.6 Phases of Fertilization

Phases of Fertilization

1. Penetration of corona radiata
2. Penetration of zona pellucida
3. Fusion of oocyte and sperm cell membranes



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Notes:

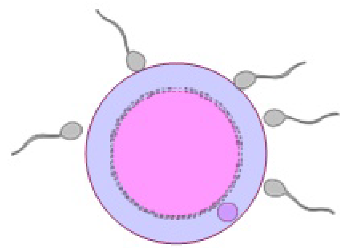
Only 300 to 500 (out of 200 to 300 million) sperm reach the site of fertilization. Fertilization is a process rather than just one event.
The sperm must breach 3 barriers for successful conception: the expanded cumulus (corona radiata), the zona pellucida, and the plasma membrane of the egg (called the oolemma or vitelline membrane). Before the sperm are able to penetrate these barriers, they must undergo a series of preparatory steps to permit fertilization. Sperm processing, including capacitation, occurs in the fallopian tube due to epithelial interactions between the sperm and the mucosal surface of the tube.
There are three general phases of fertilization starting at the three o'clock position in the figure and moving clockwise. These will be addressed in the next slides:

- Phase 1: Penetration of corona radiata
- Phase 2: Penetration of zona pellucida
- Phase 3: Fusion of oocyte and sperm cell membranes

1.7 Phase 1: Penetration of Corona Radiata

Phase 1: Penetration of Corona Radiata

- Period of condition of female reproductive tract to allow fertilization
- **Capacitation**
  - Removal of glycoprotein coat & seminal plasma proteins overlying acrosomal regions of sperm
- Hyperactivation of sperm
  - Increased flagellar motility
- Digestion of **hyaluronic acid**
  - **PH-20** enzyme



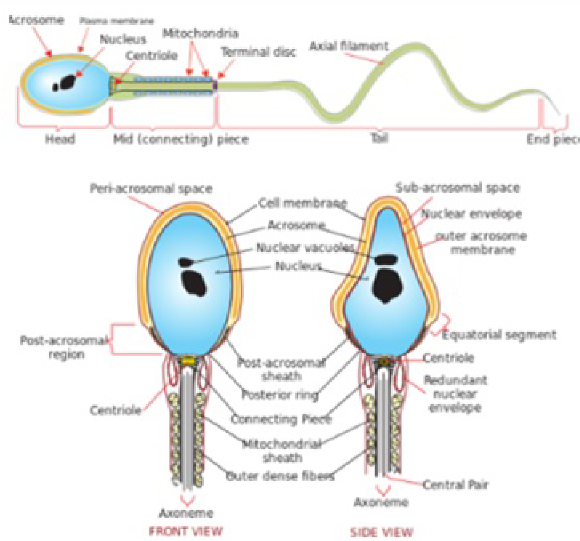
Notes:

As mentioned, there are 3 main barriers that the sperm must penetrate to succeed in fertilizing the oocyte: the expanded cumulus (corona radiata), the zona pellucida, and the oolemma. In order for the gametes to complete the first phase of fertilization (penetration of the corona radiata barrier), a few reactions must occur. This period of conditioning in the female reproductive tract also involves the removal of a glycoprotein coat and seminal plasma proteins from the plasma membrane overlying the acrosomal region of the sperm. **Capacitation** consists of alterations in metabolism, membrane biophysical characteristics, changes in protein phosphorylation state, elevations of intracellular pH and calcium levels, and hyperpolarization of membrane potential resulting in altered flagellar motility (required for penetrating the zona pellucida). The significant increase in sperm motility, changing from a “wave-like” motion to a “whip-like” motion is also referred to as “**hyperactivation**.” The cumulus cells of the corona radiata are composed predominantly of hyaluronic acid, which must be digested in order for the sperm to penetrate. This is accomplished by the membrane-bound enzyme **PH-20** on the sperm once it has undergone capacitation and hyperactivation. After approximately 7 hours of capacitation and digestion of hyaluronic acid, the sperm are able to freely pass through the corona radiata cells surrounding the oocyte.

1.8 Phase 2: Penetration of Zona Pellucida

Phase 2: Penetration of Zona Pellucida

- Continuing to prepare sperm to fertilize oocyte
- Acrosome reaction
  - Mediated by **ZP3** glycoprotein
- Sustained Ca^{2+} influx stimulates release of lysosomal enzymes from head of sperm
  - **Acrosin** is main enzyme
- Allows sperm to penetrate zona pellucida



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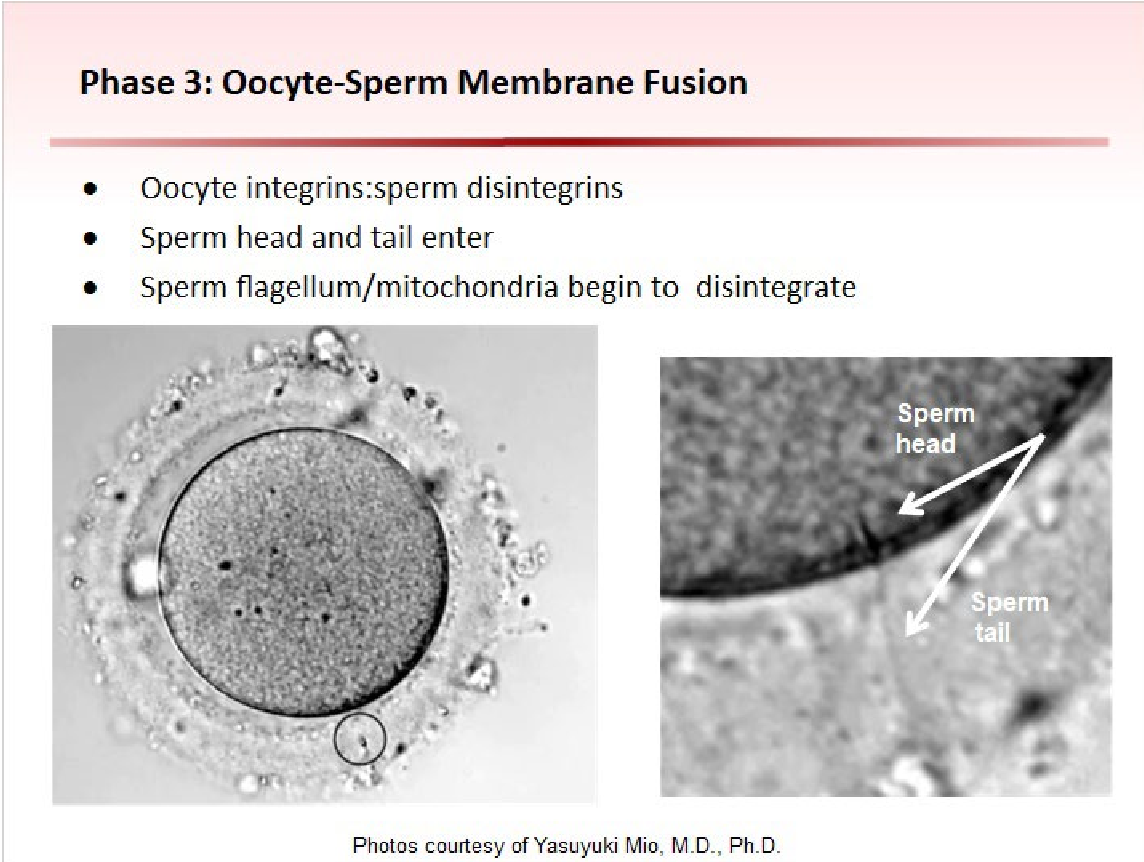
Notes:

The acrosome reaction is another important preparatory step required to initiate fertilization. The zona pellucida is a glycoprotein shell surrounding the egg; it is one of the protective layers that helps to facilitate and maintain the binding of sperm. ZP3 is a constituent glycoprotein of the zona pellucida that will bind to receptors in the anterior head of the sperm. Once bound, ZP3 induces the acrosome reaction. Due to a sustained calcium influx, this reaction releases lysosomal enzymes, including acrosin, from the acrosome region on the head of the sperm to allow the sperm to penetrate the zona pellucida and come in contact with the plasma membrane of the oocyte. Zona binding is species specific.

1.9 Phase 3: Oocyte-Sperm Membrane Fusion



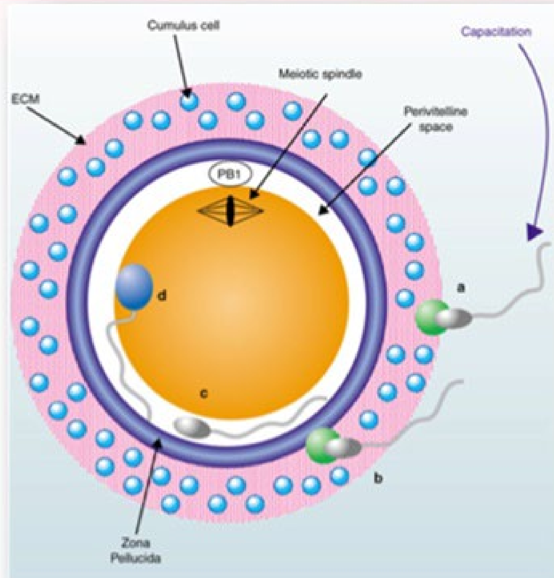
Notes:

The initial adhesion between the oocyte and sperm is possible due to the interaction between **integrins** on the membrane of the oocyte and **disintegrins** on the membrane of the sperm. Remember, the acrosome reaction that prepared the sperm to penetrate the zona pellucida removed the plasma membrane that was covering the head of the sperm. Both the head and the tail of the sperm must enter the cytoplasm of the oocyte, leaving the plasma membrane behind on the surface of the oocyte. Because of this, the actual fusion between the plasma membrane of the oocyte and the membrane of the sperm occurs on the posterior region of the sperm head. The molecular mechanisms involving fusion of the egg and sperm are not fully understood at this time.
Shortly after the sperm head and tail enter the ooplasm, the flagellum and mitochondria of the sperm disintegrate, leaving only maternal mitochondrial DNA to be passed to the offspring.

1.10 The Zona Reaction

The Zona Reaction

- Block to additional sperm penetration
- Enzymes released from cortical granules
- Depolarization of membrane receptor



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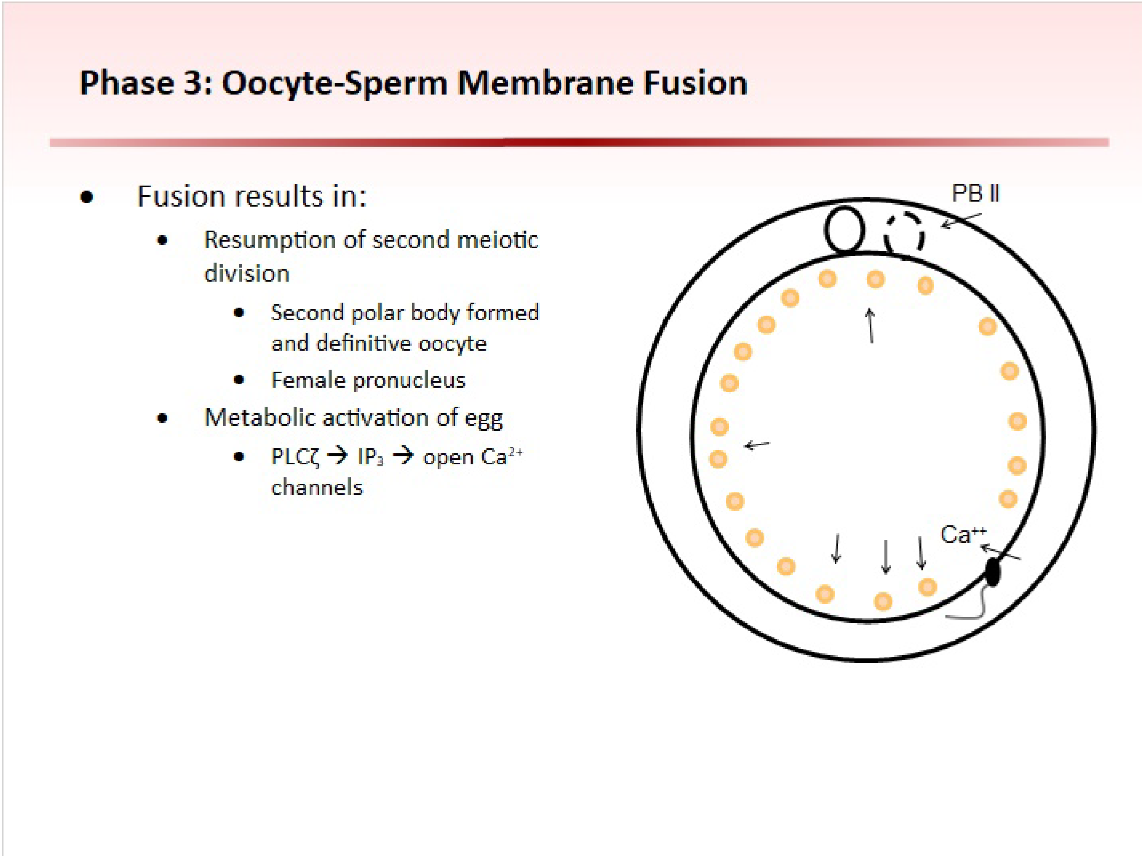
Notes:

Once the sperm comes in contact with the oocyte's plasma membrane, the zona pellucida becomes impermeable to additional sperm-an important protective mechanism to ensure viability of the developing embryo. In a series of events called the **zona reaction**, lysosomal enzymes are released from specialized secretory vesicles called the **cortical granules** that surround the cortex of the ooplasm inside the plasma membrane of the oocyte. These enzymes cause alterations of membrane characteristics through depolarization and inactivation of species-specific receptor sites for sperm in the zona.

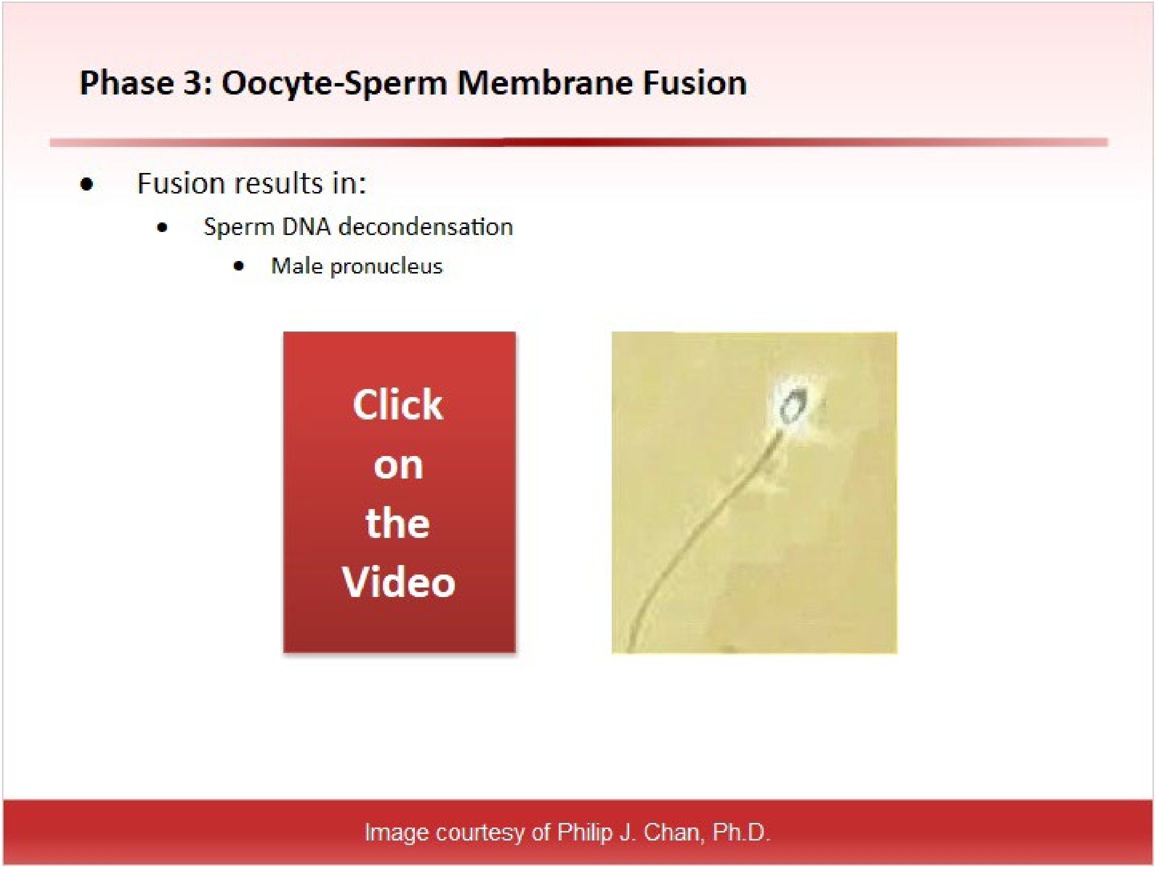
1.11 Phase 3: Oocyte-Sperm Membrane Fusion



Notes:

The resumption of meiosis and activation of the quiescent oocyte are both dependent upon a large intracellular release of Ca^{2+} in the egg. This occurs via the activation of the phospholipase C enzyme pathway by the sperm enzyme, **PLCζ (Phospholipase C Zeta)**. This enzyme produces inositol 1,4,5-triphosphate (**IP_3**) which binds to its receptor on the endoplasmic reticulum to open Ca^{2+} channels and release intracellular stores of calcium. In response to the influx of calcium, **cortical granules** from the cortical layer of the oocyte are released via exocytosis as illustrated by the tan circles. The granules contain enzymes that modify the membrane of the egg to prevent more than one sperm from binding and penetrating. In addition to the zona reaction and release of cortical granules, the enzymes released function to harden the zona pellucida and digest the ZP3 glycoproteins, making it impenetrable to sperm and preventing polyspermy. Many sperm may become embedded in the zona pellucida, but only one will penetrate to fuse with the oocyte. The release of calcium also stimulates the oocyte to resume the **second meiotic division**, forming a **pronucleus** around the female chromosomes and expelling the second polar body.

1.12 Phase 3: Oocyte-Sperm Membrane Fusion



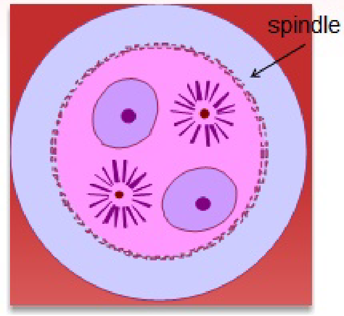
Notes:

As shown in this animated series of images, the cytosolic environment of the oocyte uncoils the sperm DNA in a process called **decondensation**. The uncoiled DNA is then surrounded by a new membrane to form the **male pronucleus** as the oocyte resumes its second meiotic division and is metabolically activated by a series of cortical reactions.

1.13 Syngamy

Syngamy

- Sperm contributes centromere
  - Becomes microtubule-organizing center
- Pronuclei migrate towards each other
- Male and female DNA replicates as two pronuclei are pulled together
- Nuclear membranes break down and chromosomes align on common metaphase plate
- Formation of one-celled zygote



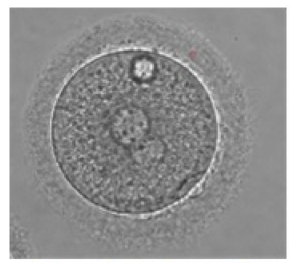


Photo courtesy of Fertility Clinic of the Carolinas

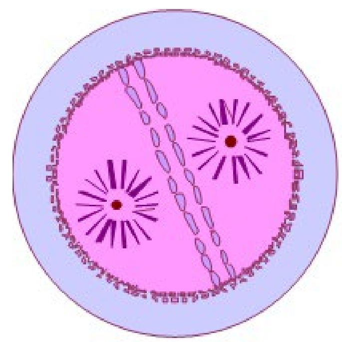
Notes:

The sperm centriole leads in organizing the microtubules to direct pronuclear migration and rotation within the cytoplasm. Pronuclear migration and rotation results in positioning their axis toward the second polar body to achieve a proper orientation at syngamy. During pronuclear formation, nuclear precursor bodies migrate and merge into nucleoli in a time-dependent manner. Nucleoli are the sites of synthesis of pre-rRNA and its availability is necessary for the translational processes when the embryonic genome becomes active sometime between the 4-cell and 8-cell stage. Following the pronuclear alignment onto the polar axis, the chromosomes will separate in preparation for mitosis (mitotic potential is inherited paternally through the centrosome which is delivered by the sperm).

1.14 Main Results of Fertilization

Main Results of Fertilization

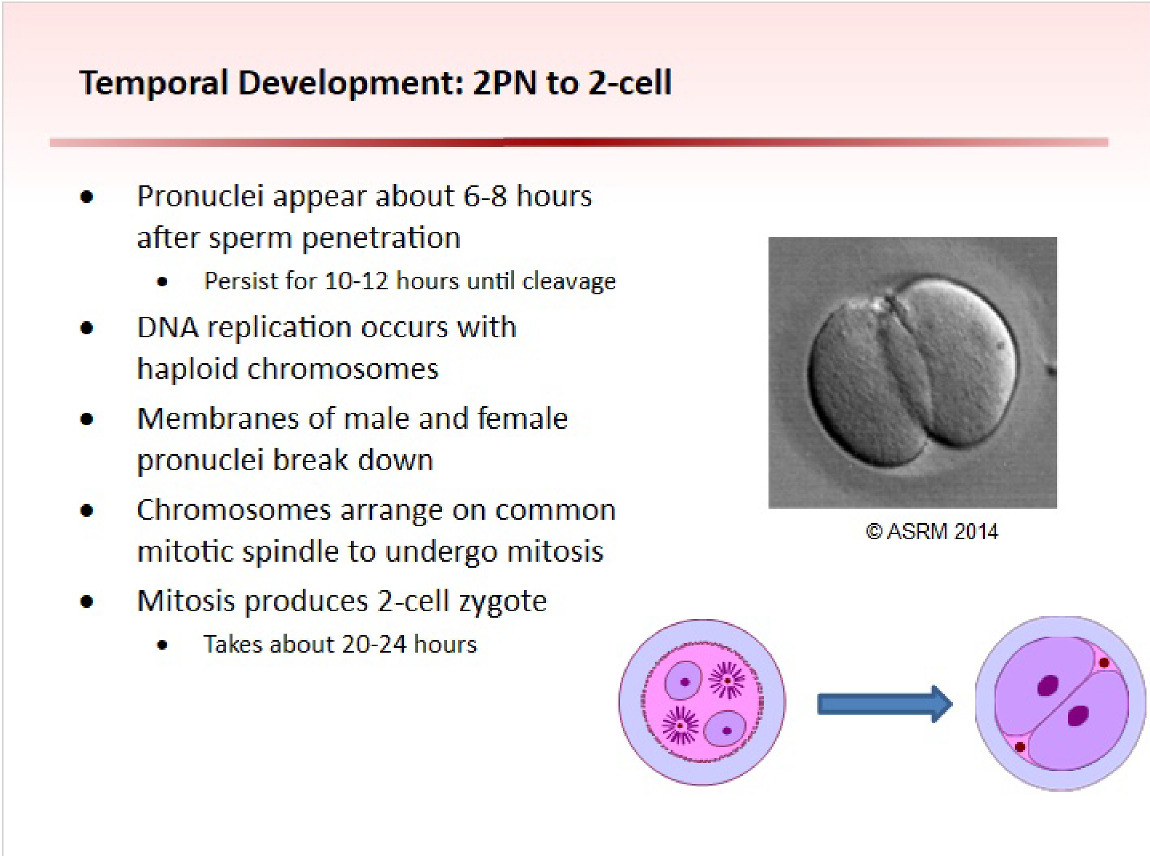
- Initiation of cleavage
- Restoration of diploid number of chromosomes
- Determination of sex of new individual



Notes:

Once the pronuclei come in contact with each other, the nuclear membrane surrounding each group of chromosomes breaks down. The chromosomes align on a common metaphase plate to initiate the first embryonic cleavage. Without fertilization the oocyte typically degenerates about 24 hours after ovulation. With the formation of the zygote, the haploid gametes' chromosomes combine to create a diploid number. The zygote will contain a combination of genetic material from both parents, half from the mother and half from the father. During the process of fertilization, the sex of the offspring is also determined from the X- or Y-bearing sperm.

1.15 Temporal Development: 2PN to 2-cell



Notes:

The male and female pronuclei appear about 6-8 hours after the sperm penetrates the egg, and can persist for 10-12 hours until cleavage. The haploid chromosomes contributed from both the mother and father undergo DNA replication. During replication, the male and female pronuclei are pulled together. Once replication has occurred, the pronuclei membranes come into contact and will break down to allow the chromosomes to arrange on a common mitotic spindle. The chromosomes that are arranged on the metaphase plate begin mitosis. The first embryonic cleavage occurs to produce a 2-cell zygote. This first cleavage takes about 24 hours post-fertilization.

1.16 Video: Fertilization to 2 Cell

Video: Fertilization to 2 Cell

Click on the Video

Video courtesy of Yasuyuki Mio, M.D., Ph.D.

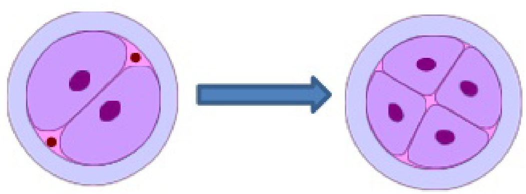
Notes:

This video illustrates the fertilization process with the sperm entering the zona pellucida, then immediately attaching to the surface of the oocyte membrane. Forty minutes later the equatorial segment of the sperm has begun its incorporation into the oocyte and the sperm head sinks beneath the oocyte membrane. Finally, the second polar body is extruded shortly after sperm fusion. One can see the fertilization cone, which is a transient protrusion of the oocyte membrane. The two pronuclei form and concurrently a translucent zone, called the halo, appears in the peripheral ooplasm. The embryo undergoes syngamy and the first cleavage occurs.

1.17 Temporal Development: 2-cell to 4-cell

Temporal Development: 2-cell to 4-cell

- Complete by about 40 hours postfertilization
- Mitotic division produces 4 equally sized cells called blastomeres
- Takes place in fallopian tube (oviduct)
- Divisions occur without embryo increasing in size



Notes:

The second cleavage from the 2-cell to 4-cell stage is complete by about 40 hours after fertilization occurs. The first embryonic cleavage produces 2 daughter cells, and the second cleavage results in 4 equally sized cells known as blastomeres. At this time, the embryo is still located in the fallopian tube as it continues its journey into the uterine cavity. It is important to note that all of the initial divisions (until the 5^{th} day when the blastocyst develops) are contained within the zona pellucida and occur without increasing the size of the embryo.

1.18 Temporal Development: 4-cell to 8-cell

Temporal Development: 4-cell to 8-cell
Pre-rRNA

- Nucleoli are sites of synthesis of pre-rRNA (ribosomal ribonucleic acid)
- Essential for translational processes in which embryonic genome becomes activated
- Occurs sometime between the 4-cell and 8-cell stage, ~72 hours

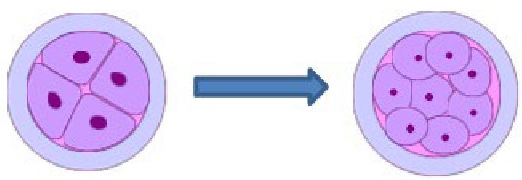
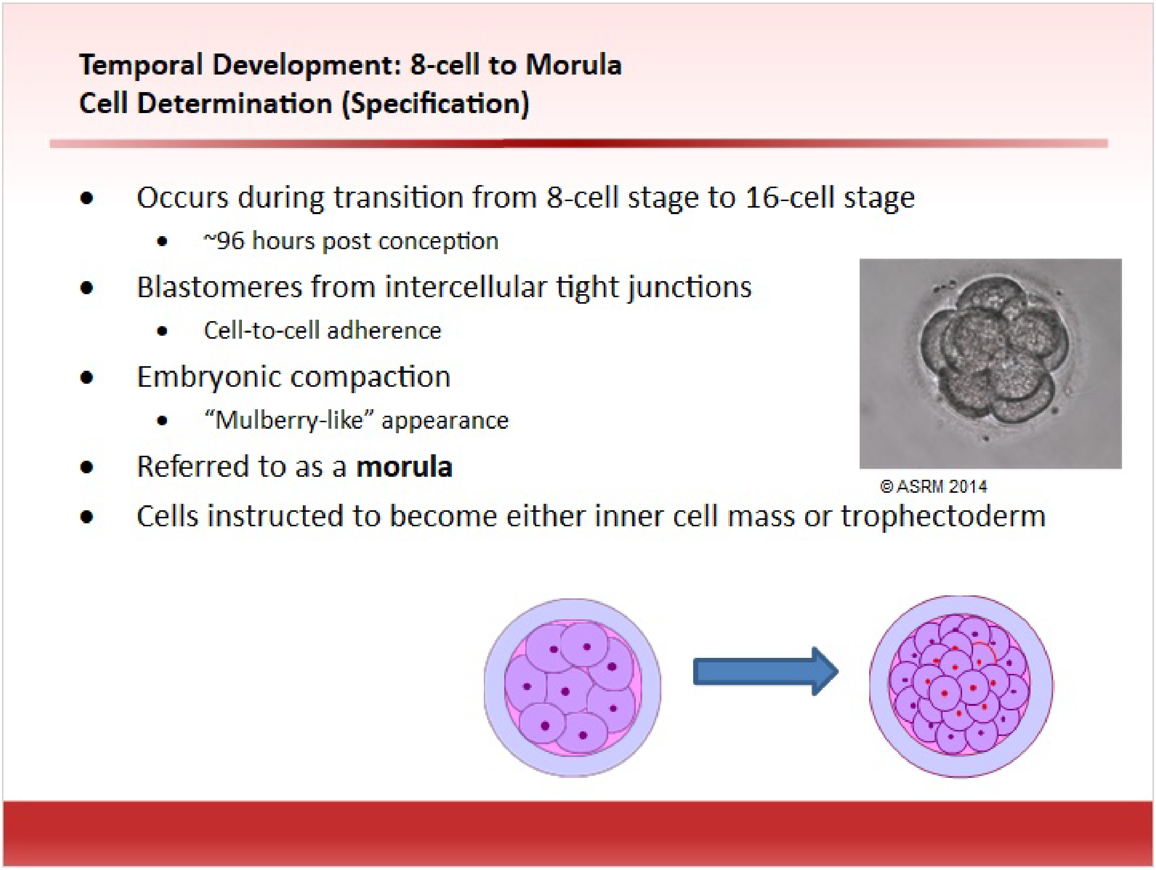


Photo courtesy of Fertility Clinic of the Carolinas

Notes:

Nucleoli are the sites of synthesis of pre-ribosomal ribonucleic acid (rRNA). Its availability is necessary for the translational processes when the embryonic genome becomes active sometime between the 4-cell and 8-cell stage. This takes place about 72 hours after fertilization occurs. The blastomeres are ‘totipotent', that is, any single blastomere should be capable of differentiated into any cell type of the developing embryo.

1.19 Temporal Development: 8-cell to Morula



Notes:

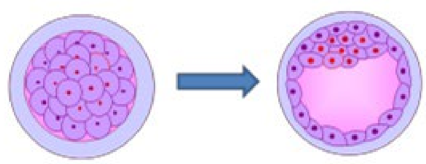
Once the embryo reaches the 8-cell stage, blastomeres will begin to form intercellular tight junctions resulting in an increase in cell-to-cell adherence, which begins the process of embryonic compaction. Embryonic compaction advances during the next division until the boundaries between the cells are barely detectable and the embryo takes on a "mulberry-like" appearance, referred to as a morula. Some point during this phase, individual cells are "instructed" to become either the **inner cell mass** or the **trophectoderm** during an undefined process referred to as **cell determination**, also known as cell specification. Generally, those cells found within the interior will become the inner cell mass while peripheral cells become the trophectoderm. This process is finished after approximately 96 hours post-conception.

1.20 Temporal Development: Morula to Blastocyst

Temporal Development: Morula to Blastocyst
Blastocyst Formation and Hatching

- During morula stage of development (days 4 & 5)
- Fluid accumulates between cells to form blastocoel
  - Na⁺/K⁺-ATPase channels
- Mass of cells now referred to as blastocyst

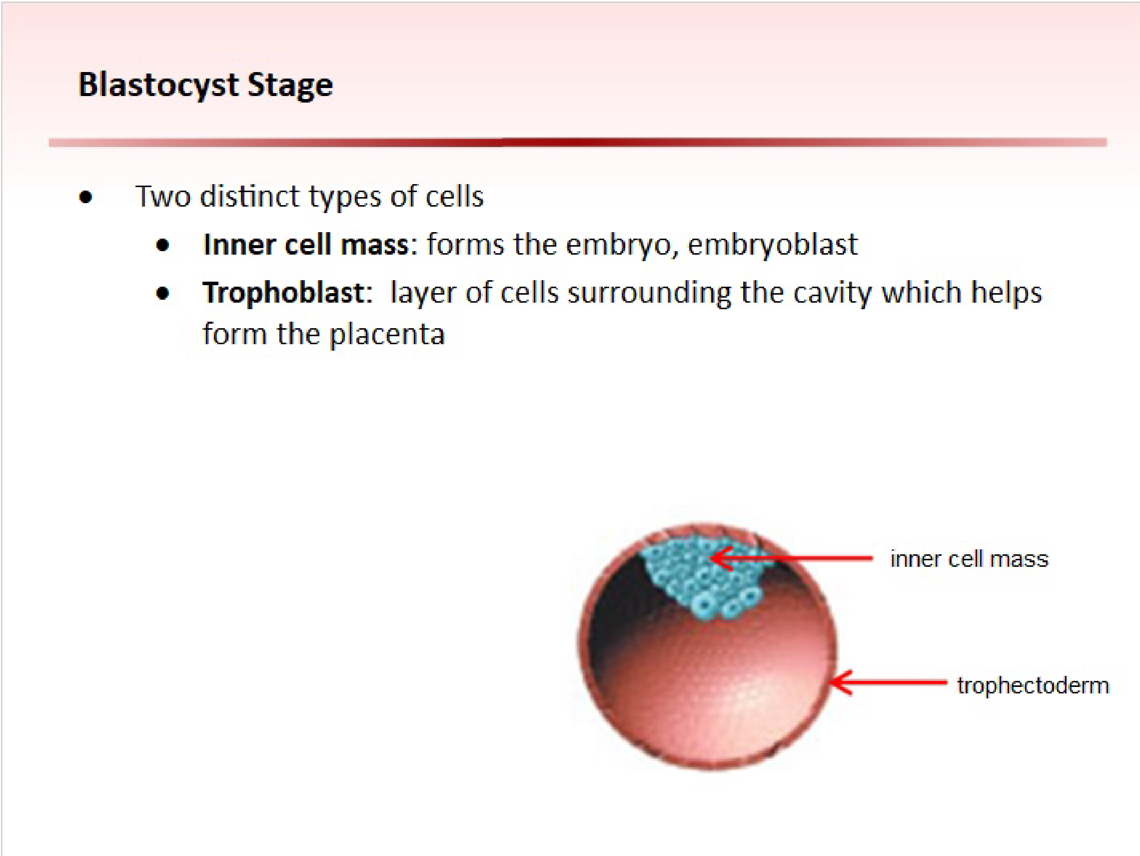
Photo courtesy of Fertility Clinic of the Carolinas



Notes:

During the morula stage of development (days 4 and 5) fluid will begin to accumulate between the cells and volume will increase to the point where a definitive cavity (blastocoel) is formed as shown by the black arrow, resulting in the formation of the early blastocyst. The blastocoel cavity further increases via membrane channel Na+/K+-ATPase, which raises the salt concentration resulting in an increase of fluid.

1.21 Blastocyst Stage



Notes:

Once the **blastocoel** forms, the embryo is called a blastocyst. As has been noted, the blastocyst has two distinct cell types: the **inner cell mass** and the **trophectoderm**. The inner cell mass forms the embryo and is referred to as the **embryoblast** while the outer cells of the **trophectoderm** will eventually form the placenta. The small group of embryoblast cells orient on one pole of the blastocyst.

1.22 Blastocyst Stage

Blastocyst Stage

- Increase of cell number and fluid accumulation thins zona pellucida
- Hatching of the blastocyst

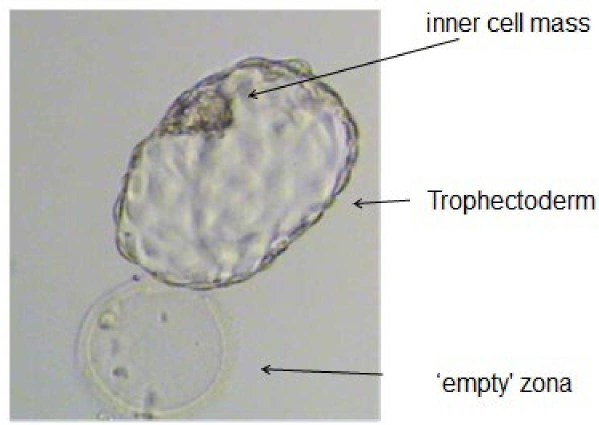


Photo courtesy of Dawn Kelk, PhD

Notes:

As the fluid inside the blastocyst increases, so does the cell number. Through the combined increase in blastocyst cell numbers, continued fluid accumulation, and zona pellucida thinning (due to embryonic enzymes), the blastocyst breaks free through a process referred to as hatching. At this time the embryo has entered the uterine cavity and will begin to prepare for implantation. At the time of implantation the outer trophoblast flattens to form the epithelial wall of the blastocyst.

1.23 Video: 2-Cell to Hatched Blastocyst

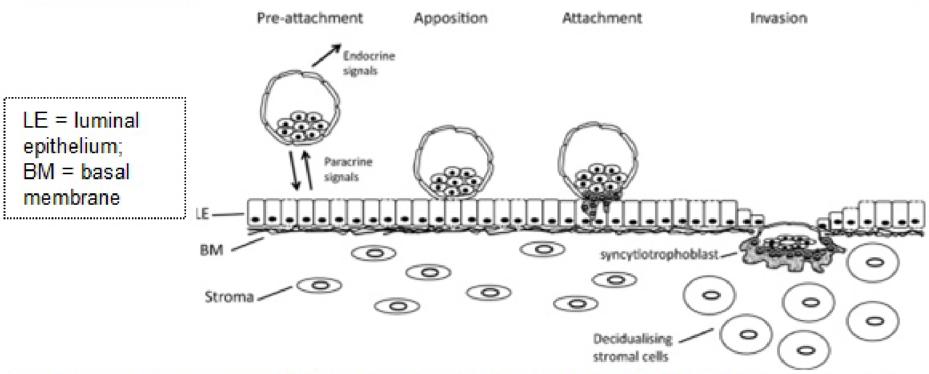
Notes:

This video demonstrates the process of a 2-cell human embryo cleaving to the hatching blastocyst stage less than 5 days later. Notice that fragment formation during the cleavage stage appears to be a dynamic and not a static process allowing a good quality hatching blastocyst to form.

1.24 Blastocyst Stage and Implantation

Blastocyst Stage and Implantation

- **Implantation** on ~day 6 post conception
  - Trophoblast erodes uterine wall
  - Takes 1 week to complete
  - Integrins and selectins
- Roles of hormones
  - Progesterone, hCG

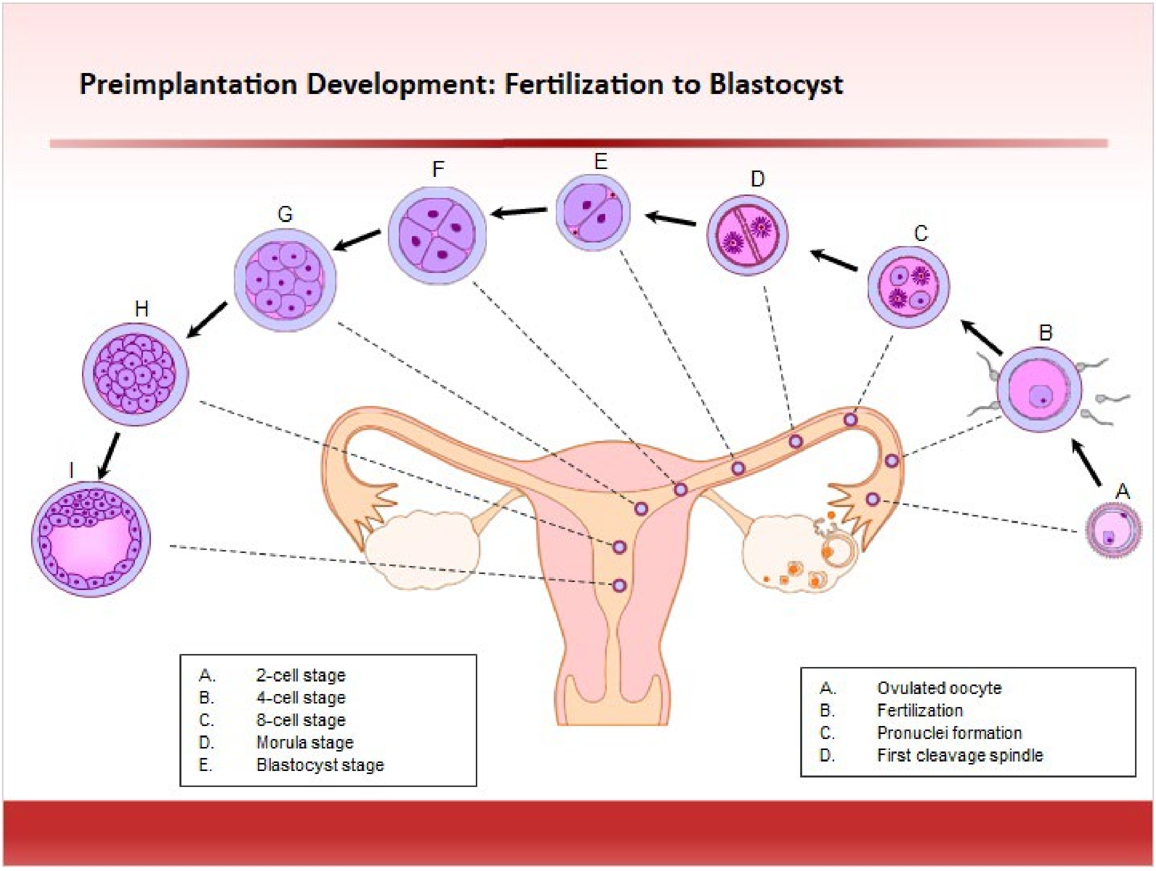


With permission from Sharkey & Macklon. Reproductive BioMedicine Online 2013; 27:453-460

Notes:

The trophoblastic cells of the embryo penetrate the epithelium of the uterine wall to allow implantation by the embryo. Attachment between the embryo and the uterine wall is possible with the help of proteins called **integrins** and **selectins** that promote adhesion between the embryo and endometrium. The process of preparation and thickening the endometrial lining, known as **decidualization**, is maintained due to hormonal secretion of **progesterone** by the **corpus luteum**. Remember that the corpus luteum is the structure of follicular cells that slowly degenerates after the oocyte is expelled from the ovarian follicle (ovulation). If the oocyte is fertilized, the production of **human chorionic gonadotropin (hCG)** by the developing embryo prevents corpus luteum degeneration. The job of progesterone production is taken over by the placenta after about 11 or 12 weeks.

1.25 Preimplantation Development: Fertilization to Blastocyst



Notes:

In summary, Fertilization typically occurs on day 15 or 16 of a woman's menstrual cycle. The actual implantation of the embryo into the uterine lining occurs about 6 days later, meaning that the first week of embryogenesis takes place primarily in the lumen of the fallopian tube and uterus.
It is important to distinguish the difference between gestational and embryonic age. Embryonic age (or fetal/fertilization age) usually begins within a day of ovulation, typically about 14 days after the beginning of a woman's last menstrual period (LMP). Gestational age (or menstrual age) measures the age of a pregnancy starting from the first day of a woman's LMP. Often difficult to arrive upon a precise date, it is a common practice to add 14 days to the known day of fertilization. This can still be variable by a few days or even weeks, depending on when a woman becomes aware of conception. For the purposes of studying embryology, embryonic or fertilization age, not gestational age is used to describe the processes of development.

1.26 Suggested Resources

Suggested Resources

1. BRS Embryology
2. Color Atlas of Embryology
3. Langman's Medical Embryology
4. http://www.embryology.ch/genericpages/moduleembryoen.html

Notes:

The resources shown here are recommended for a better understanding of the events of fertilization and early embryo development.

1.27 Thank you!

Notes:

Thank you for participating in this educational activity.