



Fertilization: The Sequence of Events

Introduction

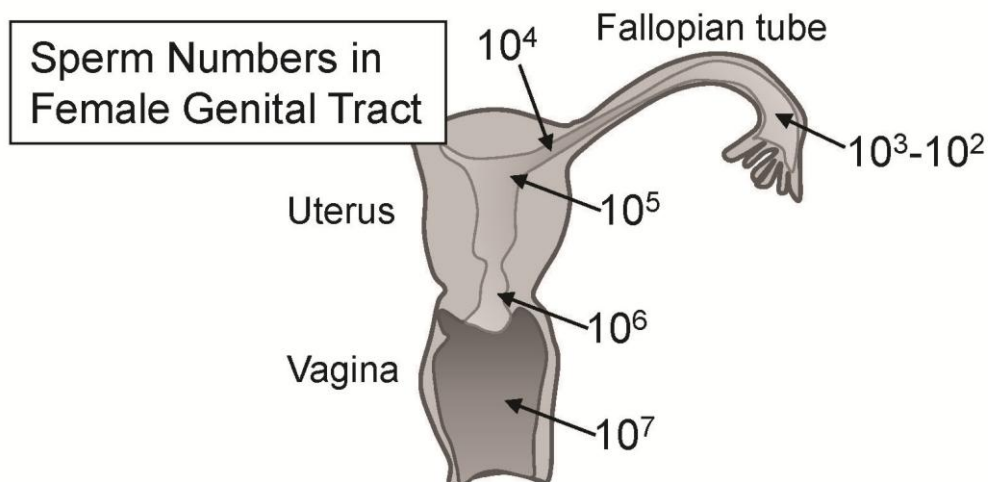
- Sperm transport in the male: adds fluid, regulatory elements, nutrients to ejaculate
- Sperm transport in the female is facilitated by muscular movements/ciliary activity
- Chemotaxis likely guides the sperm in the final stages of its trek to meet the egg
- Fertilization involves a precise sequence of events
- The acrosome contains enzymes for penetrating the egg coats
- Pronuclear swelling, migration and fusion lead to the formation of the zygote nucleus
- Development has been initiated and will be evident by the onset of cleavage

Sperm Transport in Males

- Sperm are produced in seminiferous tubules but are not motile (i.e., they can't move) and can't fertilize an egg (i.e., they are functionally immature although they appear mature morphologically)
- The sperm which are surrounded by testicular fluid passively move into the epididymus where they are stored and undergo biochemical maturation
- 4-12 day maturation phase in epididymus
- Upon ejaculation, sperm rapidly move to vas deferens
- Fluid is added increasing volume of semen and altering its characteristics: seminal vesicles--fructose for energy; prostate gland--ions, citric acid, acid phosphatase
- 2-6mL of semen (pH 7-8.3) is ejaculated
- Ejaculate normally contains 40-250 million sperm (250 million sperm)
- An ejaculate with as few as 25×10^6 sperm can still result in normal fertility

Sperm Transport in Females

The following figure shows the numbers of sperm in a normal ejaculate that are deposited in the female vagina. As the sperm move through the female genital tract, their numbers decrease rapidly.

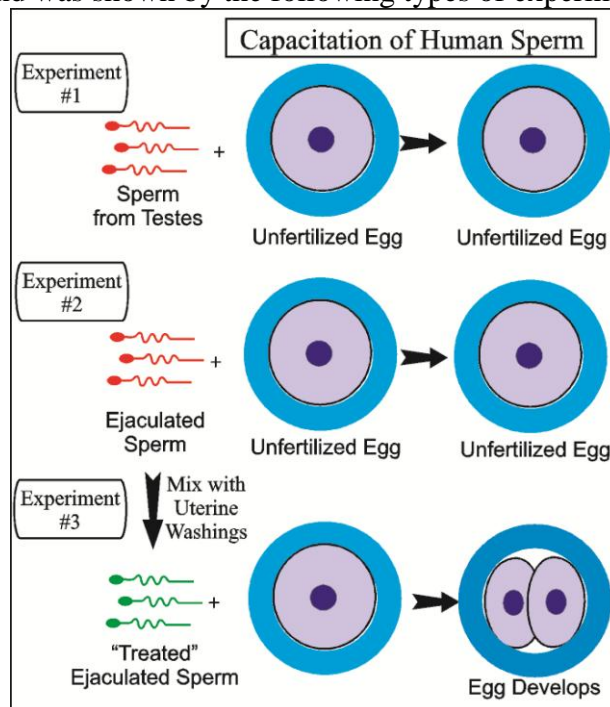


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- Sperm 20-250 million are normally deposited in the upper vagina
- Short term buffering of semen protects the sperm against the antibacterial acidity of vaginal fluid
- Swimming of sperm allows them to penetrate cervical mucous which is least viscous during days 9-16 of the menstrual cycle thus enhancing the chance for sperm to make their way through the female tract
- Muscular actions of female genital tract (fast) and sperm motility (slow; 2-3 mm/hr) are involved in sperm movements through the female genital tract
- Mode of movement through the uterus is unknown but during the movements from the vagina through the uterus many sperm lose their way
- About 200 sperm enter fallopian tubes
- Cilia line fallopian tubes and their actions move the fluid surrounding them to assist sperm movement up the tubes
- Fertilization usually occurs at ampulla region
- Sperm remain capable of fertilization for up to 80 hours (3 1/3 days) within the female genital tract--one reason unprotected sex based on the timing of the menstrual cycle can lead to pregnancy

Capacitation of Human Sperm

When experimenters first began their research on fertilization in animals, they found that for many lower animals they simply could mix egg and sperm together to get fertilization. But when mammalian sperm and eggs were mixed together, without any special treatment, fertilization did not occur. It took many years before developmental biologists began to understand why this happened. The sperm of humans, and other mammals, have to undergo some special change before they are able to fertilize the egg. This change is referred to as "capacitation" and was shown by the following types of experiment.



Capacitation Definition: Essential changes in spermatozoa that enables them to fertilize the egg

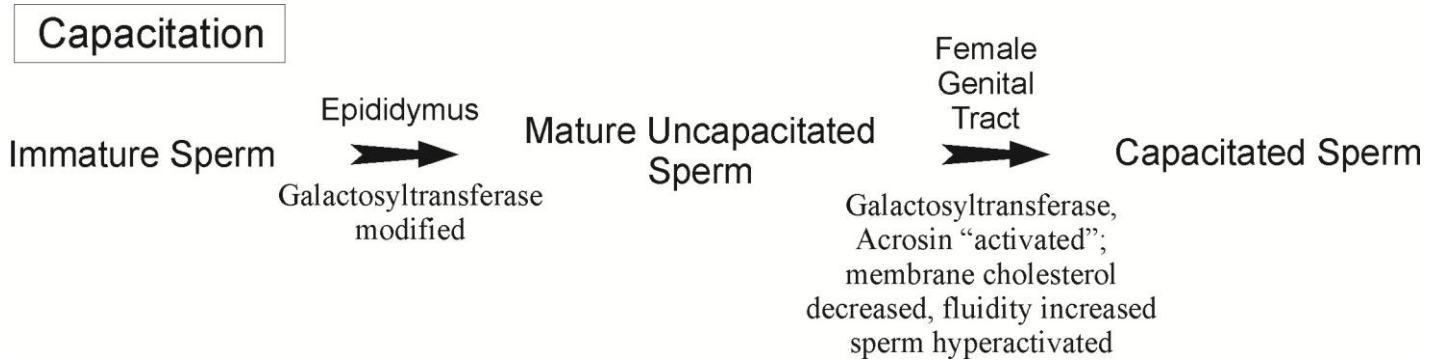
Capacitation occurs during transport in the female genital tract. It involves a number of changes including changes in the sperm cell membrane and signal transduction events. As mentioned elsewhere, only capacitated sperm are capable of chemotaxis of follicular factors.

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Major changes Occur in Sperm Cell Membrane during Capacitation

- Changes in surface glycoproteins caused by secretions of the female genital tract
- Cholesterol is removed possibly leading to an increase fluidity of the sperm cell membrane
- Glycoproteins are lost which may expose the zona binding proteins
- Proteins are phosphorylated

Current Model of Human Capacitation



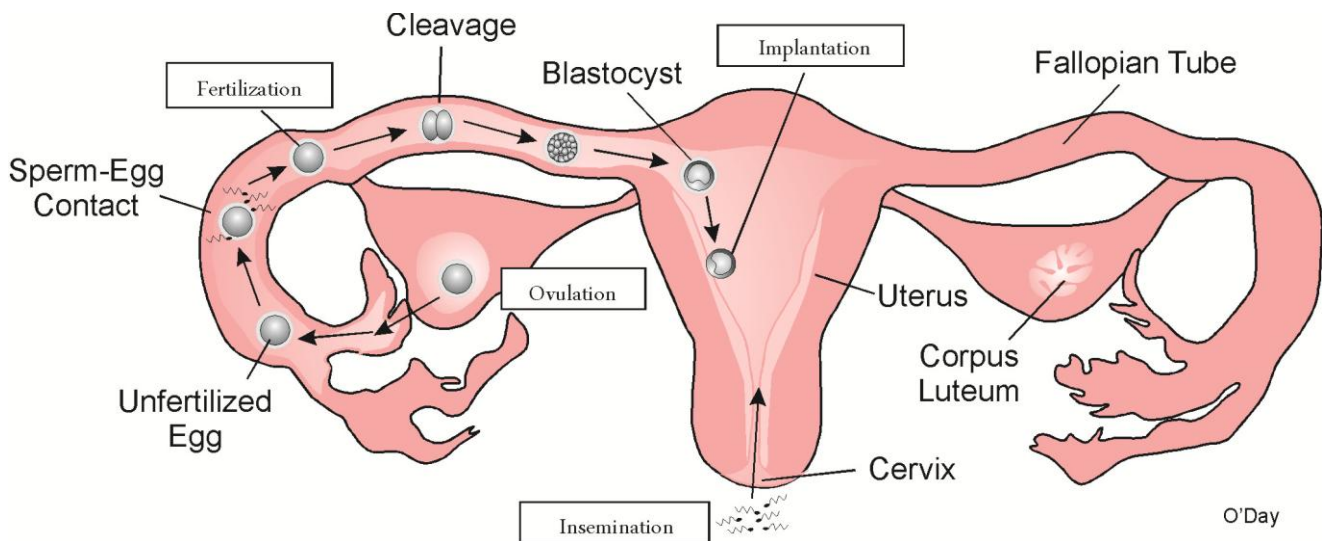
Signal Transduction Events during Capacitation

- Fluctuations occur in the intracellular levels of calcium ions; essential for hyperactivation
- Certain proteins are phosphorylated by tyrosine kinases under the influence of cAMP which has been shown to be absolutely required for human sperm cell capacitation

Effects of Capacitation on Sperm

- Increased rate of metabolism
- Hyperactivation: flagellum beats more rapidly
- Changes in sperm glycoproteins allow sperm-egg binding
- Pro-Acrosin (inactive) is converted to acrosin (active)

Site of Human Fertilization & Early Embryonic Development



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Sperm Chemotaxis

Since only a few hundred sperm reach the ampulla region of the fallopian tube, how do they find their miniscule target? Chemotaxis towards a chemical released by the egg and/or follicle cells appears to orient the movement of the sperm towards the egg to ensure that fertilization occurs. In vitro studies have shown that sperm can orient towards follicular fluid but as yet the factors involved and the mechanism by which chemotaxis occurs remains unknown. It is known that only capacitated sperm can chemotactically respond to the factors in the follicular fluid. Knowledge of how sperm chemotaxis occurs may provide more insight for improving the success of in vitro fertilization and may provide new routes to developing contraceptives.

Some Major Acrosomal Enzymes

Remember, the acrosome lies at the front end of the sperm cell just below the cell membrane. It is derived from the Golgi and, because of its content of digestive enzymes, is now considered to be a specialized lysosome-related organelle. The enzymes contained within the acrosome are designed to penetrate the egg coats during fertilization. Here's a list of those that have been characterized in humans.

Protein Digestion

Acid Proteinase: a general protease that hydrolyzes protein at an acid pH

Collagenase: a protease that digests collagen

Acrosin: A special human acrosomal protease that digests zona pellucida proteins may also mediate sperm-zona pellucida binding. However, mouse null acrosin mutants can still penetrate the zona indicating that acrosin may not be critical in the mouse.

Novel Serine Proteases: Found in mice but not yet in humans.

Carbohydrate Digestion

Beta-glucuronidase: An enzyme that removes Beta-glucuronic acid residues from carbohydrates

Hyaluronidase: An enzyme that digests carbohydrates containing hyaluronic acid residues

Neuraminidase: An enzyme that cleaves neuraminic (sialic) acid residues from carbohydrates

Lipid Digestion

Phospholipase C: A lipase that hydrolyzes phosphoinositol phospholipids, in this case, in the egg membrane

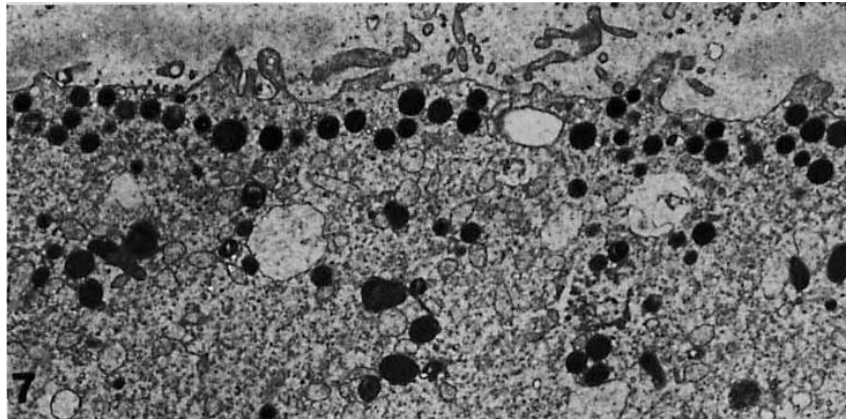
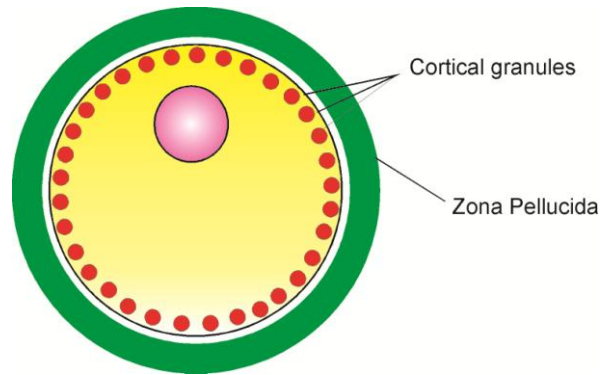
Human Acrosin

- Acrosomal serine protease
- Bound to the inner surface of acrosomal membrane
- Digests pathway through the zona pellucida which is primarily made up of three proteins: ZP1, ZP2, ZP3
- May act with other proteins to mediate the species-specific binding of sperm to the zona and hold the sperm cell in place as it digests its way through the zona

Cortical Granules

- The cortical granules are membrane bound vesicles that lie just below egg cell membrane
- Many enzymes for protein, carbohydrate digestion
- Exocytosis occurs at fertilization
- Enzymes contained in the cortical granules will digest the proteins of the zona pellucida (i.e., ZP3, ZP2) to prevent more sperm binding which could lead to polyspermy

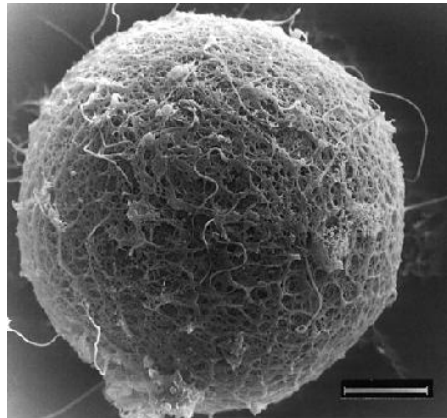
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EM picture of human cortical granules (Fig. 7, Sathanansan and Trouson, 1980. *Gamete Res.* 5: 191-198.)

Sequence of Events in Fertilization

Below is a photomicrograph of human sperm bound to the zona pellucida.

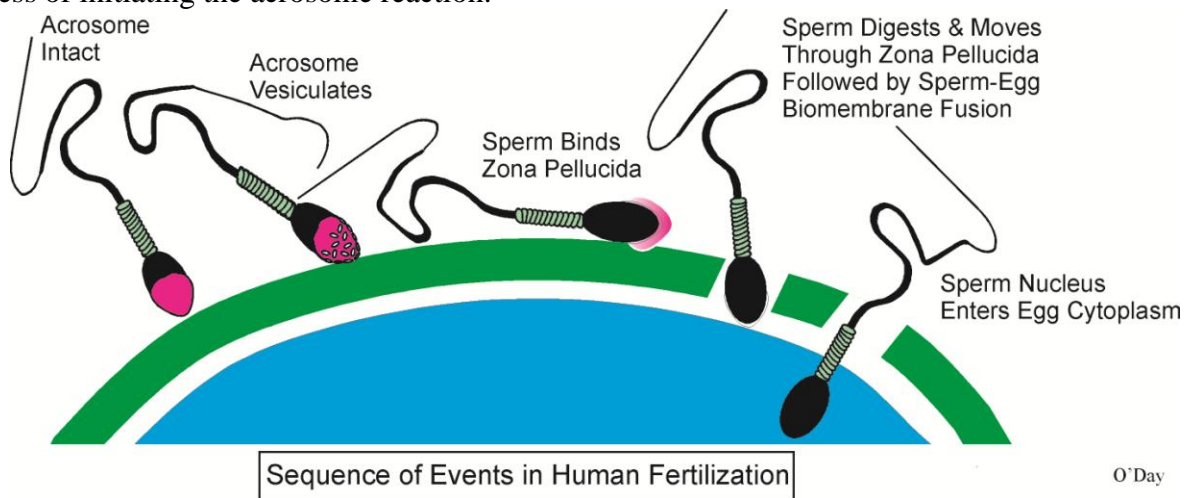


Picture from: Magerkurth, C., et al. (1999). Scanning electron microscopy analysis of the human zona pellucida: influence of maturity and fertilization on morphology and sperm binding pattern, *Human Reproduction*, 14(4). 1057-1066.

The sperm must make its way to the surface of the egg where the sperm cell membrane will contact the egg cell coats. As we covered in an earlier lecture, the egg is surrounded by both cellular and acellular layers. Most research has been done on eggs in which the cellular layers are stripped off so the egg is simply surrounded by the zona pellucida. It should be noted that the cellular layers appear to be important in activating the sperm to undergo the acrosome reaction among other things. So, while the cellular layers are

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not shown in the following figure, the first two steps below indicate the importance of the corona radiata in the process of initiating the acrosome reaction.

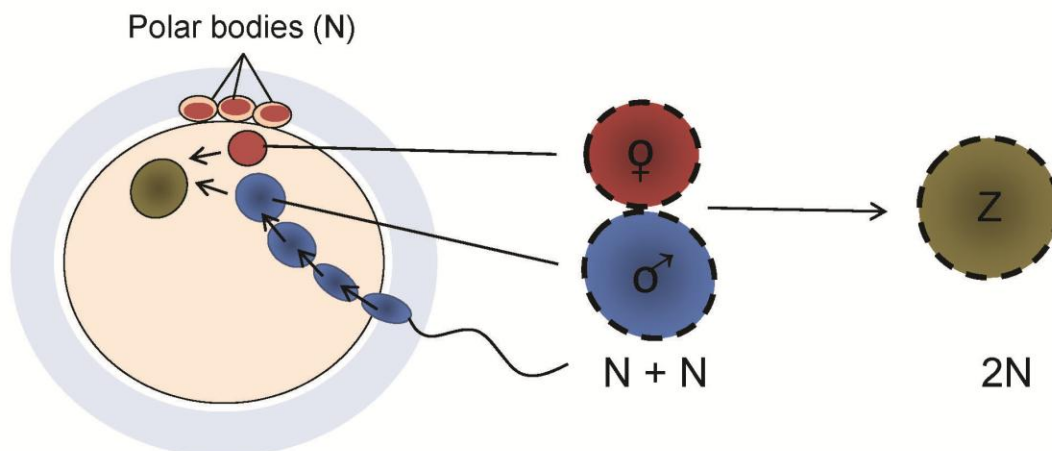


Here is the sequence of events as seen *in vitro*:

- Acrosome intact sperm contacts zona pellucida
- Acrosome vesiculates: enzymes are released
- Sperm binds to zona pellucida at post-acrosomal region; enzymes continue to digest route to egg
- Sperm-egg membrane contact & fusion
- Sperm nucleus enters egg cytoplasm

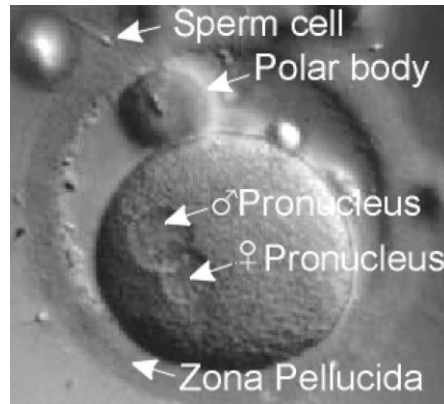
Pronuclear Events

When the sperm enters, the egg is released from meiotic arrest and will complete meiosis II prior to the fusion of the pronuclei. The male pronucleus is larger than the female pronucleus in humans, so it's easy to identify them.



- Haploid egg pronuclear forms when the sperm to activates the egg; sperm contact with the egg membrane stimulates the completion of meiosis with the release of a polar body
- The haploid sperm nucleus enters the egg cytoplasm and becomes the sperm pronucleus
- The annulate lamellae may be important for pronuclear formation and fusion because they've been shown to play a role in these processes in other mammals
- Sperm pronucleus continues to swell as it migrates towards the egg pronucleus
- The pronuclei will fuse and this involved a special series of events which differs in different organisms

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Pronuclear Fusion

- When they are in close proximity, the pronuclear envelopes vesiculate: the nuclear membranes break up to form a circle of smaller vesicles that surround the chromatin of each nucleus
- The chromatin from each pronucleus intermixes to form the diploid zygote nucleus
- The nuclear envelope reforms around the zygote nucleus and embryonic development will begin with the onset of cleavage (cell divisions of the zygote).

Parthenogenesis

One new approach to generating embryonic stem cells is to start development parthenogenetically. What is parthenogenesis? It is the development of an individual from an unfertilized egg. In some insects parthenogenesis is a way of life. For example in the honeybee, parthenogenesis generates haploid males called drones. In mammals, it is generally accepted that natural parthenogenesis doesn't occur. In frogs and sea urchins various experimental approaches can be used to induce parthenogenesis and complete development in the laboratory. Such artificial parthenogenesis is believed to result when the artificial treatments somehow evoke egg responses normally caused by the sperm. However, attempts to stimulate parthenogenetic development in mouse eggs lead to incomplete development. Experiments have shown that, in mammals, both male and female pronuclei need to be present because each contributes critical genes required for normal development. To date, there is no evidence that human embryos can be stimulated to develop parthenogenetically which might explain why the topic is not covered in any textbooks on human development or embryology. For more details you should refer to a general book on animal development.

References

Lawson et al, 2007. Identification and localization of SERCA 2 isoforms in mammalian sperm. *Molecular Human Reproduction* 13: 307-316.

Moreno and Alvarado, 2006. The mammalian acrosome as a secretory lysosome: New and old evidence. *Molecular Reproduction and Development* 73: 1430-1434.

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