# Chapter 25
# How are the sperm and egg prepared for fertilization and how does fertilization take place?

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Fertilization is a complex, multi-step process involving sperm and egg maturation, transport, and interactions that culminate in fusion of the two cells and their haploid genomes. This fascinating biological event begins with both sperm and eggs undergoing a series of preparatory steps to make fertilization possible (Fig. 1). At ovulation, the egg (or multiple eggs, depending on the species) leaves the ovary for the oviduct. Concurrently, the egg progresses through meiosis, known as "oocyte maturation" or "meiotic maturation," from prophase of meiosis I where it has been arrested since before birth, to an arrest in metaphase of meiosis II in most species. Sperm are morphologically mature upon leaving the testis, but they must undergo several steps to acquire fertilization competence. During epididymal maturation (Chapter 18), sperm develop the ability to (1) be motile and (2) undergo capacitation (addressed below). Next, upon ejaculation, the sperm are mixed with seminal fluid, which includes buffering components that help sperm survival in the acidic environment of the vagina. Seminal fluid has additional functions in reproduction as well, including providing proteins that attach to the sperm and mediate sperm binding to the oviduct, and signaling factors (e.g., cytokines, prostaglandins, hormones) that induce changes in the female reproductive tract that facilitate fertilization and reproductive success.

During sperm transit through the female reproductive tract, sperm are modified by interactions with secreted fluids, extracellular vesicles, and epithelial cells. In the female tract, sperm undergo capacitation, which are the physiological changes and reorganization of the sperm surface that confer the ability to fertilize an egg. It is notable that the discovery in the 1950s of methods to support sperm capacitation *in vitro* is one of the advances that made

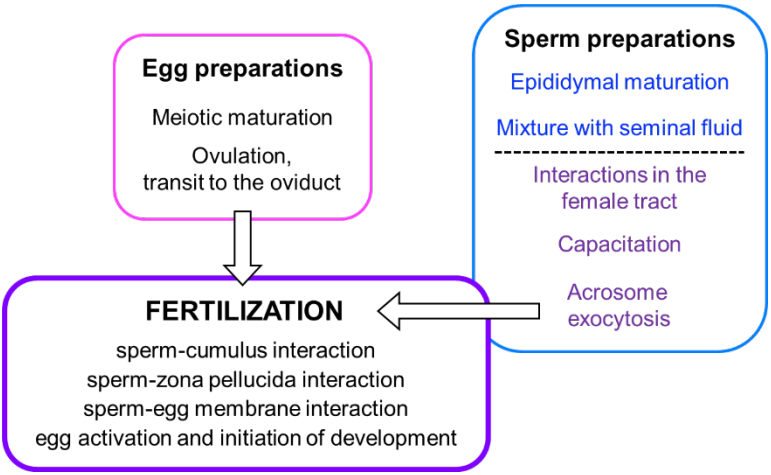


**Figure 1.** This schematic diagram shows the steps discussed in this chapter, including the egg's preparatory steps (pink box), the sperm's preparatory steps (blue box), and the steps of fertilization itself (purple box). The steps of the sperm's preparations in blue text and above dotted line occur in the male, and the steps in purple text below the dotted line occur in the female. Acrosomal exocytosis, the last step of the sperm's preparatory changes, occurs after capacitation. There are multiple possible triggers for acrosome exocytosis, originally thought to be binding of sperm to the zona pellucida (ZP) during fertilization, but newer data suggest that acrosome exocytosis can occur while the sperm are in the upper isthmus of the oviduct, stimulated by factors such as progesterone and/or mechanical shear force (see main text).

*in vitro* fertilization possible. Capacitation-associated changes include the loss of cholesterol from the sperm plasma membrane, activation of a soluble adenylate cyclase in the sperm, generation of the second messenger cyclic AMP, and activation of a number of protein kinases. Capacitated sperm are also now capable of undergoing acrosomal exocytosis. In many mammalian species, capacitation is associated with a change in the sperm's swimming pattern to a non-progressive, whiplash motion, which characterizes hyperactivated motility. This change in motility is thought to help sperm bind and transit through the vestments surrounding the egg, which include the cumulus cell layer and the zona pellucida (Fig. 2).

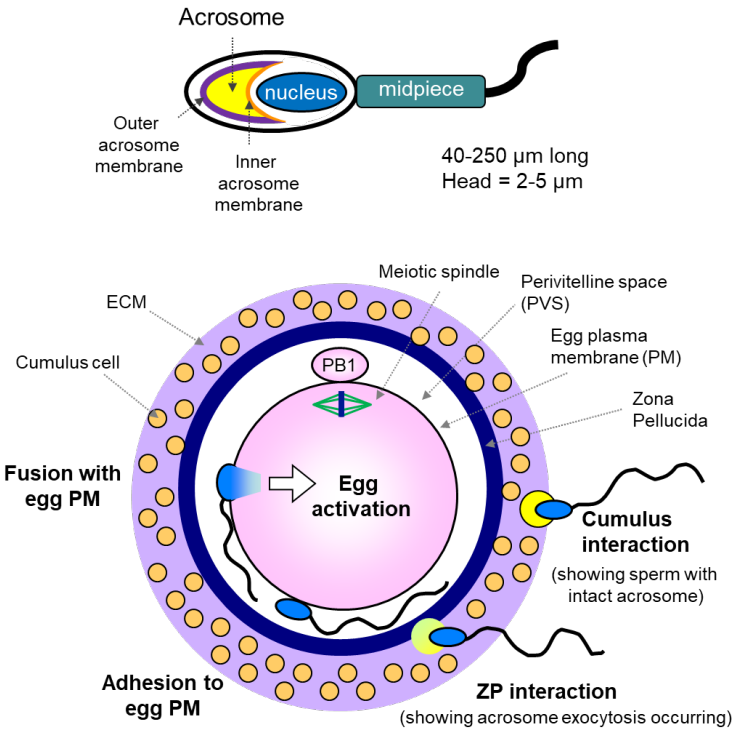


**Figure 2.** Upper panel: Schematic diagram of a mammalian sperm, indicating the two main domains, the head and the tail. The flagellar tail contains microtubules, and includes the midpiece, which is where the mitochondria are located. The head includes the sperm chromatin in the nucleus (blue), and the acrosome (yellow). Acrosomal exocytosis is achieved by the formation of fusion pores between the outer acrosomal membrane (purple) and plasma membrane (black). After acrosomal exocytosis is complete, the inner acrosomal membrane (orange) is exposed on the surface of the sperm. Lower panel: Schematic diagram showing the structures of the mammalian egg and the steps of fertilization (bold text). This diagram illustrates acrosomal exocytosis occurring with sperm-ZP interaction, although newer data suggest that there are other triggers that can induce acrosomal exocytosis prior to ZP penetration (see main text). Abbreviations: ECM, extracellular matrix in which the cumulus cells are embedded; ZP, zona pellucida; PM, plasma membrane; PVS, perivitelline space, the space between the zona pellucuda and the egg plasma membrane; PB1, first polar body, the product of cytokinesis resulting from the first meiotic division. The second meiotic division will not be completed until egg activaton. occurs.

Sperm can be stored in the female reproductive tract by interacting with epithelial cells that line the oviduct. In most mammals, including humans, sperm can survive in the female tract for up to several days. In other species, sperm can even be stored in specialized sperm storage tubules in the female tract for weeks, months, or years. Functions of sperm storage in the female tract include prevention of polyspermy (fertilization of the egg by more than one sperm), maintenance of sperm fertility and viability, and regulation of capacitation and hyperactivated motility. Eventually, sperm travel to the site of fertilization, the ampulla region or the ampullary-isthmic junction of the oviduct, depending on the species. The transit of the sperm up the oviduct could be mediated by different factors, including muscular contraction of the female tract to push sperm upward, sperm motility propelling migration up the tract, and/or sperm motility possibly directed toward a follicular or egg chemoattractant.

Fertilization occurs as a continuous process with several identifiable steps: (1) sperm-cumulus interactions, (2) sperm-zona pellucida interactions, (3) sperm-egg plasma membrane interactions, and (4) egg activation and initiation of development (Figs. 1, 2). The ovulated egg is surrounded by cumulus (granulosa) cells embedded in an extracellular matrix made of hyaluronic acid. Sperm penetration through this matrix is mediated by the sperm's motile force and may also be facilitated by hyaluronidases on the sperm surface.

The sperm next interacts with the egg's coat, the zona pellucida (ZP), which is synthesized during oogenesis by the developing egg and is composed of three or four glycoproteins, depending on the species. The identity of the "receptor" on the sperm for the ZP has been debated for decades, but sperm-ZP binding is likely mediated by a group of proteins and may depend on the 3D conformation of a protein complex. The ZP supports sperm binding in a species-selective manner but whether and how the ZP induces the sperm to undergo acrosome exocytosis is debatable. Also known as the acrosome reaction, acrosomal exocytosis involves (1) the release of the contents from the acrosome, the large secretory vesicle on the head of the sperm, and (2) additional reorganization of the sperm surface (Fig. 2). The release of the acrosome's contents is accomplished by the formation of numerous fusion pores between the outer acrosome membrane and the head plasma membrane, and is thought to proceed in a sequential manner, with soluble proteins being released more readily than acrosomal matrix proteins that are proteolytically released, at least in part, by acrosin and other

proteases. As acrosomal exocytosis progresses to completion, new arrangements of proteins on the sperm surface are exposed, which render the sperm capable of interacting with the egg plasma membrane. Thus, acrosomal exocytosis is a necessary prerequisite for the next step of fertilization, interaction with the egg plasma membrane (see below). The signal triggering the sperm to undergo acrosomal exocytosis was thought to be binding to a specific ZP component, but recent studies in mice have cast doubt on this model and instead suggest that acrosomal exocytosis is triggered in the upper isthmus prior to reaching the oocyte by other, and perhaps multiple, factors (e.g. progesterone, mechanical shear force). Downstream from this initial trigger, calcium is a key second messenger that induces the sperm to complete acrosomal exocytosis.

Once the sperm has penetrated through the ZP, it reaches the perivitelline space where it gains access to the the egg plasma membrane. The interaction of the sperm with the egg plasma membrane is a multi-step process, beginning with adhesion of the sperm to the egg plasma membrane, bringing the membranes in close contact, and culminating in membrane fusion, which creates cytoplasmic continuity between the gametes (Fig. 2). Studies using mouse knockout models have identified multiple sperm and egg molecules that are critical for sperm-egg membrane interactions. Upon the formation of cytoplasmic continuity between the gametes, a sperm-specific form of phospholipase C (PLCζ) is among the intracellular components introduced into the egg from the sperm. PLCζ plays a crucial role in inducing the egg-to-embryo transition based on studies of mouse knockout models and certain male infertile patients with sperm that are defective in initating embryo development, although some data suggest that compensatory factors may exist in sperm. Once introduced into the egg cytoplasm, PLCζ generates IP3 (inositol triphosphate) from PIP2 (phosphatidylinositol 4,5-bisphosphate). IP3 in turn binds to IP3 receptors on the egg's intracellular Ca2+ stores, the endoplasmic reticulum. This causes release of Ca2+ into the cytosol, and the activation of this Ca2+-dependent signaling pathway induces the egg-to-embryo transition, also known as egg activation. Thus, the egg must be capable of responding to this sperm-borne PLCζ,; interestingly, there may be an egg factor(s) that mediates this responsiveness, as PLCζ induces increased cytosolic calcium in eggs but not in other cell types. The main events of egg activation are the establishment of blocks at the level of the ZP and/or the egg membrane to prevent

polyspermic fertilization, the completion of meiosis (exit from metaphase II arrest) and progression to embryonic mitosis.

In summary, sperm and egg maturation involve several highly orchestrated processes that occur in the male and female reproductive tracts, culminating with gamete fusion and triggering the egg-to-embryo transition. Fertilization is a continuous series of processes that result in the delivery of the paternal chromatin to form an embryo and ultimately, competent offspring.

## Suggested reading

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