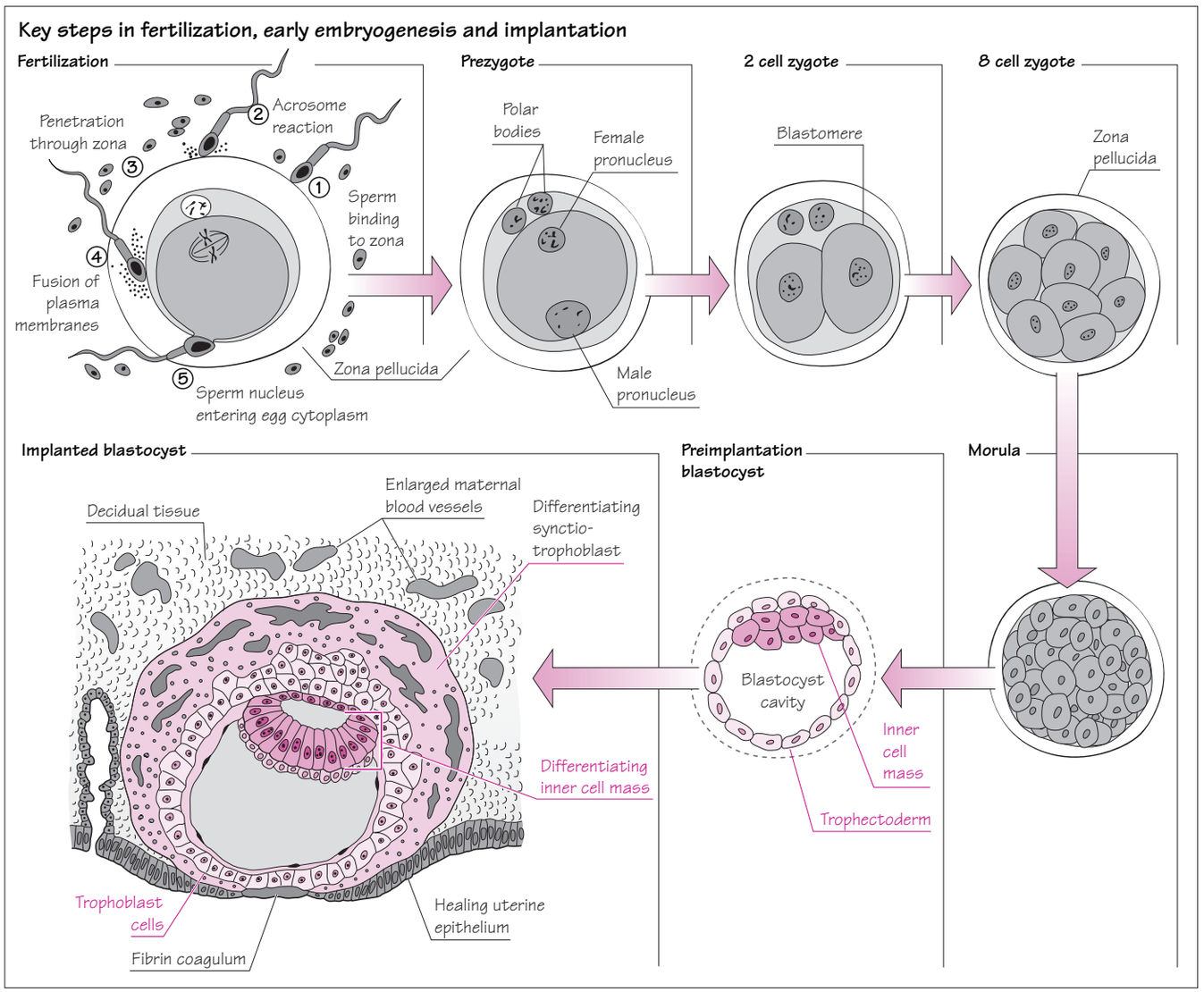
17 Fertilization and the establishment of pregnancy



The egg

At ovulation, the egg is arrested in metaphase of the second meiotic division. It is surrounded by a proteinaceous sphere called the **zona pellucida**. Those granulosa cells that adhered to the surface of the zona pellucida and were expelled with the egg from the ovary remain attached as the cumulus. Sperm that ultimately fertilize the egg must first negotiate these surrounding layers before they can penetrate the egg cell membrane. The oocyte will remain viable for 6–24 h once ovulated.

The sperm

With coitus, millions of sperm are deposited in the upper vagina. Most will never arrive at the site of fertilization. Abnormal sperm can rarely make this long trip successfully and even most of the healthy spermatozoa die along the way. The vast majority leak from the vagina upon liquification of the semen. Only a small proportion enter the cervix, where they will be found within minutes of coitus. Here they can survive within the epithelial crypts for hours. Sperm cannot traverse the cervix into the uterine cavity unless the cervical mucous is receptive. This typically occurs at midcycle when oestrogen levels are high and progesterone is low. Oestrogen softens the cervical stroma and makes cervical secretions thin and watery. Progesterone has opposite effects, a combination hostile to spermatozoa.

In the best of conditions, it takes 2–7 h for sperm to move through the uterus to the site of fertilization within the oviduct. Sperm transport results from self-propulsion, aided by the ciliary beating of cells within the uterine lining. Typically only several hundred sperm reach the oviducts, where they will linger in a quiet state until ovulation occurs. After ovulation, these spermatozoa are reactivated and begin moving toward the egg. The signal that attracts the sperm to the egg is unknown. Human spermatozoa can survive for a total of 24–48 h in the female reproductive tract.

Freshly ejaculated spermatozoa are not capable of fertilizing an egg. They acquire the ability to penetrate the cell layers surrounding the oocyte through a process known as **capacitation**. Although capacitation can be induced *in vitro* under the proper culture conditions, it occurs *in vivo* within the female reproductive tract. During capacitation, the glycoprotein coat that adheres to the spermatozoa cell membranes is initially removed, initiating changes in the surface charge of the sperm membrane and reorganization of that membrane. Capacitated sperm change their tail movements from regular undulating waves to whip-like, thrashing movements that propel the sperm forward. At the biochemical level, capacitated sperm acquire increased calcium sensitivity and elevated internal cAMP levels. Capacitation takes several hours both *in vivo* and *in vitro*.

Sperm capacitation allows for the **acrosome reaction**. In the absence of an acrosome reaction, a sperm is incapable of penetrating the zona pellucida. Contact of an intact, capacitated sperm with the zona pellucida of an egg allows interaction of a specific sperm cell surface glycoprotein, ZP3, with specific zona protein. ZP3-binding induces further calcium influx into the spermatozoa and intracellular cAMP levels rise. The acrosome swells, its outer membrane fuses with the sperm plasma membrane, and the enzymatic contents of the acrosome are released into the extracellular space surrounding the head of the sperm. This also exposes the inner acrosomal membrane and another zona-binding protein, ZP2, to the oocyte zona. ZP2-binding holds sperm near the egg. Proteolytic enzymes released from the acrosome then facilitate penetration of the zona pellucida by the whiplashing sperm. Complete penetration of the zona takes about 15 min.

Fertilization

Penetration of the zona pellucida allows contact between spermatozoa and the oocyte membrane. The germ cell membranes fuse almost immediately and the sperm cell stops moving. The sperm nucleus enters the egg cytoplasm.

Three important events are triggered within the oocyte by the rise in intracellular calcium that occurs in the oocyte upon fusion of sperm and egg cell membranes. The egg cell membrane depolarizes, preventing membrane fusion with additional spermatozoa. This is the **primary block to polyspermy**. It assures that only one male pronucleus is available for fusion with the female pronucleus and protects the diploid status of the zygote. The second event is known as the **cortical reaction**. Cortical granules lie just beneath the egg cell membrane, and with the cortical reaction they fuse with the membrane and release their contents into the zona pellucida. This hardens the zona and impairs the ability of sperm to bind the zona—a **secondary block to polyspermy**. The third event involves resumption of the second meiotic division of the egg. The second polar body is formed and extruded from the egg, thereby assuring that the female pronucleus is haploid. Again, the diploid zygote is protected. Failure to preserve the diploid state of the conceptus is a frequent cause of early pregnancy failure (Chapter 36).

Upon entry into the egg, sperm cytoplasm mixes with that of the egg and the sperm nuclear membrane breaks down. A new membrane forms around the sperm chromatin, forming the **male pronucleus**. A new oocyte nuclear membrane also forms around the **female pronucleus**. DNA synthesis begins during this period as the haploid pronuclei prepare for the first mitotic division of the zygote. The pronuclear membranes break down, the parental chromosomes mix and the metaphase mitotic spindle forms. At about 24 h after fertilization, the chromosomes separate and the first cell division occurs.

During the first few embryonic cell divisions, no new mRNA is synthesized from the nuclear DNA of the conceptus. The embryo stays the same total size and the size of each individual cell decreases accordingly. Thus, the early embryo uses only maternal cell components to develop and important signals must be transmitted to the embryo through the oocyte cytoplasm. These signals likely reside in **mitochondrial DNA**, which *is* replicated during early embryonic cell division. In fact, mitochondrial DNA is quite stable and can be traced through generations to determine maternal lineage.

The establishment of pregnancy

After fertilization, a successful pregnancy must implant within the wall of the uterus and inform the mother that pregnancy adaptations must occur. Without these two important events, the zygote will simply wash out of the uterus with the next menses.

The cleaving zygote floats in the oviduct for approximately 1 week, progressing from the 16-cell stage through the solid **morula** (mulberry) stage to the 32–64-cell **blastocyst stage**. The latter stage requires formation of the fluid-containing blastocyst cavity. The blastocyst contains two distinct differentiated embryonic cell types: the outer **trophectoderm** cells and the **inner cell mass**. The trophectoderm cells will eventually form the placenta. The inner cell mass will form the fetus and fetal membranes. It is at the blastocyst stage that the conceptus enters the uterus.

During the time that it spends in the oviduct, the conceptus remains surrounded by the zona pellucida. After about 2 days in the uterus, the blastocyst will lose or 'hatch' from the zona pellucida. With hatching, the trophectodermal cells of the blastocyst begin to differentiate into **trophoblast** cells. These simultaneous processes allow trophoblast cells to make direct contact with the uterine luminal epithelial cells. The blastocyst attaches to and invades the uterine lining. Within hours, the surface epithelium immediately underlying the conceptus becomes eroded and nearby cells lyse, releasing primary metabolic substrates for use by the blastocyst. The endometrium undergoes dramatic biochemical and morphological changes called **decidualization**, a process that begins at the point of attachment and spreads out in a concentric wave from the point of implantation. The endometrium will heal over the conceptus so that the entire implantation becomes buried within the endometrium.

As the embryo invades maternal tissues the trophoblast cells further differentiate into two cell types: **cytotrophoblast cells** and **syncytiotrophoblast cells**. Syncytiotrophoblast cells are large, multinucleated cells that develop from the cytotrophoblast layer. They are active in placental hormone secretion and in nutrient transport from mother to fetus. A subset of cytotrophoblast cells acquire invasive properties, traversing endometrial stroma to reach maternal blood vessels, including the spiral arteries of the endometrium. Appropriate invasion of the spiral arteries is key to a normal pregnancy outcome (Chapter 37).

A number of growth factors are integral to successful implantation. These include: (i) leukaemia inhibitory factor, a cytokine; (ii) the integrins, which mediate cell–cell interactions; and (iii) transforming growth factor beta (TGF-β), which stimulates syncytium formation and inhibits trophoblast invasion. Epidermal growth factor and interleukin 1β are important mediators of trophoblast invasion.

Implantation occurs about 7–10 days after ovulation. If the conceptus is to survive more than 14 days after ovulation, the ovarian corpus luteum must continue to secrete progesterone. **Human chorionic gonadotropin** (hCG) produced by the developing trophoblast and secreted into the maternal bloodstream acts like luteinizing hormone, supporting the corpus luteum by inhibiting luteal regression (Chapter 18).