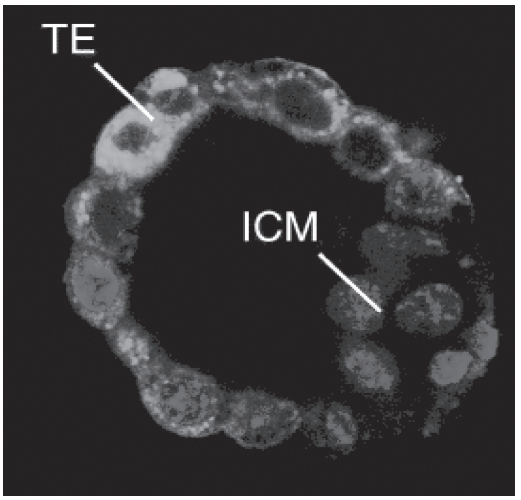
SECTION 3

DEVELOPMENTAL ASPECTS OF BIOREGENERATIVE ENGINEERING

7

FERTILIZATION AND EARLY EMBRYONIC DEVELOPMENT



Fluorescent micrograph showing a mouse blastocyst. TE: trophoblast. ICM: inner cell mass. Cells were labeled for mitochondria (green) and nuclei (blue). (Reprinted from Houghton FD: Energy metabolism of the inner cell mass and trophectoderm of the mouse blastocyst, *Differentiation* 74:11–8, 2006 by permission of Blackwell Publishing.) See color insert.

Bioregenerative Engineering: Principles and Applications, by Shu Q. Liu

An animal undergoes a developmental cycle composed of a series of biological processes: the initiation, development, maturation, and reproduction of the animal. The repetition of these processes is the foundation for the continuation of the life. The initiation and development occur during the embryonic period and are collectively referred to as *embryogenesis*. It is in this period that a new generation of animals forms. In tradition, the embryonic development of an animal is divided into two stages: the embryonic and fetal stages based on distinct markers of functional anatomy.

The *embryonic stage* is defined in the human as the period from the conception to the formation of primary organs at about the eighth week. This stage is divided into two substages: germinal and embryonic development. Germinal development takes place in the human from the conception to the formation of the three germinal layers, including the ectoderm, mesoderm, and endoderm, at the end of the second week. During this period, a male gamete (sperm) fuses into a female gamete (oocyte) to form a zygote, a process known as *fertilization*. The fusion of the two gametes allows the integration of the genomes from both parents, an essential process for transmitting genetic information from the parents to the progeny and for initiating the development of a new individual. Fertilization triggers an early mitotic segmentation process, known as *cleavage*, by which a fertilized egg is divided continuously into smaller cells. When reaching a certain cell density, the cells are organized into various patterns, which undergo dynamic changes through different stages. The embryonic cells are subsequently committed to *gastrulation*, a process leading to the formation of a three-layered structure known as *gastrula*, composed of the ectoderm, mesoderm, and endoderm. The formation of a gastrula takes place during the first two weeks and is indicative of the ending of the germinal period.

Embryonic development takes place from the second to the eighth week. During this period, the basic forms of major organs are developed from the three germ layers: ectoderm, mesoderm, and endoderm. The *ectoderm* gives rise to the central nervous system (brain and spinal cord), peripheral nerve structures, and the epidermis of the skin, teeth, nose, and external ear. The *mesoderm* is the origin of the heart, vascular system, blood, muscle, bone, cartilage, and connective tissue. The *endoderm* develops into the gastrointestinal tract, liver, pancreas, bladder, and lung. By the end of the eighth week, tissues and organs are assembled into the primary form of a fetus. The embryonic stage is the period during which most dynamic changes take place in morphogenesis.

The *fetal stage* is the remaining period from the formation of the fetus to the parturition or birth of a new individual. During this stage, the fetus gains size rapidly from several centimeters to about half a meter (~0.5 m), but the form of the tissues and organs does not change as vigorously as that during the embryonic stage. The entire embryonic period from conception to parturition is about 40 weeks.

In this chapter, the developmental processes during embryogenesis will be introduced with emphasis on the morphogenesis of embryonic structures and related regulatory mechanisms during each stage. We will see that, although bioregeneration is defined as a process occurring during the adulthood for the repair and reconstruction of lost tissues and organs, it is similar to embryonic development in many aspects.

THE SPERM [7.1]

The male and female gametes are essential components for the initiation of embryogenesis and the development of a new individual. A male gamete, known as *sperm* or *spermato-*

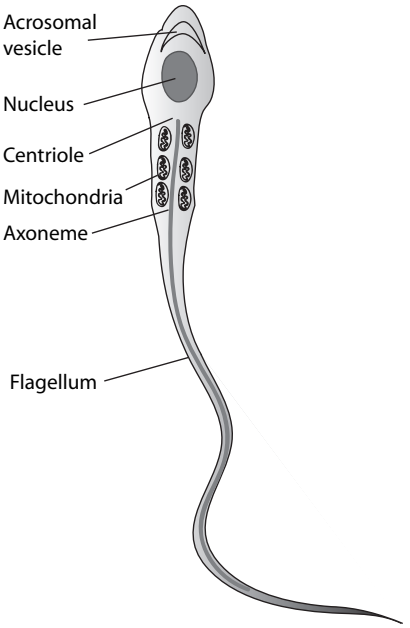


Figure 7.1. Schematic representation of a sperm cell. Based on bibliography 7.1.

zoon, is composed of several systems, including a haploid nucleus, a propulsion apparatus, and protein enzymes that are required for its interaction and fusion with a female gamete. During sperm maturation, the volume of the cytoplasm is minimized to reduce the size of the sperm, but the structures that regulate the sperm–egg interaction are evolved. These include the acrosomal vesicle and the flagellum (Fig. 7.1). The *acrosomal vesicle* is originated from the Golgi apparatus and located in front of the nucleus. This structure contains enzymes that are necessary for degrading the external layer of the egg during sperm–egg fusion. The *flagellum* is developed on the basis of the centrioles and is responsible for sperm movement, which is driven by a motile apparatus known as *axoneme*. The motile apparatus is composed of three-dimensionally organized microtubules, consisting of dimeric tubulins, and a type of motor protein known as *dynein*. A dynein molecule is attached to the microtubule and serves as an enzyme that hydrolyzes ATP. The hydrolysis of ATP provides energy necessary for the motile activity of dynein molecules and the flagellar propulsion, which induces sperm movement.

Sperms are generated from a cell type called *primordial germ cell* in the testes, the male reproductive organs. The process of sperm generation is referred to as *spermatogenesis* (Fig. 7.2). The primordial germ cells are specified during the early embryonic cell cleavage stage. A fraction of cells at the 8/16-cell stages develop into primordial germ cells. These cells usually contain mRNAs and proteins that are necessary for the development of gametes. The cytoplasm of these cells is called *germ plasm*. Sperms are formed via meiosis of the primordial germ cells. These cells are formed in the epiblast, an early embryonic structure that develops from the inner cell mass of a blastocyst and gives rise to the ectoderm, mesoderm, and endoderm. When the epiblast is developed into the three germ layers, the primordial germ cells are localized to the endoderm. With further development, these germ cells migrate into the genital ridges, where gonads (ovaries and testes)

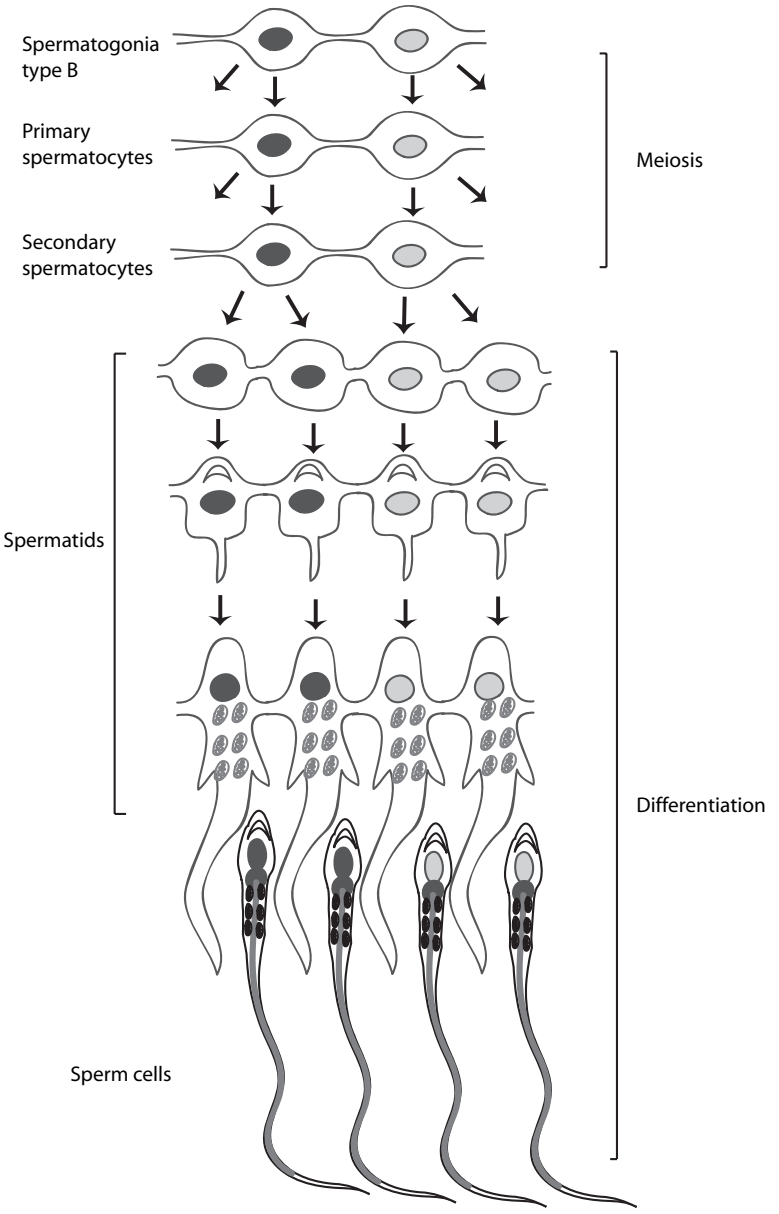


Figure 7.2. Schematic demonstration of spermatogenesis. Based on bibliography 7.1.

are developed, and remain relatively quiescent in a structure called sex cord until reaching maturity.

In the sex cord, which develops into the seminiferous tubules of the testis at puberty, the primordial germ cells differentiate into type A1 spermatogonia, which can subsequently differentiate into several levels of spermatogonia, including types A2, A3, and A4 spermatogonia (type A2 differentiates into A3, and A3 into A4). These type A

spermatogonia are stem cells in nature and can self-renew themselves as well as differentiate into specified cell types. Type 4 spermatogonia can further differentiate into a hierarchy of several cell types, including intermediate spermatogonia, spermatozoa, type B spermatogonia, and primary spermatocytes. Up to this point, mitosis is the basic form of cell division. The primary spermatocytes can divide to form another hierarchy of sperm progenitor cells via meiosis, including secondary spermatocytes and spermatids. The spermatids give rise to sperms (Fig. 7.2).

THE EGG [7.1]

The *egg* is the female gamete and is also known as the *ovum*. A developing egg before its complete meiotic division or formation of a haploid nucleus is called an *oocyte*. An egg is composed of a nucleus and an enormous volume of cytoplasm. The egg stores a large amount of food and energy-producing materials in its cytoplasm for the growth and development of the embryo. In addition, the egg cytoplasm contains a variety of proteins (structural, regulatory, morphogenetic, and protective factors), rRNAs, tRNAs, and mRNAs. The egg cytoplasm is enclosed within the cell membrane. The egg cell membrane is surrounded by an extracellular matrix layer known as *zona pellucida*. Outside the zona pellucida, there exists a thick cellular structure called *cumulus*, composed of a large number of *ovarian follicular cells* (Fig. 7.3). The follicular cells provide soluble factors and mechanical protection to the egg cell.

The process of egg formation is referred to as *oogenesis*. This process is different from spermatogenesis. Whereas spermatogenesis produces a nucleus-predominant sperm with strong motility, oogenesis gives rise to eggs with materials and factors necessary for embryonic development. In the human, there exist a limited number of female primitive germ cells (about thousand) called *oogonia*. These cells can divide into several millions

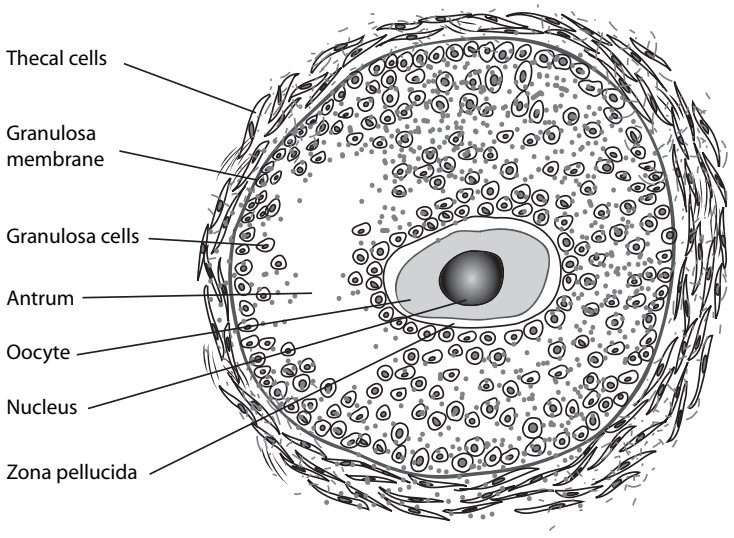


Figure 7.3. Schematic representation of an ovarian follicle. Based on bibliography 7.1.

of secondary germ cells via mitosis from the second to the seventh month of gestation. A large number of germ cells are committed to apoptosis afterward. The surviving germ cells give rise to primary oocytes via the first meiotic cycle. The primary oocytes are prompted to enter the prophase and metaphase of meiosis and maintain a quiescent state until puberty. When a human female individual reaches maturity, oocytes are periodically committed to meiosis and formation of mature eggs. Such an activity usually begins at the age of ~13 years and disappears at the age of ~50 years; these events are termed *menarche* and *menopause*, respectively. About 400 eggs can be formed through the lifespan of a female individual.

FERTILIZATION [7.1]

Fertilization is a process by which a sperm fuses into an oocyte to form a zygote, which develops into a new individual through embryogenesis. Note that the term *oocyte* is used here instead of *egg* because a sperm usually fuses with an oocyte or developing egg, which is arrested in the metaphase of meiosis and has not completed meiosis. Fertilization takes place via several steps, including: (1) attraction of sperm to an oocyte by chemotactic factors released from the oocyte, (2) interaction between the sperm and the oocyte, (3) penetration of the sperm through the extracellular layers of the oocyte, (4) the entrance of the sperm into the oocyte, and (5) fusion of the sperm with the oocyte. These steps are briefly outlined here.

Attraction of Sperm Cells to the Oocyte

In mammals, oocytes are produced and released from the ovary and moved to the oviduct. The interaction of the oocyte with sperm occurs in the ampulla region of the oviduct, which is near the ovary (Fig. 7.4). Sperm are capable of migrating toward an oocyte. Such an activity is induced and controlled by chemotaxis, or chemical gradient-directed cell movement. An oocyte can produce and release sperm-attracting proteins, which can act on specific membrane receptors in the sperm. An example of such sperm-attracting protein is resact, discovered in the sea urchin oocytes. This molecule can induce strong chemotactic activity of the sperm in vitro (Fig. 7.5). It is important to address that the chemotactic activity of sperm is dependent on animal species. Sperm can be activated and committed to directed migration only in response to a chemoattractant released from the oocytes of the same species. Furthermore, the sperm attraction activity is dependent on the developmental stage. Sperm rarely move toward oocytes that have not yet committed to the second meiosis, since these oocytes do not release sufficient chemoattractants.

Sperm–Oocyte Interaction

When a sperm approaches an oocyte, the physical interaction of the sperm with the extracellular layer of the oocyte induces the activation of the acrosomal vesicle of the sperm. In mammals, the sperm first passes through the follicular cell layer. When reaching the zona pellucida, the acrosomal vesicle undergoes exocytosis, a process that releases proteolytic enzymes. These enzymes degrade the matrix of the zona pellucida and thus help the sperm approach the oocyte membrane. The exocytosis of the acrosomal vesicle is induced by the binding of a zona pellucida protein, zona protein 3 (ZP3), to a specific

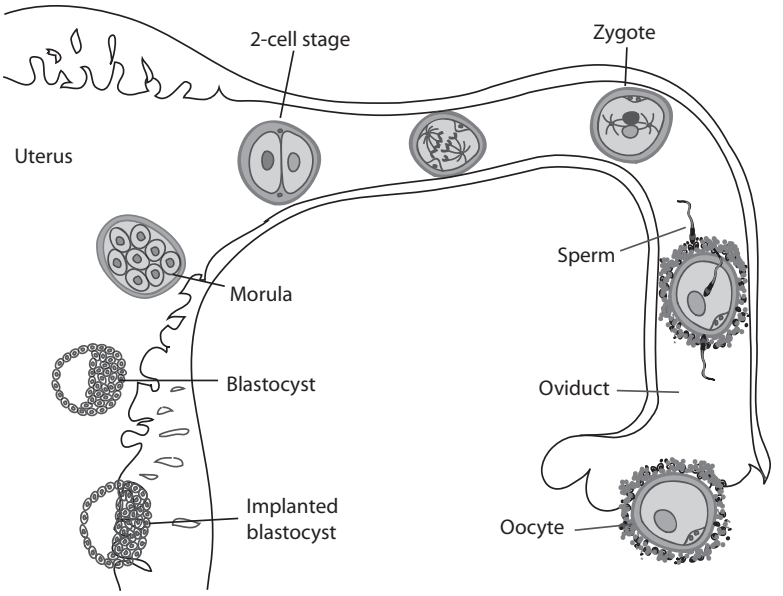


Figure 7.4. Schematic representation of the locations for oocyte fertilization, morula formation, and blastocyst formation and implantation in the human oviduct and uterus. Based on bibliography 7.1.

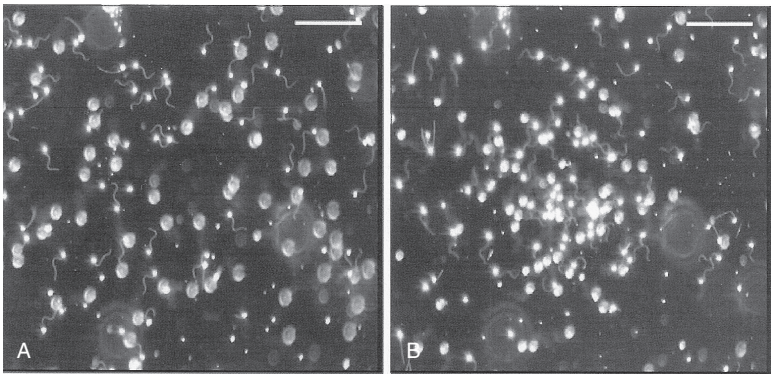


Figure 7.5. Resact-mediated movement of sperm cells. (A) Dispersed control sperm cells without the release of resact. (B) Chemotactic accumulation of sperm cells in response to the release of resact. Scale bar: 100 µm. (Reproduced from Solzin J et al: *J General Physiol* 124:115–24, 2004 copyright by permission of The Rockefeller University Press.)

sperm surface receptor, galactosyltransferase-I. The binding activity stimulates a G-protein-mediated signaling pathway and results in the release of calcium, which in turn induces the release of proteolytic enzymes from the acrosomal vesicle. Since the structure of the ligand and receptor is specific to an animal species, sperm can interact only with the egg zona pellucida of the same species.

Sperm–Oocyte Fusion

After a sperm passes through the zona pellucida, it approaches and attaches to the cell membrane of the oocyte. The cell membrane of the sperm and that of the oocyte can fuse together, bringing the sperm into the oocyte cytoplasm (Fig. 7.6). Gamete membrane fusion is a process regulated by fusion proteins. While the regulatory mechanisms of fusion are not completely understood, an oocyte membrane protein, CD9 (Table 7.1), has been suggested to play a role in the mediation of gamete fusion. The knockout of the CD9 gene is associated with impaired interaction between sperm and oocyte, resulting in infertility. When CD9 mRNA is delivered into the egg with CD9 gene knockout, infertility can be reversed. In addition, a molecule found in the membrane of sperm cells, known as the *Izumo immunoglobulin superfamily protein*, plays a critical role in regulating sperm fusion into the oocyte. In Izumo −/− mice, the sperm cells can develop to maturity and

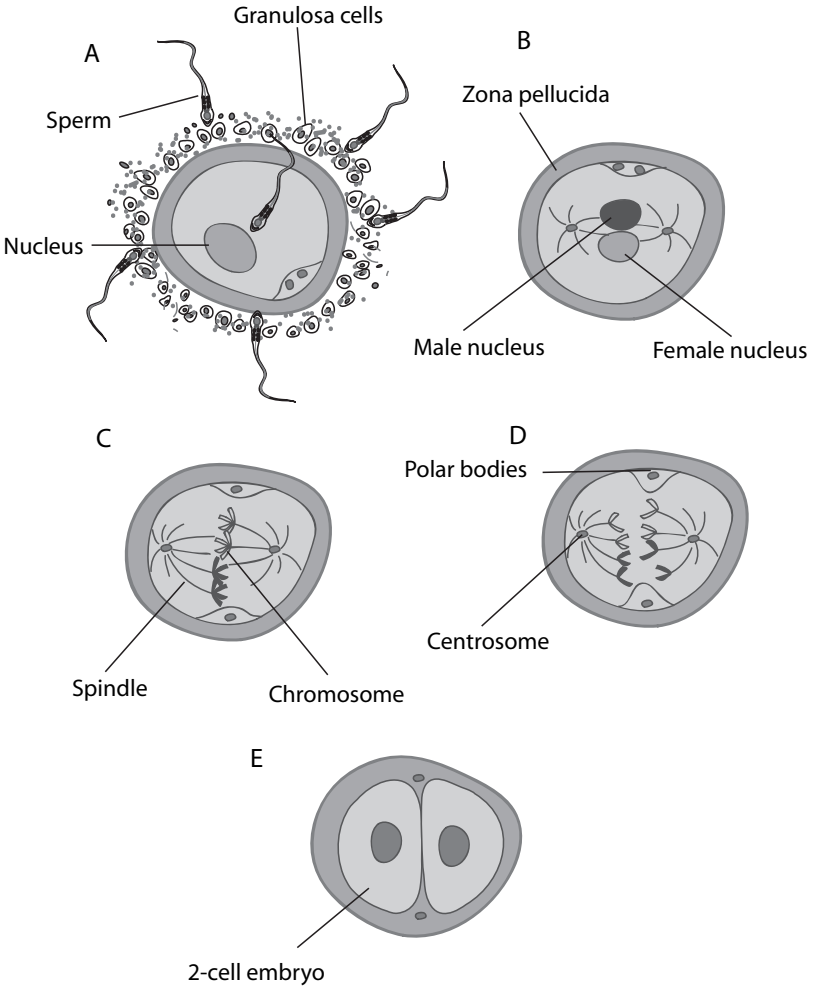


Figure 7.6. Schematic representation of sperm fusion into an oocyte. Based on bibliography 7.1.

can penetrate the zona pellucida, but cannot fuse into the oocyte (Fig. 7.7). However, the exact mechanisms of Izumo action remains poorly understood.

When a sperm enters an oocyte, the oocyte immediately becomes resistant to further sperm fusion, a mechanism that prevents polyspermy. The antipolyspermy activity is controlled by a rapid shift of cell membrane potential. A sperm can fuse with an oocyte at the normal resting membrane potential (about −90 mV). Immediately after a sperm fuses into the oocyte, the normally negative resting membrane potential is reversed to a positive value (about 20 mV), induced by the opening of the sodium channels and sodium flux into the oocyte. Such a rapid change in membrane potential prevents the interaction of other sperm cells with the oocyte during the early period after a sperm fuses with the oocyte. The shift of membrane potential is a transient process that lasts only about one minute. There exists another mechanism, by which a sperm-fused oocyte prevents further sperm fusion. The oocyte cell contains a large number of cortical granules, which contain proteolytic enzymes (Fig. 7.8). The fusion of a sperm to the oocyte triggers the release of calcium, which in turn induces the exocytosis of the cortical granules, releasing the enzymes into the zona pellucida. These enzymes cleave regulatory proteins, such as zona pellucida glycoprotein 3 (ZP3; Table 7.2), which mediate sperm–oocyte interaction. Thus, sperm can no longer bind to the zona pellucida and enter the sperm-fused oocyte.

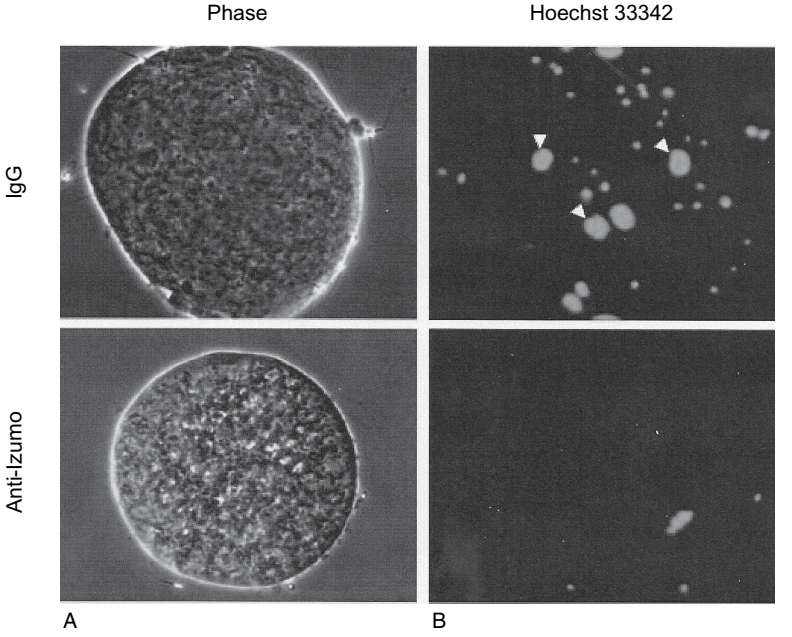


Figure 7.7. Role of the the Izumo protein in the regulation of sperm fusion to oocytes. The Izumo protein is expressed in the membrane of the sperm cell and regulates sperm fusion into eggs. (A) Izumo$^{-/-}$ mouse sperm cells are not able to fuse into a mouse egg compared to Izumo$^{+/-}$ mouse sperm cells. Note that fused sperm cells show enlarged cell nuclei. (B) Anti-human Izumo antibody (anti-hIzumo) significantly suppressed human sperm fusion into a human egg. The blue color represents cell nucei labeled with Hoechst 33342. (Reprinted by permission from Macmillan Publishers Ltd: Inoue N et al: *Nature* 434:234–8, copyright 2005.)

TABLE 7.1. Characteristics of CD9*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
CD9	CD9 antigen, leukocyte antigen MIC3, p24 antigen	228	25	Sperm, oocyte, leukocyte, skin, intestine	A membrane glycoprotein that interacts with integrins and regulates cell adhesion, migration, and fusion

*Based on bibliography 7.1.

TABLE 7.2. Characteristics of ZP3*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Gene Locus	Expression	Functions
ZP3	Zona pellucida glycoprotein 3A, sperm receptor, zona pellucida protein C, zona pellucida sperm-binding protein 3	424	47	7q11.23	Oocyte	A zona pellucida component that regulates sperm–oocyte interaction and activation of fertilization processes

*Based on bibliography 7.1.

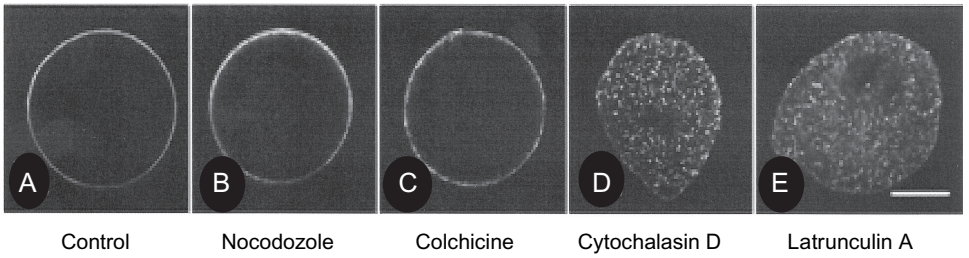


Figure 7.8. Translocation of cortical granules in oocytes. The cortical granules of oocytes are translocated to the cortical layer following germinal vesicle breakdown. This process is dependent on the organization and function of the actin filaments. A. Distribution of cortical granules in a control egg. B and C. Distribution of cortical granules in eggs treated with microtubule inhibitors nocodozole and colchicine, respectively. Note that these inhibitors did not influence the distribution of the cortical granules, suggesting that microtubules do not play a significant role in regulating the translocation of cortical granules. D and E. Distribution of cortical granules in eggs treated with actin filament inhibitors cytochalasin D and latrunculin A, respectively. Note that these inhibitors significantly influenced the distribution of the cortical granules, suggesting that actin filaments play a role in regulating the translocation of cortical granules. Reprinted from Wessel GM et al. Development 129:4315–4325, 2002 by permission of The Company of Biologists Ltd.

Activation of Embryonic Development

The fusion of a sperm cell into an oocyte triggers the activation of several important cellular activities, including DNA synthesis, restoration of mitosis, protein synthesis, and membrane synthesis. All these activities are directly or indirectly related to changes in the intracellular concentration of calcium. Since sea urchin oocytes are used extensively in the study of sperm–oocyte fusion, these cells are used here as an example. The fusion of a sperm with an oocyte, especially the binding of ZP3 to its receptor on the sperm, triggers the activation of G proteins, which further activate phospholipase C. Activated phospholipase C can cleave phosphatidylinositol biphosphate (PIP_2) to form inositol triphosphate (IP_3) and diacylglycerol. IP_3 can act on calcium channels to induce calcium release and an increase in the level of intracellular calcium. Calcium is involved in the regulation of mitosis, DNA synthesis, protein synthesis, and membrane synthesis. Diacylglycerol can further activate protein kinase C, which is involved in the regulation of DNA and protein synthesis. These activities are essential for initiating the developmental processes of the zygote or the fertilized oocyte.

Integration of Gamete Genomes

In mammals, a sperm usually fuses with an oocyte that is arrested in the metaphase of meiosis (note that an oocyte is a developing egg before completing meiosis). The interaction of the sperm with the oocytes triggers a number of events: (1) separation of the sperm nucleus and centrioles from the flagellum, (2) degradation of the sperm mitochondria and flagellum, (3) migration of the sperm nucleus to the oocyte nucleus, (4) completion of oocyte meiosis, and (5) fusion of the sperm and oocyte genetic materials (Fig. 7.6). These

processes are critical to the embryonic development. Since the sperm mitochondria and microtubules are mostly degraded, the mitochondrial and microtubular systems in each individual are derived from the oocyte.

The sperm chromatids are organized and packed within the nucleus by DNA-binding proteins through disulfide bonds. When the sperm enters the oocyte cytoplasm, the oocyte is able to reduce the disulfide bonds and loose the packed chromatids. The loose sperm DNA then migrates toward the oocyte nucleus and is prepared for fusion with the oocyte DNA. At the same time, the oocyte activates its signaling pathways that stimulate the reactivation and completion of meiosis, resulting in the formation of the haploid egg nucleus (note that the oocyte has now matured to an egg). The microtubules of the egg are connected with the sperm and the egg nucleus. This process allows the migration of both nuclei toward each other. The interaction of the nuclei induces the integration of the genetic materials.

CLEAVAGE [7.2]

Cleavage is a process of zygote separation, which is accomplished through two events: mitotic division of the nucleus (karyokinesis) and cytoplasmic division (cytokinesis). The mitotic division takes place immediately following fertilization. Cytokinesis occurs subsequently. These events generate individual nucleated cells, known as *blastomeres*. The zygote is cleaved into two cells, which are subsequently cleaved into four cells, and so on, although the mammalian blastomeres may not cleave symmetrically and some cells may skip a round of cleavage (Fig. 7.9). The zygote moves along the oviduct during cleavage. The first cleavage process occurs approximately at the end portion of the oviduct. With further cleavage, the embryo moves to the uterus and is ready for implantation (Fig. 7.4).

Blastomere cleavage represents the most rapid process of cell division through the entire lifespan, including the embryonic period and adulthood. A *Drosophila* egg, for example, can be cleaved to generate about 50,000 blastomeres within about 12 h. The cleavage of the egg is initiated by a molecule known as *mitosis-promoting factor* (MPF). The fertilization process induces the activation of MPF, which stimulates the fertilized egg to enter the mitosis cycle. The mitotic process during the early cleavage undergoes only two phases: DNA synthesis (S) and mitosis (M). During the periodic cleavage cycles, the level of MPF changes cyclically: it reaches the maximal level during the M phase and reduces to the minimal level during the S phase. The periodic change in the MPF level controls the cyclically procession of cell division.

FORMATION OF THE BLASTOCYST [7.2]

Blastocyst is an embryonic structure composed of an *inner cell mass* of about 30 stem cells, which can differentiate into all specified cell types, and an external sac known as *trophoblast*, which develops into an embryo-supporting tissue called *chorion* (the external layer of the extraembryonic membrane and the embryonic part of the placenta) (see chapter-opening figure). Since the inner cell mass and the trophoblast serve as the origins of distinct embryonic and extraembryonic tissues, the formation of blastocyst is considered a milestone of embryogenesis.

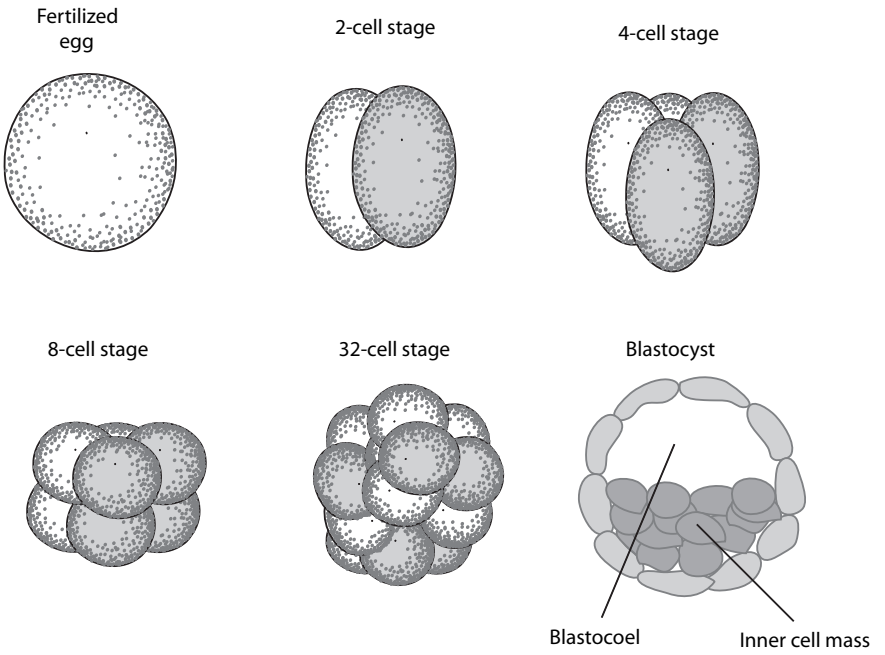


Figure 7.9. Schematic demonstration of developmental stages. Based on bibliography 7.1.

There are several key steps for the formation of the blastocyst: zygote cleavage, formation of morula, and cavitation of the morula (Fig. 7.9). Zygote cleavage has been discussed above. When cells are cleaved to a stage of 16 cells at about day 4–5, all cells are highly compacted into a solid ball-like structure. This structure is defined as a *morula*. With further cell divisions, the external cells of the morula transform into trophoblast cells at about day 6–7, while the internal cells transform into cells of the inner cell mass (Fig. 7.10). The inner cell mass further develops into two layers: the epiblast and the hypoblast. These layers eventually develop into embryonic and extraembryonic tissues as discussed below.

GASTRULATION [7.2]

Gastrulation is a process by which early embryonic cells are organized into three distinct layers: ectoderm, mesoderm, and endoderm (Fig. 7.11). These layers eventually develop into specified tissues and organs for the new individual (see page 346 for details). When cell cleavage generates a sufficient number of cells, the embryonic cells are organized into a blastocyst, composed of the *inner cell mass* and *trophoblast*. The inner cell mass gives rise to *epiblast* and *hypoblast.* The former develops into the *embryonic epiblast* and *amnionic ectoderm*, and the latter develops into *extraembryonic endoderm*. The embryonic epiblast develops into the embryonic *ectoderm* and *primitive streak*. The primitive streak gives rise to *embryonic endoderm* and *mesoderm*. The amnionic ectoderm gives rise to the *amnionic sac*. The hypoblast-derived extraembryonic endoderm develops into the *yolk sac*. The trophoblast develops into extraembryonic supporting structures.

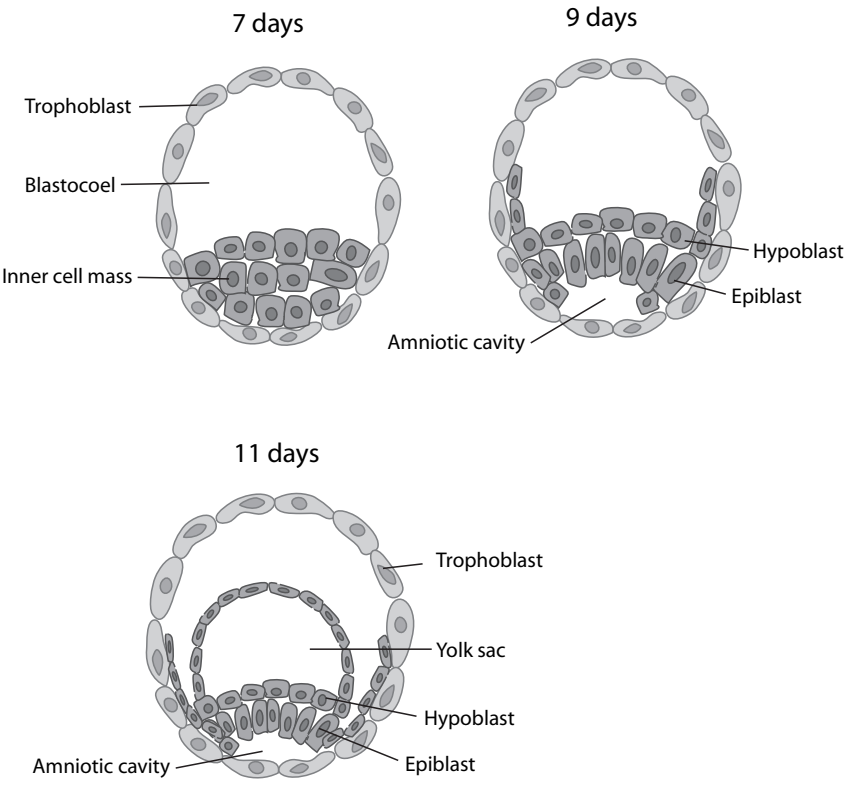


Figure 7.10. Schematic demonstration of the formation of the inner cell mass and the epiblast and hypoblast layers. Based on bibliography 7.2.

The extraembryonic supporting structures include the chorion, amnionic sac or amnion, and yolk sac. The *chorion* is the outmost extraembryonic membrane developed from the trophoblast of the blastocyst. The chorionic membrane is composed of chorionic cells, a vascular system, and connective tissue. A region of the chorion gives rise to the embryonic placenta, which is composed of two types of cellular structure called *cytotrophoblast* and *synctiotrophoblast*. The cells of these structures can interact with the maternal uterine epithelial cells via adhesion molecules, which assist in the attachment and implantation of the blastocyst into the uterine wall. Furthermore, these cells produce and release enzymes that degrade the uterine tissue, facilitating the invasion of the blastocyst into the uterine wall. Such a uterine degradation process also reorganizes the uterine vascular system in favor of the transport of nutrients and oxygen from the maternal blood to the embryo and fetus. The cytotrophoblast and synctiotrophoblast eventually develop into placenta structures including chorionic villi and intervillus space. The chorionic villi contain embryonic/fetal arteries, capillaries, and veins, which absorb and transport nutrients and oxygen from the maternal placenta to the embryo/fetus.

The *amnionic sac* is a tough extraembryonic membrane that lines the internal surface of the chorion and, when fully developed, encloses the fetus. The amnionic sac is developed from the inner cell mass-derived epiblast. During the early embryonic stage, the amnionic sac is located between the chorionic embryonic placenta and the ectoderm. It

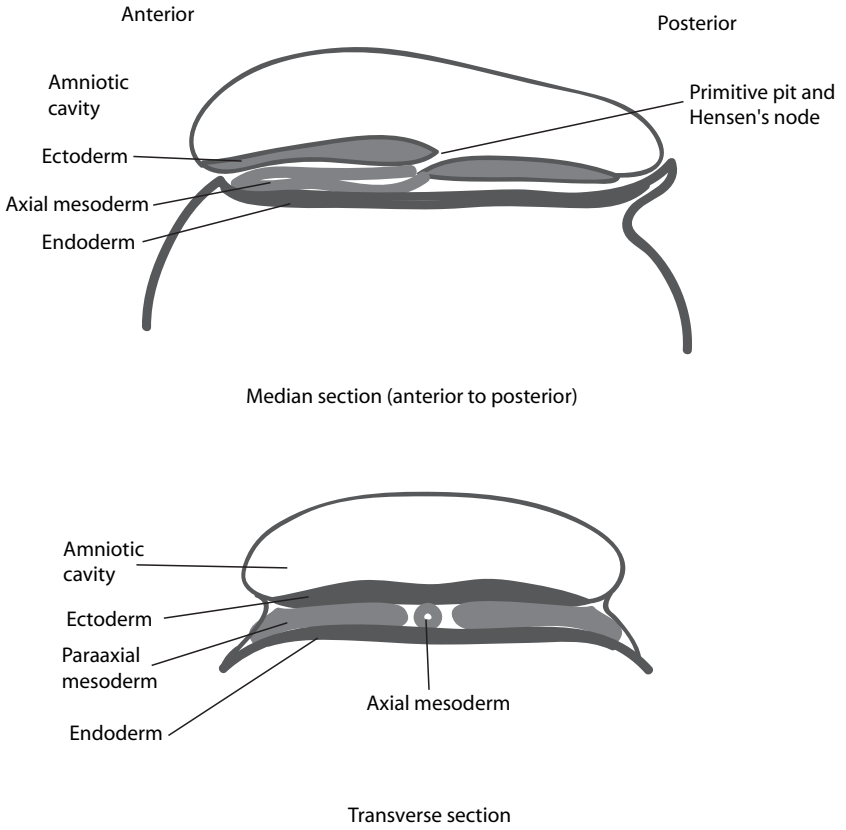


Figure 7.11. Formation of the three embryonic layers. Based on bibliography 7.2.

folds and extends gradually around the embryo, and eventually lines the chorionic membrane and encloses the embryo/fetus. The amnionic cavity is filled with a fluid called the *amnionic fluid*, in which the embryo/fetus resides. Together with the chorionic membrane, the amnionic membrane mediates the interaction of the embryo/fetus with the mother and protects the embryo/fetus from harmful environment. The *yolk sac* is a membrane structure that stores nutrients in the yolk cavity for the early development of the embryo. This structure is developed from the inner cell mass-derived hypoblast, and is located near the endoderm. The yolk cavity is relative large during the early embryonic stage, but is reduced in size and is gradually diminished during embryogenesis.

BIBLIOGRAPHY

7.1. Sperm, Egg, and Fertilization

Gilbert Scott F: *Developmental Biology*, 7th edn, Sinauer Associates, Sunderland, UK, 2003.

Inoue N, Isotani A, Okabe M: The immunoglobulin superfamily protein Izumo is required for sperm to fuse with eggs, *Nature* 434:234–8, 2005.

Gutierrez-Lopez MD, Ovalle S, Yanez-Mo M, Sanchez-Sanchez N, Rubinstein E et al: A functionally relevant conformational epitope on the CD9 tetraspanin depends on the association with activated beta1 integrin, *J Biol Chem* 278:208–18, 2003.

Zhu GZ, Miller BJ, Boucheix C, Rubinstein E, Liu CC et al: Residues SFQ (173–175) in the large extracellular loop of CD9 are required for gamete fusion, *Development* 129:1995–2002, 2002.

Benoit P, Gross MS, Frachet P, Frezal J, Uzan G et al: Assignment of the human CD9 gene to chromosome 12 (region p13) by use of human specific DNA probes, *Hum Genet* 86:268–72, 1991.

Kaji K, Oda S, Shikano T, Ohnuki T, Uematsu Y et al: The gamete fusion process is defective in eggs of Cd9-deficient mice, *Nature Genet* 24:279–82, 2000.

Le Naour F, Rubinstein E, Jasmin C, Prenant M, Boucheix C: Severely reduced female fertility in CD9-deficient mice, *Science* 287:319–21, 2000.

Miyado K, Yamada G, Yamada S, Hasuwa H, Nakamura Y et al: Requirement of CD9 on the egg plasma membrane for fertilization, *Science* 287:321–4, 2000.

Tachibana I, Hemler ME: Role of transmembrane 4 superfamily (TM4SF) proteins CD9 and CD81 in muscle cell fusion and myotube maintenance, *J Cell Biol* 146:893–904, 1999.

Waterhouse R, Ha C, Dveksler GS: Murine CD9 is the receptor for pregnancy-specific glycoprotein 17, *J Exp Med* 195:277–82, 2002.

Dean J: Biology of mammalian fertilization: Role of the zona pellucida, *J Clin Invest* 89:1055–9, 1992.

Lunsford RD, Jenkins NA, Kozak CA, Liang LF, Silan CM et al: Genomic mapping of murine Zp-2 and Zp-3, two oocyte-specific loci encoding zona pellucida proteins, *Genomics* 6:184–7, 1990.

van Duin M, Polman JEM, Suikerbuijk RF, Geurts van Kessel AHM, Olijve W: The human gene for the zona pellucida glycoprotein ZP3 and a second polymorphic locus are located on chromosome 7, *Cytogenet Cell Genet* 63:111–3, 1993.

van Duin M, Polman JEM, Verkoelen CCEH, Bunschoten H, Meyerink JH et al: Cloning and characterization of the human sperm receptor ligand ZP3: Evidence for a second polymorphic allele with a different frequency in the Caucasian and Japanese populations, *Genomics* 14:1064–70, 1992.

Zhao M, Gold L, Ginsberg AM, Liang LF, Dean J: Conserved furin cleavage site not essential for secretion and integration of ZP3 into the extracellular egg coat of transgenic mice, *Mol Cell Biol* 22:3111–20, 2002.

7.2. Cleavage, Formation of Blastocyst, and Gastrulation

Wassarman PM: Contribution of mouse egg zona pellucida glycoproteins to gamete recognition during fertilization, *J Cell Physiol* 204:388–91, 2005.

Shur BD, Ensslin MA, Rodeheffer C: SED1 function during mammalian sperm-egg adhesion, *Curr Opin Cell Biol* 16:477–85, 2004.

Santella L, Lim D, Moccia F: Calcium and fertilization: The beginning of life, *Trends Biochem Sci* 29:400–8, 2004.

Shur BD, Rodeheffer C, Ensslin MA: Mammalian fertilization, *Curr Biol* 14:R691–2, 2004.

Klonisch T, Fowler PA, Hombach-Klonisch S: Molecular and genetic regulation of testis descent and external genitalia development, *Dev Biol* 270:1–18, 2004.

Dean J: Reassessing the molecular biology of sperm-egg recognition with mouse genetics, *Bioessays* 26:29–38, 2004.

Lenart P, Ellenberg J: Nuclear envelope dynamics in oocytes: From germinal vesicle breakdown to mitosis, *Curr Opin Cell Biol* 15:88–95, 2003.

Evans JP, Florman HM: The state of the union: The cell biology of fertilization, *Nat Cell Biol* 4 (Suppl):s57–63, 2002.

Primakoff P, Myles DG: Penetration, adhesion, and fusion in mammalian sperm-egg interaction, *Science* 296:2183–5, 2002.

Matzuk MM, Burns KH, Viveiros MM, Eppig JJ: Intercellular communication in the mammalian ovary: oocytes carry the conversation, *Science* 296:2178–80, 2002.

Runft LL, Jaffe LA, Mehlmann LM: Egg activation at fertilization: Where it all begins, *Dev Biol* 245:237–54, 2002.

Wassarman PM, Jovine L, Litscher ES: A profile of fertilization in mammals, *Nat Cell Biol* 3: E59–64, 2001.

Wassarman PM: Mammalian fertilization: molecular aspects of gamete adhesion, exocytosis, and fusion, *Cell* 96:175–83, 1999.

Snell WJ, White JM: The molecules of mammalian fertilization, *Cell* 85:629–37, 1996.

Wassarman PM: Towards molecular mechanisms for gamete adhesion and fusion during mammalian fertilization, *Curr Opin Cell Biol* 7:658–64, 1995.

Nothias JY, Majumder S, Kaneko KJ, DePamphilis ML: Regulation of gene expression at the beginning of mammalian development, *J Biol Chem* 270:22077–80, 1995.

Schultz RM, Kopf GS: Molecular basis of mammalian egg activation, *Curr Top Dev Biol* 30:21–62, 1995.