

Chapter 1

Epigenetics of Mammalian Gamete and Embryo Development

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SUMMARY

Roots of mammalian development stem from successful gametogenesis and embryogenesis. Many aspects of developmentally regulated events in gamete and embryo biology involve epigenetic changes that impact gene expression and thus function. From the moment an oocyte and a sperm cell come together to form a zygote, up to the formation of the blastocyst, there are dramatic epigenetic changes that determine the success of the developmental program. This chapter will first provide an overview of oogenesis, spermatogenesis, and the process of fertilization,

which, when successfully accomplished, leads to embryogenesis. We will then review the epigenetic mechanisms regulating life at the onset of development, particularly DNA methylation, posttranslational modifications of core histones, chromatin remodeling, and a related concept, noncoding RNAs. We will address the influence of epigenetic mechanisms on the developmental competency of spermatozoa, oocytes, and embryos in mammals. A better understanding of the epigenome of gametes and embryos will lead to identification of biological networks that play important roles in disease and development and help improve fertility and health.

“... at fertilization, the diploid genome contains all the information necessary to regulate (or cause) individual ontogenesis, requiring only an appropriately permissive and supportive environment for full genomic expression to occur.” (Moss, 1981)

GAMETOGENESIS

Gametes, spermatozoa, and oocytes are formed in the **gonads** (testes and ovaries), through a process that starts during the embryonic and fetal development of the animal, comes to a halt during the animal's infancy, and is resumed once the individual reaches puberty. The formation of gametes is called gametogenesis and is a complex process that involves a series of common events for both males and females followed by a very distinctive pathway in the formation of sperm cells or eggs.

For male and female mammalian embryos, gametes are formed as a cell line that differentiates from the somatic cells early in development (Surani et al., 2004). Surprisingly, these “primitive gametes,” known as **primordial germ cells** (PGCs), are not originated in the primitive gonads or urogenital ridges; they are actually a group of cells from the epiblast that are located in the extraembryonic mesoderm, at the base of the allantois in the posterior part of the embryo (Gardner and Rossant, 1979). This group of cells later migrates into the left and right urogenital ridges (Hahnel and Eddy, 1986; McLaren and Lawson, 2005). The migration of PGCs has been extensively characterized due to their peculiar and high alkaline phosphatase activity, which allows us to identify them and follow their ameboid migratory movements (Ginsburg et al., 1990). The migration process occurs during days 7–14 of gestation in the mouse embryo (De Felici, 2009; De Miguel et al., 2009) and between days 30 and 64 of gestation in the bovine embryo (Aerts and Bols, 2008). During their migration, PGCs actively proliferate through mitosis; in the mouse, the population of PGCs reaching the gonads is estimated at several thousand (Tam and Snow, 1981). The migration of PGCs is completed toward the end of embryonic gastrulation (Matsui, 2010). Therefore, the complex series of developmental events in PGCs should proceed precisely in a spatially and temporally dependent manner (Matoba and Ogura, 2011).

Several genes are thought to be involved in PGC differentiation and in their migration. Recent evidence suggests that members of the bone morphogenetic protein (BMP) family play important roles in early development of PGC precursors. BMP4 and BMP8B, secreted from the extraembryonic ectoderm, and BMP2, from the visceral endoderm, seem to be crucial for early specification of PGC precursors from other somatic cells (De Felici, 2009; Hayashi et al., 2007; Kurimoto et al., 2008; Ohinata et al., 2009; Saitou, 2009). The protein Prdm14 (PRDI-BF1-RIZ domain containing 14) also plays a key role in germ cell specification and differentiation of PGC precursors. In Prdm14 null embryos, PGC-like cells are initially formed; however, they do not undergo differentiation and cannot undergo proper epigenetic reprogramming into PGCs. Therefore, Prdm14 null female and male mice are infertile (Edson et al., 2009). E-cadherin is also important in primordial germ cell formation, and migration treatment of PGC precursors with a blocking monoclonal antibody for E-cadherin, ECCD-1, prevented the formation of PGCs, indicating that E-cadherin-mediated cell-cell interaction among the precursors is essential for PGC formation (Okamura et al., 2003). RNA binding proteins, cell adhesion proteins, tyrosine kinase receptors, and G protein-coupled receptors facilitate PGC migration and early

colonization of the gonads; PGCs express NANOG and the cell surface markers SSEA1, EMA1, and TG1 (Nicholas et al., 2009).

The beginning of gametogenesis is identical for both male and female embryos. Before their arrival to the urogenital ridge, XX-female and XY-male PGCs appear to behave identically in all aspects. They both originate from epiblastic cells at the base of the allantois, and they both migrate toward the urogenital ridges. This suggests that formation, migration, and entry of the PGCs into the genital ridge is not a sexually dimorphic process (Edson et al., 2009). However, the next steps of meiosis and gamete formation are initiated at very different time points and in a different way in males and females (Kocer et al., 2009).

SPERMATOGENESIS

In the male embryo, primitive germ cells soon stop their divisions after colonizing the primitive testicle and enter a period of mitotic quiescence. Quiescent male germ cells are called **prospermatogonia** or gonocytes (De Felici, 2009), and they remain in their mitotic “slumber” until the male reaches puberty, when the spermatogenic cycle is initiated within the seminiferous tubule, the functional unit of the testis. Serial cross-sections of a seminiferous tubule show that sperm cells differentiate in distinctive associations. Each association is a stage of the seminiferous epithelial cycle. In other words, a spermatogenic cycle is the time it takes for the recurrence of the same cellular stage within the same segment of the tubule. Each stage of the cycle follows in an orderly sequence along the length of the tubule. The number of stages in the spermatogenic cycle is species-specific with 12 stages in the mouse and bull and 6 stages in man (Phillips et al., 2010). During each spermatogenic cycle, spermatogonia proliferate by mitosis, and, after several stages, primary spermatocytes are formed. Each primary spermatocyte will enter meiosis and through the first meiotic division will produce two secondary spermatocytes, each of which will finish meiosis becoming round haploid spermatids. The last part of the process is spermiation, characterized by the loss of most of the cytoplasm and organelles, the formation of a tail, and the delivery of these tailed cells into the seminiferous tubule lumen (Lie et al., 2009). Spermatozoa will then be transported into the epididymis, where they will be stored and acquire forward motility. However, final maturation of sperm cells is only completed in the female reproductive tract.

OOGENESIS

Contrary to the mitotic arrest of the male germ cells, PGCs in the female embryo continue to divide mitotically for a while until they enter meiosis and pass through leptotene, zygotene, and pachytene stages before arresting in diplotene stage (McLaren, 2003). The peak number of female PGCs is reached at the transition from mitosis to meiosis (Gondos, 1981), but this number is drastically reduced before birth as a result of apoptosis (Hartshorne et al., 2009; Morita and Tilly, 1999). In the cow, the maximum number of PGCs was estimated at 2,100,000 during the mitosis-to-meiosis transition, but it is reduced to around 130,000 at birth (Erickson, 1966). In humans, the maximum number of PGCs is considered to be established during the fifth month of fetal development at 7,000,000, but only around 2,000,000 are thought to remain at birth (Tilly, 1996). The concept that female mammals are born with a fixed supply of oocytes that are depleted during each estrous (or menstrual in the human) cycle declining with age has been an accepted dogma of reproductive biology for many years. However, in 2004, a controversial study published by Johnson and collaborators suggesting that neo-oogenesis takes place during adult life in the mouse ovary from germline stem cells in the surface epithelium of the ovary challenged this dogma (Johnson et al., 2004). To this day, several studies have supported this theory (Abban and Johnson, 2009; De Felici, 2010; Fu et al., 2008; Lee et al., 2007; Tilly et al., 2009; Virant-Klun and Skutella, 2010), whereas others failed to find evidence that any cells contribute to the formation of new oocytes in the adult (Begum et al., 2008; Bristol-Gould et al., 2006; Eggen et al., 2006; Notarianni, 2011).

Table 1.1. Timeline of oogenesis in some mammals.^a

Species Cell type	Mouse	Cattle	Human
PGCs	13.5 days	90 days	30 days
Oogonium	18–20 days	80–90 days	112–130 days
Primary oocyte	21 days	210 days	140 days
Secondary oocyte	Puberty	Puberty	Puberty
Ootid	Fertilization	Fertilization	Fertilization
Ovum	After fertilization	After fertilization	After fertilization

^aThe stages from PGC migration until primary oocyte formation are expressed in days during the female embryo development. Although secondary oocytes are observed before puberty, the majority of oocyte maturation occurs after puberty.

Mammalian oogenesis is accomplished through three developmental stages: the initiation of meiosis, the formation of a **follicle** around each oocyte during the perinatal period, and the cyclic growth of the follicles and the maturation of the oocytes within. The events that coordinate the initiation of meiosis are not completely understood; however, several studies have proposed that retinoic acid is the molecular switch that determines meiotic entry in the developing ovary (Bowles et al., 2006; Koubova et al., 2006; Wang and Tilly, 2010). Once each oocyte has arrested meiosis in the diplotene stage, a single layer of pregranulosa cells surrounds the oocyte, forming a primordial follicle (Hirshfield, 1991). The formation of primordial follicles is known as **ovarian follicular assembly** and occurs at around day 112–130 of gestation in humans (Hartshorne et al., 2009) and 80–90 days of gestation in the bovine fetus (Braw-Tal and Yossefi, 1997; Nilsson and Skinner, 2009), but it occurs in the days immediately following birth in rodents (Pepling, 2006; Pepling and Spradling, 2001). Oocytes remain in their meiotic arrest until the female reaches puberty. During each estrous or menstrual cycle, a cohort of follicles is recruited; these follicles will grow and develop an antrum or cavity, therefore being known as **antral follicles**. From this cohort, only a subset of follicles (in polytocous species) or only one follicle (in monotocous species) is selected for dominance and ovulation, becoming preovulatory follicles (McGee and Hsueh, 2000). Prior to ovulation, oocytes resume meiosis; this can be recognized by dissolution of the nuclear envelope, known as germinal vesicle breakdown. However, meiosis is stopped again and oocytes are ovulated at the metaphase of the second meiotic division; therefore, they are known as **MII oocytes**. The final stage of meiosis will only be completed if the oocyte is fertilized (see *Oocyte activation* below). Thus, after being formed in the embryo and remaining in “meiotic stand by” for months or even years, the oocyte can only complete its journey with fertilization. The main processes during oogenesis in mammals and the differences between some of the model species are summarized in Table 1.1.

FERTILIZATION AND EGG ACTIVATION

Once delivered into the female reproductive tract, sperm cells have to travel a long distance and swim against a series of obstacles (the low vaginal pH, the cervix, and the presence of macrophages in the uterus) that serve as the selection machinery preventing abnormal spermatozoa from reaching the egg. During their transit from the uterus to the oviduct, their last destination, spermatozoa go through a process called **capacitation**, or the acquisition of fertilization capability, described independently by both Chang (1951) and Austin (1951, 1952). When spermatozoa reach the oviduct, there are still two more barriers they need to overcome in

order to reach the egg. First, oocytes are surrounded by layers of cells, which together form the **cumulus cell oocyte complex**. Sperm cells need to pass the cumulus cell layers in order to reach the last barrier separating them from the oocyte, the **zona pellucida**, a transparent glycoprotein coat that surrounds and protects the oocyte.

Fertilization consists of a series of events that begins when the sperm makes contact with the cumulus cells and ends with the fusion of paternal and maternal chromosomes at metaphase of the first mitotic division of the zygote. The events of fertilization require just over 24 hours and include a series of steps, the first of which is the passage through the cumulus cells. The second step is the penetration of the zona pellucida, a receptor–ligand interaction with a high degree of species specificity, in which the zona pellucida glycoproteins ZP1, ZP2, and ZP3 (that were formed during oocyte maturation) play the leading role (Wassarman et al., 2004). The last step is the binding and fusion of the sperm and oocyte.

The zona pellucida is made out of three glycoproteins in rodents (Wassarman, 1988) and four in humans and bovines (Conner et al., 2005; Goudet et al., 2008; Lefievre et al., 2004). One of the zona pellucida glycoproteins, ZP3, is a well-known, species-specific receptor. Although ZP3 is highly conserved in mammals, its differential glycosylation pattern in each species only allows the entry of spermatozoa from the same species (Goudet et al., 2008; Litscher et al., 2009). Interaction of spermatozoa with ZP3 causes the **acrosome reaction**, which is characterized by the loss of the acrosome from the sperm head and the liberation of several enzymes that allow the sperm the final entry through the zona pellucida. The ability of ZP3 to induce the acrosome reaction resides in its C-terminal fragment; whereas in rodents O-linked glycans are critical for ZP3-induced acrosome reaction, in humans N-linked glycans are the ones involved in ZP3-mediated acrosome reaction (Gupta and Bhandari, 2011).

Only acrosome-reacted sperm can fuse with the oocyte. However, after all the obstacles that they encounter, only a few (probably <10) sperm cells do actually reach the egg. The complete molecular control of the sperm–oocyte binding and fusion is not known; however, several molecules have been implicated in this process, including the tetraspanin protein family members CD9 and CD81, GPI-anchored proteins (Evans, 2002; Primakoff and Myles, 2007), and the ADAM family of proteins, now known as fertilin (Primakoff and Myles, 2000) and the protein Izumo1 (Ikawa et al., 2008; Inoue et al., 2005, 2011).

When the mammalian oocyte is fertilized, it is still arrested at metaphase II and would remain so, unless the sperm's entrance triggers a release of calcium from storage sites into the ooplasm in a wave-like pattern (Kline and Kline, 1992; Swann and Yu, 2008). The repeated oscillations of cytosolic Ca^{2+} give rise to a set of events collectively known as **oocyte activation**. Oocyte activation includes mainly two very important events for zygote formation. First, the release of cortical granules (enzyme-filled vesicles that lie just below the oocyte's plasma membrane) modifies the zona pellucida glycoproteins rendering the zona impenetrable by any other spermatozoa (Abbott and Ducibella, 2001); this is known as the **block to polyspermy** and has been widely studied in several species (Gardner and Evans, 2006; Gardner et al., 2007; White et al., 2010). Second, the calcium oscillations trigger the **cell cycle resumption**, which is the culmination of meiosis and the extrusion of the second polar body. Several studies point to the importance of sperm phospholipase C zeta in oocyte activation (Kashir et al., 2010). Within around 24 hours of fertilization, the paternal and maternal genomes are assembled into pronuclei, which replicate their DNA, and their chromosomes come together at **syngamy**, the last step of fertilization that culminates with the formation of the one-cell embryo, the **zygote**.

EMBRYOGENESIS

From a cytological perspective, early embryo development is a process of series of repeated cell divisions known as **cleavage**. Each cell in an early mammalian embryo is called a **blastomere**, and there are no morphologic differences among individual blastomeres (Chen et al., 2010).

However, from a molecular point of view, there are many complex processes taking place in each individual blastomere in order to attain cell division and begin cell differentiation.

During the first cell cycles, the preimplantation embryo is controlled by maternal genomic information that is accumulated during oogenesis (Telford et al., 1990). Around the 2-cell stage in the mouse embryo (Schultz, 1993, 2002), the 4- to 8-cell stage in the human embryo (Schultz, 2002; Telford et al., 1990), and the 8-cell stage in the bovine embryo (Memili and First, 2000), the embryo starts transcribing its own RNA. This process has been called embryonic genome activation (EGA), maternal-to-zygotic transition, or zygotic gene activation. The developmental program initially directed by oocyte-stored proteins and transcripts is replaced by a new program controlled by the expression of the new embryonic genes. The goal of the EGA is to transform the highly differentiated oocyte into a set of totipotent blastomeres. The EGA is a crucial condition for following successful embryonic development (Kanka, 2003). The length of the first cell cycles is particularly variable. Around 18–36 hours are needed to complete the first cell cleavage. The second- and third-cell stages are shorter, taking from 8 to 12 hours to be accomplished. The fourth cell cycle may span from 9 to 40 hours (Lequarre et al., 2003).

At about the 8-cell stage in mouse embryos (Hamatani et al., 2004) and 16-cell stage in human and bovine embryos, an amorphous mass of cells undergoes “compaction,” in which loosely associated cells adhere tightly to generate an organized mulberry-shaped cell mass called the **morula**. Then, the cells on the outside of the sphere begin to express membrane transport molecules, including sodium pumps causing fluid to accumulate inside the embryo, which signals the formation of a **blastocyst**. The blastocyst is composed of two distinctive groups

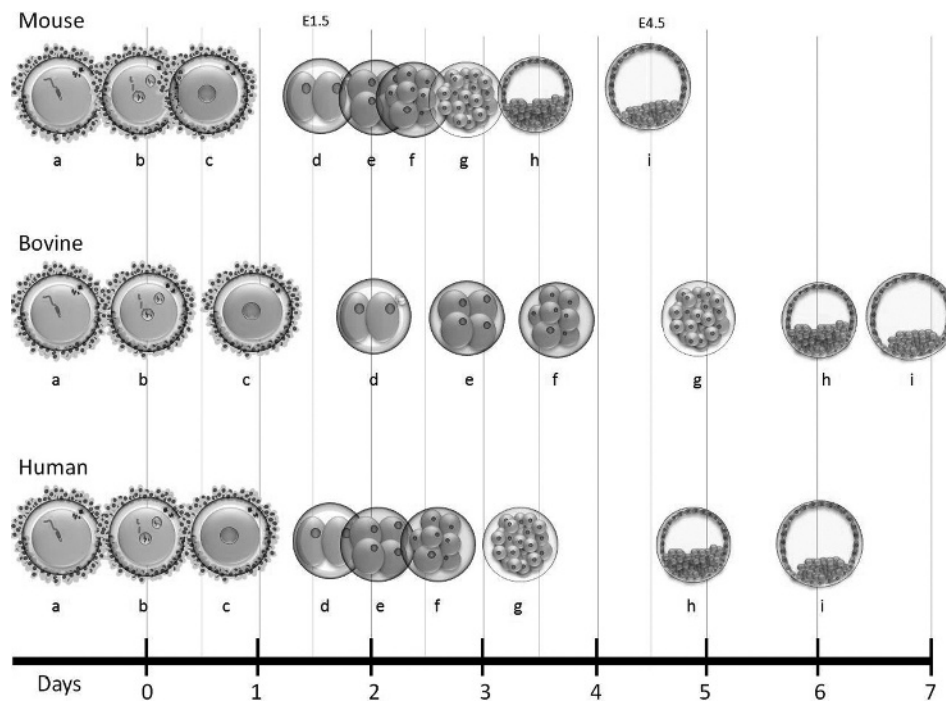


Figure 1.1. Timeline of preimplantation development in the mouse, bovine, and human embryos. Pronuclear formation is assumed as day zero. (a) Fertilization; (b) pronuclear formation; (c) zygote; (d) two-cell embryo; (e) four-cell embryo; (f) eight-cell embryo; (g) Morula; (h) early blastocyst; (i) late blastocyst. (For color detail, please see the color plate section.)

of cells: the outside cells, called the **trophectoderm** (TE), that will give rise to extraembryonic tissues, particularly those forming the placenta, and the inside pluripotent cells of the **inner cell mass** (ICM) that will originate the embryo proper. A bovine blastocyst contains around 100 cells, whereas a mouse blastocyst is formed by only around 60 cells (Cockburn and Rossant, 2010). The cells that form the blastocyst continue to divide mitotically and produce more fluid, which causes the blastocyst to expand, stretching the zona pellucida. Eventually, the stretched zona pellucida cracks and the blastocyst “hatches.” The hatched blastocyst will rapidly undergo implantation in human and mouse embryos, but in bovine embryos, blastocysts will elongate, becoming several centimeters long before they implant (Goossens et al., 2007). The main processes during preimplantation development in mouse, human, and bovine embryos are summarized in Figure 1.1.

EPIGENETIC REPROGRAMMING DURING EMBRYOGENESIS AND GAMETOGENESIS

The main types of epigenetic mechanisms include DNA methylation, histone modification, chromatin remodeling, and RNA-mediated silencing. We will briefly review each one of these mechanisms and then address particular aspects of epigenetic reprogramming in sperm cells, oocytes, and early embryos.

DNA methylation

DNA methylation is the most widely studied epigenetic mechanism used by the cell for the establishment and maintenance of a controlled pattern of gene expression (Quina et al., 2006). The methylation of DNA is mediated by a family of DNA methyltransferases, which catalyze the transfer of a methyl group to the 5 position of cytosine residues in CpG islands located near or within promoter regions. In mammals, silencing of genes is frequently correlated with DNA methylation as it provides a genome-wide means of regulation associated with the inheritance of lineage-specific gene silencing between cell generations (Robertson and Wolffe, 2000). The patterns of DNA methylation are distinct for each cell type and confer cell type identity (Szyf, 2005a). With few exceptions, unmethylated DNA is associated with an active chromatin configuration and methylated DNA is associated with inactive chromatin (Szyf, 2005b).

The family of DNA methyltransferases includes DNMT1, which maintains the methylation after each DNA replication by using the parental DNA strand as a template to methylate the daughter strand (Bestor et al., 1992; Pradhan et al., 1999). DNMT2, the smallest mammalian DNA methyltransferase, has an enigmatic role in DNA methylation with studies pointing toward its DNA methylation role (Kunert et al., 2003; Liu et al., 2003; Tang et al., 2003), whereas others have failed to detect DNA methylation activity for this enzyme (Hermann et al., 2003; Rai et al., 2007). DNMT3a and DNMT3b are two members of this protein family that are considered *de novo* methyltransferases because they establish new DNA methylation patterns by adding methyl groups to unmethylated DNA, particularly during early embryonic development and gametogenesis (Okano et al., 1998, 1999). DNMT3L is a protein associated with the DNA methyltransferase family, although it lacks the methyltransferase motifs and therefore cannot methylate DNA. However, DNMT3L interacts with DNMT3A forming a dimer, which ensures DNMT3A *de novo* methylation activity (Jia et al., 2007). Additionally, DNMT3L activates histone deacetylase 1 (HDAC1) (Deplus et al., 2002; Turek-Plewa and Jagodzinski, 2005) and recognizes histone H3 tails that are unmethylated at lysine 4 (Ooi et al., 2007).

Gametes and early embryos have to erase their epigenetic program in order to accomplish their future fate by removing the genomic imprints and reestablishing them in a sex-specific way. The first stage in gamete formation and PGC differentiation requires the repression of

the mesodermal cell program, the reacquisition of potential pluripotency, and a genome-wide epigenetic reprogramming (Yamaji et al., 2008), which includes the modification of histones associated with the repressed or active chromatin state (Hajkova et al., 2008; Seki et al., 2007), the erasure of genomic imprints by demethylation, and the reestablishment of new methylation patterns (De Miguel et al., 2009). Global remethylation of DNA is thought to coincide with imprint reestablishment, which occurs after PGCs have committed to a sex-specific developmental program (Durcova-Hills et al., 2006). Sex-specific DNA methylation patterns are reestablished during male and female gametogenesis, although at different times. In the male germline, prospermatogonia become methylated at paternal imprint loci during embryonic development, whereas oocytes only initiate maternal imprint methylation after birth (Lucifero et al., 2002). The mechanisms involved in these epigenetic changes are not completely elucidated, but it is evident that they are key for the formation of functional gametes (De Felici, 2009).

Prior to fertilization, the genomes of both spermatozoa and oocytes are transcriptionally inactive and are highly methylated (Reik et al., 2001). Within hours of fertilization, a dramatic genome-wide loss of DNA methylation has been reported in the male pronucleus (Mayer et al., 2000; Oswald et al., 2000). Several mechanisms have been suggested for the active demethylation of the paternal genome: first, the removal of the methyl group from the cytosine; second, the removal of the methyl-cytosine base by glycosylation; and, third, the removal of a number of nucleotides (excision repair) (Dean et al., 2003). The nature of the mechanisms involved in the active demethylation of the paternal genome remains unknown. After several cleavage divisions, the female pronucleus is also demethylated. However, this process seems to be passively caused by a loss of methyl groups during each round of DNA replication due to the lack of DNMT1 (Mayer et al., 2000; Oswald et al., 2000). The only methylation marks preserved in the embryonic genome are thought to be the ones on the imprinted genes (Oswald et al., 2000; Reik et al., 2001; Young and Beaujean, 2004).

By the blastocyst stage, the embryo is hypomethylated (Monk et al., 1987). New methylation patterns are established, around the blastocyst stage, by the *de novo* DNA methyltransferases DNMT3a and DNMT3b, which add methyl groups to unmethylated CG dinucleotides (Rodriguez-Orsorio et al., 2009). Once the new patterns of methylation are established, they can be propagated through rounds of DNA replication by DNMT1. Oocytes express an exclusive shorter isoform of DNMT1 called DNMT1o, which lacks 114 amino acids from the N-terminal domain because its translation initiation lies on exon 4 instead of exon 1 (Bestor, 2000). DNMT1o is stored in the cytoplasm of oocytes and early embryos (Ratnam et al., 2002) and is transiently translocated to the nucleus only at the eight-cell stage (Kurihara et al., 2008). After implantation, maternal DNMT1o is soon replaced by the somatic DNMT1, expressed by the embryonic genome. The absence of DNMT1o from the nucleus during early embryonic development is in accordance with the global hypomethylation of the embryonic genome (Oliveri et al., 2007). Figure 1.2 is a schematic representation of the demethylation of paternal and maternal genomes after fertilization.

Modulation of DNA methylation during early embryogenesis is a dynamic process that is developmentally regulated and crucial for the establishment of gene expression during embryonic development (Eden and Cedar, 1994; Jones et al., 1998). However, other studies suggest that DNA methylation may only affect genes that are already silenced by other mechanisms in the embryo, indicating that DNA methylation could be a consequence rather than a cause of gene silencing during development (Bestor, 2000; Nan et al., 1998; Walsh and Bestor, 1999). Mutations in either the maintenance or the *de novo* methyltransferases result in early embryonic death in mice (Li et al., 1992; Young and Beaujean, 2004), indicating that the establishment and maintenance of appropriate methylation patterns are crucial for normal development.

DNA methylation has also been shown to be important for X-chromosome inactivation. In female mammalian embryos, at about the morula stage, nearly all genes in one of the two X chromosomes are inactivated by a dosage compensation mechanism known as X-chromosome inactivation (Lyon, 1961). In fetal tissues, this inactivation is random; in some cells, the

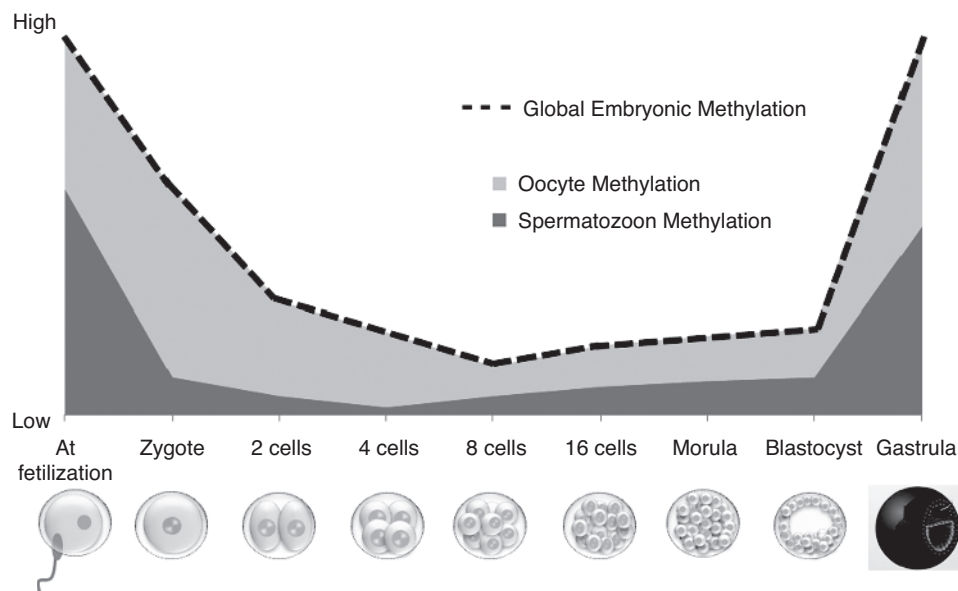


Figure 1.2. Schematic representation of the changes in DNA methylation in the bovine embryo throughout preimplantation development. DNA methylation is shown as arbitrary units on the y-axis. The DNA methylation level of the preimplantation embryo is the sum of the spermatozoon (blue) and oocyte (pink) methylation. Before the first mitotic division, the sperm genome undergoes active demethylation, and the oocyte genome undergoes passive demethylation throughout several cell divisions. Paternal and maternal genomes remain separated until after the four-cell stage. After the eight-cell stage, a small wave of de novo methylation is observed. By the blastocyst stage, the DNA methylation level in the trophectoderm cells is markedly lower compared with cells of the ICM. At the perigastrulation stage, de novo DNA methylation is completed throughout the entire embryo. (For color detail, please see the color plate section.)

inactivated X chromosome is paternal, whereas in others it is maternal. However, in the trophectodermal cells, the paternal X chromosome seems to be the only inactivated one (Heard et al., 1997).

Histone modifications

Core and linker histones are the main protein components of chromatin. Core histones have the capacity to undergo posttranslational modifications, including acetylation, methylation, phosphorylation, ubiquitination, sumoylation, citrullination, and ADP-ribosylation, which alter their interaction with DNA and nuclear proteins. Histone methylation is associated with the addition of methyl groups to certain lysine residues and may cause gene activation or silencing, depending on the lysine residue methylated.

A characteristic of specified PGCs is the increase in methylation of H3-K27 and H3-K4 as well as the reduction in H3-K9 methylation, which determines their chromatin organization and gene expression patterns (Hajkova et al., 2008). Histone phosphorylation is important during spermatogenesis (Meetei et al., 2002), and histone acetylation appears to be the main modification in oogenesis. Maturing oocytes have low acetylation levels, which might influence gene expression during oocyte growth and development (Kim et al., 2003). In oocytes, the somatic histone H1 is replaced by the maternal histone variant H1FOO (H1 histone family,

oocyte-specific) that could play a role in chromatin condensation and transcriptional repression (Teranishi et al., 2004).

Obvious histone modifications during preimplantation embryo development occur in the male pronucleus. Right after fertilization, protamines associated with the paternal chromatin are rapidly replaced by acetylated histones, which are not methylated at H3K4, K9, or K27 (Adenot et al., 1997). After the male pronucleus protamines have been replaced by histones, mono-methylation of H3K4, K9, and K27 can be detected (Lepikhov and Walter, 2004). Contrary to what happens in the male pronucleus, the maternal chromatin maintains histone H3 methylation throughout early embryonic development (Santos et al., 2005).

Chromatin remodeling

Chromatin remodeling is extensive and occurs during early embryogenesis. A particular property of the embryonic chromatin structure is that it prevents the promoters in the genome from accessing the embryo's transcriptional machinery. Embryonic chromatin structure allows the expression of only some genes, mediated by chromatin remodeling proteins, which may change the overall pattern of expression, allowing transcription factors and signaling pathways to produce different genomic transcriptional responses to common signals (Olave et al., 2002).

For the male pronucleus, a crucial chromatin remodeling event is the removal of histones, which are replaced by protamines; however, recent data indicate that some histones remain associated with specific sequences of the sperm DNA, implying a pattern that suggests a programmed distribution rather than a residual deposition (Yamauchi et al., 2011). This could indicate that paternal chromatin has a greater role in the regulation of transcriptional activity during preimplantation development than what has been considered before (Bui et al., 2011). Before male and female pronuclei come together to form the nucleus of the totipotent zygote, each pronucleus undergoes distinct programs of transcriptional activation and chromatin remodeling. The highly packaged paternal chromatin delivered by the sperm is decondensed and acquires a number of specific epigenetic marks, but markedly remains devoid of those usually associated with constitutive heterochromatin. During this period, the maternal chromatin remains relatively stable except for marks associated with transcription and/or replication, such as arginine methylation and H3/H4 acetylation. These changes in chromatin structure generate activation of the transcriptional machinery and gene expression occurring during early embryo development, leading to a unique chromatin structure capable of maintaining totipotency during embryogenesis and differentiation during postimplantation development (Misirlioglu et al., 2006).

The chromatin remodeling events during early embryonic development are likely to be important in establishing the nuclear foundations required for later triggers of differentiation. There are three key moments during the earlier reprogramming events: (1) a relatively stable maternal chromatin after fertilization, (2) a rapid acquisition of specific histone marks by the paternal chromatin a few hours post fertilization, and (3) a rapid remodeling of heterochromatic marks in the core of the nucleosome from the first mitotic division. These characteristics may be necessary for the maintenance of a chromatin state compatible with cellular reprogramming and plasticity (Burton and Torres-Padilla, 2010) and are particularly important for preimplantation embryos, which start cell differentiation cascades that will lead to tissue and organogenesis. Changes in chromatin structure lead to the activation of the embryonic transcriptional machinery, leading to a unique chromatin structure capable of totipotency in the early embryo and differentiation after implantation (Uzun et al., 2009).

If the embryo cannot overcome the chromatin repression and activate transcription of important developmental genes, an embryonic block will occur. This developmental block is observed at different stages depending on the species: second cell cycle in the mouse embryo, third to fourth cell cycle in the human embryo, and fourth to fifth cell cycle in the bovine embryo (Memili and First, 2000).

RNA-mediated silencing

Aravin and Bourc'his (2008) have suggested that the methylation of DNA might occur in a sequence-specific manner, which could be mediated through small RNAs that direct DNA methylation to target loci. By interacting with the DNA methylation machinery and histone-modifying proteins, PIWI-piRNA complexes are thought to have the ability to detect the synthesis of transposable element transcripts during gametogenesis (Nicholas et al., 2009; Tam et al., 2008).

NONCODING RNAs

In mammals, almost 35% of their genome has repeated DNA sequences called transposon elements, and ~1.2% of the eukaryotic genome consist of noncoding RNAs (Figure 1.3). Surprisingly, only 2% of the human genome is translated into proteins; and these nontranslated RNAs, called noncoding RNAs (ncRNAs) (Mattick and Makunin, 2006), have several functions in the cell, including gene regulation. In addition to its intermediate function from DNA to protein, RNA plays a critical role in gene regulation via coding RNAs and ncRNAs. Ribosomal RNA (rRNA) and transfer RNA (tRNA) have housekeeping functions during translation in which messenger RNA (mRNA) plays a key role. However, neither tRNA nor rRNA is coded for its proteins, and thus they are considered ncRNAs (Brosnan and Voinnet, 2009). Recently, small nuclear RNAs (snRNAs) and "snurps" (small nuclear riboproteins) were characterized as ncRNAs. Key coding RNAs and ncRNAs are listed in Figure 1.3 and Table 1.2. Breathtaking discoveries have recently been made in the areas of both short and long ncRNAs that regulate gene expression in development and disease. Long interspersed nuclear elements (LINEs) and short interspersed nuclear elements (SINEs) are abundantly found in eukaryotes. In mammals, SINEs are derived from tRNAs and 7SL RNAs, whereas LINEs arise from tRNAs. These ncRNAs are not expressed into their protein, but they are derived from the long terminal repeats of LINE (Athanasius et al., 2007). These SINEs and LINEs may be sources for novel ncRNAs, such as 4.5SH RNA and 4.5SI RNA in rodents.

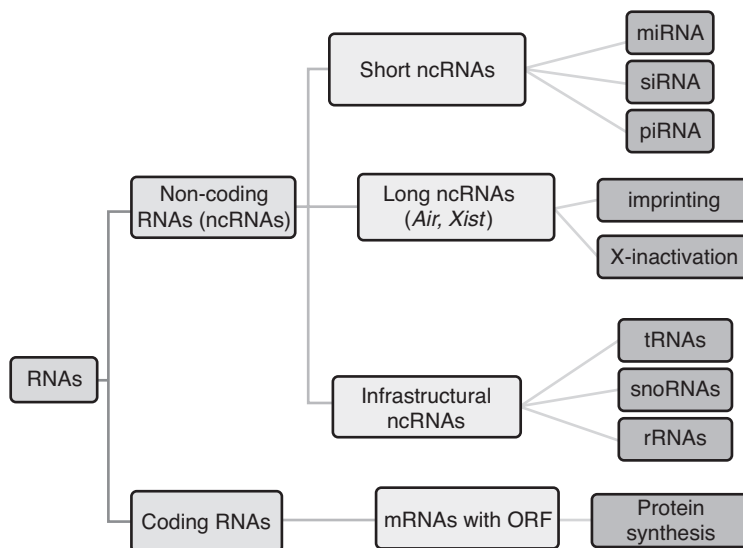


Figure 1.3. Classification of RNAs. (This representation is generated according to Brosnan and Voinnet, 2009; Mattick and Makunin, 2006; and Ørom et al., 2010.) (For color detail, please see the color plate section.)

Table 1.2. Short noncoding RNAs.

Cell/tissue type	Organism	Noncoding RNAs	Function	Author
HeLa cell	Human	scaRNAs	?	Jády et al., 2004
Oocytes	Mouse	miRNA and endo-siRNA	Oocyte maturation	Suh et al., 2010
ES cell (mWS244.6)	Human	<i>Xist</i>	X inactivation	Ng et al., 2007
Placenta ES cells	M. musculus	miR-467 family	?	(See Related References in Noguer-Dance et al., 2010)
Early mouse embryos	mice	<i>Xist</i>	random X inactivation	Huynh and Lee, 2003
Oocytes	mouse	miRNA	?	Ma et al., 2010
Hela cell	Mammalian tissue culture	siRNA	MicroRNA like function, mRNA repression	Doench et al., 2003
Placenta (paternally inherited chromosome) hES	Human	C19MC primates-microRNA gene cluster	?	(Noguer-Dance et al., 2010)
ES cells Placenta Embryos	Human	C14MC primates-microRNA gene cluster	Placenta development	(See Related References in Noguer-Dance et al., 2010)
Embryos	Mouse	TERRA/TelRNA	Telomeric function	Reviewed in Schoeftner and Blasco, 2010
Cell lines Keratinocyte Fibroblast	Human	3,019 Long ncRNA	?	Ørom et al., 2010
oocyte, one-cell zygote, two-cell embryo, four-cell embryo, and eight-cell embryo	Mice	miRNAs	Developmental function	Tang et al., 2007 (See References in Carletti and Christenson, 2009).
MII, 8- to 16-cell embryos, blastocysts and ICM	Mice	miR-16 and -200c miRNAs, sn/snoRNA135/sc/srp/RNAs tRNA and rRNA	Retrotransposon silencing and Gametogenesis	Ohnishi et al., 2010.

Table 1.2. (Continued)

Cell/tissue type	Organism	Noncoding RNAs	Function	Author
d30 bovine embryos	Bovine	miRNAs (miR-122a and miR-199a)	Pro-apoptotic function miRNA mediated post-transcriptional gene regulation during early embryonic development	Coutinho et al., 2007 (See References in Carletti and Christenson, 2009).
Sperm cells	Bovine	miRNAs	Fertility marker	Robertson et al., 2009
Male germline, spermatocytes,	Mammals	sncRNAs	?	See References in Johnson et al., 2011

Long Noncoding RNAs

Long ncRNAs are the products of protein-coding genes without open reading frame; they are the transcripts that carry out more than 100 nt (200 nt to >100 kb) and are not coded (Brosnan and Voinnet, 2009; Ørom et al., 2010). In contrast to rapid evolution of ncRNAs, lack of conservation does not mean lack of function (Pang et al., 2006). One of the long ncRNAs is the long functional *Xist* RNA, which is unique to placental mammals and silences one of the X chromosomes in mammals to ensure gene dosage between males and females (Ng et al., 2007).

Short Noncoding RNAs

Short ncRNAs are involved in several biological processes in mammalian cells, such as development and gene regulation. There are three classes of short ncRNAs: microRNAs (miRNAs), small interfering RNAs (siRNAs), and piwi-interacting RNAs (piRNAs). Short ncRNAs are <100 nt in length and are not translated into proteins. Discovery of RNA interference (RNAi) has led to exogenous use of these RNAs to understand gene functions and mechanisms of regulation; therefore, therapeutic applications for treatment of diseases have been developed.

MicroRNAs

MicroRNAs (miRNAs) are 20–24 nt long and are processed by Dicer enzymes, which are endoribonucleases of the RNase III family (Brosnan and Voinnet, 2009). They were first described in *Caenorhabditis elegans* (Lee et al., 1993) and down-regulate gene expression through RNAi pathway (Fire et al., 1998; Ørom et al., 2010). miRNAs are endogenously expressed and bind to the 3' untranslated region of mRNAs to repress their functions (Gangaraju and Lin, 2009; Ørom et al., 2010). Following the first discovery of the miRNA *lin-4* in *C. elegans* (Lee et al., 1993), it has been shown that miRNAs are conserved among some species; for example, known miRNAs are highly conserved (>90%) between mice and humans (Pang et al., 2006). miRNAs have critical roles in gene regulation; for example, they have been proposed to modulate gene expression in mouse oocytes (Ma et al., 2010). A mammalian miRNA cluster controls DNA methylation

and telomere recombination via Rbl2-dependent regulation of DNA methyltransferases (Benetti et al., 2008).

miRNAs are expressed in diverse sets of tissues and developmental stages in agriculturally important organisms (Liu et al., 2010). They are essential during meiotic maturation of mouse oocytes in which their functions are different from those in somatic cells. Endogenous miRNAs in somatic cells repress mRNA reporters compared with their counterparts in oocytes. Recently, a study revealed that miRNA activity is down-regulated in oocyte development due to the reprogramming of gene expression (Ma et al., 2010). Dgcr8 has been shown to have a function in miRNA processing, encoding an RNA-binding protein that is deleted in normal meiotic maturation of oocytes. miRNAs were shown to be differently expressed between TE and the ICM (Ohnishi et al., 2010). In addition to oocytes, it is known that sperm cells express microRNAs and these ncRNAs might be an indicator of male infertility. For example, it was reported that seven microRNAs are differently expressed in sperm from bulls with varying fertility (Robertson et al., 2009). However, functions of sperm-borne miRNAs either before or after fertilization have been poorly defined (Carletti and Christenson, 2009).

Small interfering RNAs

siRNAs result from the processing of long dsRNAs via Dicer enzymes and have recently become popular by virtue of their external use in gene silencing (Brosnan and Voinnet, 2009). siRNAs bind to their complementary mRNA target sequences, which are then degraded by nucleases within the RNA-induced silencing complex. Endogenous siRNAs have been discovered in flies, mice, and humans (Azzalin et al., 2007; Ghildiyal and Zamore, 2009; Ma et al., 2010; Suh et al., 2010). Recently, siRNAs have been exogenously used in gene knock-down and knock-out studies; therefore, gene regulation and protein expression can easily be controlled via RNAi pathway. For example, the functions of eight ncRNAs were identified via exogenous siRNA; it was shown that they have functions in cell viability, repressing Hedgehog signaling and regulation of nuclear trafficking (Mattick, 2005).

Piwi-interacting RNAs

piRNAs are 25–30 nt long and were named by virtue of their PIWI proteins, which make their transcripts different from siRNAs and miRNAs with Ago proteins. Although piRNAs can be found in both female and male germline in *Drosophila*, they are present only in male germline in mammals (Brosnan and Voinnet, 2009). piRNAs are expressed in developing spermatocytes as prepachytene and pachytene piRNAs. It was shown that prepachytane piRNAs are associated with direct DNA methylation of transposons (Ghildiyal and Zamore, 2009). Recently, the transient up-regulation of piRNAs/siRNAs to the zygotic miRNAs was detected during early mouse embryonic development. Therefore, a proposed schema regarding transition of small RNAs during mammalian oogenesis and preimplantation was established (Ohnishi et al., 2010).

Major differences between these three short ncRNAs are listed in Table 1.3. The basic function of Argonaute proteins is to cooperate with small RNAs to target their mRNA precursors or other related molecules, which leads to gene silencing. The Argonaute proteins have PAZ (Piwi Argonaut and Zwiller) and PIWI domains, and are classified into two groups: Ago members and Piwi members. DICER is important in both miRNA and siRNA processing. However, piRNA processing is DICER-independent and involves piRNA complex (piRC), including proteins functioning as DNA helicases. Piwi members were established both in mice and in humans (Ghildiyal and Zamore, 2009). Functions and locations of major ncRNAs are summarized in Table 1.3.

Table 1.3. Noncoding RNAs: functions and locations.

miRNA	siRNA	piRNA
20–24 nt in length	Approximately 21nt in length	25–30 nt in length
Generated from its precursor; primary miRNA (pri-miRNAs), than it is formed an intermediate form; pre-miRNAs (Gangaraju and Lin, 2009)	Generated from long dsRNA (Ghildiyal and Zamore, 2009)	Not generated from long dsRNA (Ghildiyal and Zamore, 2009)
Processed by Dicer	Processed by Dicer but not Drosha	—
Translational repression Translational activation (Gangaraju and Lin, 2009)	Target mRNA cleavage Somatic cell transposon silencing (Gangaraju and Lin, 2009)	Translational control (?) Epigenetic regulation Germline silencing (Gangaraju and Lin, 2009)
Ago proteins	Ago Proteins	Piwi proteins

IMPLICATIONS OF CHANGES IN EPIGENOME FOR ANIMAL BIOLOGY

Early mammalian reproduction involves a series of developmentally regulated events during gametogenesis and embryogenesis that set the stage for later development. As a complex trait that is economically important, fertility is defined as the quality of the sperm and oocyte for successful fertilization to sustain development at the embryonic stage and beyond. Thus, fertility is the single most important factor controlling animal reproduction and development.

Despite a wealth of genetic information on livestock, many fundamental questions related to animal development remain unanswered. In addition, the use of genomics to predict fertility, a complex trait that is economically important, has yet to enhance production efficiency of livestock. This is because phenotype is a result of genetics, environment, and gene interactions. Gene–environment interactions therefore have direct influence on fertility.

In the male, epigenetic errors during spermatogenesis are known to occur, and they influence developmental competency of the gamete, that is, changes in the chromatin structures due to packaging of sperm DNA and dynamics of DNA methylation. For example, abnormal profiles of DNA methylation in sperm were associated with infertility in men (Houshdaran et al., 2007). Environmental toxicants, nutrition, and herd management are all expected to influence sperm viability and thus fertility.

In the female, oogenesis involves well-orchestrated and developmentally regulated events in which epigenome plays an important role in generating quality gametes. For example, maternal nutrition, endocrine disruptors, and in vitro culture conditions are all known to influence “epigenetic health” of the developing oocyte and its viability. These external factors can cause alterations of kinds of transcripts and proteins that are stored in the oocyte, and these would directly influence the ability of the oocyte to undergo fertilization, egg activation, and sustain embryonic development.

In the embryo, which is the union of sperm and egg, epigenetics plays a vital role in activation of the egg and development of the resultant embryo. In the preimplantation embryo, specific sets of genes are activated at specific levels during specific stages of embryogenesis. DNA methylation and chromatin remodeling as well as synthesis of specific ncRNAs all contribute

to successful development. Research shows that assisted reproductive technologies (ART), such as in vitro fertilization and somatic cell nuclear transfer or cloning, cause abnormalities in some aspects of the epigenome, thereby resulting in less efficient production of animals and production of animals with health problems.

In summary, alterations in key epigenetic aspects, including DNA methylation, chromatin remodeling, and noncoding transcripts, influence viability of gametes and embryos. This is vitally important in ART both for humans and livestock. Plans for animal management, nutrition, environmental factors, and in vitro culture conditions should be carefully designed with epigenetic changes in mind.

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