Physiology of Gametogenesis

1

Ying-Hui Ye, Le-Jun Li, Yue-Zhou Chen, He-Feng Huang, and Zhong-Yan Liang

Abstract

Gametogenesis is a biological process by which diploid or haploid precursor cells undergo cell division and differentiation to form mature haploid gametes. The biology of gamete production is different between males and females. In human, gametogenesis is the development of diploid germ cells into either haploid sperm (spermatogenesis) or eggs (oogenesis). Gamete production in females is intimately part of the endocrine responsibility of the ovary. If there are no gametes, then hormone production is drastically curtailed. Depletion of oocytes implies depletion of the major hormones of the ovary. In the male this is not the case. Androgen production will proceed normally without a single spermatozoon in the testes. This chapter reviews some of the basic structural morphology and physiology of the testes and the ovaries.

1.1 Spermatogenesis

The male reproductive tract in human consists of testes, epididymides, ductus deferentes, accessory sex glands, and penis. The differentiation of testes takes place from indifferent gonads after expression of the sex-determining region Y gene (SRY) on the short arm of the Y chromosome. The fetal Leydig cells then secrete androgens that induce differentiation of the mesonephric (or wolffian) duct to form the epididymis, ductus deferens, and, accessory sex glands, as well as the indifferent external genitalia into a penis and a scrotum. There are two components to the testis;

Y.-H. Ye (✉) • L.-J. Li • Y.-Z. Chen • H.-F. Huang • Z.-Y. Liang
The Key Laboratory of Reproductive Genetics, Zhejiang University, Ministry of Education, Hangzhou, People's Republic of China

Department of Reproductive Endocrinology, Women's Hospital, School of Medicine, Zhejiang University, Hangzhou, People's Republic of China
e-mail: yeyh1999@hotmail.com

H.-F. Huang, J.-Z. Sheng (eds.), *Gamete and Embryo-fetal Origins of Adult Diseases*,
DOI 10.1007/978-94-007-7772-9_1,

the interstitial (intertubular) component and the seminiferous tubule. The interstitial component is well vascularized and contains Leydig cells clustered around the vessels. The seminiferous tubules contain epithelium consisting of germ cells which form numerous concentric layers penetrated by a single type of somatic cells first identified by Enrico Sertoli in 1865 [1]. The cytoplasm of Sertoli cells extends around germ cells to provide nutrition and support while the germ cells undergo progressive differentiation and development into mature spermatozoa.

Spermatogenesis is a dynamic process in which stem spermatogonia become mature spermatozoa throughout the reproductive lifetime of the individual [2, 3]. By the process of mitosis, stem spermatogonia produce two types of cells, additional stem cells and differentiating spermatogonia. The differentiating spermatogonia then undergo rapid, successive mitotic divisions to form primary spermatocytes, which are then followed by a lengthy meiotic phase as preleptotene spermatocytes proceed through two cell divisions (meiosis I and II) to give rise to haploid spermatids. These, in turn, produce mature spermatozoa which undergo a complex process of morphological and functional differentiation. To study such complex and lengthy processes, spermatogenesis has been organized into different "stages" and "phases" that include mitosis, meiosis and spermiogenesis.

1.1.1 Spermatogonia and Mitosis

Spermatogenesis can be divided into three phases, each of which involves a class of germ cells [2–4]. The initial phase, spermatocytogenesis, is the proliferative or spermatogonial phase during which stem spermatogonia undergo mitosis and produce two types of cells; additional stem cells and differentiating spermatogonia. In rat, there are three types of spermatogonia; stem cell spermatogonia (Ais or A isolated), proliferative spermatogonia (Apr or A paired and Aal or A aligned), and differentiated spermatogonia (Al, A2, A3, A4 and B). The stem cells, Ais, divide sporadically to replicate themselves and to produce pairs of Apr spermatogonia, which engage in a series of synchronous divisions, resulting in the formation of chains of Aal spermatogonia. The Aal spermatogonia differentiate into A1 spermatogonia which divide to give rise to more differentiated (A2, A3, A4, and B) cells. In man, three different types of spermatogonia [the dark type A (Ad), pale type A (Ap) and B type] have been identified [5]. The Ap cells have the capacity to give rise to new Ap cells as well as to more differentiated B spermatogonia, and, that are the renewing stem cells. The Ad spermatogonia are reserve stem cells which rarely divide under normal circumstances. The precise mechanisms of differentiation, and, renewing their own population of stem spermatogonia remain unknown.

1.1.2 Meiosis

The meiotic, or spermatocyte, phase deals with the formation of haploid spermatids and divides into five sequential stages including leptotene, zygotene, pachytene, diplotene, and diakinesis. DNA synthesis is involved in each of these five stages of

the primary spermatocytes (preleptotene), and, RNA synthesis in the diplotene stage. Elaborate morphological changes occur in the chromosomes as they pair (synapse) and then begin to unpair (desynapse) during the first meiotic prophase. Some of the changes during this stage include: (1) initiation of intimate chromosome synapsis at zygotene stage, when the synaptonemal complex begins to develop between the two sets of sister chromatids in each bivalent; (2) completion of synapsis with fully formed synaptonemal complex and crossing over at pachytene stage; (3) dissipation of the synaptonemal complex and desynapsing at the diplotene stage. Following the long meiotic prophase, the primary spermatocytes rapidly complete their first meiotic division, resulting in formation of two secondary spermatocytes, each contains 22 duplicated autosomal chromosomes and either a duplicated X or a duplicated Y chromosome. These cells, after a short interphase with no DNA synthesis, undergo a second maturation division to produce four spermatids, each of them has a haploid number of single chromosomes.

1.1.3 Spermiogenesis

The spermiogenic phase, also known as spermiogenesis, involves morphological and functional differentiation of newly formed spermatids into mature spermatozoa. During this phase, the spherical, haploid spermatids transform into elongated, highly condensed, mature spermatozoa, which are released into the lumen of seminiferous tubules. The differentiation of spermatids passes through four phases including Golgi, cap, acrosomal, and maturation phases.

Golgi apparatus is important during the early steps of spermiogenesis [1, 6, 7]. The Golgi apparatus creates vesicles and granules containing enzymes that will become the acrosome. During the capping phase, the acrosome covers the developing sperm nucleus. The acrosomic granule touches the nuclear envelope and the vesicle begins to flatten into a small cap over the nuclear surface. Then, the acrosomic vesicle becomes very thin and the granule flattens. Finally, the acrosome flattens over approximately one third of the nuclear surface. The acrosomal phase involves migration of the acrosomal system over the ventral surface of the elongating spermatid nucleus. Maturation shows fewer changes in nuclear shape and acrosomal migration. The nucleus continues to condense and the acrosome matures into a thin periodic Acid-Schiff stain positive (PAS+) structure that protrudes at the apex but covers nearly all the nucleus, except for that portion connected to the tail [1]. After the formation of prominent cytoplasmic lobes and residual bodies, the excess cytoplasm, which contains unused mitochondria, ribosomes, lipids, vesicles and other cytoplasmic components, is removed [1, 8, 9].

1.1.4 Sperm Production

Spermatogenesis has the greatest number of cell divisions compared to other self-renewing cells in the body. Both the kinetics and rate of germ cell loss affect sperm production. The total duration of spermatogenesis based on 4.5 spermatogenic

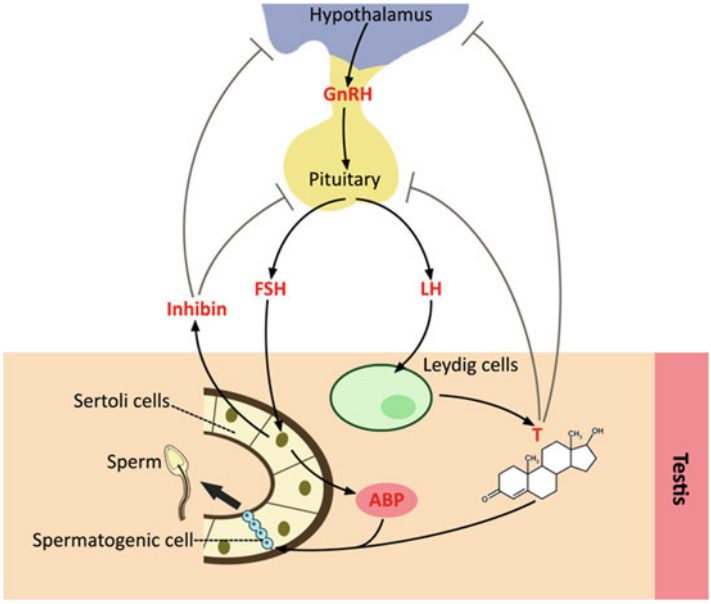

Fig. 1.1 The hypothalamic-pituitary-testicular axis

cycles ranges from approximately 30–78 days in mammals [1, 10–12], and is under the control of the germ cell genotype [13]. Similar results were found utilizing porcine and ovine testis xenografts [14]. However, temperature and some drugs may influence the duration of spermatogenesis [15–17], probably by altering the cell cycle [18, 19]. In humans, the entire spermatogenic process lasts more than 70 days. Germ cell loss (apoptosis) also occurs during spermatogenesis [20], playing a critical role in determining total sperm output. However, the greatest influence on germ cell production is the capacity for mitosis. Significant germ cell apoptosis occurs during the spermatogonial phase, called "density-dependent regulation", primarily during mitotic divisions of spermatogonia. Apoptosis is also frequent during meiosis, especially in humans, and is probably related to chromosomal damage. However, missing generations of spermatocytes and spermatids in the seminiferous epithelium, plus apoptosis, contribute to the low efficiency of human spermatogenesis [20, 21].

1.1.5 Regulation of Spermatogenesis

Androgen production and spermatogenesis are two key functions of the testis that rely on regulation by pituitary gonadotrophins; luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Other hormones contribute to testicular regulation, and there is a plethora of paracrine and autocrine regulatory effects between, and within, different testicular cell compartments. The hypothalamic-pituitary-testicular (HPT) axis plays a key role in the regulation of spermatogenesis (Fig. 1.1) [2, 22–26].

The HPT axis is a classical example of an endocrine regulatory circuit, with cascades of positive and negative regulatory events at multiple functional levels. The highest level is at the hypothalamus, where the cells of specific nuclei synthesize the decapeptide gonadotropin-releasing hormone (GnRH), giving the positive stimulus for gonadotrophin secretion from the anterior pituitary. The same GnRH peptide is responsible for the release of both gonadotrophins, although evidence for a separate FSH-releasing hypothalamic principle also exists [27]. The axon terminals of GnRH neurons make contact with hypophyseal portal vessels, which transport the releasing hormone, secreted in pulses of 1–2 h intervals, to the anterior pituitary gland [28].

In pituitary gonadotroph cells, GnRH stimulates the synthesis and release of LH and FSH. The secretory peaks are more distinct with LH, due to its shorter circulatory half-life compared to FSH [29]. Targets of LH action in the testis are interstitial Leydig cells, whereas FSH regulates Sertoli cells in seminiferous tubuli. LH stimulates steroidogenesis in mature Leydig cells, and is responsible for the supply of testosterone for the maintenance of spermatogenesis, and, for extragonadal androgen effects. FSH maintains the functional capacity of Sertoli cells in the support of spermatogenesis. It is well established that LH stimulates testicular steroidogenesis and maintains the high intratesticular steroid concentration that is necessary for spermatogenesis [30]. In contrast, it is not known how exactly high levels of intratesticular testosterone regulate this process. Androgen receptors are present in Leydig cells, peritubular myoid cells and Sertoli cells, but apparently not in spermatogenic cells [2].

The Sertoli cells are the only target of FSH action in the testis. The FSH stimulates the proliferation of Sertoli cells in the prenatal and prepubertal periods [31]. Sertoli cells become differentiated at puberty, and rarely differentiate thereafter. Thus, FSH determines the spermatogenic capacity before the onset of puberty. Both testicular steroid and peptide hormones regulate FSH secretion. Inhibin and activin originate in Sertoli cells, and, are the main regulators of FSH secretion at the pituitary level. Inhibin is a heterodimer of an α subunit and a PA (inhibin A) or PB (inhibin B) subunit, which inhibits both the synthesis and secretion of FSH. Activin is a homodimer of two P subunits which stimulate FSH. Follistatin, also inhibits FSH by binding and inactivating activin. Androgens and estrogens inhibit FSH action by affecting the functions of hypothalamus. Inhibin B is the product of Sertoli cells and is also important in feedback regulation [32].

The HPT axis plays a role in the negative feedback link between gonadal steroid and peptide hormones [33]. In the male, testosterone mainly controls GnRH secretion, by suppressing GnRH secretion at the hypothalamic level, and, gonadotropin synthesis in the pituitary gland. Testicular steroids also have effects on FSH through the two Sertoli cell proteins, activin and inhibin.

Some part of the steroid feedback is directed to inhibition of gonadotropin synthesis at the pituitary level [34] by both androgenic and oestrogenic components. This feedback occurs mainly at the hypothalamic or pituitary level. Steroids affect GnRH neurons indirectly, and, are mediated by inhibitory inputs from neighboring neurons.

1.1.6 Sertoli Cell

The Sertoli cell also plays an important role in spermatogenesis through (1) support and nutrition of the active germ cells; (2) compartmentalization of the seminiferous tubule by tight junctions; (3) controlled release of mature spermatids into the tubular lumen; (4) secretion of fluid, proteins and several growth factors; (5) phagocytosis of degenerating germ cells and phagocytosis of excess cytoplasm [34]; (6) mediation of the actions of FSH- and LH-stimulated testosterone production in the testis [2]. Some investigations of the Sertoli cell-specific, knockout of androgen receptor (SCARKO) found that spermatogenesis rarely advanced beyond diplotene spermatocytes [35]. Thus, at least in mouse, androgens are crucial for late meiosis and spermiogenesis.

1.1.7 Sperm Maturation in the Human Epididymis

1.1.7.1 Sperm Transit

Based on thymidine labelling of spermatozoa [36], sperm migrate through the human epididymis in 1–21 days. Measurements of extragonadal sperm reserves suggest shorter values of 3–4 days [37, 38]. Faster transit (up to 2 days) may occur in men with large, daily sperm production [38]. However, such rapid transit may result from poor production of spermatozoa, because human epididymal fluid is not viscous [39]. Interestingly, rapid sperm transit associated with large testicular size and high sperm production also occurs in the chimpanzee [40].

1.1.7.2 Sperm Heads and Tails

Morphological analysis of human epididymal sperm following air-drying of smears results in artefactual swelling of the heads of sperm from the caput region, but not those from the caudal epididymidis [41, 42]. These changes demonstrate maturational changes that affect primate spermatozoa [43]. Thus, some spermatozoa with ‘acorn-shaped’ heads in human semen indicate the appearance of immature spermatozoa, because of abnormal epididymal function [44]. The percentage of spermatozoa with normal heads still increases with maturation, not as a result of removal of abnormal cells by epididymal epithelium [45, 46], but by processes of maturation. During transit through the epididymis the sperm increase intramolecular disulphide bonding [47] to withstand the stresses associated with air drying. The dimensions of heads of non-swollen sperm also change with maturation. This dehydration caused by high, intraluminal, osmotic pressures results in cell shrinkage, however, little is known about this in man. The osmotic pressure for fluid obtained from the human vas deferens is 342 mmol/kg [48], while the osmolality of fluid entering the human epididymis is 280 mmol/kg. An increase in compactness of nuclear contents may also explain the decrease in head size. The abnormal morphology of sperm tails is related to human epididymal dysfunction [49], however, the mechanisms are still unclear.

Cytoplasmic Droplets

There is difference between the cytoplasmic droplets of morphologically normal cells and excess residual cytoplasm of abnormal cells [50]. The air-drying procedure of seminal smears routinely is used for the morphological analysis of human spermatozoa. However, this has been changed based on the insufficiency for the preservation of normal cytoplasmic droplets on the majority of spermatozoa in fixed preparations [50]. A neck droplet on human epididymal spermatozoon was shown on the electron micrographs and well fixed seminal preparations display the same [51]. Spermatozoa leaving the human testis present in fluid collected from epididymal spermatocoeles of accumulations of testicular fluid mainly [52], also shows droplets at the neck. The failure of droplet migration along the midpiece in man may indicate a heat stress, since spermatozoa from an inguinal testis and epididymis display neck droplets after exposure of abdominal temperature of cryptorchidism [53]. The failure of migration could results on the low sperm concentration within the epididymal lumen, which reduces the shear forces incumbent upon more highly packed spermatozoa, since the migration of the droplet along the midpiece can be induced by centrifugation of porcine and caprine testicular spermatozoa [54, 55].

1.1.7.3 Motility

Spermatozoa are kept immotile, not by the viscosity of the fluid in the human epididymis [39] but possibly because of low pH [56]. They are activated to become motile after release into the female genital tract or physiological fluids. Sperm obtained from epididymal spermatocoeles and caput epididymidis are weakly motile [52, 57]. Examination of the duration and intensity of motility in vitro of sperm retrieved from epididymides obtained from 21 to 44 year-old men within 1–5 h of death demonstrated that the duration was far higher for sperm from the cauda (18.7 h) than those from the caput (4.6 h) or testis (2.1 h) [58]. The percentage of motile sperm increases as sperm pass through the epididymis. However, in old men, a decrease in motility was observed when recovered from the cauda, which may be associated with reduced ejaculation and sperm storage. Sperm obtained from the testis and caput are only motile in men with ductal occlusion [59] while sperm retrieved by testicular biopsy become motile after incubation in vitro [60], suggesting that the initiation of motility may be time-dependent rather than a result of epididymal secretions. The epididymal region where sperm motility normally develops is shifted proximally after vasectomy [61] or ductal occlusion [62].

1.1.7.4 Sperm Numbers

The small storage capacity of the human epididymis of about 3 days worth of testicular production is demonstrated by the small size of the cauda region [63, 64], and, the rapidity with which sperm reserves can diminish after multiple ejaculation [65]. After epididymal emptying by providing three ejaculates within 4 h, sperm numbers in the first of three subsequent ejaculates is increased from 50 millions after 1 day

abstinence to over 300 millions after 10 days and beyond this time ejaculated sperm numbers remain constant as the epididymis is filled and sperm begin to enter the urine [66].

1.1.7.5 Sperm Protection

Mammals have both adaptive (acquired) immunity, and innate (natural) immunity. Mechanisms of innate immunity include the generation of disinfectants (H_2O_2, NO), large antimicrobial proteins (lysozyme, cathepsins, lactoferrin, phospholipase A2) and small antimicrobial peptides (cathelicidins e.g. glycodelins, and, defensins) [67–69]. A wide range of defensins have been identified in human epididymis. As defensins are more active in the low ionic strength of epididymal fluid, they are situated to prevent the migration of invading micro-organisms into the male tract. A variety of anti-oxidant enzymes are present in human seminal plasma but most are not of epididymal origin [70], although some anti-oxidant activity does originate there [71].

1.1.8 Epigenetic Patterns in Male Germ Cells

Epigenetics refers to non-sequence based mechanisms that control gene expression. The paternal epigenome plays an important role in the developing embryo which is not limited to nucleosome retention data. To date three main mechanisms, DNA methylation, histone modifications and RNA-associated silencing, have been associated with epigenetic silencing of gene expression [72]. The sperm epigenetic program is unique, and, tailored to meet the needs of this highly specialized cell. Chromatin changes in sperm contribute to virtually every function that the male gamete must perform throughout spermatogenesis and in the mature cell [73]. But the requisite replacement of canonical histones with sperm-specific protamine proteins has called into question the utility of the paternal epigenome in embryonic development [74]. The protamination of sperm chromatin provides the compaction necessary for safe delivery to the oocyte, but removes histones which are capable of eliciting gene activation or silencing via tail modifications (methylation, acetylation, etc.) [75]. In effect, protamination removes a potentially informative epigenetic layer from the paternal chromatin, leading to the previously held belief that sperm are incompetent to drive epigenetic changes in the embryo and suggesting that their utility is found only in the delivery of an undamaged DNA blueprint. In fertile patients, histone retention is found at the promoters of genes important in the embryo including developmental gene promoters, microRNA clusters, and imprinted loci, suggesting that the nucleosome retention is programmatic in nature [76].

1.1.8.1 DNA Methylation

DNA methylation, one of the best-characterized DNA modifications associated with the modulation of gene activity is a common regulatory mark found on the 5 carbon of cytosine residues (5-mC) at cytosine–phosphate–guanine dinucleotides

(CpGs), which exert strong epigenetic regulation in many cell types [77]. During gametogenesis, non-imprinted genes acquire their methylation similarly to imprinted genes, however, after fertilization both the maternal and paternal genomes become demethylated while imprinted genes retain their methylation status [78]. Some repeat sequences appear to escape demethylation completely during gametogenesis, and, retain a high proportion of their initial methylation marking during preimplantation development [79]. Founding cells of the germ line, the primordial germ cells (PGCs), are thought to carry full complements of parental methylation profiles when they begin migrating towards the genital ridge [80]. Upon entry into the genital ridge, around 10.5 days of gestation, they undergo extensive genome-wide demethylation [81]. Early studies employing methylation-sensitive, restriction enzymes, Southern blot and PCR approaches indicated that PGCs have completely demethylated genomes by 13.5 days of gestation [82–84]. A number of imprinted genes, including Peg3, Kcnq1ot1 (also known as Lit1), Snrpn, H19, Rasgrf1 and Gtl2, as well as non-imprinted genes such as α-actin, become demethylated between 10.5 and 13.5 days of gestation [85]. However, certain sequences (at least some repetitive elements) appear to be treated differently: IAP, LINE-1 and minor satellite sequences are only subject to partial demethylation, whereas most imprinted and single-copy genes become demethylated [86, 87]. Rapid, and possibly active, genome-wide erasure of methylation patterns takes place between 10.5 and 12.5 days of gestation, leaving PGCs of both sexes in an equivalent epigenetic state by embryonic day 13.5 [88, 89]. Following demethylation in PGCs, male and female gametes acquire sex- and sequence-specific genomic methylation patterns. For nonimprinted genes and repeat sequences, DNA methylation can be assessed directly. For imprinted genes, determination of DNA methylation status and assessment of mono- or biallelic expression of the genes of interest in the resulting embryos, are necessary. A second genome-wide demethylation occurs in the early embryo. Marks established on imprinted genes and some repeat sequences must be faithfully maintained during preimplantation development at a time when the methylation of non-imprinted sequences is lost (Fig. 1.2).

In humans, abnormalities of DNA methylation have been linked to infertility, imprinting disorders in children, and, cancer. Recent studies have suggested that assisted reproductive technologies (ARTs) may be associated with an increased incidence of epigenetic defects in children, and, it is unclear whether the etiology is related to infertility with an underlying epigenetic cause, or, the specific experimental techniques.

1.1.8.2 Retained Histones

Protamination creates a highly condensed nuclear structure that helps to enable proper motility and protects DNA from damage. Although incorporation of this unique, sperm-specific protein results in a quiescent chromatin structure, some regions retain histones and their associated modifications. Recent studies have found this nucleosome retention to be programmatic, and not a result of random distribution [76]. In theory, this selective retention in sperm may allow for targeted gene activation or silencing in the embryo. Multiple histone variants found in

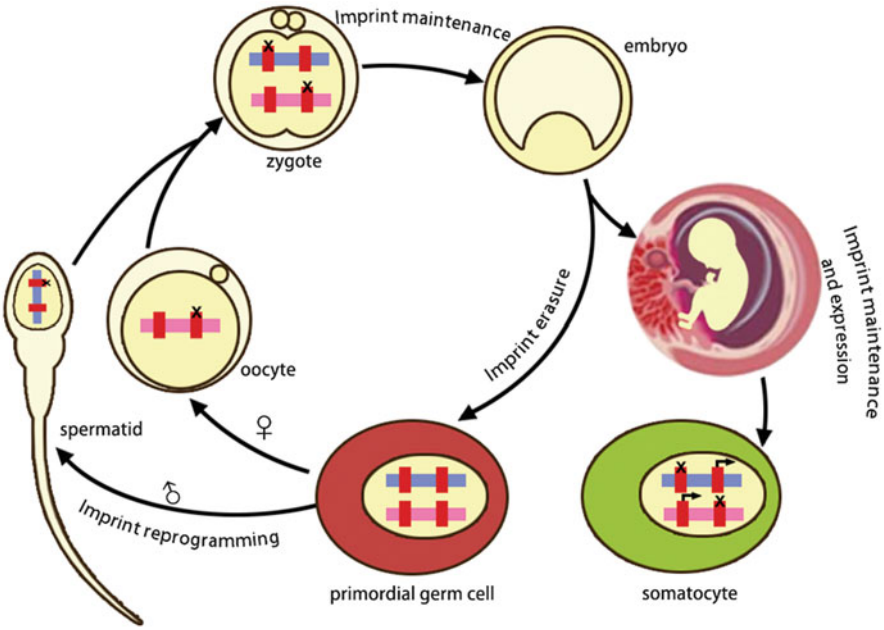

Fig. 1.2 Genes and their imprinting

sperm play an essential role throughout spermatogenesis as well as in the mature spermatozoa. Among these, important nuclear proteins are histone 2A and B (H2A and H2B), histone 3 (H3), histone 4 (H4), and the testes variant (tH2B) [90]. Recent studies implicate aberrant histone methylation and/or acetylation in the mature sperm in various forms of infertility. Loss-of-function mutation of JmjC-domain-containing-histone demethylase 2A (JHDM2A), an enzyme with known H3K9 demethylase activity, reveal decreased transcription of transition protein 1 and P1 during spermatogenesis [91]. Additional studies demonstrate that varying degrees of infertility, including sterility, are correlated with perturbations in histone methylation.

1.1.9 Spermatozoal RNA Transcripts

As a terminally differentiated cell, the ejaculated spermatozoon is exquisitely specialized for delivering the paternal genome to the egg. The presence of RNA in the sperm nucleus is paradoxical if one assumes that it serves no function [92]. The selective retention of mRNAs and siRNAs when most cytoplasmic RNA is lost to the residual body (normally destroyed during sperm preparation) during remodeling argues against passive trapping as does the evidence that sperm RNA can support

protein synthesis de novo during capacitation [92]. Based on a heterologous model system, we know that the spermatozoon delivers its RNA cargo to the oocyte [93]. One study showed that a c-Kit-derived heritable effect on hair color in the mouse was strongly influenced by the presence of aberrant levels of 'scrambled', noncoding c-Kit RNA transferred by the spermatozoa of the affected individual [94]. The concept of active, RNA-dependent translation in capacitating sperm is supported by reports showing that specific sperm RNAs are 'consumed' during manual processing that supports capacitation of normal viable sperm in vitro [95]. An effect of sperm RNA on non-Mendelian inheritance of coat color in mice has also been reported [94]. The mechanism appeared to be through an RNA-mediated epigenetic and heritable paramutation effect.

RNA transcripts co-localize with nucleosome-bound chromatin near the nuclear envelope in the mature sperm, as is the case with the insulin-like growth factor 2 (IGF2) locus [96]. Spermatozoal RNA transcripts are capable of inhibiting the protamination process and maintaining a histone-bound chromatin structure.

1.2 Oogenesis

Oogenesis is a complex and highly orchestrated sequence of differentiation from germ cells to mature oocyte. Human oogenesis is characterized by germ cell migration, differentiation and proliferation during the embryonic period, initiation of meiosis and follicle formation during the fetal period, and oocyte growth and maturation in the adult [97]. To accomplish its role as an oocyte, a PGC must erase the epigenetic program obtained in early embryogenesis and re-establish parental imprints during oogenesis [98]. Erasure and re-establishment of imprinted genes without the alteration of DNA sequence, is termed epigenetic reprogramming. Here, we briefly summarize the development of the oocyte and its epigenetic reprogramming during this process.

1.2.1 Primordial Germ Cells (PGCs)

1.2.1.1 Origin of PGCs

Mammalian development derives from fusion of a highly specialized oocyte with a sperm. The totipotent zygote replicates haploid parental genomes and begins to undergo cleavage divisions. At embryonic day (E) 3.5, the blastocysts include two cell types, the trophectoderm (TE) cells and the inner cell mass (ICM) cells. The trophectoderm can be classified into the polar TE (pTE), which contacts and covers the ICM, and the mural TE (mTE), which delineates the blastocoele cavity [99]. The ICM is the pluripotent cell population and is destined to become the embryo proper, including germ cells. With further development, the primitive endoderm (PE) differentiates from the ICM, located on the inner surface of the ICM at E4.5. The PE layer grows to cover the mTE and becomes the parietal endoderm. The undifferentiated cells in the ICM maintain pluripotency and become the

primitive ectoderm. During the process of implantation, pTE will grow into a thick column of extraembryonic ectoderm. The primitive ectoderm cells eventually will give rise to a cup-liked epithelial sheet, called the epiblast [99]. All the somatic cells including the germ cells derive from these epiblast cells. Although the exact time and place of origin of PGCs in human is unknown, mouse germ cell lineage recruits from pluripotent epiblast cells in response to extraembryonic signals at E6.25 [100]. PGCs in both males and females can be first identified within the wall of the human yolk sac, an extraembryonic membrane, during the 4th week of gestation [101].

1.2.1.2 PGC Migration

PGC migration is a process shared among many species during germ cell development. PGCs migrate from their extragonadal origin to the developing gonad (genital ridge), where they interact with gonadal somatic cells that will support germ cells development and maturation into functional gametes [97]. Between 4th and 6th weeks, PGCs migrate by amoeboid movement from the yolk sac to the wall of the gut tube, and then via the mesentery of the gut to the dorsal body wall. In the dorsal body wall, these cells come to rest on either side of the midline in the loose mesenchymal tissue just deep to the membranous lining of the coelomic cavity. Most of the PGCs populate the region of the body wall at the level that will form the gonads [101].

A series of proteins facilitate PGC migration. SDF1 is expressed along the migratory route of the hindgut and genital ridge, providing a guidance signal for PGC migration by binding to CXCR-4 receptor expressed on PGCs [97, 102]. PGCs also express β1/2 integrin that can interact with extracellular matrix along their journey [103]. Furthermore, the interactions themselves are important for PGC migration and clonization. PGCs express the CX43 gap-junction protein during migration, and PGCs up-regulate E-cadherin on leaving the gut and colonizing the genital ridge. E-cadherin mediated, cell-cell adhesion may also support PGC migration arrest in the developing gonad [104, 105].

During PGC migration there is a reduction in genome-wide, DNA methylation. The process includes loss of methylation marks that control somatic gene expression and also removes DNA methylation imprints from imprinted gene loci [106]. Several imprinted genes exhibit earlier demethylation; for example, *Igf2r* begins demethylation in some PGCs before colonization of the genital ridge [107]. The genome-wide DNA methylation level declines at around E8.0 in the mouse [108]. The DNA methyltransferases (Dnmts) are responsible for the methylation of DNA. Dnmt1 methylates hemimethylated CpG di-nucleotides in the genome, and, is the key maintenance methyltransferase during cell division. Dnmt3 are responsible for de novo methylation by targeting the unmethylated CpG site [109]. Dnmt3 are actively repressed in PGCs at E6.75 to E7.5, suggesting that the loss of Dnmts activities may contribute to genome-wide DNA demethylation in PGCs [108, 110]. From around E7.75 onwards, PGCs undergo genome-wide demethylation of H3K9me2 [108]. All PGCs at E8.75 show low H3K9me2 levels. Levels of H3K9me2 halve their original levels during this period [108]. Following the genome-wide loss of DNA methylation and H3K9me2, genomic-wide H3K27me3

becomes upregulated during PGC migration from around E8.25 onwards [111]. Upregulation of genomic-wide H3K27me3, however, may not depend on de novo transcription of some specific factors. The crosstalk between H3K27 methylation and H3K9 methylation may contribute to this process. And the hypomethylation of H3K9me2 in migrating PGCs may be sensed and rescued by the methylation of H3K27me3 [112].

Taken together, DNA methylation and H3K9me2 play critical roles in the stable maintenance of the repressed state of unused genes during cell fate specification. H3K27me3 may be a more plastic repression of the lineage-specific genes in pluripotent cells [99]. Upregulation of H3K27me3 in PGCs may contribute to the creation of an ES cell-like genome organization. Indeed, it is possible to derive pluripotent mice stem cells from PGCs from E8.5 to E12.5 [113].

1.2.1.3 PGC Proliferation

Classically, once PGCs reach approximately 40 in number in the mouse, they proliferate constantly with a doubling time of about 16 h [114]. PGCs continue to multiply by mitosis during the migration. However, it has been shown that PGCs do not increase their number constantly [111]. From around E7.75 to E9.25, coincident with their migration in the developing hindgut endoderm, their increase in number is relatively slow compared to that after E9.5. Through calculation of DNA contents of PGCs, a majority of PGCs (approximately 60 %) from E7.75 to E8.75 are in the G2-phase of the cell cycle. After E9.75, PGCs exhibit a cell cycle distribution with a clear G1 peak, a wide S-phase, and less prominent G2 peak, which is indicative of a rapidly cycling state. These results indicate that a majority of PGCs migrating in the hidgut endoderm arrest at the G2-phase of the cell cycle [99].

When PGCs arrive in the presumptive gonad region, they stimulate cells of the adjacent coelomic epithelium to proliferate and form somatic support cells. Proliferation of the somatic support cells creates a swelling just medial to each mesonephros on both the right and left sides of the gut mesentery. These swellings, the genital ridges, represent the primitive gonads. In both male and female, PGCs undergo further mitotic division within the gonads. PGCs are usually called gonocytes during this period [115].

1.2.1.4 Imprinted Genes in PGC Development

The vast majority of genes possess a bi-allelic pattern of expression. Imprinting corresponds to specific epigenetic regulation leading to expression of only one parental allele of a gene. Some imprinted genes exhibit paternal expression while others exhibit maternal expression. Imprinting is mediated by epigenetic modifications including DNA methylation and histone modifications imposed on either maternal or paternal alleles [99]. Currently, about 75 imprinted genes have been identified to date in humans, although it is estimated that from 100 to 600 imprinted genes may exist in the human genome [109].

Imprinting marks from their parents generally maintain at somatic lineages through specific mechanisms, however, newly-formed germ cells need to erase the

imprints of the previous generation and established new ones depending on the sex of the new generation [116]. Numerous studies examined DNA methylation states of imprinted genes in migrating and postmigrating PGCs from E9.5 to E12.5 [117, 118]. Allele-specific DNA methylation in nearly all imprinted genes is maintained in migrating PGCs until about E9.5, indicating that imprinted genes are resistant to the global DNA demethylation in migrating PGCs. The erasure of imprinting commences in a part of the migrating PGCs at E9.5, progresses gradually afterward, and completes in PGCs at E12.5 [99].

Despite the mechanism of imprint erasure being unknown, it has been suggested that erasure is an active process involving the direct removal of DNA methylation [119]. A recent study showed that some imprinted loci, *H19* and *Lit1*, are resistant to the demethylation process in *Aid*-deficient PGCs [120]. Another study showed that several imprinted regions undergo complete demethylation in the Dnmt3a and 3b knockout ES cells [121]. The results indicated that the absence of Dnmt3a and 3b may be a critical requirement for erasure of imprints.

1.2.2 From Gonocytes to Primary Oocytes

In the primitive gonads, PGCs (now called gonocytes) undergo more mitotic divisions after they are invested by the somatic support cells and then differentiate into oogonia. By 12 weeks development, oogonia in the genital ridges enter the first meiotic prophase and then almost immediately become dormant.

1.2.2.1 Meiotic Arrest

Meiosis is a specialized process of cell division that occurs only in germ cell lines. In mitosis, a diploid cell replicates its DNA and undergoes a single division to yield two diploid cells. In meiosis, a diploid germ cell replicates its DNA and undergoes two successive cell divisions to yield four haploid gametes [101]. In females, the meiotic divisions of oogonia are unequal and yield a single, massive, haploid definitive oocyte and three, small, nonfunctional, haploid, polar bodies. Oocytes enter meiosis prenatally but do not finish the second meiotic division until fertilization, thus the process can take several decades to complete [122].

The preliminary step in meiosis, as in mitosis, is the replication of each chromosomal DNA molecule; thus, the diploid cell converts from 2N to 4N. This event marks the beginning of gametogenesis. The oogonium is now called a primary oocyte. Once the DNA replicates, each consists of two chromatids joined together at the centromere. In the next step, prophase, the chromosomes condense into compact, double-stranded structures. During the later stages of prophase, the double-stranded chromosomes of each homologous pair match up to form a joint structure, or, chiasma. Chiasma formation makes it possible for two homologous chromosomes to exchange large segments of DNA by "crossing over". The resulting recombination of genetic material on homologous maternal and paternal chromosomes is largely random, and, increases the genetic variability of the future gametes. Once primary oocytes enter meiotic "arrest" during the first meiotic prophase, they

enter a state of dormancy and remain in meiotic arrest as primary oocytes until sexual maturity [123].

1.2.2.2 Meiosis and Epigenetic Transition

In PGCs, the genomes are wiped clean of most of their DNA methylation and other chromatin modifications, so that germ cells can acquire the capacity to support postfertilization development. This process also prepares germ cells for meiosis, during which homologous chromosomes become aligned to allow synapsis and recombination [106].

A functional link between DNA methylation and meiosis has been discovered in female germ cells. Specifically, ablation of ATP-dependent chromatin remodeling factor *Lsh* (lymphoid specific helicase) gives rise to DNA demethylation and activation of transposable elements in mouse female germ cells. Reduced methylation occurs at tandem repeats and pericentric heterochromatin. These changes are associated with incomplete synapsis of homologous chromosomes and developmental arrest at the pachytene stage [106]. The use of antibodies against methylated cytosines (5mC) has shown low methylation levels in both nuclei and chromosomes of proliferating oogonia in female mice. In early oocytes at both late zygotene and late pachytene/diplotene stages of meiosis, overall DNA methylation remains low [107].

1.2.3 Folliculogenesis

1.2.3.1 Primordial Follicle

By the 5th month of human fetal development all oogonia begin meiosis, after which they are called primary oocytes [101]. The nucleus of each of these dormant primary oocytes, containing the partially condensed prophase chromosomes, becomes very large and watery and is referred to as a germinal vesicle. The swollen condition of the germinal vesicle may protect the oocyte's DNA during the long period of meiotic arrest [101].

A single-layered, squamous capsule of epithelial follicle cells derived from the somatic support cells tightly encloses each primary oocyte. This capsule and its enclosed primary oocyte constitute a primordial follicle. The first primordial follicles appear in the human fetus as early as 15th week of gestation, and, are complete by 6 months after birth [124]. Studies in human and rodents identify a variety of genes involved in primordial follicle assembly, such as transcription factors, zona proteins, meiosis-specific enzymes and nerve growth factors [125].

Initiation of meiosis in oogonia with investment of granulosa cells to form the primordial follicle appear to protect from atresia, as they cannot persist beyond the 7th month of gestation without entering meiosis. Therefore, ovaries in the newborn are usually devoid of oogonia [125, 126]. By 5 months, the number of primordial follicles in the ovaries peaks at about seven million. Most of these follicles subsequently degenerate. By birth only 700,000 to 2 million remain, and by puberty, only about 400,000 [101].

1.2.3.2 Primordial-to-Primary Follicle Transition

Primordial follicles remain in a dormant phase until being recruited into the primary stage for growth. The transition process is an orchestrated multi-directional communication among the oocytes and somatic cells (granulosa cells and thecal cells), and certain matrix components and growth factors play roles in this process and subsequent growth of follicles [127].

Recent studies on genetically modified mice confirm inhibitory signals that maintain primordial follicles in the dormant state. Loss of function of any of the inhibitory molecules for follicular activation, including tumor suppressor tuberous sclerosis complex 1 (Tsc-1), phosphatase and tensin homolog deleted on chromosome 10 (PTEN), Foxo3a, p27 and Fox12, leads to premature activation of the primordial follicle pool [128–130]. Global activation of primordial follicles inevitably causes early exhaustion of the follicle pool and premature ovarian failure (POF) though only the Fox12 mutation has been linked to POF in human [131]. The Foxo3a knockout mouse is infertile owing to total depletion of follicles, however, mutation or common single nucleotide polymorphisms in the Foxo3a gene are not associated with POF in human [132, 133].

Anti-Mullerian hormone (AMH) is a member of the transforming growth factor-beta (TGF-β) family, produced by granulosa cells of growing preantral and small antral follicles as a dimeric glycoprotein [125]. In fetal ovaries, AMH is first detected at 36 weeks of gestation in granulosa cells of developing preantral follicles and reaches its highest levels in puberty, becoming undetectable after menopause [134, 135]. Increased recruitment of primordial follicles into the growing pool in AMH null mice suggests a negative effect of AMH on the primordial-to-primary follicle transition [136, 137]. Flattened granulosa cells of primordial follicles become cuboidal during transition into their primary stages along with an increase in oocyte diameters and acquisition of zona pellucidae [138]. The transcription factor Fox12 appears to play a critical role in this transition. Although mice with interruption of Fox12 gene had normal formation of primordial follicles, the granulosa cells did not complete their squamous-to-cuboidal transition, which led to the absence of secondary follicles [139].

Other signals in the ovary promote transition of primordial follicles to primary follicles. Studies on animal models and the human ovary confirm that BMP-4, BMP-7 and GDF-9 are involved in these processes [140, 141]. Mice with the GDF-9 null gene are infertile owing to arrested follicle development at the primary stage with no further growth beyond that stage [142]. Furthermore, FSH is not required for this transition as primordial follicles do not express FSH receptors [143]. Other growth factors and cytokines, such as kit-ligand (KL) and leukemia inhibitory factor (LIF) also act at a paracrine level in the formation of primary follicles [144, 145].

1.2.3.3 Preantral and Antral Follicle

With mitotic expansion of granulosa cells, single-layer primary follicles are transformed into multi-layered ones. An increase in oocyte diameter, and formation of basal lamina, zona pellucida and theca cell layer are among other changes that characterize this developmental stage [146]. During this phase follicle diameter

increases from 40–60 μm at the primary stage to 120–150 μm at the preantral stage. With further growth, follicles reach a diameter of 200 μm, and, enter the antral stage. It is during this stage that the follicle begins to develop fluid-filled spaces within the granulosa cell layers, which will coalesce to form the antral cavity. Development of a multi-layered secondary follicle from a primary follicle with a single layer of granulosa cells is a lengthy process that takes months in humans. This process appears to be independent of the actions of gonadotrophins. Even though preantral follicles may express FSH receptors, FSH may have a permissive role rather than being essential to preantral follicle growth [143].

A series of growth factors and cytokines, such as GDF-9, BMP-15, BMP-4, BMP-7 and TGF-β play crucial roles in the growth of primary follicles into preantral and antral stages. In vitro experiments show that GDF-9 increases the number of primary and secondary follicles in human and rodents [147]. Mice null for the GDF-9 gene show arrested follicle growth at the primary stage, which further confirms the growth-promoting effects of GDF-9 on follicles beyond the primary stage [148]. Another positive regulator of follicle growth into preantral and antral stage is BMP-15. This oocyte-derived growth factor stimulates the proliferation of granulosa cell mitosis in pre-antral follicles during the FSH-independent stages of early follicular growth [149]. Theca cells play an important role in follicle growth. Theca-derived BMP-4 and -7 promote follicle growth beyond the primary stage in rodents [150]. During rapid growth of preantral-antral follicles, BMP-4 and -7 also modulate FSH signaling in a way that promotes estradiol production while inhibiting progesterone synthesis, and acting as a luteinization inhibitor. BMP-4 and -7 do not affect granulosa cell steroidogenesis in the absence of FSH [151]. The actions of TGF-β include proliferation of granulosa cells, progesterone production and FSH-induced estradiol production [152].

In contrast to the positive effects of these growth factors and cytokines, AMH appears to have a negative effect on preantral follicle development beyond primordial-primary transition [153]. Its expression is first detected on granulosa cells of primary follicles and continues until mid-antral stages of follicle development in humans. The highest level of expression occurs in granulosa cells of secondary, preantral and small antral follicles less than 4 mm in diameter [154]. These findings suggest a negative effect of AMH on preantral follicle development beyond the primordial-primary transition.

1.2.3.4 Premature Follicle and the Selection of Dominant Follicle

Follicle growth after the antral stage is characterized by further proliferation of granulosa and theca cells, increased vascularization, oocyte growth and formation of the antral space. FSH becomes a critical determinant of further follicle growth and survival at this stage. Cyclical recruitment of a cohort of antral follicles for further growth and selection of a dominant follicle from among this cohort are the characteristic features of this phase. When a selected cohort of antral follicles grow, modulation of their steroidogenetic activity and their response to gonadotrophins are necessary to sensitize certain follicles for further growth and select a dominant

follicle [125]. Current evidence suggests that these goals are accomplished by locally produced growth factors, such as activin, GDF-9 and BMPs.

Activin A promotes FSH receptor expression in rat granulosa cells and suppresses the growth of primary follicles while inducing follicular growth at later stages [155, 156]. Activin A also involves regulation of estrogen synthesis, LH receptor expression and oocyte maturation [157]. When activin signalling is disrupted, follicle development arrests [158]. However, AMH negatively affects cyclic recruitment and dominant follicle selection by reducing the responsiveness of preantral and small antral follicles to FSH [159].

Another characteristic of follicular development is a switch from an activin A-dominant to inhibin A-dominant environment. Small follicles tend to produce more activin A relative to inhibin A, whereas large, selected, antral follicles secrete more inhibin A [160]. Activin A can attenuate LH-dependent androgen production by theca cells of small preantral follicles [161]. On the other hand, inhibin A released in large quantities by selected antral follicles counteracts the inhibitory effects of activin A and increases LH-induced androgen secretion from theca cells. This provides a sufficient supply of androgen for conversion into estrogens in granulosa cells as there will be a great demand for estrogen synthesis during the peri-ovulatory period. The expression pattern of activin A subunits and its receptors suggests a role for activin in acquisition of maturational competence in oocytes of growing pre-antral follicles [161]. By contrast inhibin A can function as a meiotic inhibitor and therefore impair oocyte maturation and developmental competence [162].

1.2.3.5 Folliculogenesis and Epigenetic Modification

During follicle maturation, appropriate epigenetic modifications are necessary for normal oocyte growth and development [163]. Methylation marks are re-established with oocyte growth and follicle development. The methylation pattern is related to oocyte size and increases throughout the entire nucleus until it reaches its maximal level in the fully grown GV-stage oocyte [107]. Several studies show that DNA methylation levels increase with oocyte growth, with a slight increase on day 10 (post-partum) and marked increases thereafter until the later GV stage in mice [163]. DNA methylation of imprinting genes establishes between days 10 and 15 in oocytes [164]. In addition, de-novo expression of Dnmt3a and Dnmt3b mRNA was observed in late GV-stage and 15 day-old oocytes [163]. These enzymes may catalyze DNA methylation in regions of the genome other than the imprinted genes until the late GV stage. The role of global DNA methylation is still unclear, but it may involve transiently repressing transposon mobilization to maintain genome stability during oocyte growth [165].

Maternal imprinting is also reset during oocyte growth and maturation. This modification may be dependent on the development stage of the oocyte. Various imprinted genes receive an imprinting mark asynchronously at particular stages during the primordial to antral follicle transition [166]. These imprints are not established at the same time, and each imprinted gene is methylated at a specific time. Meanwhile, the imprinting pattern of the oocyte is not fully laid down until the oocyte is within a mature follicle ready to ovulate [167]. Remethylation of

maternally expressed imprinted genes starts at the secondary follicle stage and completes at the antral follicle stage [166]. Another study shows that *Snrpn*, *Znf127*, and *Ndn* genes are imprinted during the process of primordial-to-primary follicle transition, whereas imprinting of *Peg3*, *Igf2r*, and $p57^{KIP2}$ occurs at the secondary follicle stage [167]. Additionally, there is species-specific difference in the establishment of methylation imprinting. For example, *Snrp* is methylated in late GV-stage, metaphaseI(MI), and metaphaseII (MII) human oocytes [168]. The establishment of maternal methylation imprints for some genes may be later in humans than in mice during oocyte development.

1.2.4 Oocyte Maturation

To become competent for normal fertilization and embryonic development, oocytes in preovulatory follicles undergo both nuclear and cytoplasmic maturation. Nuclear maturation includes at least two steps: oocytes resume meiosis, undergo GVBD and progress to metaphase I; the first polar body is extruded and the oocytes proceed to metaphase II. In addition to nuclear maturation, oocytes also undergo cytoplasmic maturation characterized by cytoplasmic changes essential for monospermic fertilization, processing of the sperm, and preparation for development to preimplantation embryos [169].

1.2.4.1 Meiotic Arrest Maintenance

Before reproductive maturity, oocytes arrest in prophase I of meiosis. Maintenance of oocyte meiotic arrest is dependent upon follicular somatic cells, because oocytes or cumulus-oocyte-complexes released from preovulatory follicles resume meiosis spontaneously [170].

Meiotic arrest of oocytes is maintained by high concentrations of the second messenger cyclic AMP (cAMP) [171]. cAMP levels within the oocyte affect the activity of maturation promoting factor (MPF), a complex of Cdc2 and cyclin B, via cAMP-dependent protein kinase A (PKA). High cAMP levels in oocytes result in phosphorylation of Cdc2 on Thr14 and Tyr15, keeping the MPF complex inactive. A decrease of cAMP levels within oocytes leads to dephosphorylation of Thr14 and Tyr15, and MPF becomes active so that oocytes can resume meiosis. The phosphorylation is catalyzed by Wee1 kinases, whereas dephosphorylation is dependent on Cdc25 phosphatases. The activity of Wee1 kinases and Cdc25 phosphatases is directly regulated by PKA [172, 173].

Because oocyte meiotic arrest is dependent on the interaction between oocyte and follicular somatic cells, cAMP in oocytes may derive from somatic cells through gap junctions which are present between cumulus cells and oocytes. However, cAMP levels remain constant when gap junctions are closed, suggesting that cAMP can be produced by the oocyte. Recent studies found that G-protein coupled receptors (GPCRs), GPR3 and GPR12, are expressed by rodent oocytes and these receptors play a role in maintaining high cAMP levels within oocyte and meiotic arrest. Other intracellular components required for cAMP generation such as Gs protein

and adenylyl cyclases are present in oocytes and are functional [173]. Thus, cAMP may also be generated autonomously by the oocyte at levels sufficient to maintain meiotic arrest.

Cyclic GMP is also important for maintaining meiotic arrest. Increasing cGMP levels in the oocyte maintains meiotic arrest. Phosphodiesterase 3A (PDE3A), an oocyte-specific phosphodiesterase, is crucial for meiotic resumption. This enzyme decreases cAMP levels within oocyte after LH surge, and, initiates pathways leading to meiotic resumption. Before the LH surge, the activity of PDE3A is inhibited by cGMP [174]. Recent studies show that mural granulosa cells express natriuretic peptide precursor type C (NPPC), whereas cumulus cells surrounding oocytes express NPR2, the receptor of NPPC. NPPC can increase cGMP levels in cumulus cells and oocytes in cultured cumulus-oocyte-complexes. And meiotic arrest is not sustained in Graafian follicles of Nppc or Npr2 mutant mice [175]. It is likely that NPPC produced by mural granulosa cells activates NPR2 in cumulus cells, and activated NPR2 increases cGMP levels in cumulus cells. Since NPR2 is not detectable in oocytes, high levels of cGMP within oocytes may be a result of transferring cGMP from cumulus cells to oocytes through gap junctions. High, cGMP levels in oocytes suppress PDE3A activity, resulting in elevated cAMP levels within oocytes that block meiotic progression.

1.2.4.2 Meiotic Resumption Triggered by LH

The preovulatory LH surge triggers a cascade of events in ovarian follicles, including resumption of meiotic maturation, luteinization, expansion or maturation of the cumulus cells, and follicle rupture. The LH receptor belongs to the group of GPCRs that depend on interaction with Gsα and activation of adenylyl cyclase to produce cAMP. It is accepted that cAMP is the primary signal mediating LH stimulation, but numerous signals activate after the LH surge, and, are involved in the resuming oocyte meiosis.

Regulation of meiotic maturation may include mitogen activated protein kinase (MAPK), also termed extracellular regulation kinase (ERK). The most widely studied MAPKs in oocytes are ERK1 and ERK2, which can be phosphorylated by upstream MEK (MAPK kinase). Artificial activation of MAPK re-initiates meiosis. LH-dependent ERK 1/2 is activated downstream of cAMP and dependent on PKA activation. Recent studies show that MAPK in cumulus cells plays a critical role in resumption of oocyte meiosis [176]. Multiple signaling cascades, including PKC, gap junctions, epidermal growth factor (EGF) and endothelin-1 pathways may also mediate ERK 1/2 activation.

Phosphatidylinositol 3-kinase (PI3K) phosphorylates the D-3 position of the inositol ring of phosphatidylinositol to produce phosphatidylinositol-3-phosphate and its analogues. The role of PI3K in resuming meiosis in mouse, rat, porcine and bovine includes regulating oocyte maturation through gap junctions between cumulus cells and oocytes [177]. PI3K may also regulate oocyte maturation by activation of RAS since PI3K related enzyme is crucial for RAS-induced, MPF activation [178].

Mammalian oocytes express few LH receptors and are insensitive to direct LH stimulation [179]. Thus, factors released by somatic follicular cells convey LH

stimuli to oocytes. Recently, the discovery that LH surge activates EGF signaling network has provided new insights on how the LH signal is conveyed from mural granulosa cells to the cumulus-oocyte-complexes to induce oocyte maturation. LH induces a rapid and transient expression of EGF-like factors, amphiregulin (AREG), epiregulin (EREG) and betacellulin (BTC), in somatic cells of preovulatory murine follicles. In vitro culture of follicles with EGF-like factors promotes cumulus expansion and oocyte maturation [180]. EGF-like factor influences LH induction of oocyte maturation in several other species, including pig, horse, monkey and human [181]. In addition to EGF-like factors, insulin-like 3 (INSL3) and endothelin-1 also mediate the actions of LH in controlling GVBD of the oocyte. These factors transmit ovulatory LH signals from theca or granulosa cells to cumulus cells or oocytes via their receptors in a paracrine manner. Binding to their receptors in cumulus cells or oocytes, activates downstream signaling cascades to resume meiosis. EGF-like factors activate EGF receptors on cumulus cells and induce resumption of oocyte meiosis via PI3K and ERK 1/2 signal pathways, probably by disrupting gap junctions [182–184]. LH stimulates INSL3 transcripts in ovarian theca cells and INSL3 binds a GPCR, LGR8 (leucine-rich repeat-containing G protein-coupled receptor 8), expressed in oocyte to activate the inhibitory G protein, thus leading to decreases in cAMP production [185]. Endothelin-1 promotion of GVBD is mediated by cumulus cells through EDNRA, involving MAPK/ERK pathway [186].

Intercellular communications are essential for oocyte responses to the LH surge. Gap junctions are responsible for transmitting signals between somatic cells of the follicle, as well as the cumulus cells and oocytes. Gap junctions consisting of intercellular channels are located at points of very close cell-cell contact. The junctional channel is composed of two end-to-end hemichannels, each of which is a hexamer of connexin subunits [187]. Different types of gap junctions are distinguishable on connexins and permeable to molecules of different sizes and charge. Of the 20 connexins encoded in the mammalian genome, connexin 43 (CX43) and connexin 37 (CX37) play critical roles in oocyte maturation. CX43 is the primary connexin in the follicular somatic cell junctions, by contrast, CX37 is found at the oocyte surface in oocyte-somatic cell gap junctions. LH causes a decrease in the gap junction permeability between the somatic cells, resulting from MAP kinase-dependent phosphorylation of CX43 [188]. CX43 phosphorylation induces gap junction closure sufficient for initiating resumption of meiosis. However, a parallel meiosis-stimulatory mechanism may be present, as the inhibition of MAP kinase activation, which prevents the LH-induced channel closure, does not prevent the meiotic progression in response to LH. Oocytes of CX37 mutant mice fail to undergo GVBD when released from the follicles and granulosa cells prematurely became luteal cells, suggesting CX37 is responsible for transmitting signals from oocyte to granulosa cells to maintain the differentiated status of granulosa cells [187].

1.2.4.3 Cytoplasmic Oocyte Maturation

The processes of oocyte nuclear maturation and cytoplasmic maturation are usually coordinated. However, some oocytes competent to complete nuclear maturation are unable to develop to blastocyst stage, which is indicative of deficient

or defective cytoplasmic maturation [169]. Cytoplasmic maturation involves organelle redistribution and storage of mRNA, proteins and transcription factors which are crucial for completion of meiosis, fertilization and early embryo development [189].

Ultrastructural changes and organelle redistribution occur during oocyte maturation. Cytoskeletal microfilaments and microtubules present in the cytoplasm promote the movements of these organelles and act on chromosome segregation. Ultrastructural examination shows that mitochondria in bovine oocytes move from a peripheral distribution to a more dispersed distribution after LH surge. Upon reaching metaphase II, the mitochondria move to the central area of oocytes and produce ATP necessary for synthesis of proteins supporting oocyte maturation and subsequent embryonic development [189]. Mouse oocytes from small antral follicles, matured in vitro, have reduced number of mitochondria, reduced amounts of ATP and abnormal mitochondrial distributions, suggesting compromised developmental potential [189]. Ca^{2+} oscillation, triggered by a fertilizing spermatozoon, is essential for activation of the embryonic development. The ability to generate Ca^{2+} oscillation develops during the oocyte maturation process and involves several cytoplasmic changes [190]. Endoplasmic reticulum (ER) plays an important role in regulation of Ca^{2+} gradients. Storing and releasing Ca^{2+} are mediated by inositol 1,4,5 tri-phosphate receptor (IP3R), which is located on the ER membrane [190]. Thus, biochemical and structural changes in the ER during maturation are critical for generation of Ca^{2+} oscillation. In GV stage oocytes, the ER is uniformly distributed in the ooplasm. However, following maturation to MII, the ER moves to cortical regions and accumulates in 1~2 μm wide clusters. Morphology of the ER also characteristically changes during oocyte maturation [191]. The exocytosis of cortical granules is an important mechanism used by the oocyte to prevent polyspermy. Cortical granules of GV oocytes are distributed in cluster throughout the cytoplasm. When the oocytes reach MII stage, cortical granules aggregate at the inner surface close to the plasma membrane.

To acquire developmental competence, oocytes complete “molecular maturation” consisting of transcription, storage and processing of maternal mRNA. The ribosomes further translate these mRNAs into proteins involved in maturation, fertilization and embryogenesis. Since global transcriptional silencing in fully grown oocytes is a critical event during mammalian oogenesis, these mRNAs will be stored in an inactive form and sustain the very early stages of preimplantation development through timely translation. Altered expression of maternal genes such as Zar1, Npm2, stella, Smarca4 and Oct4 leads to developmental arrest at the time of zygotic genome activation [192].

1.2.5 Epigenetic Regulation During Oogenesis and Oocyte Maturation

The molecular changes that occur during oogenesis are important for oocytes to acquire its developmental competence. In addition to transcription factor

binding to promoters, regulation of transcription may be achieved through epigenetic mechanisms. Epigenetic mechanisms in oocyte and early embryo include chromatin remodeling, DNA methylation, histone modification and non-coding RNAs [193].

1.2.5.1 Chromatin Remodeling

During mammalian oocyte growth, the nucleus of oocyte arrests at diplotene stage, termed the germinal vesicle (GV), and undergoes chromatin remodeling for control of gene expression. GV oocytes from murine antral follicles divide into two groups according to the chromatin distribution in the nucleus. (I) surrounded nucleolus (SN) oocytes, with rather condensed chromatin surrounding the nucleolus; (II) non-surrounded nucleolus (NSN) oocytes, with more dispersed chromatin not surrounding the nucleolus [194]. Other mammalian GV oocytes, including rats, monkeys, pigs and humans also possess similar SN/NSN chromatin organization.

During oocyte growth and maturation, GV chromatin configuration varies in different species. In mice, oocytes in preantral follicles with a diameter between 10 and 40 μm have NSN configuration. SN oocytes can only be found in antral follicles and the proportion of SN configuration increases as the size of the oocytes becomes larger. SN oocytes are silent during transcription, while NSN oocytes are actively transcribing. Transcriptional repression is associated with meiotic competence in fully grown GV oocytes [195]. The percentage of oocytes that resume meiosis is higher in SN oocytes than in NSN oocytes. After fertilization, NSN oocytes cannot develop beyond the 2-cell stage while a proportion of SN oocytes can develop to blastocyst [196]. Thus, the chromatin configuration is highly related to oocyte developmental competence.

1.2.5.2 DNA Methylation

In mammals, maternal and paternal alleles of most genes are expressed at similar levels, but some genes behave differently depending on their parent of origin. These genes are called imprinted genes and regulated by DNA methylation in differentially methylated region (DMR) during gametogenesis. Up to now, more than 100 imprinted genes are identified in mammals and most of them are maternally imprinted. In female germlines, DNA methylation is erased during the differentiation of primordial germ cells, and de novo DNA methylation initiates asynchronously during the growth phase of diplotene-arrested oocyte [197]. DNA methylation is established in an oocyte size-dependent manner, and the maternal methylation imprints become fully established by the fully grown oocyte stage [198].

DNA methylation occurs at the C5 position of cytosine, mostly within CpG dinucleotides. CpG methylation regulates the expression of imprinted and non-imprinted genes. DNA methyltransferases are a family of enzymes that catalyze the transfer of a methyl group to DNA. Dnmt3a and Dnmt3b are responsible for establishing de novo CpG methylation, while Dnmt1 maintains the methylation pattern during chromosome replication. The activity of Dnmt3a and Dnmt3b is catalyzed by a related protein, Dnmt3L. Dnmt3L is highly expressed in germ cells and forms a complex with Dnmt3a and Dnmt3b [197]. Although Dnmt3b is dispensable for

the establishment of maternal imprints, Dnmt3a and Dnmt3L are both necessary to establish maternal imprints in growing oocytes. Dnmt1o, the oocyte-specific isoform of Dnmt1s methyltransferase, is produced in oocytes and maintains the CpG methylation in oocytes and preimplantation embryos [192].

1.2.5.3 Histone Modification

In addition to DNA methylation, histone modification plays an important role in controlling gene expression in gametes and early embryos. Nucleosome is the fundamental building component of chromatin and it is composed of 147 base pairs of genomic DNA and an octamer of two subunits of each of the core histones H2A, H2B, H3 and H4. The amino-terminal portion of the histone protein contains a flexible and highly-basic tail region, which is subject to various post-translational modifications, including acetylation, phosphorylation, methylation, ADP-ribosylation, ubiquitination, sumoylation, biotinylation and proline isomerization [199].

Histone acetylation is associated with enhanced transcriptional activity, whereas histone deacetylation is correlated with repression of gene expression. Acetylation of H3 and H4 is more extensively studied than H2A and H2B. The level of acetylation on histone H3 and H4 increases during oocyte growth and generally all the lysine residues are acetylated in fully grown GV oocytes. However, with the resumption of meiosis, deacetylation will take place in several lysine residues and reaches its peak in MII oocytes. Histone (de)acetylation is related to chromatin remodeling during oocyte growth and is necessary for the binding of a chromatin remodeling protein to the centromeric heterochromatin, an essential step for the correct alignment of the chromosomes [192].

Although all core histones contain phosphor-acceptor sites, the phosphorylation of serine 10 and 28 residue on histone H3 (H3/Ser10ph and H3/Ser28ph) is the most extensively characterized. However, studies on distribution and expression of H3/Ser10ph and H3/Ser28ph during oocyte maturation are discordant. The phosphorylation level of both H3/Ser10ph and H3/Ser28ph increases as oocytes proceed to MI stage, but the distribution pattern are different between them. Although phosphorylation of H3/Ser10ph correlates with chromosome condensation in mitotic cells, recent studies demonstrate that there are no relationships between H3/Ser10ph phosphorylation and chromosome condensation. However, H3/Ser28ph may be associated with chromosome condensation in oocytes [199].

In contrast to acetylation and phosphorylation, histone methylation is relatively stable during oocyte maturation. The main methylation sites are the basic amino acid side chains of lysine (K) and arginine (R) residues. Histone methylation may contribute to establishment and maintenance of an imprinted pattern of gene expression together with DNA methylation [192, 199].

1.2.5.4 Small RNAs Regulation of Oocyte Maturation

From the fully grown oocyte stage until zygotic genome activation (ZGA), the genome is transcriptionally silent. During this period, all mRNA regulation must occur post-transcriptionally. Transcripts expressed by oocytes will support its

maturation, fertilization and early stages of embryonic development [200]. By MII stage, more than half of the mRNA stored in oocytes will be degraded. Small, non-coding RNAs are implicated in the elimination of maternal mRNAs [201].

Small, non-coding RNAs range in size from 18 to 32 nucleotides (nt) in length and play a critical role in post-transcriptional regulation. Three major classes of small, non-coding RNAs have been identified in mammals: microRNAs (miRNAs), endogenous small interfering (endo-siRNAs) and Piwi-interacting RNAs (piRNAs) [34]. There are two subclasses of miRNAs, canonical and non-canonical miRNAs. Canonical miRNAs will be processed by Drosha-Dgcr8 complex to form pre-miRNAs transported from the nucleus into cytoplasm while non-canonical miRNAs can bypass the Drosha-Dgcr8 step [202]. Both miRNAs and endo-siRNAs involve RNase III enzyme Dicer processing with Dicer products being assembled into ribonucleoprotein complexes called RNA-induced silencing complexes (RISC). RISC binds the target RNA and silences gene expression by cleaving the target RNA [33]. The key components of RISC are proteins of Argonaute (Ago) family. In mammals, four Ago proteins function in miRNA repression but only Ago2 functions in siRNA repression. Ago2 is maternally expressed and plays an essential role in degradation of maternal mRNAs. In contrast, piRNAs don't require Dicer processing and are expressed predominantly in germ lines in mammals. They are able to interact with the piwi proteins, a distinct family of Argonaute family [202].

Previous studies demonstrate that miRNAs, such as Let-7, Mir22, Mir16-1 and Mir29, are present in oocytes. Furthermore, mRNA profiling and bioinformatic analyses show that many targets for the expressed miRNAs are also present in oocytes. However, there is little miRNA function in mature oocytes [203] and the mRNA levels of oocytes from Dgcr8 mutant mice are unchanged [204]. These results suggest that miRNA function is downregulated during oocyte development. In contrast, endo-siRNAs may play an essential role in oocyte meiosis. Dicer loss in oocytes show hundreds of misregulated transcripts and results in meiosis arrest with abnormal spindles and severe chromosome congression defects [205]. Ago2-deficient oocytes have similar phenotypes [206]. These results suggest a role for endo-siRNA in regulating oocyte meiosis. piRNAs have been identified in growing oocytes, but deletion of Piwi proteins have no oocyte phenotype [202]. The role of piRNAs in oogenesis remains to be defined.

1.3 Fertilization

Fertilization is the union of a humanoid egg and sperm, that usually occurs in the ampulla of the uterine tube. Fertilization is more a chain of events than a single, isolated phenomenon. Indeed, interruption of any step in the chain will almost certainly cause fertilization failure. Successful fertilization requires not only that a sperm and egg fuse, but that only one sperm fuses with the egg. Fertilization by more than one sperm, polyspermy, almost inevitably leads to early embryonic death. The eggs of many species adopt different strategies to avoid polyspermy.

1.3.1 Sperm Capacitation

Mammalian sperm are unable to fertilize eggs immediately after ejaculation. They acquire fertilization capacity after residing in the female tract for a finite period of time that varies depending on the species. The sperm of many mammals, including humans, can also be capacitated by incubation in certain fertilization media. Molecular events implicated in the initiation of capacitation include lipid rearrangements in the sperm plasma membrane, ion fluxes resulting in alteration of sperm membrane potential [207], and increased tyrosine (tyr) phosphorylation [208] of proteins involved in induction of hyperactivation and the acrosome reaction. Sperm that have undergone capacitation are said to become hyperactivated, and, display hyperactivated motility. Most importantly however, capacitation appears to destabilize the sperm's membrane to prepare it for the acrosome reaction.

1.3.2 Sperm-Zona Pellucida Binding

The egg is encased within a zona and surrounded by layers of cells of the cumulus. Sperm must penetrate these vestments to fuse with the egg. The cumulus acts as a selective barrier, arresting both uncapacitated sperm as well as sperm that have completed the acrosome reaction, while permitting capacitated, acrosome-intact sperm to penetrate and engage in the later steps of fertilization [209]. Exposure of sperm to either cumulus cells or to cumulus-conditioned medium increase the duration of motility, the forward velocity, and the force generated by flagellar beating. Binding of sperm to the zona pellucida is a receptor-ligand interaction with a high degree of species specificity [210]. The carbohydrate groups on the zona pellucida glycoproteins function as sperm receptors. The sperm molecule that binds this receptor is not known with certainty, and indeed, there may be several proteins that can serve this function. In addition, cumulus cells may release a sperm chemo-attractant [211]. To reach the egg plasma membrane, sperm adhere to the outer edge of the zona, undergo acrosomal exocytosis that is regulated by the zona, and then move into, and through, the zona matrix.

1.3.3 The Acrosome Reaction

The acrosome is a Golgi-derived, exocytotic organelle that covers the tip of the sperm head. Acrosomal exocytosis, the so-called acrosome reaction, happens only in capacitated sperm and is a prerequisite for a sperm to fuse with an egg [212]. The acrosome reaction provides the sperm with an enzymatic “drill” to get through the zona pellucida. The same zona pellucida protein that serves as a sperm receptor also stimulates a series of events that lead to many areas of fusion between the plasma membrane and outer acrosomal membrane. Membrane fusion (actually an

exocytosis) and vesiculation expose the acrosomal contents, leading to leakage of acrosomal enzymes from the sperm's head [213].

The mechanism of the acrosome reaction itself has been well characterized. In brief, transient calcium influx leads to activation of phospholipase C (PLC), and activated PLC generates IP3 and diacylglycerol (DAG) from PIP2. IP3 releases calcium from intracellular stores, and DAG mediates PKC activation and phosphorylation of substrate proteins [214]. These early events promote a subsequent calcium influx via transient receptor potential cation channels (TRPCs), which induces the complete acrosome reaction. Disruption of Plcd4 impairs the in vitro ZP-induced acrosome reaction, while the Ca^{2+} ionophore A23187-induced acrosome reaction occurs normally [215].

Sperm that lose their acrosomes before encountering the oocyte are unable to bind to the zona pellucida and thereby unable to fertilize. Assessment of acrosomal integrity of ejaculated sperm is commonly used in semen analysis.

1.3.4 Penetration of the Zona Pellucida

After passing through the cumulus oophorus, sperm encounter the ZP, their last hurdle before meeting the egg. The major components of the ZP are three glycosylated proteins, Zp1, Zp2, and Zp3. Zp3 functions as the primary sperm receptor and can induce the acrosome reaction [216].

The ZP not only functions as a receptor for sperm but also acts as a species-specific barrier. One study replaced mouse Zp2 and Zp3 with their human homologs and examined the fertilization potential of eggs surrounded with a mouse Zp1/human ZP2/human ZP3 chimeric ZP [217]. Mouse sperm, but not human sperm, were able to bind to the chimeric ZP and fertilize the eggs. As Zp3 is thought to be the primary sperm receptor, these results suggest that oligosaccharides attached to the ZP proteins, rather than the peptide sequences themselves, are critical for species-specific sperm binding.

Five knockout mouse strains (Clgn-, Ace-, Adam1a-, Adam2-, and Adam3-knockout mice) have sperms that show defective sperm-ZP binding [218–222]. And sperm share another notable phenotype: they are unable to migrate into the oviduct. This suggests that oviduct migration and ZP binding might share a common mechanism.

1.3.5 Sperm-Oocyte Binding

After penetration of the ZP, sperm immediately meet and fuse with the egg plasma membrane. Once a sperm penetrates the zona pellucida, it binds to and fuses with the plasma membrane of the oocyte. Binding occurs at the posterior (post-acrosomal) region of the sperm head.

The molecular nature of sperm-oocyte binding is not completely resolved. A leading candidate in some species is a dimeric sperm glycoprotein called

fertilin, which binds to a protein in the oocyte plasma membrane and may also induce fusion. Interestingly, humans and apes have inactivating mutations in the gene encoding one of the subunits of fertilin, suggesting that they use a different molecule to bind oocytes. Izumo1$^{-/-}$ male mice are completely sterile, even though the mutant sperm can penetrate the ZP and contact the egg plasma membrane. When the fusion step was bypassed by intracytoplasmic sperm injection into unfertilized eggs, Izumo1$^{-/-}$ sperm activated eggs, and the fertilized eggs developed to term normally when transferred to the uterus of female mice. Therefore, the Izumo1 protein is essential for sperm-egg fusion [223]. And the Cd9-knockout mouse shows a defect restricted to eggs, in which the protein was found to be essential for sperm-egg fusion [224]. If these proteins do indeed interact, it is likely that they both require associating proteins on the sperm and egg cell surface, and the identity of these putative factors is being intensively investigated.

1.3.6 Egg Activation and the Cortical Reaction

Prior to fertilization, the egg is in a quiescent state, arrested in metaphase of the second meiotic division. The transition of an unfertilized, MII-arrested egg into a developing embryo requires completion of a sequence of events known as egg "activation". These events include cortical granule (CG) exocytosis, modifications of the zona pellucida and plasma membrane that prevent polyspermy, completion of meiosis, recruitment of maternal mRNAs into polysomes for translation, and formation of male and female pronuclei [225].

Upon binding of a sperm, the egg rapidly undergoes a number of metabolic and physical changes. Prominent effects include a rise in the intracellular concentration of calcium, completion of the second meiotic division and the so-called cortical reaction.

The cortical reaction refers to a massive exocytosis of cortical granules seen shortly after sperm-oocyte fusion [226]. Cortical granules contain a mixture of enzymes, including several proteases, which diffuse into the zona pellucida following exocytosis from the egg. These proteases alter the structure of the zona pellucida, inducing what is known as the zona reaction. Components of cortical granules may also interact with the oocyte plasma membrane.

1.3.7 The Zona Reaction

The zona reaction refers to an alteration in the structure of the zona pellucida catalyzed by proteases from cortical granules. The critical importance of the zona reaction is that it represents the major block to polyspermy in most mammals [227]. This effect is the result of hardening of the zona pellucida. "Runner-up" sperm that have not finished traversing the zona pellucida by the time the hardening occurs, cannot proceed further.

1.3.8 Post-fertilization Events

Following fusion of the fertilizing sperm with the oocyte, the sperm head incorporates into the egg cytoplasm. The nuclear envelope of the sperm disperses, and the chromatin rapidly loosens from its tightly packed state in a process called de-condensation. In vertebrates, other sperm components, including mitochondria, are degraded rather than incorporated into the embryo.

Chromatin from both the sperm and egg become encapsulated in a nuclear membrane, forming pronuclei. Each pronucleus contains a haploid genome [228]. They migrate together, their membranes break down, and the two genomes condense into chromosomes, thereby reconstituting a diploid organism.

References

1. Hess RA, Franca LR. History of the Sertoli cell discovery. In: Griswold M, Skinner M, editors. Sertoli cell biology. New York: Academic; 2005. p. 3–14.
2. Russell LD, Etllin RA, Sinha Hikim AP, et al. Histological and histopathological evaluation of the testis. Clear Water: Cache River Press; 1990. p. 1–40.
3. Sharpe RM. Regulation of spermatogenesis. In: Knobil E, Neil JD, editors. The physiology of reproduction. Raven Press: New York; 1994. p. 1363–434.
4. Kerr JB. Functional cytology of the human testis. Bailliere's Clin Endocrinol Metab. 1992;6:235–50.
5. Heller CG, Clermont Y. Kinetics of the germinal epithelium in man. Recent Prog Horm Res. 1964;20:545–75.
6. Hess RA. Quantitative and qualitative characteristics of the stages and transitions in the cycle of the rat seminiferous epithelium: light microscopic observations of perfusion-fixed and plastic-embedded testes. Biol Reprod. 1990;43:525–42.
7. Leblond CP, Clermont Y. Spermiogenesis of rat, mouse, hamster and guinea pig as revealed by the "periodic acid-fuchsin sulfurous acid" technique. Am J Anat. 1952;90: 167–215.
8. Hess RA, Miller LA, Kirby JD, et al. Immunoelectron microscopic localization of testicular and somatic cytochromes c in the seminiferous epithelium of the rat. Biol Reprod. 1993;48:1299–308.
9. Franca LR, Ye SJ, Ying L, et al. Morphometry of rat germ cells during spermatogenesis. Anat Rec. 1995;241:181–204.
10. Franca LR, Avelar GF, Almeida FF. Spermatogenesis and sperm transit through the epididymis in mammals with emphasis on pigs. Theriogenology. 2005;63:300–18.
11. Franca LR, Russell LD. The testis of domestic mammals. In: Martinez-Garcia F, Regadera J, editors. Male reproduction: a multidisciplinary overview. Madrid: Churchill Communications Europe Espa; 1998. p. 197–219.
12. Hess RA, Schaeffer DJ, Eroschenko VP, et al. Frequency of the stages in the cycle of the seminiferous epithelium in the rat. Biol Reprod. 1990;43:517–24.
13. Franca LR, Ogawa T, Avarbock MR, et al. Germ cell genotype controls cell cycle during spermatogenesis in the rat. Biol Reprod. 1998;59:1371–7.
14. Zeng W, Avelar GF, Rathi R, et al. The length of the spermatogenic cycle is conserved in porcine and ovine testis xenografts. J Androl. 2006;27:527–33.
15. Hess RA, Chen P. Computer tracking of germ cells in the cycle of the seminiferous epithelium and prediction of changes in cycle duration in animals commonly used in reproductive biology and toxicology. J Androl. 1992;13:185–90.

16. Creasy DM. Evaluation of testicular toxicity in safety evaluation studies: the appropriate use of spermatogenic staging. Toxicol Pathol. 1997;25:119–31.
17. Creasy DM. Evaluation of testicular toxicology: a synopsis and discussion of the recommendations proposed by the Society of Toxicologic Pathology. Birth Defects Res B Dev Reprod Toxicol. 2003;68:408–15.
18. Liu Y, Nusrat A, Schnell FJ, et al. Human junction adhesion molecule regulates tight junction resealing in epithelia. J Cell Sci. 2000;113:2363–74.
19. Vilela DAR, Silva SGB, Peixoto MTD, et al. Spermatogenesis in teleost; Insights from the Nile tilapia (*Oreochromis niloticus*) model. Fish Physiol Biochem. 2003;28:187–90.
20. Russell LD, Chiarini-Garcia H, Korsmeyer SJ, et al. Bax-dependent spermatogonia apoptosis is required for testicular development and spermatogenesis. Biol Reprod. 2002;66:950–8.
21. Johnson L, Chaturvedi PK, Williams JD. Missing generations of spermatocytes and spermatids in seminiferous epithelium contribute to low efficiency of spermatogenesis in humans. Biol Reprod. 1992;47:1091–8.
22. Saez JM. Leydig cells: endocrine, paracrine, and autocrine regulation. Endocr Rev. 1994;5: 574–611.
23. Huhtaniemi I, Toppari J. Endocrine, paracrine and autocrine regulation of testicular steroidogenesis. Adv Exp Med Biol. 1995;377:33–54.
24. Jégou B, Pineau C. Current aspects of autocrine and paracrine regulation of spermatogenesis. Adv Exp Med Biol. 1995;377:67–86.
25. Weinbauer GF, Gromoll J, Simoni M, et al. Physiology of testicular function. In: Nieschlag E, Behre H, editors. Andrology. Berlin/Heidelberg: Springer; 1997. p. 23–57.
26. Griffin JE, Wilson JD. Disorders of the testis and the male reproductive tract. In: Wilson JD, Foster DW, Kronenberg HM, Larsen PR, editors. Williams textbook of endocrinology. Philadelphia: Saunders; 1998. p. 19–875.
27. Yu WH, Karanth S, Walczewska A, et al. A hypothalamic follicle-stimulating hormone-releasing decapeptide in the rat. Proc Natl Acad Sci U S A. 1997;94:9499–503.
28. Hotchkiss J, Knobil E. The hypothalamic pulse generator: the reproductive core. In: Adashi EY, Rock JA, Rosenwaks Z, editors. Reproductive endocrinology, surgery, and technology. Philadelphia: Lippincott-Raven; 1996. p. 123–62.
29. Thorner MO, Vance ML, Laws Jr ER, et al. The anterior pituitary. In: Wilson JD, Foster DW, Kronenberg HM, Larsen PR, editors. Williams textbook of endocrinology. Philadelphia: Saunders; 1998. p. 249–340.
30. Rao CV. The beginning of a new era in reproductive biology and medicine: expression of low levels of functional luteinizing hormone/human chorionic gonadotropin receptors in nongonadal tissues. J Physiol Pharmacol. 1996;47:41–53.
31. Orth JM. The role of follicle-stimulating hormone in controlling Sertoli cell proliferation in testes of fetal rats. Endocrinology. 1984;115:1248–55.
32. Burger HG, Robertson DM. Editorial: inhibin in the male – progress at last. J Clin Endocr Metab. 1997;138:1361–2.
33. Evans WS, Griffin ML, Yankov VI. The pituitary gonadotroph: dyna111ics of gonadotropin release. In: Adashi EY, Rock JA, Rosenwaks Z, editors. Reproductive endocrinology, surgery, and technology. Philadelphia: Lippincott-Raven; 1996. p. 181–210.
34. Russell LD, Griswold MD, editors. The Sertoli cell. Clearwater: Cache River Press; 1993.
35. De Gendt K, Atanassova N, Tan KA, et al. Development and function of the adult generation of Leydig cells in mice with Sertoli cell-selective or total ablation of the androgen receptor. Endocrinology. 2005;146:4117–26.
36. Rowley MJ, Teshima F, Heller CG. Duration of transit of spermatozoa through the human male ductular system. Fertil Steril. 1970;21:390–6.
37. Amann RP, Howards SS. Daily spermatozoal production and epididymal spermatozoal reserves of the human male. J Urol. 1980;124:211–15.
38. Johnson L, Varner DD. Effect of daily sperm production but not age on transit time of spermatozoa through the human epididymis. Biol Reprod. 1988;39:812–17.

39. Turner TT, Reich GW. Cauda epididymal sperm motility: a comparison among five species. Biol Reprod. 1985;32:120–8.
40. Smithwick EB, Gould KG, Young LG. Estimate of epididymal transit time in the chimpanzee. Tissue Cell. 1996;28:485–93.
41. Soler C, Pérez-Sánchez F, Schulze H, et al. Objective evaluation of the morphology of human epididymal sperm heads. Int J Androl. 2000;23:77–84.
42. Yeung CH, Perez-Sanchez F, Soler C, et al. Maturation of human spermatozoa (from selected epididymides of prostatic carcinoma patients) with respect to their morphology and ability to undergo the acrosome reaction. Hum Reprod Update. 1997;3:205–13.
43. Gago C, Soler C, Perez-Sanchez F, et al. Effect of cetrorelix on sperm morphology during migration through the epididymis in the cynomolgus macaque (*Macaca fascicularis*). Am J Primatol. 2000;51:103–17.
44. Ludwig G, Frick J. Spermatology: atlas and manual. Berlin: Springer; 1987.
45. Cooper TG, Yeung CH, Jones R, et al. Rebuttal of a role for the epididymis in sperm quality control by phagocytosis of defective sperm. J Cell Sci. 2002;115:5–7.
46. Sutovsky P, Moreno R, Ramalho-Santos J, et al. A putative, ubiquitin-dependent mechanism for the recognition and elimination of defective spermatozoa in the mammalian epididymis. J Cell Sci. 2001;114:1665–75.
47. Bedford JM, Calvin H, Cooper GW. The maturation of spermatozoa in the human epididymis. J Reprod Fertil. 1973;l18:199–213.
48. Hinton BT, Pryor JP, Hirsch AV, et al. The concentration of some inorganic ions and organic compounds in the luminal fluid of the human ductus deferens. Int J Androl. 1981;4:457–61.
49. Haidl G, Badura B, Hinsch K-D, et al. Disturbances of sperm flagella due to failure of epididymal maturation and their possible relationship to phospholipids. Hum Reprod. 1993;8:1070–3.
50. Cooper TG, Yeung CH, Fetic S, et al. Cytoplasmic droplets are nor-mal structures of human sperm but are not well preserved by routine procedures for assessing sperm morphology. Hum Reprod. 2004;19:2283–8.
51. Holstein AF, Roosen-Runge EC. In: Holstein AF, Roosen-Runge EC, editors. Atlas of human spermatogenesis. Berlin: Grosse Verlag; 1981.
52. Cooper TG, Raczek S, Yeung CH, et al. Composition of fluids obtained from human epididymal cysts. Urol Res. 1992;20:275–80.
53. Bedford JM, Berrios M, Dryden GL. Biology of the scrotum. Testis location and temperature sensitivity. J Exp Zool. 1982;224:379–88.
54. Kato S, Yasui T, Nano I, et al. Migration of cytoplasmic droplet in boar spermatozoa by centrifugation. Jpn J Anim AI Res. 1984;6:15–8.
55. Kato S, Yasui T, Kanda S. Migration of cytoplasmic droplet in goat testicular spermatozoa by centrifugation. Jpn J Anim Reprod. 1983;29:214–16.
56. Carr DW, Usselman MC, Acott TS. Effects of pH, lactate, and viscoelastic drag on sperm motility: a species comparison. Biol Reprod. 1985;33:588–95.
57. Yeung CH, Cooper TG, Oberpenning F, et al. Changes in movement characteristics of human spermatozoa along the length of the epididymis. Biol Reprod. 1993;49:274–80.
58. Belonoschkin B. Biologie der Spermatozoe n im menschlichen Hoden und Nebenhoden. Arch M Gynakoel. 1943;174:357–68.
59. Jow WW, Steckel J, Schlegel PN, et al. Motile sperm in human testis biopsy specimens. J Androl. 1993;14:194–8.
60. Emiliani S, Van den Bergh M, Vannin AS, et al. Increased sperm motility after in-vitro culture of testicular biopsies from obstructive azoospermic patients results in better post-thaw recovery rate. Hum Reprod. 2000;15:2371–4.
61. Mooney JK, Horan AH, Lattimer JK. Motility of spermatozoa in the human epididymis. J Urol. 1972;108:443–5.
62. Mathieu C, Guerin JF, Cognat M, et al. Motility and fertilizing capacity of epididymal human spermatozoa in normal and pathological cases. Fertil Steril. 1992;57:871–6.

63. Bedford JM. Sperm dynamics in the epididymis. In: Asch RA, Balmaceda JP, Johnston I, editors. Gamete physiology. Norwell: Serono Symposia; 1990. p. 53–67.
64. Schoysman RJ, Bedford JM. The role of the human epididymis in sperm maturation and sperm storage as reflected in the consequences of epididymovasostomy. Fertil Steril. 1986;l46:293–9.
65. Cooper TG, Keck C, Oberdieck U, et al. Effects of multiple ejaculations after extended periods of sexual abstinence on total, motile and normal sperm numbers, as well as accessory gland secretions, from healthy normal and oligozoospermic men. Hum Reprod. 1993;8:1251–8.
66. Barratt CLR, Cooke ID. Sperm loss in the urine of sexually rested men. Int J Androl. 1988;11:201–7.
67. Bourgeon F, Evrard B, Brillard-Bourdet M, et al. Involvement of semenogelin-derived peptides in the antibacterial activity of human seminal plasma. Biol Reprod. 2004;70:768–74.
68. Com E, Bourgeon F, Evrard B, et al. Expression of antimicrobial defensins in the male reproductive tract of rats, mice, and humans. Biol Reprod. 2003;68:95–104.
69. Yang D, Biragyn A, Kwak LW, et al. Mammalian defensins in immunity: more than just microbicidal. Trend Immunol. 2002;23:291–6.
70. Yeung CH, Cooper TG, DeGeyter M, et al. Studies on the origin of redox enzymes in seminal plasma and their relationship with results of in-vitro fertilisation. Mol Hum Reprod. 1998;4:835–9.
71. Perey B, Clermont Y, Leblond CP. The wave of the seminiferous epithelium in the rat. Am J Anat. 1961;108:47–77.
72. Egger G, Liang G, Aparicio A, et al. Epigenetics in human disease and prospects for epigenetic therapy. Nature. 2004;429:457–63.
73. Jenkins TG, Carrell DT. The sperm epigenome and potential implications for the developing embryo. Reproduction. 2012;143:727–34.
74. Orsi GA, Couble P, Loppin B. Epigenetic and replacement roles of histone variant H3.3 in reproduction and development. Int J Dev Biol. 2009;53:231–43.
75. Strahl BD, Allis CD. The language of covalent histone modifications. Nature. 2000;403: 41–5.
76. Hammoud SS, Nix DA, Zhang H, et al. Distinctive chromatin in human sperm packages genes for embryo development. Nature. 2009;460:473–8.
77. Portela A, Esteller M. Epigenetic modifications and human disease. Nat Biotech. 2010;28: 1057–68.
78. Reik W. Stability and flexibility of epigenetic gene regulation in mammalian development. Nature. 2007;447:425–32.
79. Lucifero D, Chaillet JR, Trasler JM. Potential significance of genomic imprinting defects for reproduction and assisted reproductive technology. Hum Reprod Update. 2004;10:3–18.
80. Shiota K. DNA methylation profiles of CpG islands for cellular differentiation and development in mammals. Cytogenet Genome Res. 2004;105:325–34.
81. Kafri T, Gao X, Razin A. Mechanistic aspects of genome-wide demethylation in the pre-implantation mouse embryo. Proc Natl Acad Sci U S A. 1993;90:10558–62.
82. Brandeis M, Ariel M, Cedar H. Dynamics of DNA methylation during development. BioEssays. 1993;15:709–13.
83. Chaillet JR, Vogt TF, Beier DR, et al. Parental-specific methylation of an imprinted transgene is established during gametogenesis and progressively changes during embryogenesis. Cell. 1991;66:77–83.
84. Kafri T, Ariel M, Brandeis M, et al. Developmental pattern of gene-specific DNA methylation in the mouse embryo and germ line. Genes Dev. 1992;6:705–14.
85. Li E. Chromatin modification and epigenetic reprogramming in mammalian development. Nat Rev Genet. 2002;3:662–73.
86. Hajkova P, Erhardt S, Lane N, et al. Epigenetic reprogramming in mouse primordial germ cells. Mech Dev. 2002;117:15–23.
87. Lane N, Dean W, Erhardt S, et al. Resistance of IAPs to methylation reprogramming may provide a mechanism for epigenetic inheritance in the mouse. Genesis. 2003;35:88–93.

88. Szabo PE, Mann JR. Biallelic expression of imprinted genes in the mouse germ line: implications for erasure, establishment, and mechanisms of genomic imprinting. Genes Dev. 1995;9: 1857–68.
89. Szabo PE, Hubner K, Scholer H, et al. Allele-specific expression of imprinted genes in mouse migratory primordial germ cells. Mech Dev. 2002;115:157–60.
90. Gatewood JM, Cook GR, Balhorn R, et al. Isolation of four core histones from human sperm chromatin representing a minor subset of somatic histones. J Biol Chem. 1990;265:20662–6.
91. Okada Y, Scott G, Ray MK, et al. Histone demethylase JHDM2A is critical for Tnp1 and Prm1 transcription and spermatogenesis. Nature. 2007;450:119–23.
92. Millar MR, Sharpe RM, Maguire SM, et al. Localization of mRNAs by in-situ hybridization to the residual body at stages IX-X of the cycle of the rat seminiferous epithelium: fact or artefact? Int J Androl. 1994;17:149–60.
93. Ostermeier GC, Dix DJ, Miller D, et al. Spermatozoal RNA profiles of normal fertile men. Lancet. 2002;360:772–7.
94. Rassoulzadegan M, Grandjean V, Gounon P, et al. RNA-mediated non-Mendelian inheritance of an epigenetic change in the mouse. Nature. 2006;441:469–74.
95. Lambard S, Galeraud-Denis I, Martin G, et al. Analysis and significance of mRNA in human ejaculated sperm from normozoospermic donors: relationship to sperm motility and capacitation. Mol Hum Reprod. 2004;10:535–41.
96. Boissonnas CC, Abdalaoui HE, Haelewyn V, et al. Specific epigenetic alterations of IGF2-H19 locus in spermatozoa from infertile men. Eur J Hum Genet. 2010;18:73–80.
97. Nicholas CR, Chavez SL, Baker VL, et al. Instructing an embryonic stem cell-derived oocyte fate: lessons from endogenous oogenesis. Endocr Rev. 2009;30:264–83.
98. Reik W, Dean W, Walter J. Epigenetic reprogramming in mammalian development. Science. 2001;293:1089–93.
99. Rousseaux S, Khochbin S. Epigenetic reprogramming associated with primordial germ cell development. Epigenetics and human reproduction. Berlin/Heidelberg: Springer; 2011. p. 99–117.
100. Ohinata Y, Payer B, O'Carroll D, et al. Blimp1 is a critical determinant of the germ cell lineage in mice. Nature. 2005;436:207–13.
101. Schoenwolf GC, Bleyl SB, Brauer PR, et al. Ganetogenesis, fertilization, and first week. Larsen's human embryology. 4th ed. Philadelphia: Churchill Livingstone; 2009. p. 15–50.
102. Ara T, Nakamura Y, Egawa T, et al. Impaired colonization of the gonads by primordial germ cells in mice lacking a chemokine, stromal cell-derived factor-1 (SDF-1). Proc Natl Acad Sci U S A. 2003;100:5319–23.
103. Anderson R, Fassler R, Georges-Labouesse E, et al. Mouse primordial germ cells lacking beta1 integrins enter the germline but fail to migrate normally to the gonads. Development. 1999;126:1655–64.
104. Bendel-Stenzel MR, Gomperts M, Anderson R, et al. The role of cadherins during primordial germ cell migration and early gonad formation in the mouse. Mech Dev. 2000;91:143–52.
105. Juneja SC, Barr KJ, Enders GC, et al. Defects in the germ line and gonads of mice lacking connexin43. Biol Reprod. 1999;60:1263–70.
106. Kota SK, Feil R. Epigenetic transitions in germ cell development and meiosis. Dev Cell. 2010;19(5):675–86.
107. Pan Z, Zhang J, Li Q, et al. Current advances in epigenetic modification and alteration during mammalian ovarian folliculogenesis. J Genet Genomics. 2012;39(3):111–23.
108. Seki Y, Hayashi K, Itoh K, et al. Extensive and orderly reprogramming of genome-wide chromatin modifications associated with specification and early development of germ cells in mice. Dev Biol. 2005;278(2):440–58.
109. Paoloni-Giacobino A. Epigenetics in reproductive medicine. Pediatr Res. 2007;61(5 Pt 2): 51R–7.
110. Yabuta Y, Kurimoto K, Ohinata Y, et al. Gene expression dynamics during germline specification in mice identified by quantitative single-cell gene expression profiling. Biol Reprod. 2006;75(5):705–16.

111. Seki Y, Yamaji M, Yabuta Y, et al. Cellular dynamics associated with the genome-wide epigenetic reprogramming in migrating primordial germ cells in mice. Development. 2007; 134:2627–38.
112. Peters AH, Kubicek S, Mechtler K, et al. Partitioning and plasticity of repressive histone methylation states in mammalian chromatin. Mol Cell. 2003;12:1577–89.
113. Matsui Y, Zsebo K, Hogan BL. Derivation of pluripotential embryonic stem cells from murine primordial germ cells in culture. Cell. 1992;70:841–7.
114. Tam PP, Zhou SX, Tan SS. X-chromosome activity of the mouse primordial germ cells revealed by the expression of an X-linked lacZ transgene. Development. 1994;120:2925–32.
115. Raz E. Primordial germ-cell development: the zebrafish perspective. Nat Rev Genet. 2003;4: 690–700.
116. Surani MA. Reprogramming of genome function through epigenetic inheritance. Nature. 2001;414:122–8.
117. Yamazaki Y, Low EW, Marikawa Y, et al. Adult mice cloned from migrating primordial germ cells. Proc Natl Acad Sci U S A. 2005;102:11361–6.
118. Sato S, Yoshimizu T, Sato E, et al. Erasure of methylation imprinting of Igf2r during mouse primordial germ-cell development. Mol Reprod Dev. 2003;65:41–50.
119. Hajkova P, Erhardt S, Lane N, et al. Epigenetic reprogramming in mouse primordial germ cells. Mech Dev. 2002;117:15–23.
120. Popp C, Dean W, Feng S, et al. Genome-wide erasure of DNA methylation in mouse primordial germ cells is affected by AID deficiency. Nature. 2010;463:1101–5.
121. Chen T, Ueda Y, Dodge JE, et al. Establishment and maintenance of genomic methylation patterns in mouse embryonic stem cells by Dnmt3a and Dnmt3b. Mol Cell Biol. 2003;23: 5594–605.
122. Lynn A, Ashley T, Hassold T. Variation in human meiotic recombination. Annu Rev Genomics Hum Genet. 2004;5:317–49.
123. Strich R. Meiotic DNA, replication. Curr Top Dev Biol. 2004;61:29–60.
124. Maheshwari A, Fowler PA. Primordial follicular assembly in humans–revisited. Zygote. 2008;16:285–96.
125. Oktem O, Urman B. Understanding follicle growth in vivo. Hum Reprod. 2010;25:2944–54.
126. Abir R, Orvieto R, Dicker D, et al. Preliminary studies on apoptosis in human fetal ovaries. Fertil Steril. 2002;78:259–64.
127. Oktay K, Briggs D, Gosden RG. Ontogeny of follicle-stimulating hormone receptor gene expression in isolated human ovarian follicles. J Clin Endocrinol Metab. 1997;82:3748–51.
128. Rajareddy S, Reddy P, Du C, et al. p27kip1 (cyclin-dependent kinase inhibitor 1B) controls ovarian development by suppressing follicle endowment and activation and promoting follicle atresia in mice. Mol Endocrinol. 2007;21:2189–202.
129. Reddy P, Liu L, Adhikari D, et al. Oocyte-specific deletion of Pten causes premature activation of the primordial follicle pool. Science. 2008;319:611–13.
130. Adhikari D, Zheng W, Shen Y, et al. Tsc/mTORC1 signaling in oocytes governs the quiescence and activation of primordial follicles. Hum Mol Genet. 2010;19:397–410.
131. De Baere E, Beysen D, Oley C, et al. FOXL2 and BPES: mutational hotspots, phenotypic variability, and revision of the genotype-phenotype correlation. Am J Hum Genet. 2003;72: 478–87.
132. Castrillon DH, Miao L, Kollipara R, et al. Suppression of ovarian follicle activation in mice by the transcription factor Foxo3a. Science. 2003;301:215–18.
133. Gallardo TD, John GB, Bradshaw K, et al. Sequence variation at the human FOXO3 locus: a study of premature ovarian failure and primary amenorrhea. Hum Reprod. 2008;23: 216–21.
134. Rajpert-De ME, Jorgensen N, Graem N, et al. Expression of anti-Mullerian hormone during normal and pathological gonadal development: association with differentiation of Sertoli and granulosa cells. J Clin Endocrinol Metab. 1999;84:3836–44.
135. De Vet A, Laven JS, de Jong FH, et al. Antimullerian hormone serum levels: a putative marker for ovarian aging. Fertil Steril. 2002;77:357–62.

136. Durlinger AL, Gruijters MJ, Kramer P, et al. Anti-Mullerian hormone attenuates the effects of FSH on follicle development in the mouse ovary. Endocrinology. 2001;142:4891–9.
137. Durlinger AL, Gruijters MJ, Kramer P, et al. Anti-Mullerian hormone inhibits initiation of primordial follicle growth in the mouse ovary. Endocrinology. 2002;143:1076–84.
138. Rankin T, Familari M, Lee E, et al. Mice homozygous for an insertional mutation in the Zp3 gene lack a zona pellucida and are infertile. Development. 1996;122:2903–10.
139. Schmidt D, Ovitt CE, Anlag K, et al. The murine winged-helix transcription factor Foxl2 is required for granulosa cell differentiation and ovary maintenance. Development. 2004;131: 933–42.
140. Nilsson E, Skinner MK. Bone morphogenetic protein-4 acts as an ovarian follicle survival factor and promotes primordial follicle development. Biol Reprod. 2003;69:1265–72.
141. Vitt UA, McGee EA, Hayashi M, et al. In vivo treatment with GDF-9 stimulates primordial and primary follicle progression and theca cell marker CYP17 in ovaries of immature rats. Endocrinology. 2000;141:3814–20.
142. Carabatsos MJ, Elvin J, Matzuk MM, et al. Characterization of oocyte and follicle development in growth differentiation factor-9-deficient mice. Dev Biol. 1998;204:373–84.
143. Oktay K, Briggs D, Gosden RG. Ontogeny of follicle-stimulating hormone receptor gene expression in isolated human ovarian follicles. J Clin Endocrinol Metab. 1997;82:3748–51.
144. Nilsson EE, Skinner MK. Kit ligand and basic fibroblast growth factor interactions in the induction of ovarian primordial to primary follicle transition. Mol Cell Endocrinol. 2004;214: 19–25.
145. Nilsson EE, Kezele P, Skinner MK. Leukemia inhibitory factor (LIF) promotes the primordial to primary follicle transition in rat ovaries. Mol Cell Endocrinol. 2002;188:65–73.
146. Knight PG, Glister C. TGF-beta superfamily members and ovarian follicle development. Reproduction. 2006;132:191–206.
147. Wang J, Roy SK. Growth differentiation factor-9 and stem cell factor promote primordial follicle formation in the hamster: modulation by follicle-stimulating hormone. Biol Reprod. 2004;70:577–85.
148. Hanrahan JP, Gregan SM, Mulsant P, et al. Mutations in the genes for oocyte-derived growth factors GDF9 and BMP15 are associated with both increased ovulation rate and sterility in Cambridge and Belclare sheep (*Ovis aries*). Biol Reprod. 2004;70:900–9.
149. Otsuka F, Yao Z, Lee T, et al. Bone morphogenetic protein-15. Identification of target cells and biological functions. J Biol Chem. 2000;275:39523–8.
150. Lee WS, Yoon SJ, Yoon TK, et al. Effects of bone morphogenetic protein-7 (BMP-7) on primordial follicular growth in the mouse ovary. Mol Reprod Dev. 2004;69:159–63.
151. Shimasaki S, Zachow RJ, Li D, et al. A functional bone morphogenetic protein system in the ovary. Proc Natl Acad Sci U S A. 1999;96:7282–7.
152. Saragueta PE, Lanuza GM, Baranao JL. Autocrine role of transforming growth factor beta1 on rat granulosa cell proliferation. Biol Reprod. 2002;66:1862–8.
153. Durlinger AL, Gruijters MJ, Kramer P, et al. Anti-Mullerian hormone attenuates the effects of FSH on follicle development in the mouse ovary. Endocrinology. 2001;142:4891–9.
154. Visser JA, Themmen AP. Anti-Mullerian hormone and folliculogenesis. Mol Cell Endocrinol. 2005;234:81–6.
155. Xiao S, Robertson DM, Findlay JK. Effects of activin and follicle-stimulating hormone (FSH)-suppressing protein/follistatin on FSH receptors and differentiation of cultured rat granulosa cells. Endocrinology. 1992;131:1009–16.
156. Oktem O, Oktay K. The role of extracellular matrix and activin-A in in vitro growth and survival of murine preantral follicles. Reprod Sci. 2007;14:358–66.
157. Findlay JK. An update on the roles of inhibin, activin, and follistatin as local regulators of folliculogenesis. Biol Reprod. 1993;48:15–23.
158. Matzuk MM, Kumar TR, Bradley A. Different phenotypes for mice deficient in either activins or activin receptor type II. Nature. 1995;374:356–60.
159. Visser JA, Themmen AP. Anti-Mullerian hormone and folliculogenesis. Mol Cell Endocrinol. 2005;234:81–6.

160. Yamoto M, Minami S, Nakano R, et al. Immunohistochemical localization of inhibin/activin subunits in human ovarian follicles during the menstrual cycle. J Clin Endocrinol Metab. 1992;74:989–93.
161. Hsueh AJ, Dahl KD, Vaughan J, et al. Heterodimers and homodimers of inhibin subunits have different paracrine action in the modulation of luteinizing hormone-stimulated androgen biosynthesis. Proc Natl Acad Sci U S A. 1987;84:5082–6.
162. Silva CC, Groome NP, Knight PG. Demonstration of a suppressive effect of inhibin alpha-subunit on the developmental competence of in vitro matured bovine oocytes. J Reprod Fertil. 1999;115:381–8.
163. Kageyama S, Liu H, Kaneko N, et al. Alterations in epigenetic modifications during oocyte growth in mice. Reproduction. 2007;133:85–94.
164. Lucifero D, Mann MR, Bartolomei MS, et al. Gene-specific timing and epigenetic memory in oocyte imprinting. Hum Mol Genet. 2004;13:839–49.
165. Kato M, Miura A, Bender J, et al. Role of CG and non-CG methylation in immobilization of transposons in Arabidopsis. Curr Biol. 2003;13:421–6.
166. Hiura H, Obata Y, Komiyama J, et al. Oocyte growth-dependent progression of maternal imprinting in mice. Genes Cells. 2006;11:353–61.
167. Obata Y, Kono T. Maternal primary imprinting is established at a specific time for each gene throughout oocyte growth. J Biol Chem. 2002;277:5285–9.
168. Geuns E, De Rycke M, Van Steirteghem A, et al. Methylation imprints of the imprint control region of the SNRPN-gene in human gametes and preimplantation embryos. Hum Mol Genet. 2003;12:2873–9.
169. Eppig JJ. Coordination of nuclear and cytoplasmic oocyte maturation in eutherian mammals. Reprod Fertil Dev. 1996;8:485–9.
170. Pincus G, Enzmann EV. Can mammalian eggs undergo normal development in vitro? Proc Natl Acad Sci U S A. 1934;20:121–2.
171. Conti M, Andersen CB, Richard F, et al. Role of cyclic nucleotide signaling in oocyte maturation. Mol Cell Endocrinol. 2002;187:153–9.
172. Mehlmann LM. Stops and starts in mammalian oocytes: recent advances in understanding the regulation of meiotic arrest and oocyte maturation. Reproduction. 2005;130:791–9.
173. Conti M, Hsieh M, Zamah AM, et al. Novel signaling mechanisms in the ovary during oocyte maturation and ovulation. Mol Cell Endocrinol. 2012;356:65–73.
174. Norris RP, Ratzan WJ, Freudzon M, et al. Cyclic GMP from the surrounding somatic cells regulates cyclic AMP and meiosis in the mouse oocyte. Development. 2009;136:1869–78.
175. Zhang M, Su YQ, Sugiura K, et al. Granulosa cell ligand NPPC and its receptor NPR2 maintain meiotic arrest in mouse oocytes. Science. 2010;330:366–9.
176. Liang CG, Su YQ, Fan HY, et al. Mechanisms regulating oocyte meiotic resumption: roles of mitogen-activated protein kinase. Mol Endocrinol. 2007;21:2037–55.
177. Shimada M, Terada T. Phosphatidylinositol 3-kinase in cumulus cells and oocytes is responsible for activation of oocyte mitogen-activated protein kinase during meiotic progression beyond the meiosis I stage in pigs. Biol Reprod. 2001;64:1106–14.
178. Gaffre M, Dupre A, Valuckaite R, et al. Deciphering the H-Ras pathway in Xenopus oocyte. Oncogene. 2006;25:5155–62.
179. Peng XR, Hsueh AJ, LaPolt PS, et al. Localization of luteinizing hormone receptor messenger ribonucleic acid expression in ovarian cell types during follicle development and ovulation. Endocrinology. 1991;129:3200–7.
180. Park JY, Su YQ, Ariga M, et al. EGF-like growth factors as mediators of LH action in the ovulatory follicle. Science. 2004;303:682–4.
181. Zamah AM, Hsieh M, Chen J, et al. Human oocyte maturation is dependent on LH-stimulated accumulation of the epidermal growth factor-like growth factor, amphiregulin. Hum Reprod. 2010;25:2569–78.
182. Hsieh M, Lee D, Panigone S, et al. Luteinizing hormone-dependent activation of the epidermal growth factor network is essential for ovulation. Mol Cell Biol. 2007;27:1914–24.

183. Gilchrist RB. Recent insights into oocyte-follicle cell interactions provide opportunities for the development of new approaches to in vitro maturation. Reprod Fertil Dev. 2011;23: 23–31.
184. Li M, Liang CG, Xiong B, et al. PI3-kinase and mitogen-activated protein kinase in cumulus cells mediate EGF-induced meiotic resumption of porcine oocyte. Domest Anim Endocrinol. 2008;34:360–71.
185. Kawamura K, Kumagai J, Sudo S, et al. Paracrine regulation of mammalian oocyte maturation and male germ cell survival. Proc Natl Acad Sci U S A. 2004;101:7323–8.
186. Kawamura K, Ye Y, Liang CG, et al. Paracrine regulation of the resumption of oocyte meiosis by endothelin-1. Dev Biol. 2009;327:62–70.
187. Kidder GM, Vanderhyden BC. Bidirectional communication between oocytes and follicle cells: ensuring oocyte developmental competence. Can J Physiol Pharmacol. 2010;88:399–413.
188. Norris RP, Freudzon M, Mehlmann LM, et al. Luteinizing hormone causes MAP kinase-dependent phosphorylation and closure of connexin 43 gap junctions in mouse ovarian follicles: one of two paths to meiotic resumption. Development. 2008;135:3229–38.
189. Ferreira EM, Vireque AA, Adona PR, et al. Cytoplasmic maturation of bovine oocytes: structural and biochemical modifications and acquisition of developmental competence. Theriogenology. 2009;71:836–48.
190. Ajduk A, Malagocki A, Maleszewski M. Cytoplasmic maturation of mammalian oocytes: development of a mechanism responsible for sperm-induced Ca^{2+} oscillations. Reprod Biol. 2008;8:3–22.
191. Stricker SA. Structural reorganizations of the endoplasmic reticulum during egg maturation and fertilization. Semin Cell Dev Biol. 2006;17:303–13.
192. Zuccotti M, Merico V, Cecconi S, et al. What does it take to make a developmentally competent mammalian egg? Hum Reprod Update. 2011;17:525–40.
193. Prather RS, Ross JW, Isom SC, et al. Transcriptional, post-transcriptional and epigenetic control of porcine oocyte maturation and embryogenesis. Soc Reprod Fertil Suppl. 2009;66: 165–76.
194. Tan JH, Wang HL, Sun XS, et al. Chromatin configurations in the germinal vesicle of mammalian oocytes. Mol Hum Reprod. 2009;15:1–9.
195. Liu H, Aoki F. Transcriptional activity associated with meiotic competence in fully grown mouse GV oocytes. Zygote. 2002;10:327–32.
196. Zuccotti M, Ponce RH, Boiani M, et al. The analysis of chromatin organisation allows selection of mouse antral oocytes competent for development to blastocyst. Zygote. 2002;10:73–8.
197. Li Y, Sasaki H. Genomic imprinting in mammals: its life cycle, molecular mechanisms and reprogramming. Cell Res. 2011;21:466–73.
198. Hiura H, Obata Y, Komiyama J, et al. Oocyte growth-dependent progression of maternal imprinting in mice. Genes Cells. 2006;11:353–61.
199. Gu L, Wang Q, Sun QY. Histone modifications during mammalian oocyte maturation: dynamics, regulation and functions. Cell Cycle. 2010;9:1942–50.
200. Paynton BV, Rempel R, Bachvarova R. Changes in state of adenylation and time course of degradation of maternal mRNAs during oocyte maturation and early embryonic development in the mouse. Dev Biol. 1988;129:304–14.
201. Rana TM. Illuminating the silence: understanding the structure and function of small RNAs. Nat Rev Mol Cell Biol. 2007;8:23–36.
202. Suh N, Blelloch R. Small RNAs in early mammalian development: from gametes to gastrulation. Development. 2011;138:1653–61.
203. Ma J, Flemr M, Stein P, et al. MicroRNA activity is suppressed in mouse oocytes. Curr Biol. 2010;20:265–70.
204. Suh N, Baehner L, Moltzahn F, et al. MicroRNA function is globally suppressed in mouse oocytes and early embryos. Curr Biol. 2010;20:271–7.
205. Murchison EP, Stein P, Xuan Z, et al. Critical roles for Dicer in the female germline. Genes Dev. 2007;21:682–93.

206. Kaneda M, Tang F, O'Carroll D, et al. Essential role for Argonaute2 protein in mouse oogenesis. Epigenet Chromatin. 2009;2:9.
207. Flesch FM, Gadella BM. Dynamics of the mammalian sperm plasma membrane in the process of fertilization. Biochim Biophys Acta. 2000;1469:197–235.
208. Ficarro S, Chertihin O, Westbrook VA, et al. Phosphoproteome analysis of capacitated human sperm. Evidence of tyrosine phosphorylation of a kinase-anchoring protein 3 and valosin-containing protein/p97 during capacitation. J Biol Chem. 2003;278:11579–89.
209. Legendre LM, Stewart-Savage J. Effect of cumulus maturity on sperm penetration in the golden hamster. Biol Reprod. 1993;49:82–8.
210. Fetterolf PM, Jurisicova A, Tyson JE, et al. Conditioned medium from human cumulus oophorus cells stimulates human sperm velocity. Biol Reprod. 1994;51:184–92.
211. Sun F, Bahat A, Gakamsky A, et al. Human sperm chemotaxis: both the oocyte and its surrounding cumulus cells secrete sperm chemoattractants. Hum Reprod. 2005;20:761–7.
212. Chapman N, Kessopoulou E, Andrews P, et al. The polypeptide backbone of recombinant human zona pellucida glycoprotein-3 initiates acrosomal exocytosis in human spermatozoa in vitro. Biochem J. 1998;330(Pt 2):839–45.
213. Tesarik J, Drahorad J, Peknicova J. Subcellular immunochemical localization of acrosin in human spermatozoa during the acrosome reaction and zona pellucida penetration. Fertil Steril. 1988;50:133–41.
214. Roldan ER, Shi QX. Sperm phospholipases and acrosomal exocytosis. Front Biosci. 2007;12:89–104.
215. Fukami K, Nakao K, Inoue T, et al. Requirement of phospholipase Cdelta4 for the zona pellucida-induced acrosome reaction. Science. 2001;292:920–3.
216. Beebe SJ, Leyton L, Burks D, et al. Recombinant mouse ZP3 inhibits sperm binding and induces the acrosome reaction. Dev Biol. 1992;151:48–54.
217. Rankin TL, Coleman JS, Epifano O, et al. Fertility and taxon-specific sperm binding persist after replacement of mouse sperm receptors with human homologs. Dev Cell. 2003;5:33–43.
218. Ikawa M, Inoue N, Benham AM, et al. Fertilization: a sperm's journey to and interaction with the oocyte. J Clin Invest. 2010;120:984–94.
219. Hagaman JR, Moyer JS, Bachman ES, et al. Angiotensin-converting enzyme and male fertility. Proc Natl Acad Sci U S A. 1998;95:2552–7.
220. Nishimura H, Kim E, Nakanishi T, et al. Possible function of the ADAM1a/ADAM2 Fertilin complex in the appearance of ADAM3 on the sperm surface. J Biol Chem. 2004;279:34957–62.
221. Yamaguchi R, Muro Y, Isotani A, et al. Disruption of ADAM3 impairs the migration of sperm into oviduct in mouse. Biol Reprod. 2009;81:142–6.
222. Ikawa M, Wada I, Kominami K, et al. The putative chaperone calmegin is required for sperm fertility. Nature. 1997;387:607–11.
223. Inoue N, Ikawa M, Isotani A, et al. The immunoglobulin superfamily protein Izumo is required for sperm to fuse with eggs. Nature. 2005;434:234–8.
224. Le Naour F, Rubinstein E, Jasmin C, et al. Severely reduced female fertility in CD9-deficient mice. Science. 2000;287:319–21.
225. Ducibella T, Kurasawa S, Duffy P, et al. Regulation of the polyspermy block in the mouse egg: maturation-dependent differences in cortical granule exocytosis and zona pellucida modifications induced by inositol 1,4,5-trisphosphate and an activator of protein kinase C. Biol Reprod. 1993;48:1251–7.
226. Lee SH, Ahuja KK, Gilburt DJ, et al. The appearance of glycoconjugates associated with cortical granule release during mouse fertilization. Development. 1988;102:595–604.
227. Sato K. Polyspermy-preventing mechanisms in mouse eggs fertilized in vitro. J Exp Zool. 1979;210:353–9.
228. McGrath J, Solter D. Completion of mouse embryogenesis requires both the maternal and paternal genomes. Cell. 1984;37:179–83.