MAMMALIAN GAMETOGENESIS TO IMPLANTATION

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Contents

Summary

The origin of new live beings and the events that occur before birth have been the object of fascination and discussion over the centuries. Our concept of two cells, ovum and sperm, fusing to generate a zygote, then an embryo and finally a new individual, is relatively new; as it is the idea of two sets of chromosomes carrying the genetic information inherited from each parent and being coordinated to generate something new and different. The recent advances in the cytology field, as well as in biochemistry and genetics, have allowed us to trace back the origins and formation of ovum and sperm (gametogenesis) and to follow the dramatic modifications that occur in the embryo during the first days until it is implanted in the maternal uterus in mammals. Moreover, the rapid expansion of new techniques such as *in vitro* fertilization, genetically modified organisms and cloning, and their implications in our society, have

invigorated the interest in the processes that surround fertilization.

This article summarizes the major events that occur during and after the fusion of the ovum and sperm until the embryo implants. It reviews our current knowledge about the origin of the gametes, and how such specialized cells can generate embryonic cells with the potential of generating every possible tissue type. It also discusses recent progresses, specially the latest contributions from the genetics field, as well as the questions that are still open or are object of debate.

1. Introduction

The generation of a new individual as a result of the fusion of a maternal and a paternal gamete is a complex and fascinating process. *Gametogenesis* is the production of haploid sex cells in mammals (*ovum* and *sperm*), carrying each one-half of the genetic complement of the parents. Both female and male gametogenesis provides mechanisms through which genetic information may be passed to the offspring. The fusion of sperm and ovum during *fertilization* results in a *zygote* with a fully restored diploid genome.

Errors during the processes of gametogenesis and fertilization until implantation have a major impact in fertility. 15% of couples worldwide are childless due to fertility problems. In addition, miscarriages are extremely frequent in normal couples: most of them occur so early that are not detected, but of those noticed, approximately one-third have an abnormal number of chromosomes. This is mostly due to errors during the formation of the eggs: as many as 20% of them, but only 3-4% of sperm are chromosomally abnormal in humans. Consequently, both gametogenesis and early embryogenesis have been the object of great interest, specially since the advent of the *in vitro* fertilization technologies.

In this major review, we begin presenting how germ cells are initially specified in the early embryo and the processes that lead to their differentiation in the gonads. Then we will introduce fertilization and activation of the egg -the vital step that leads to the first stages of embryogenesis. These stages will be discussed until the implantation of the embryo in the uterus, the most unique process of mammalian embryogenesis. Finally, we will review the genetic and epigenetic changes that underlie these developmental processes

2. Origin and Development of Germ Cells

Germ cells may be defined as those cells, all of whose descendents will become sperm or eggs. In all sexually reproducing animals, these cells play a uniquely important role: the transmission (after meiotic recombination) of the genetic information from one generation to the next.

The origin of the germ cell lineage, which includes the origin of the first *primordial germ cells* (PGCs), has been a topic of discussion in recent past. In many vertebrates and invertebrates, PGCs are formed in a specified location and are predetermined. But in mammals, tracking the germ cell location has been a difficult task.

PGCs appear during the embryonic development. In mammals, the fertilized egg undergoes several divisions (*cleavage*) before forming a *blastocyst* stage embryo that is ready to implant in the uterus (see details below). After implantation, the surface cells of the inner cell mass of the blastocyst forms the primitive endoderm and reminder of inner cell mass forms the primitive or embryonic ectoderm- specifically called the epiblast. Cells of the epiblast actually give rise to embryo proper.

Many studies about the origin and differentiation of PGCs have been performed in mouse (Figure 1). However, mouse embryologists have been unable to identify any cells that give rise to germ cells in cleavage embryo nor in blastocyst stage. Even after implantation, 4-5 days postcoitum (dpc), they have not identified any germ cell specific cells. But in later developmental stages (*i.e.*, by 8.5 dpc) identification of PGCs has been facilitated by detection of tissue non-specific alkaline phosphatase (TNAP) activity, which is expressed at high levels in PGCs. TNAP is not required for PGCs survival but is an invaluable marker. From the time of implantation to 8.5 dpc, a process called gastrulation takes place. Gastrulation is a process that results in the formation of the gut and the main body plan, which emerges when the cells on the outside of embryo move inwards. During this process, the mouse PGCs migrate from the base of the allantois at 8.5 dpc to their entry into the *genital ridges*, the site of the future gonads. At least in mouse, PGCs are derived from the epiblast (primitive/embryonic ectoderm), not from the endoderm. This was initially demonstrated by transplantation experiments of single epiblast cells at 6.0-6.5dpc with a lineage specific marker and following their fate of their clonal descendents. Most importantly, even at 6.5 dpc, PGCs lineage is not determined, *i.e.*, they are still not lineage-restricted. Clonal analysis establishes that germ cell fate is determined in a group of 45 progenitor cells at about 7.2 dpc. By 8.5dpc, PGCs start migrating towards the genital ridges (Figure 1).

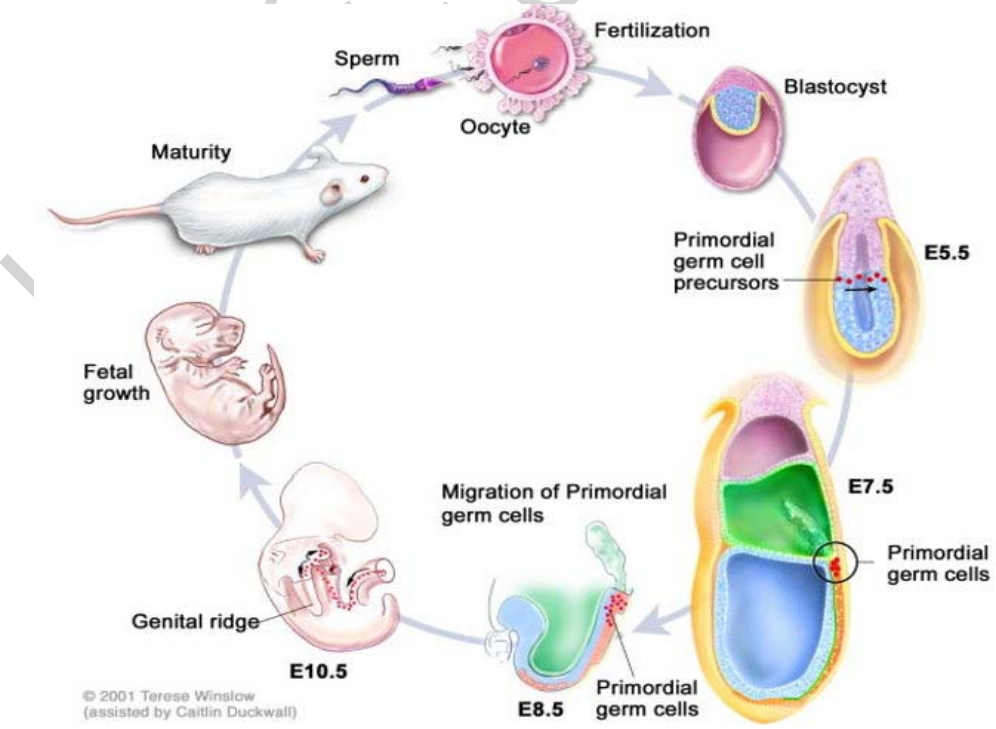

Figure 1. Migration of germ cells to the genital ridge in mouse: After fertilization, the

embryo undergoes early cleavage in the oviduct. By E5.5, the embryo implants in to the uterine wall, and precursors of PGCs are formed. By E7.5, PGCs' fate is determined. From E8.5, PGCs start migrating and, by E10.5- 11.5, they reach the genital ridge that will constitute the gonads. (©2001 Terese Winslow (assisted by Caitlin Duckwall))

2.1. Migration of the Primordial Germ Cells towards the Gonad

Since 8.5 dpc in mouse gastrula stage, germ cells can easily be detected by alkaline phosphatase staining, which allows us to follow their migration (Figure 1). At gastrulation stage, endoderm starts invaginating to form the hind gut. Thus, PGCs become incorporated into the hind gut, and then migrate into the adjacent connective tissue. By the time the hind gut is fully formed, the PGCs lie along its length and very few PGCs remain at the base of allantois. Germ cells remain in motion until they enter the genital ridge. Initially PGCs leave the hind gut individually, but then gradually they extend up to 40μm processes. With these processes they link to each other to form an extensive network. By 10 dpc, genital ridges start to form, and, by 10-11.5 dpc, networks of PGCs aggregate to form groups in the genital ridge. By this time, PGCs loose their extended processes and become non-motile. Once the germ cells reach the genital ridge, the expression of germ cell specific genes commences.

3. Germ Cell Differentiation: Gametogenesis and Meiosis

After germ cells migrate towards the gonads, they differentiate into sperm or eggs via a process called gametogenesis. The development into eggs or oocytes is known as oogenesis, while the development into sperm is known as spermatogenesis. Upon arrival to the genital ridge (which will become the gonads), the germ cells begin to differentiate into sperm or eggs. By 12.5 dpc, PGCs undergo two-three rounds of mitosis in both female and male mouse embryos. In the male genital ridge, mitosis proceeds no further, and enters G1 arrest -called prospermatogonia stage (prospermatogonia, or gonocytes, are the cells that differentiate from primordial germ cells to the first mature type of spermatogonia in the developing testis). Mitosis is resumed after birth. In the female genital ridge, germ cells enter meiotic prophase as primary oocytes and arrest at the diplotene stage at the time of birth (see details in Meiosis and Sexual Dimorphism sections.)

Germ cells have to reduce their chromosome number by half during gametogenesis, so that at fertilization the diploid chromosome number is restored. PGCs (precursors of gametes) are initially diploid, and reduction from diploid to haploid state to form gametes occurs during a process known as *meiosis*.

3.1. Meiosis: The Formation of Haploid Gametes

Meiosis is a specialized cell division that occurs in all sexually reproducing organisms. During meiosis (Figure 2), diploid cells generate haploid daughter cells. A germ cell (*2n*, diploid) divides twice after a single DNA replication event: firstly, by separating the paternally inherited from the maternally inherited (homologous) chromosomes at *meiosis I*, and secondly, by separating sister chromatids of each chromosome during *meiosis II*, thereby producing four haploid (*n*) products (Figure 2).

Unlike mitosis, homologous chromosomes first pair and then segregate into separate nuclei during the first meiotic division (MI). MI has four stages- prophase I, metaphase I, anaphase I and telophase I. The prophase I of meiosis I is the most critical event, when homologues pairing as well as double strand break (DSB) formation and resolution leads to the exchange of genetic information between homologous chromosomes (*recombination*). Prophase I is further divided into five sub stages- leptotene, zygotene, pachytene, diplotene and diakinesis. In leptotene, condensation and coiling of already replicated DNA takes place; in zygotene, pairing of homologues occurs, forming bivalents. A synaptonemal complex of proteins forms between homologue. At pachytene, crossing over between homologues occurs. Recombination by crossing over leads to genetic diversification. By diplotene, points of crossing over become visible under the microscope -called chiasma or chiasmata. By diakinesis, chromosome starts to uncoil. During metaphase I, homologous chromosomes attach to the poles of the spindle, in anaphase I homologues separate to opposite poles, and in telophase I chromosomes uncoil. Meiosis II has 4 stages- prophase II, metaphase II, anaphase II and telophase II. In prophase II, coiling of sister chromatids occurs; in metaphase II, sister chromatids align at the equatorial plate and by anaphase II, sister chromatids segregate to opposite poles. Finally, in telophase II, cytokinesis takes place to form haploid products.

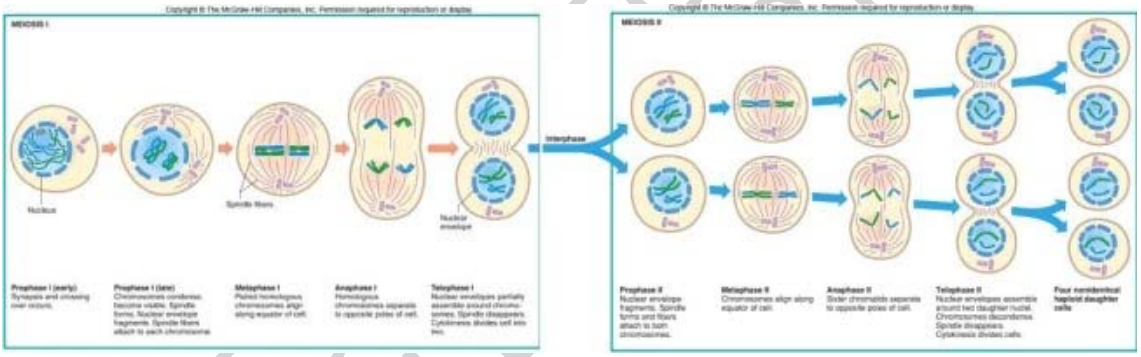

Figure 2. Meiosis. The detailed sequence of events is shown for two pairs of homologous chromosomes. At the initiation of meiosis I, chromosomes condense and each homologous pair forms a bivalent. Within the bivalents, crossing over occurs, which involves breakage of chromosome arms and exchange of DNA. Meiosis I proceeds to form two nuclei, each having one member of each homologous pair with the two sister chromatids still attached through the centromeres. During the second meiotic division, sister chromatids separate. As a result, the final products of meiosis contain a single copy of each chromosome and are haploid. (Reproduced from Human Genetics Concepts and Applications by Ricki Lewis ©2008 McGraw-Hill Companies, Inc.)

3.1.1. Crossover and Recombination: The Basis of Genetic Variability

Crossing over results in the exchange of genetic information (recombination) between chromosomes of different parental origin. Therefore, the offspring receives chromosomes with different allelic combinations than those present in any of the parents. Crossing over is one of the specialized steps of prophase I of meiosis. During pachytene, crossing over between non-sister chromatids of homologues takes place. At least one crossover is required per chromosome arm for proper segregation of the

chromosomes to the poles of the spindle. During meiosis I, crossovers hold the homologues together until they migrate toward opposite poles. Inefficient crossing over impairs chromosome segregation, resulting in aneuploid gametes and offspring. Thus, crossing over has a double role- firstly, producing genetic diversification by recombination; secondly, supporting proper chromosome segregation.

3.2. Sexual Dimorphism- Oogenesis *vs.* Spermatogenesis

In *males*, meiosis and germ cell differentiation commence after attaining puberty and continue throughout adult life. Diploid spermatogonia undergo mitosis to generate an infinite pool of primary spermatocytes, which enter meiosis to generate haploid spermatids that will differentiate into sperm. The end product of meiosis is four haploid sperm from each primary spermatocyte.

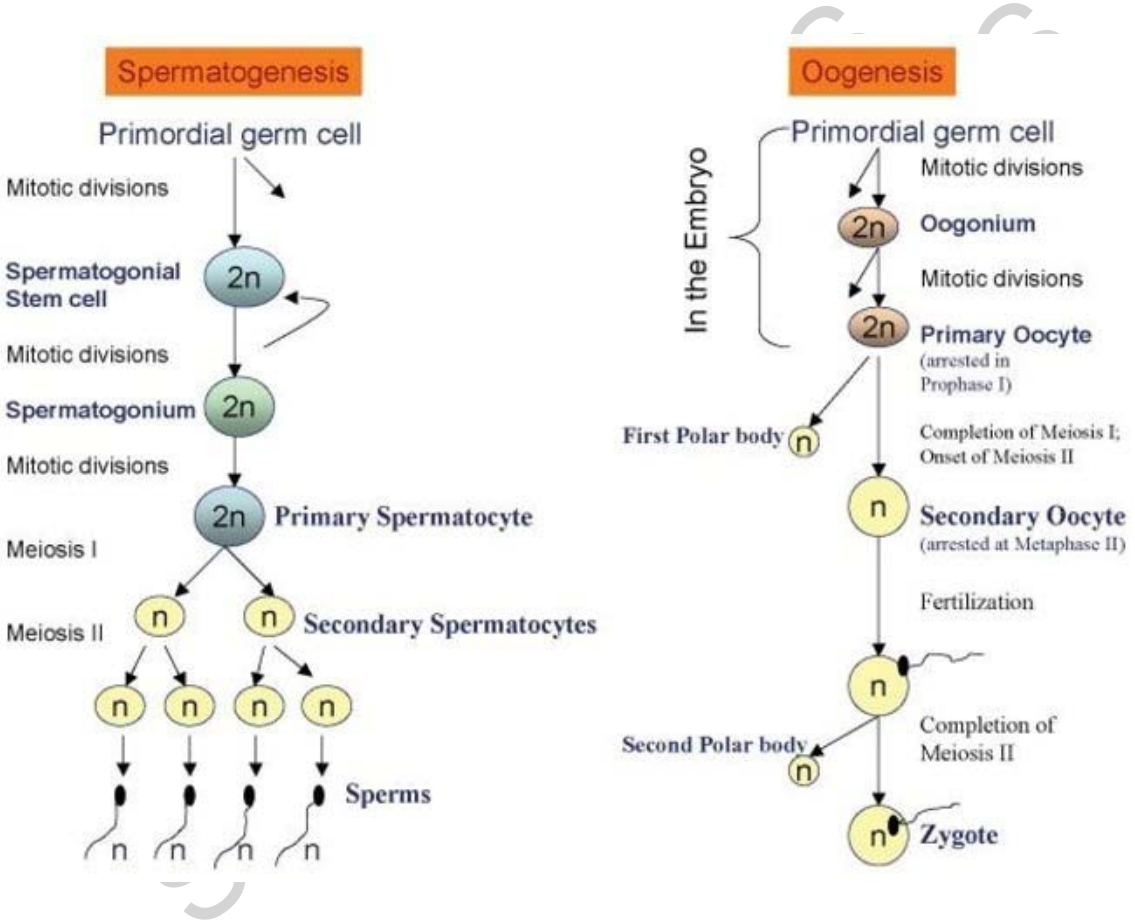

Figure 3. Mammalian Oogenesis and Spermatogenesis: Oogenesis: Fetal ovaries contain about 500,000 (in humans) primary oocytes arrested at the diplotene stage of meiosis I. Meiosis I is resumed around the time of ovulation. Meiosis II is completed only if fertilization occurs. Only one cell per meiosis serves as the functional ovum. Spermatogenesis: Spermatogonia divide by mitosis to produce primary spermatocytes. After subsequent meiotic divisions, mature sperms are released. From each primary spermatocyte, four haploid sperms are formed.

In *females*, meiosis I comences during fetal development, including recombination. At the last stage (diplotene) of prophase I, the oocyte enters the dictyate arrest. The oocyte

remains in that dormant state until the female reaches puberty. During each menstrual cycle, a selected number of oocytes are ovulated and resume meiosis. They complete the first meiotic division and half of the chromosomes are extruded to the first polar body, while the rest remain in the egg; again, the egg is arrested at metaphase II. Upon fertilization, the egg completes meiosis II and releases a second polar body containing half of the sister chromatids. Therefore, the result of female meiosis is one haploid egg, while the rest of the meiotic products are extruded to the polar bodies and, eventually, degenerate. Notice that only one of the four possible products of meiosis is transmitted to the offspring and it has been generally assumed that all four products have similar probabilities for doing so. However, unequal segregation of chromosomes or chromatids between the ovum and polar bodies has been documented in female meiosis -this is called maternal meiotic drive. The effect of meiotic drive is observed in heterozygous loci, such that certain alleles are preferentially retained in the egg during the first or second meiotic divisions and, therefore, have a selective advantage to be passed to the offspring.

OOGENESIS	SPERMATOGENESIS
1. During embryogenesis, germ cells enter the fetal ovary, where diploid oogonia multiply by mitotic divisions. Next they initiate the prophase of meiosis I and become primary oocytes. They become arrested at this stage before birth. Further growth and development of the primary oocyte is delayed until puberty.	1. Germ cells enter the testis, and arrest in G1 phase of the cell cycle. After birth, spermatogonia multiply by mitotic divisions to produce an infinite pool of spermatocytes.
2. During ovulation, one or several oocytes resume meiosis I and progress until metaphase II, but meiosis II is only completed if fertilization occurs.	2. Differentiation into sperm takes place continuously after reaching puberty, by completion of meiosis I and meiosis II.
3. The final product of meiosis is one haploid egg (ovum) and two-three small polar bodies from each primary oocyte that degenerate.	3. The final product of meiosis is four haploid sperm from each primary spermatocyte.

Table 1. Main differences between oogenesis and spermatogenesis in mammals.

3.3. Meiosis Success in Males and Females and Its Consequences

The female meiotic process is highly error prone, especially in humans compared to other species. Errors in chromosome segregation during meiosis result in the production of eggs or sperm with abnormal numbers of chromosomes, rather than the normal complement. When these gametes undergo fertilization, they produce *aneuploid* embryos. Aneuploids are cells missing chromosomes or having extra chromosomes - *e.g.*, $2n-1$(monosomics) or $2n+1$(trisomics). About 20% of human conceptions are lost as a result of meiotic errors.

Aneuploidy is the result of *non-disjunction*. In normal meiosis, homologues separate and each of the resulting gametes receives only one member of each chromosome pair. But sometimes, a chromosome pair or a sister chromatid fails to separate at anaphase of the first or second meiotic division, respectively. This produces a gamete that has two copies of a particular chromosome or none. When such gamete participates in fertilization, the resulting zygote has, in humans for instance, either 45 ($2n$-1) or 47 ($2n$+1), instead of 46 ($2n$) chromosomes. Abnormal chromosomal number has a wide range of implications in the embryonic development, depending on the chromosome affected. Most of the aneuploid embryos do not survive to birth, but those who do so (such as Down syndrome, Turner syndrome, etc.) have developmental problems that include mental retardation, sterility, stunted growth etc.

Most of the aneuploidies are caused by errors during female meiosis I. Male meiosis errors, as well as mitotic non-disjunction prior to meiosis or after fertilization, have also been reported to generate aneuploid embryos. As mentioned above, meiosis is a continuous process in males, while in females it is arrested at the dictyate stage for days, months or years. Meiotic segregation errors escalate with age in females, increasing the incidence of conceptions with developmental abnormalities such as Down syndrome (trisomy 21) and aneuploidy-related miscarriages. Is is thought that these errors are the result of the progressive deterioration of the attachments of the homologous chromosomes between themselves and to the spindle, thereby leading to non-disjunction when meiosis finally resumes. Male gametes, in contrast, appear to be more prone to carry other types of mutations due to the larger number of mitotic divisions that occurs in spermatogenesis compared to that in oogenesis. However, recent studies in mouse have revealed that cell-cycle check points are more permissive during female oogenesis than male spermatogenesis. Consequently, most spermatocytes carrying mutations that affect spermatogenesis are rapidly eliminated, while eggs with aneuploidies often survive to be fertilized.

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