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Gametogenesis, Early Embryo Development and Stem Cell Derivation

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Foreword

Science is evolving at an immense speed. Breakthroughs, which most of us had never even thought of few years ago because they clashed against cemented dogmas, are now realities. They now pave the way for further scientific endeavors and offer new solutions to important societal problems. In the area of biology, some of the greatest breakthroughs over the past years have been the birth of the cloned sheep, Dolly, in 1996, and the finding just 10 years later in 2006 that differentiated somatic cells can be reprogrammed and revert to a pluripotent stem cell state. The two achievements have commonalities: Both have demolished the biological dogma that terminally differentiated cells cannot de-differentiate, both are based upon a total reprogramming of the epigenetic control of cellular gene expression, and both have their roots in embryology. It is embryological thinking that is the background for understanding how an oocyte can be used for reprogramming of a somatic cell for the creation of a cloned animal, and it is embryological reasoning that resulted in isolation and culture of embryonic stem cells and, later, the identification of exclusive stem cell factors of such a potency that they can reprogram somatic cells into stem cells. As visualized by just these two examples, embryology stands out as a modern contemporary scientific discipline, in spite of its classical nature with a history dating all the way back to Aristotele (384–322 BC).

The immense speed by which science evolves not only reflects in spectacular breakthroughs, as alluded to above, it is also reflected in the level and depth of molecular understanding of processes. A wealth of data are generated that allows us to penetrate deeper and deeper into the molecular understanding of how life is organized, controlled, and passed from generation to generation. The mapping of the genomes of different organisms has contributed significantly to this process, but over the past years an enormously focussed penetration into the understanding of the epigenetic landscape, which controls gene expression and silencing, has been instrumental. And again, embryology is central in this aspect: The full understanding of epigenetic reprogramming can exclusively be obtained if this phenomenon is seen in an embryological perspective. Hence, the understanding of the epigenetic mechanisms, which operate when the genome is passed from one generation to the next, is crucial for normal reproduction as well as for contemporary

phenomena like fetal programming, where there is a growing body of evidence that the mother affects the epigenetic patterns of the embryo and fetus to such a degree that it is decisive for the rest of the life. It's all embryology …

The present book stands out as a contemporary appreciation of the most important aspects of embryology that are important to grasp not only in relation to stem cell biology, but also as a background for assisted reproductive technologies and several other scientific methodologies. Tiziana Brevini is an internationally highly recognized and respected embryologist who has contributed to numerous aspects of the area, from oocyte biology to stem cell culture and differentiation. It is a gift to the scientific area of embryology that she has devoted time for the book in your hands.

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Preface

In a somehow limited view of this discipline, Embryology has been considered in the past years a prerequisite and a fundamental acquisition for a better and more dynamic understanding of gross anatomy. We are certainly not denying this idea that has a solid ground and highlights the impact of the complex differentiation processes in the definition of the final architectural morphology of a tissue/organ and the related function.

At present, however, we are convinced that this view needs to be expanded considering the central role played by Embryology in a series of new scientific fields. Innovative and quickly developing research in biomedical science and modeling find solid bases in recently acquired information related to embryo induction and differentiation. Similarly, the latest exciting scientific acquisitions in stem cell research and regenerative medicine have been supported by the elucidation of the mechanisms and molecules controlling pluripotency and driving commitment and differentiation in the early embryo.

This Brief is intended as a concise, handy overview of the main concepts related to Embryology, re-visited through the novel concepts that are applied daily in stem cell research and cell therapy oriented investigations.

Milan, Italy

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Chapter 1
Gametogenesis

1.1 Primordial Germ Cells

Early embryo cells have the capability to give rise to all cell types of the body. During the process of gastrulation, however, most of them lose this ability and acquire a tissue specific fate. This loss of pluripotency is a key event in development since it results in lineage commitment and allows the definition of the three somatic germ layers from which all different tissues of the body will originate. Through a series of complex and finely orchestrated morphogenetic movements, that involve cell migration, clustering and delamination, the process leads to the formation of the ectoderm, the mesoderm, and the endoderm (Fig. 1.1).

During the process of gastrulation, however, a subpopulation of cells remains pluripotent and does not undergo any lineage commitment. These cells are the common progenitors of the male and female gametes of the developing embryo and are known as the primordial germ cells. Like all other somatic cells they are diploid and, in human embryos, they can be identified in the epiblast or primary ectoderm as early as the second week of gestation. Shortly after that, the primordial germ cells migrate from the primary ectoderm into the yolk sac wall and localize outside the embryo proper. It is believed that the primordial germ cells are moved to this location outside the developing embryo in order to release them from the differentiation cues driving gastrulation and patterning (Fig. 1.2). This seclusion would ensure them a neutral environment to preserve their pluripotency.

During these stages, primordial germ cells can be identified by their relatively big dimensions and through immunostaining carried out using antibodies specific for transcription factors such as Oct 4, Stella, VASA, Fragilis, BLIMP-1, and Alkaline phosphatase (see Table 1.1).

Recent studies have demonstrated that some of these factors are directly related to the pluripotent state and are actively repressing the expression of key genes driving differentiation of epiblast cells to the somatic lineages, thus concurring in maintaining a “non-committed” environment for primordial germ cells.

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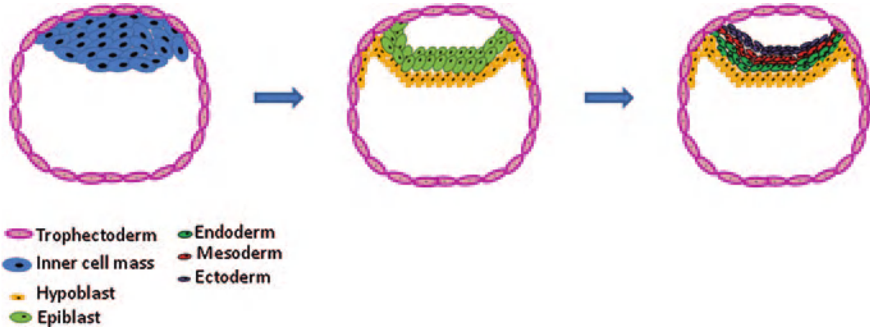


Fig. 1.1 Formation of the three germ layers. The blastocyst consists of two cell types: trophectoderm (placenta formation) and inner cell mass (embryo proper) cells. At the end of blastulation, the inner cell mass cells give rise to hypoblast (internal) and epiblast (external) cells, establishing the bilaminar embryonic disk. During the process of gastrulation, epiblast cells differentiate into ectoderm, mesoderm, and endoderm, converting the bilaminar disk into a trilaminar embryonic disk

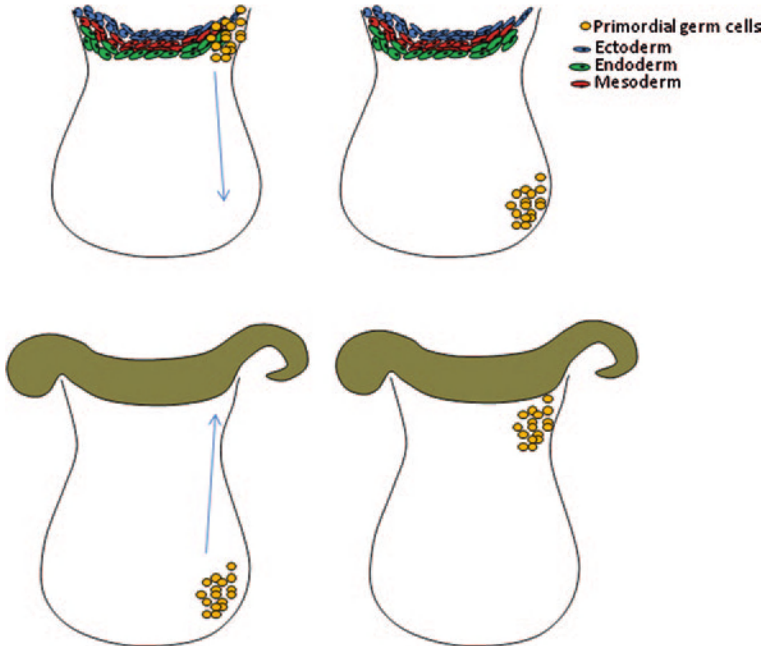


Fig. 1.2 Primordial germ cell migration. During the process of mesoderm and endoderm formation, the primordial germ cells move from the primary ectoderm into the yolk sac wall and localize outside the forming embryo. Here these cells proliferate actively, without differentiating, and then migrate again to colonize the genital ridge of the embryo

Soon, primordial germ cells start migrating along the caudal region of the yolk sac and wander back into the embryo where they colonize the gonadal ridge. It is yet unclear whether this relocation is actively or passively controlled but possibly

Table 1.1 Pluripotency-related transcription factors identified in primordial germ cells

Gene symbol	Gene name
Akp2	Alkaline phosphatase, liver/bone/kidney
Blimp1	PR domain-containing 1, with ZNF domain
BMP4	Bone morphogenetic protein 4
c-kit	Kit oncogene
c-myc	Myelocytomatosis oncogene
Dax1	Dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1
Dmc1	DMC1 dosage suppressor of mck1 homolog, meiosis-specific homologous recombination (yeast)
Figα	Factor In the Germline alpha
Fragilis	Interferon-induced transmembrane protein 3
Fgf9	Fibroblast growth factor 9
miRNAs	Different microRNA cluster
Nanog	Nanog homeobox
Nanos3	Nanos homolog 3, Drosophila
Oct4	POU domain, class 5, transcription factor 1
Scp3	Synaptonemal complex protein 3
Sox2	Sry-box containing gene 2
Sox9	Sry-box containing gene 9
Sry	Sex determining region of chromosome Y
Ssea-1	Fucosyltransferase 4
Stella	Developmental pluripotency-associated 3
Stra8	Stimulated by retinoic acid gene 8

it may be facilitated by the rostral-caudal curvature and the folding of the embryo. At present, it is widely accepted that local signals, generating outside the embryo proper, act through the Smad pathway, and involve the Bone Morphogenetic Protein 4, and 8b. Together with the many factors described above and summarized in Table 1.1, these molecules drive the germ line specification process and growth. Indeed during their journey, but also once in the gonadal ridge, primordial germ cells increase in number and multiply by mitotic divisions. These proliferation events are finely tuned and, in the mouse, eight proliferation cycles of 16 h each, with an increase in cells from about 100 to 20,000, have been reported. Furthermore, the involvement of growth factors that are directly stimulating cell proliferation and molecules like kit, KL, and LIF that down-regulate and prevent apoptosis has also been described. A few days after colonization of the genital ridge, primordial germ cells undergo mitotic arrest, associate with the surrounding somatic cells, and engage in sex-driven differentiation (Fig. 1.3). Although the sex of a mammalian embryo is genetically determined at fertilization, the genital ridges are kept in an undifferentiated state during the early phase of gestation (Figs. 1.4 and 1.5).

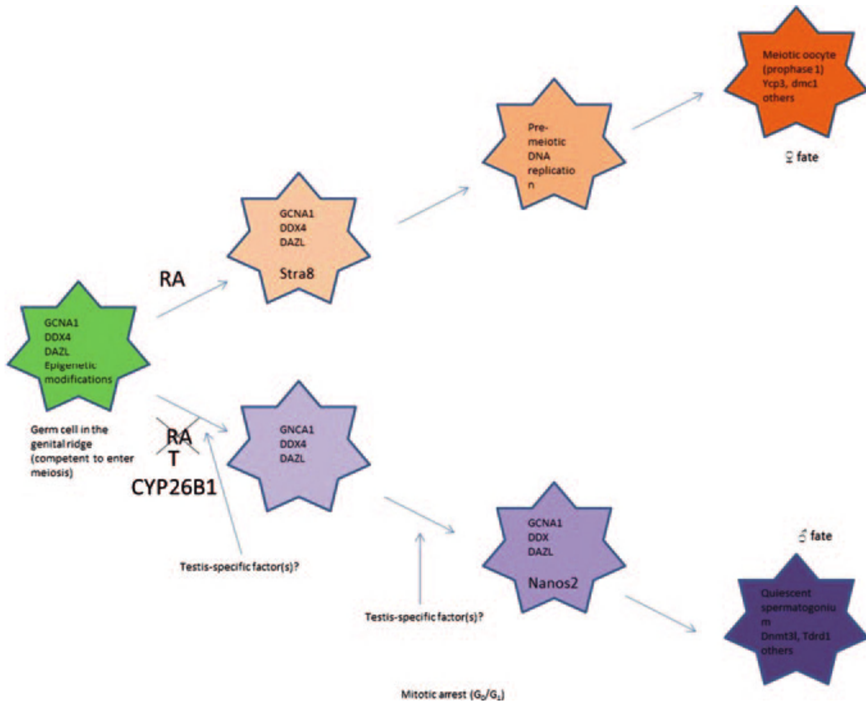
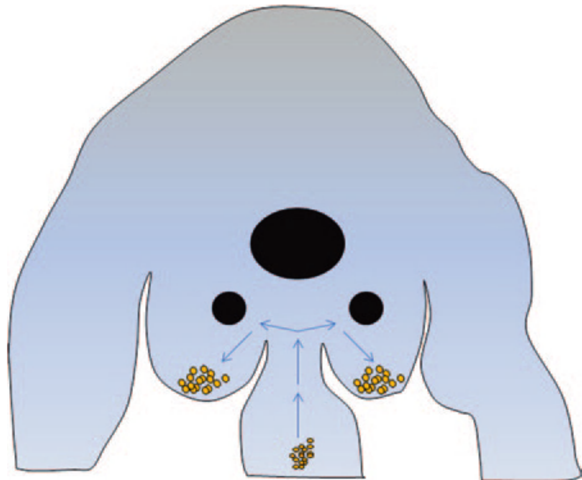


Fig. 1.3 Molecular regulation of germ line development. A complex network involving several factors regulates the acquisition of the gender-specific fate

Fig. 1.4 Scheme of indifferent female gonad



During this period, they are referred to as indifferent gonads or primitive gonadal primordium and, only following the migration of the primordial germ cells do these structures develop into the definitive and gender-specific gonads.

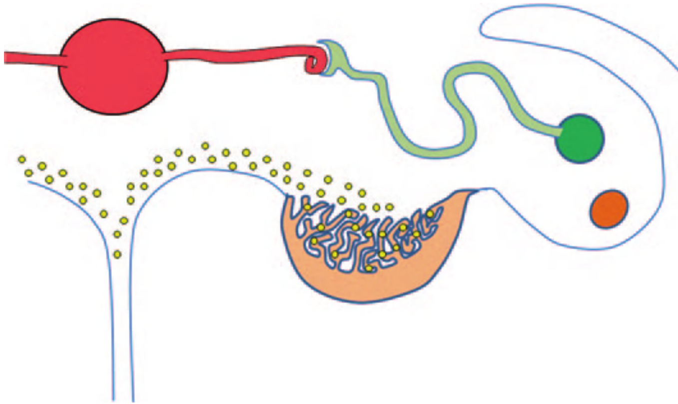


Fig. 1.5 Scheme of indifferent male gonad

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1.2 Mitosis and Meiosis

Hereditary traits are determined by specific DNA segments, identified as "genes". Together with several proteins, DNA is organized in chromosomes that are inherited from the mother and the father. Somatic cells present the full number of chromosomes. Every cell contains two of each type of chromosome, forming the diploid

chromosome complement designed as 2n. One chromosome of each homologous pair is inherited from the mother (oocytes) and the other from the father (spermatozoa), at the time of fertilization. This is possible thanks to the meiotic division of gametes that contain only one chromosome from each pair, and are therefore haploid (1n).

Meiosis is a particular cell division process that takes place in germ cells and is necessary for sexual reproduction in eukaryotes. This mechanism allows obtaining haploid cells, containing one of every pair of homologous chromosomes, and involves only one DNA replication and two distinct and consecutive nuclear reductions (meiosis I and meiosis II).

In particular, the meiotic process encompasses interphase, meiosis I and meiosis II. The interphase consists of G1 and S phases, while meiosis I and II are divided into prophase, metaphase, anaphase, and telophase stages, similarly to the corresponding phases in the mitotic cell cycle. The meiotic process therefore includes the stages of meiosis I (prophase I, metaphase I, anaphase I, telophase I), and meiosis II (prophase II, metaphase II, anaphase II, telophase II).

During interphase gametes synthesizes a vast array of proteins, including enzymes and structural proteins required for growth (G1 phase), and in order to duplicate each of the chromosomes forming a complex of two identical sister chromatids (S phase).

This is followed by the first step of the prophase of meiosis I, the leptotene stage, wherein the chromosomes condense and align in pairs along the center of the nucleus. Each chromosome therefore consists of four chromatids and is referred to as a tetrad, in which maternal chromatids become bound to its paternal counterpart by the synaptonemal complex (zygotene stage). During the pachytene stage, this coupling allows for DNA to be exchanged between homologous chromatid segments at certain points—called chiasmata—through a crossover process, resulting in a new DNA combination. This event, together with the random segregation of maternal and paternal chromosomes, represents a significant source of genetic recombination and ensures a unique combination of parental genomes for the next generations.

During the following phases, diplotene and diakinesis, the synaptonemal complex degrades, homologous chromosomes are gradually released from each other and further condense, the nucleolus disappears together with the nuclear membrane, and the mitotic spindle begins to form. Furthermore in male gametes, with the exception of the mouse, centriole pairs, which were duplicated during S phase, migrate to opposite poles of the cell, forming microtubule organizing centers. However, in the non-rodent mammalian female, the oocyte degrades its centrioles and retains a stockpile of centrosomal proteins that are used to make up the spindle poles. The microtubules occupy the nuclear region, attaching to the chromosomes at the kinetochore. During metaphase I, homologous chromosomes align along the equatorial plane; in anaphase I, kinetochore microtubules shorten, pulling homologous chromosomes toward opposing poles, thus forming two haploid groups (telophase I). At this point, each chromosome, which still contains a pair of sister chromatids, decondenses uncoiling back into chromatin, microtubules disappear and a new nuclear membrane surrounds each haploid set.

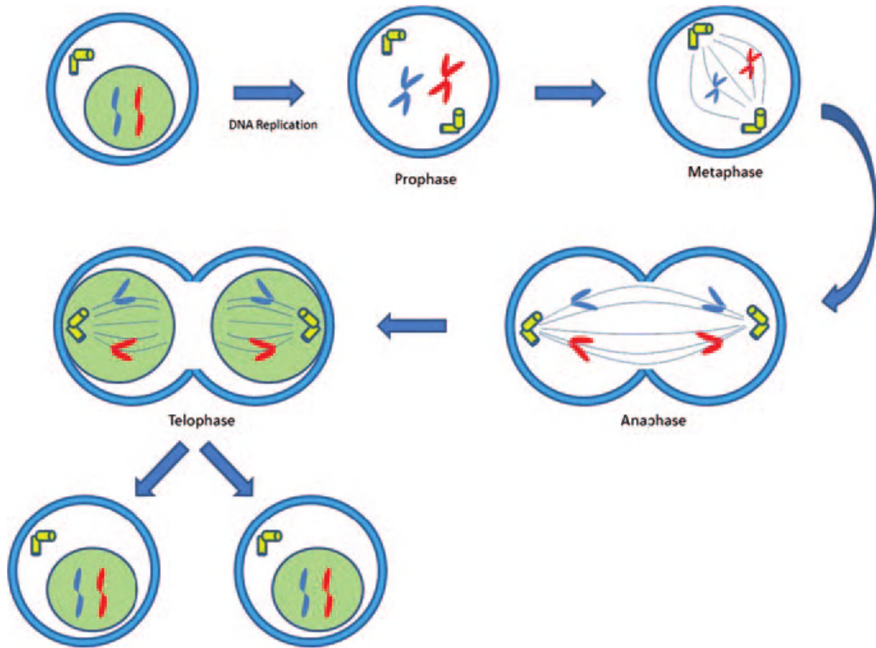

Fig. 1.6 Diagram of mitosis: the process by which one somatic cell produces two daughter cells identical to one another and to the original parent cell. Mitosis is divided into four principals stages: Prophase, Metaphase, Anaphase, and Telophase. During prophase, the chromatin condenses into chromosomes consisting of two sister chromatids. The latter then align at the equatorial plate, attaching to microtubules of the mitotic spindle (Metaphase). The sister chromatids separate and move toward opposite poles (Anaphase) and the nuclear envelope reappears (Telophase). Finally the cytoplasm divides, producing two daughter cells

At the end of meiosis I, the primary spermatocyte divides in two secondary spermatocytes, while the oocyte gives rise to one larger daughter cell (secondary oocyte) and one smaller cell without organelles (first polar body).

After completing meiosis I, the male and female gametes may begin the meiosis II process, with no DNA replication, thus going directly from telophase I to prophase II without the interphase. In prophase II, chromosomes with two chromatids again condense, nucleoli disappear, the nuclear envelope dissolves, and centrioles move to the polar regions arranging spindle fibers. The chromosomes move into the center of the cell, forming a new equatorial metaphase plate (metaphase II), next the kinetochores move toward the poles, splitting up the sister chromatids (anaphase II). The process ends with telophase II, during which the cells divide for the last time, concentrating the chromatids in the opposite poles, dissolving the spindle, reforming the nuclear envelope, and decondensing chromosomes.

At this point a total of four haploid cells, each with a half set of chromosomes, are produced. In fact, the secondary spermatocyte divides into two spermatids, while the secondary oocyte splits into one large cell (precursor of zygote) and one smaller cell (second polar body). It is important to highlight that the ovulated

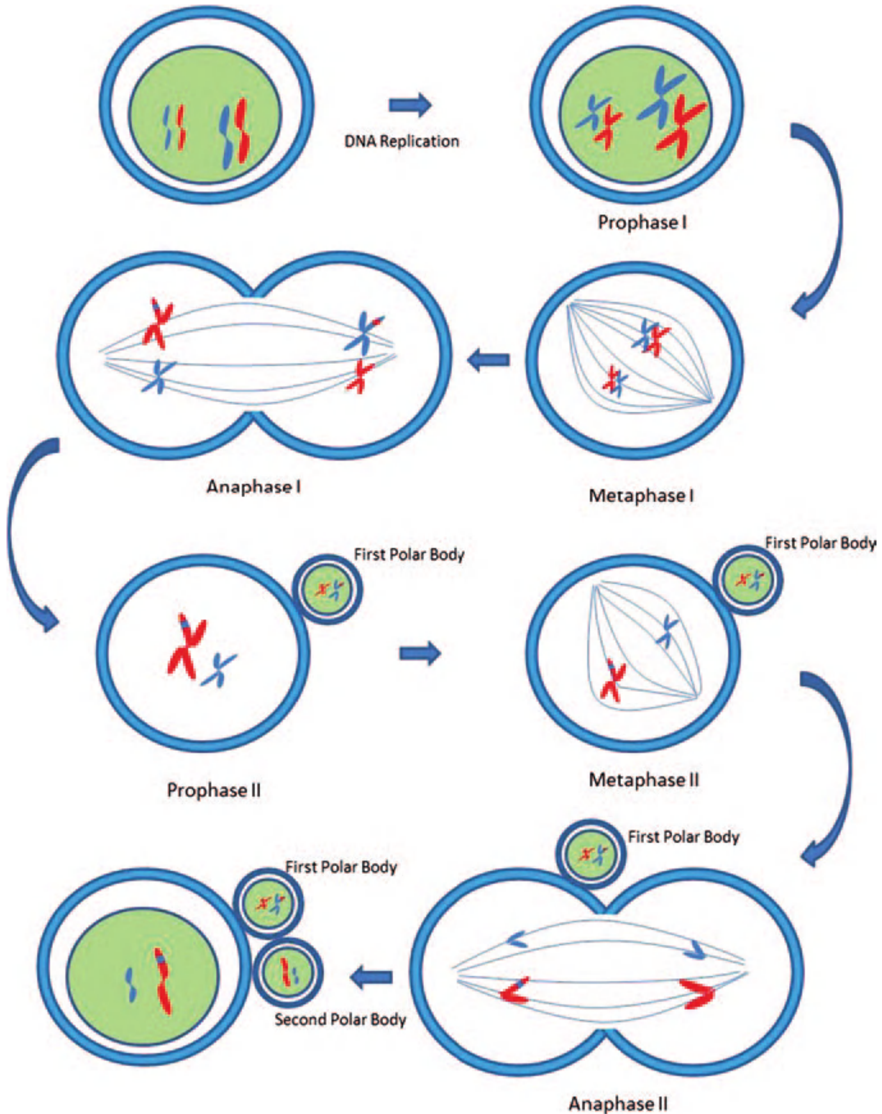

Fig. 1.7 Diagram of meiosis: the process by which a germ cell produces haploid cells, containing one of every pair of homologous chromosomes. It involves only one DNA replication and two distinct and consecutive nuclear reductions (meiosis I and meiosis II), which are divided into Prophase, Metaphase, Anaphase, and Telophase. Briefly, during prophase I the chromosomes condense. They move into the center of the cell and align in pairs along the equatorial plane, forming the tetrad, and crossover takes place (Metaphase I). During the following phases, chromosomes are pulled to opposite poles and form two haploid groups, containing a pair of sister chromatids. After completing meiosis I, gametes directly enter meiosis II, without any DNA replication. In prophase II, chromosomes with two chromatids again condense. They form a new equatorial metaphase plate (metaphase II) and then move toward the poles, splitting up the sister chromatids (anaphase II). The process ends with telophase II, during which the cells divide for the last time, producing haploid cells, thus each with a half set of chromosomes

Table 1.2 Comparison of meiosis and mitosis. Definition, steps, functions, and characteristics of the two processes

	Meiosis	Mitosis
Definition	A process in which the gamete divides into four haploid cells, reducing the number of chromosomes and separating homologous chromosomes	A process in which the cell divides into two identical diploid cells, maintaining an equal number of chromosomes
Occurs in	Human, animals, plants	All organisms
Steps	*Meiosis I*: Interphase, prophase I, metaphase I, anaphase I, and telophase I; *Meiosis II*: Prophase II, metaphase II, anaphase II, and telophase II	Interphase, prophase, metaphase, anaphase, telophase, and cytokinesis
Number of DNA replications	1	1
Number of cell divisions	2	1
Pairing of homologs	Yes	No
Crossing over	Yes	No
Number of daughter cells produced and their chromosomal assessment	4 haploid cells	2 diploid cells
Creates	Gametes only: Female egg cells or Male sperm cells	Somatic cells
Function	Sexual reproduction	Somatic cell replication

oocyte is arrested in metaphase II and only after fertilization it becomes able to conclude the meiosis II process (Figs. 1.6, 1.7 and Table 1.2).

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