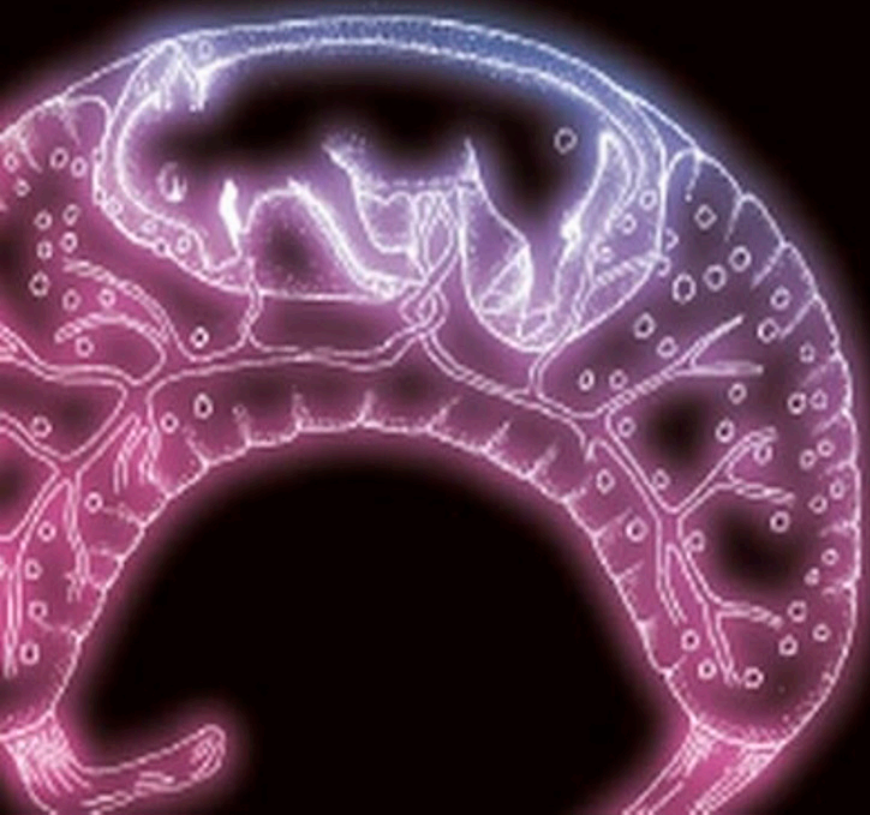
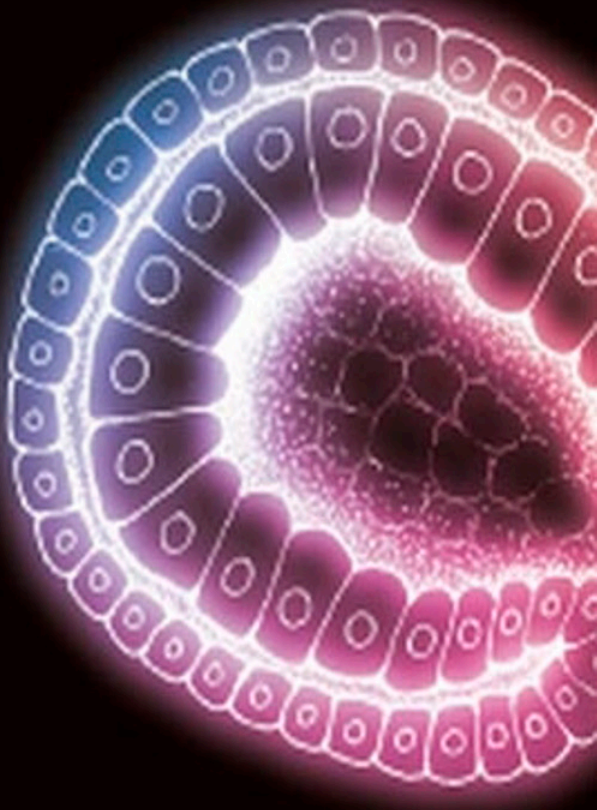
Veterinary Embryology

T. A. McGeady, P. J. Quinn
E. S. FitzPatrick and M. T. Ryan
Illustrated by S. Cahalan





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Preface

An understanding of the origin, development and maturation of cells in the developing embryo and, later, in the foetus provides veterinary students with information relevant to organ primordia and development of body systems. A study of embryology offers the student an understanding of the development, structure, final form and relationships of tissues and organs. Developmental defects and the clinical conditions to which they give rise can be more completely understood through a knowledge of the factors which control developmental processes and the deleterious affects of environmental teratogens on normal embryological development.

This book is primarily concerned with developmental aspects of cells, tissues, organs and body systems of animals. Where feasible, comparative aspects of human embryology are included. Drawings of cells, tissues and organs, along with flow diagrams and tables, are used to provide a clear understanding of information contained in the text.

There are 24 chapters in this book, each dealing with topics which are fundamental to an understanding of the sequential stages of embryological and foetal development. Cell division, gametogenesis, fertilisation, cleavage and gastrulation are presented in sequential chapters. Succeeding chapters are concerned with cell signalling, establishment of a body plan, formation of foetal membranes and placentation. Body systems are considered in separate chapters and the embryological aspects of structures associated with special senses are reviewed. Age determination and aspects of mutagenesis and teratogenesis are briefly reviewed in final chapters.

Although this book is intended primarily as a textbook for undergraduate veterinary students, it may be of value to colleagues engaged in teaching embryology, either as part of a veterinary curriculum or in courses relating to animal science or developmental biology. Research workers engaged in projects on animal reproduction and allied topics may find particular chapters relevant to their fields of investigation.

Throughout the book, emphasis is placed on the origin and differentiation of tissues and organs and their relationships to each other. This approach provides a logical foundation for acquiring an understanding of the form and relationships of cells, tissues, organs and structures in defined regions of the body. Such knowledge is a fundamental requirement for the appreciation of topographical anatomy, a cornerstone in the acquisition of clinical skills, interpretation of diagnostic imaging and the implementation of surgical procedures. Molecular aspects of embryology provide an introduction to genes and the transcription factors which promote or regulate orderly development of the embryo and foetus. Developmental defects of clinical significance are also included. The classification used throughout the book generally conforms to the *Nomina Embryologica Veterinaria* system proposed in 1994. Selected review articles and textbooks are listed in each chapter as sources of additional information.

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Dublin, September 2005

1 Division, Growth and Differentiation of Cells

The mammalian body is composed of an array of organs, tissues and individual cells which function in a specialised and highly coordinated manner. Although these cells, tissues and organs exhibit considerable diversity in both structure and function, they all derive from a single cell, a fertilised ovum. The fertilised ovum is a product of the fusion of two specialised reproductive cells, gametes, of male and female origin. Following fertilisation, the ovum undergoes a series of divisions which ultimately lead to the formation of pluripotent stem cells, from which all cells, tissues and organs of the body arise. The study of this process of growth and differentiation, beginning with the fertilisation of an ovum and progressing to a fully formed individual animal, is termed embryology.

Cells associated with tissue formation and regeneration are described as somatic cells. Specialised reproductive cells, referred to as germ cells, include gametes of male and female origin and their precursors.

Coordinated and regulated cell division is essential for embryological development. Somatic cell division consists of nuclear division, mitosis, followed by cytoplasmic division, cytokinesis. In mitotic division of somatic cells, the daughter cells produced are genetically identical. A form of cell division distinctly different from mitosis occurs in germ cells. In this form of cell division, referred to as meiosis, the cells produced contain half the number of chromosomes of the progenitor germ cell and are not genetically identical. Somatic cell division combined with other cellular processes such as progressive differentiation, migration, adhesion, hypertrophy and apoptosis are prerequisites for embryological development.

The cell cycle

Somatic cells undergo a series of molecular and morphological changes as part of the cell cycle. These changes occur in four sequential phases, namely G_1, S, G_2 and M, and also a quiescent phase, termed G_0 (Fig. 1.1). The G_1 and G_2 phases are termed resting phases. In these phases, the cell is metabolically active, fulfilling its specialised function preparatory to the next phase of the cycle, but DNA replication does not take place. During the S phase, DNA synthesis occurs prior to chromosomal replication. This is followed by mitosis which occurs during the M phase. Collectively, the G_1, S and G_2 phases constitute the interphase (Fig. 1.1). Cells which enter a G_0 state may remain transiently or permanently in that state. Certain fully differentiated cells, such as neurons, do not divide, and continue to function permanently in a G_0 state. Other cell types, such as epithelial cells and hepatocytes, can re-enter the cell cycle from G_0 and proceed to mitotic division in response to appropriate stimuli.

A number of stimuli such as growth factors, mitogens and signals from other cells and from the extra-cellular matrix can induce cells in a G_0 state to re-enter the cell cycle near the end of the G_1 phase. Growth factors which bind to cell surface receptors activate intracellular signalling pathways. In most mammalian cells, the activation of genes encoding cyclins and cyclin-dependent kinases (Cdks) specific to the G_1 phase regulate the cell cycle and commit the cell to enter the S phase. This process is initiated at the restriction point, a stage at which mammalian cells become committed to entering the S phase and are then capable of completing the cell cycle independent of extra-cellular influences.

The rate of cell division varies in different cell types and at different stages of differentiation. Variations in cell cycle length are largely attributed to differences in the length of the G_1 phase, which can range from six hours to several days. Early embryonic development is characterised by rapid cell division, but as cells become more differentiated during organ development, the rate of cell division generally decreases.

Mitosis

The nuclei of somatic cells of each mammalian species have a defined number of chromosomes (Table 1.1). A somatic cell with a full complement of chromosomes

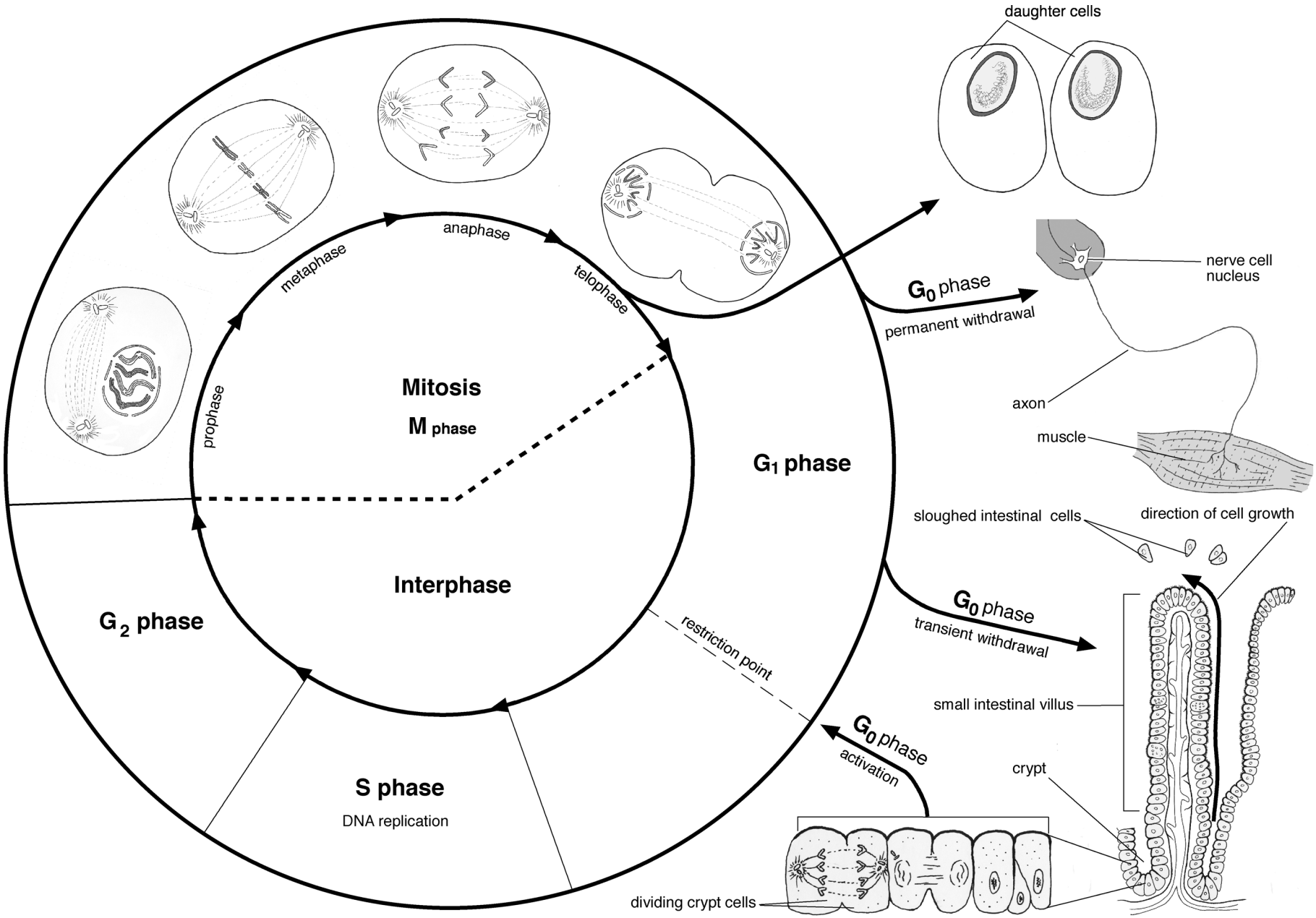

Figure 1.1 Stages in somatic cell division indicating the major phases of the cell cycle.

Table 1.1 The number of chromosomes in diploid human and animal cells.

Species	Number of chromosomes (2n)
Humans	46
Cats	38
Cattle	60
Chickens	78
Dogs	78
Donkeys	62
Goats	60
Horses	64
Pigs	38
Rabbits	44
Rats	42
Sheep	54

is referred to as diploid and given the designation 2n. The term mitosis is used to describe nuclear division of somatic cells, a process which usually results in the production of two cells, with the same chromosome complement as the progenitor cell from which they derived. Mitosis is essential for embryonic growth and development and for repair and replacement of tissue throughout life. The stages of mitosis occur as a distinct sequence of cytological events, which are part of the cell cycle.

Stages of mitosis

Preparatory to mitosis, the chromosomes are replicated in the S phase of the cell cycle forming sister chromatids. Within the nuclear envelope sister chromatids remain attached at a constricted region of the chromosome called a centromere. Following the G_2 phase, mitosis, which can be divided into four stages, prophase (Fig. 1.2B), metaphase (Fig. 1.2C), anaphase (Fig. 1.2D) and finally telophase (Fig. 1.2E), begins. The stages of mitosis are usually followed by cytoplasmic division or cytokinesis (Fig. 1.2F).

Prophase

The first stage of mitosis is prophase (Fig. 1.2B). During this period, the chromosomes, consisting of closely associated sister chromatids, condense. Outside the nucleus, the centrosomes, composed of paired centrioles previously replicated during interphase, begin to form microtubule spindles or asters. The spindles are responsible for the movement of the centrosomes to opposite poles of the dividing cell.

Microtubules, an essential part of the mitotic apparatus, are visible microscopically only during the M phase. Individual microtubules are cylindrical structures, composed of 13 parallel protofilaments consisting of alternating α-tubulin and β-tubulin subunits. An individual microtubule may grow or shrink by a process of polymerisation of α-tubulin and β-tubulin. A growing microtubule has a structure referred to as a guanidine-triphosphate (GTP) cap. The β-subunit of a microtubule contains GTP capable of being hydrolysed to guanidine-diphosphate (GDP). This, in turn, alters the conformation of the subunits, resulting in shrinking of the microtubules. If GTP hydrolysis occurs more rapidly than subunit addition, the cap is lost and the microtubule shrinks. Shrinking and growing are a dynamic process and these changes enable the microtubules to actively orientate and move chromosomes during mitosis and meiosis.

Metaphase

Events during the metaphase stage of mitosis can be divided into two phases, pro-metaphase and metaphase. Disintegration of the nuclear envelope marks the beginning of pro-metaphase. A kinetochore, a protein complex which forms on the centromeres during late prophase, acts as a platform for attachment to microtubules. Chromosomes attach to the microtubules via their kinetochores and the combination of these two latter structures is termed a kinetochore microtubule. The formation of the kinetochore microtubule enables the movement of chromosomes to take place. During metaphase, the chromosomes are positioned midway between the poles of the cell at a region termed the metaphase plate. Each sister chromatid is attached to the centrosome by its kinetochore microtubule (Fig. 1.2C).

Anaphase

During the anaphase stage, the pairs of conjoined sister chromatids synchronously separate as the centromeres split and the attached kinetochore microtubules shorten. The newly separated chromatid sets are drawn towards opposite poles of the cell (Fig. 1.2D).

Telophase

The two groups of identical chromosomes (former chromatids) clustered at their respective poles, de-condense and a nuclear envelope forms around each set. The formation of nuclear envelopes marks the end of mitosis, a process which results in equal and symmetrical division of the nucleus (Fig. 1.2E).

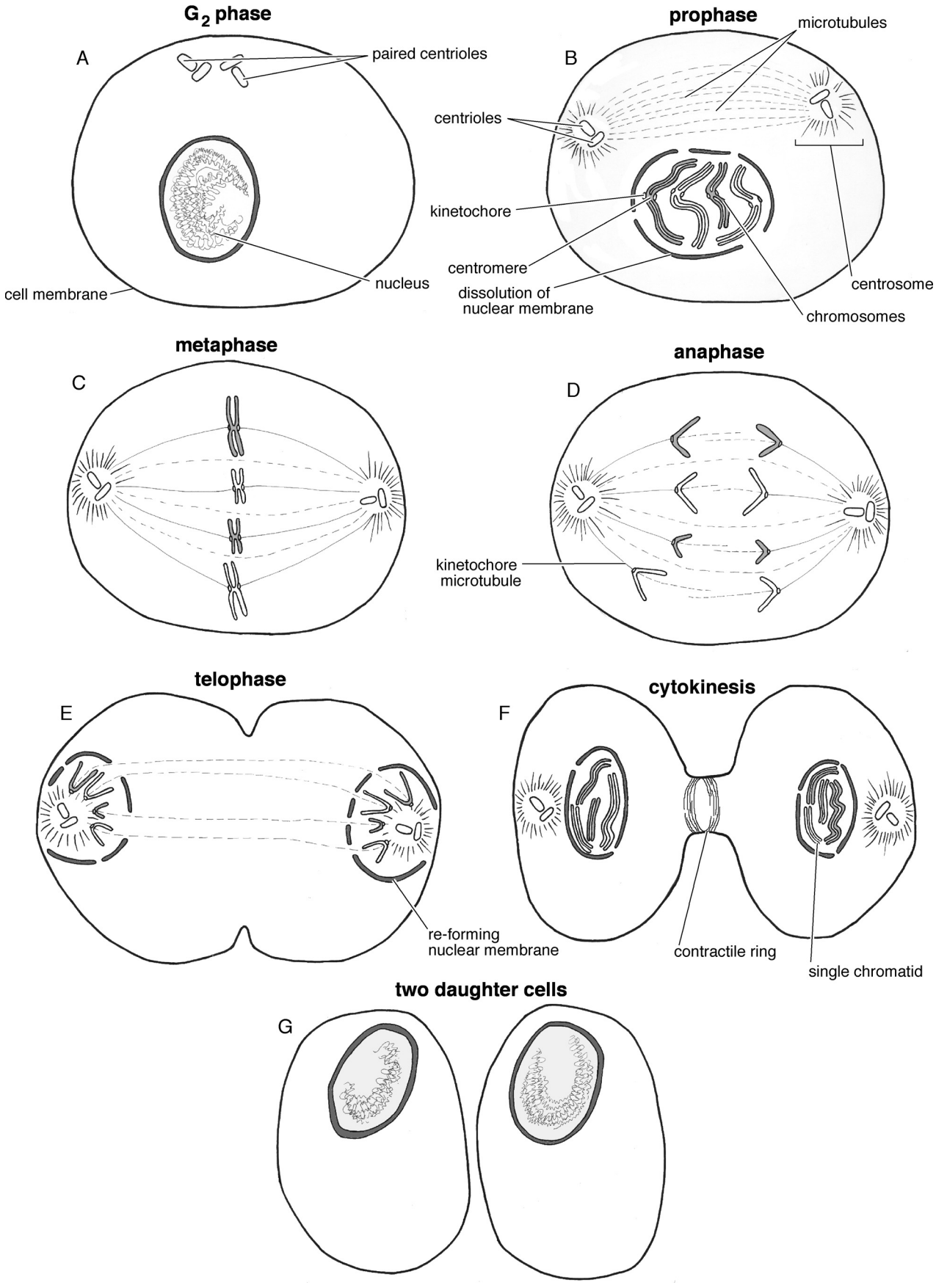

Figure 1.2 An outline of the sequential stages in mitosis (A to G). After the G_2 phase, prophase commences followed by metaphase, anaphase, telophase and cytokinesis, leading to the formation of two daughter cells.

Cytokinesis

Following the formation of the nuclear envelope, a contractile ring of actin and myosin pinches the cell wall and divides the cytoplasm, resulting in the formation of two daughter cells (Figs. 1.2F and G). This latter process, termed cytokinesis, typically results in the formation of two equally-sized daughter cells. Occasionally, unequal amounts of cytoplasm or organelles may be distributed to the daughter cells during cytokinesis. In some instances mitosis may occur without subsequent cytokinesis, resulting in the formation of binucleate or, occasionally, multinucleate cells.

In lower organisms such as amphibians, the cytokinesis which occurs early in development can generate daughter cells in which the factors which direct the fate of the cells may not be uniformly distributed. This unequal division of fate determinants results in differing developmental potential in individual daughter cells. In mammals, experimental evidence suggests that cell divisions which give rise to totipotential cells occur early in development. This suggests that, in mammals, cytoplasmic determinants are shared uniformly between daughter cells and that the initial stages of differentiation arise as a result of cell communication and microenvironmental factors.

Regulation of mitosis

The enzyme M-cyclin-dependent kinase (M-Cdk) has a central role in the initiation of mitosis following the G_2 phase of the cell cycle. This heterodimeric protein, which is a complex of Cdk1 and M-cyclin, is activated by the removal of inhibitory phosphate groups in the late G_2 phase. The M-Cdk protein induces events essential for mitosis, including phosphorylation of the proteins which control microtubule dynamics, chromatin condensation, rearrangement of both the cytoskeleton and organelles and, finally, dissolution of the nuclear envelope. Although the mitotic cell cycle is normally highly regulated, undesirable alterations in the functioning of the genes known as proto-oncogenes or tumour suppressor genes, responsible for the control of cell proliferation or differentiation, may lead to malignant transformation of normal tissue. Typically, changes in two or more of these regulatory genes appear to be required for cells to undergo malignant transformation.

Mitotic division in successive generations of cells derived from a neoplastic cell continues to give rise to abnormal cells which are not subject to normal regulatory processes. Neoplastic conditions such as leukaemia, lymphoma and myeloma can arise from gene alteration within a single cell in the bone marrow or in peripheral lymphoid tissue. With the accumulation of large populations of abnormal cells, clinical effects of neoplasia become evident.

Meiosis

This process of cell division occurs only during gametogenesis. Meiosis differs from mitosis in several respects:

(1) The resulting gametes are haploid and are given the designation ‘n’.
(2) There is a reciprocal exchange of genetic material between non-sister chromatids (Fig. 1.3).
(3) The resulting gametes are a product of the random segregation of maternally-derived and paternally-derived chromatids.

Meiosis is divided into two stages, meiosis I and II.

The first meiotic division

Meiosis I consists of prophase I (Figs. 1.4B and C), metaphase I (Fig. 1.4D), anaphase I (Fig. 1.4E) and telophase I (Fig. 1.4F). The amount of DNA in a cell entering prophase I doubles.

Prophase I

During prophase I, many crucial intracellular events occur (Figs. 1.4B and C). This process can be further subdivided into five substages: leptotene, zygotene, pachytene, diplotene, and diakinesis. At the diakinesis stage, the chromosomes become short and thick, the centrosomes are positioned at the poles and the nuclear membrane begins to disintegrate.

During prophase I, segments of chromosome are exchanged between homologous but non-sister chromatids (Fig. 1.4C). This process is referred to as crossover. At this stage, duplicated homologous chromosomes assemble side by side and assume a tetrad configuration. Chromatid arms within the tetrad may then overlap to form a chiasma, which allows crossover to take place between paternally-derived and maternally-derived chromatids (Fig. 1.3). As a consequence of crossover, recombinant chromatids acquire an allocation of genetic material derived from both paternal and maternal chromatids. The crossover events which occur during meiosis extend the genetic variation beyond that which is possible from the random segregation of maternal and paternal chromatids. It is generally accepted that the variability arising from the recombination confers evolutionary advantage on animal populations in accordance with the principles of natural selection.

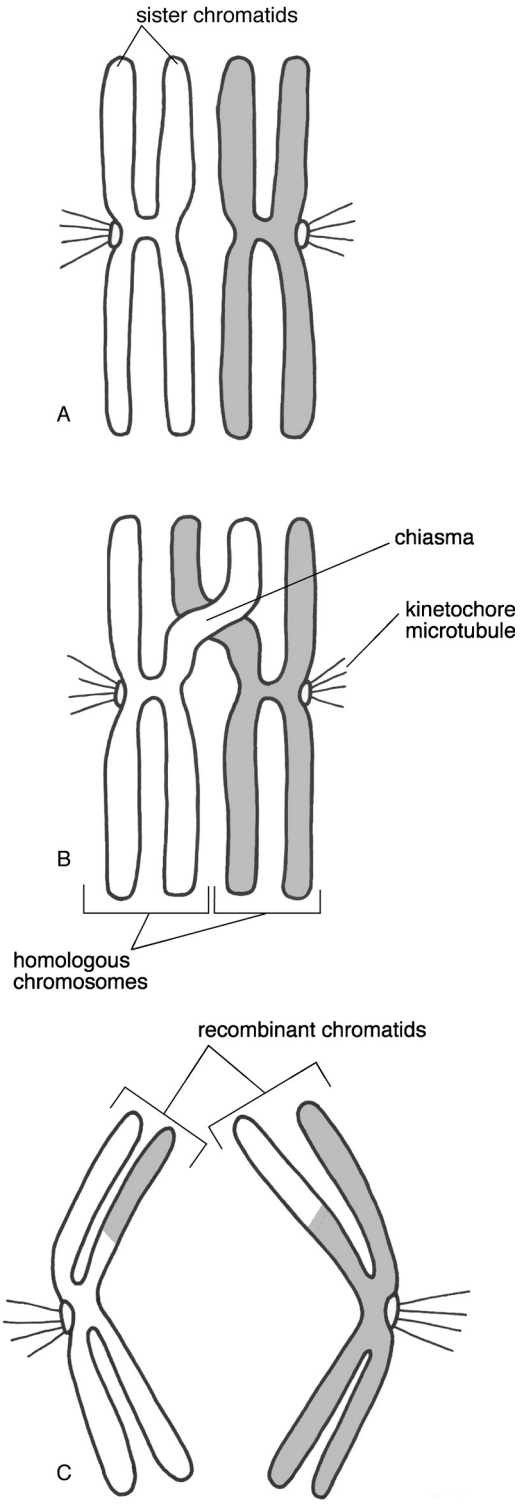

Figure 1.3 Chiasma formation and reciprocal exchange of genetic material between non-sister homologous chromatids during meiosis I.

Metaphase I

As in mitosis, homologous chromosome pairs attach via their kinetochores to the microtubules arising from the centrosomes which are located at opposite poles of the cell. During metaphase, the homologous chromosome pairs are positioned at the metaphase plate by the kinetochore microtubules (Fig. 1.4D).

Anaphase I

During anaphase I, the tetrad splits into two dyads (half a tetrad), which move to opposite poles of the cell. Unlike the anaphase stage of mitosis, splitting of the centromeres does not occur because in this instance only one kinetochore forms on each dyad. The distribution of paternally derived and maternally derived homologous chromosomes at this point is random, and it is this variable arrangement which underlies the Mendelian principle of random assortment (Fig. 1.4E).

Telophase I

In telophase I, nuclear envelopes develop around the separate chromosome sets and cytokinesis follows (Figs. 1.4F and G). In the formation of primary spermatocytes, progenitors of male gametes, the cytoplasm is divided equally between the two cells. However, during the formation of oocytes, female gametes, one of the two resulting cells retains the greater portion of cytoplasm. The smaller of the two cells is termed a polar body. A short resting phase, termed interkinesis, follows telophase I and replication of DNA does not occur during this phase.

The second meiotic division

Prophase II

The events of prophase II are similar to prophase I. The nucleus contains a set of dyads each composed of a pair of chromatids connected by a shared centromere (Fig. 1.5A).

Metaphase II

The phase termed metaphase II is similar to metaphase I in that the chromosomes are positioned at the metaphase plate by the kinetochore microtubules. In this instance, however, kinetochores form on each of the individual chromatids. This allows the microtubules to attach separately to each chromatid (Fig. 1.5B).

Anaphase II

During anaphase II, the dyads are separated into individual chromatids by the kinetochore microtubules and the sets of chromatids are drawn towards opposite poles of the dividing cell (Fig. 1.5C).

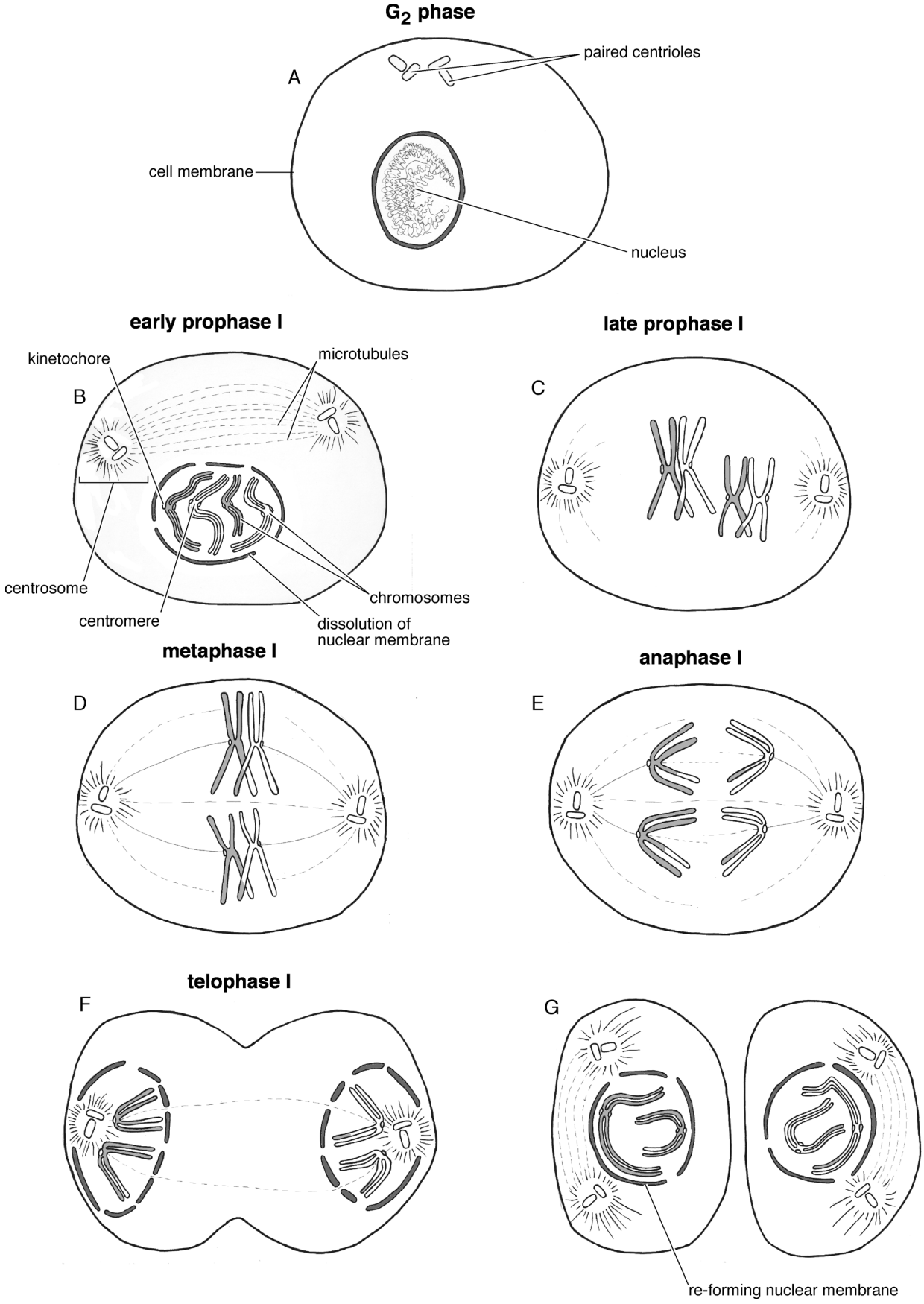

Figure 1.4 An outline of the sequential stages of the first meiotic division (A to G). After the G_2 phase, prophase I commences followed by metaphase I, anaphase I and telophase I.

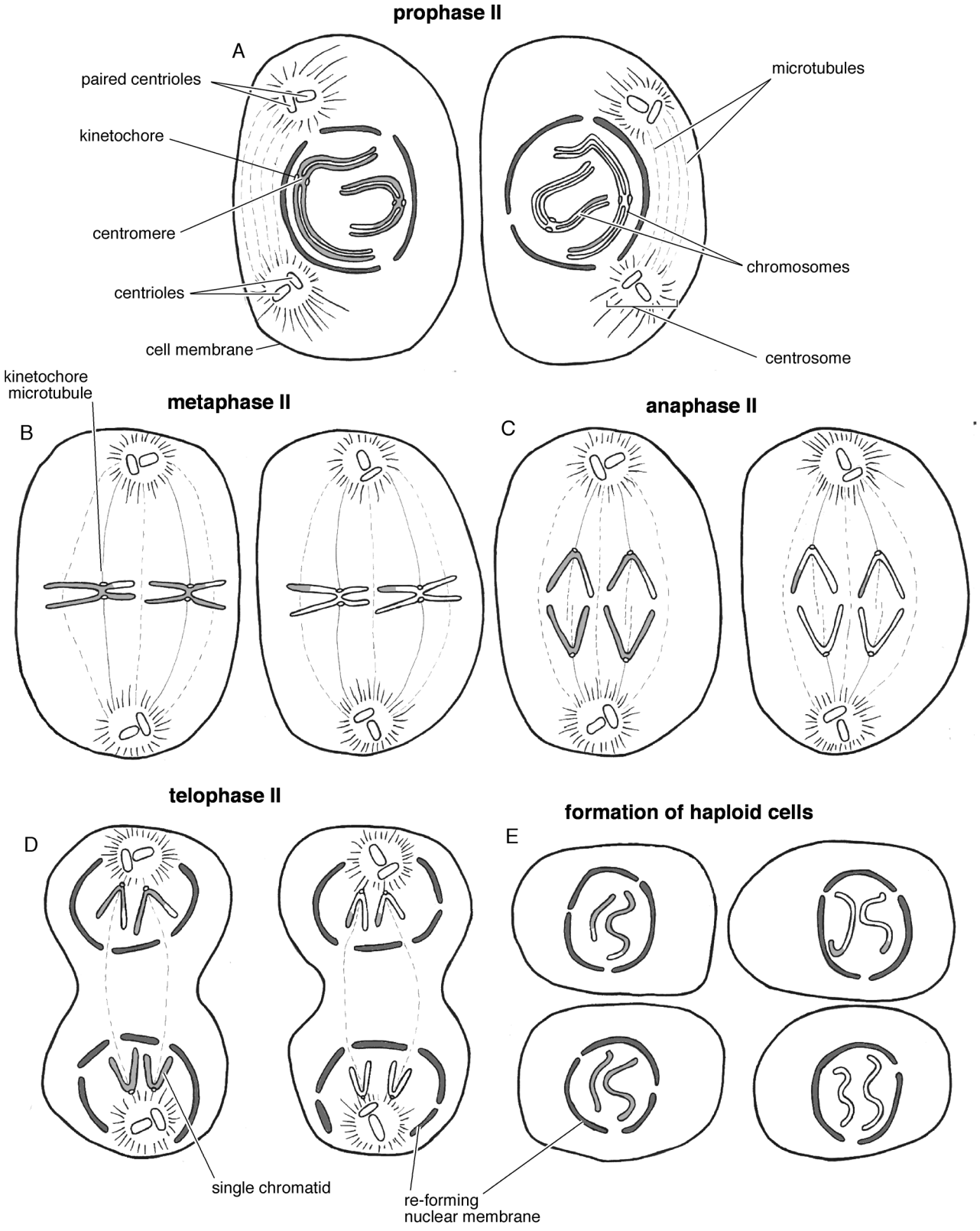

Figure 1.5 An outline of the sequential stages of the second meiotic division (A to G). After meiosis I, prophase II commences, followed by metaphase II, anaphase II, and telophase II, leading to the formation of four haploid gametes. Only two pairs of chromosomes are represented for clarity.

Telophase II

At the end of telophase II, nuclear envelopes form around each set of chromatids and the cytoplasm divides again (Fig. 1.5D). As a consequence of meiosis I and II, four haploid cells are formed from a single diploid germ cell (Fig. 1.5E).

Consequences of non-dysjunction of chromosomes during meiosis

The term non-dysjunction describes the failure of two homologous chromosomes in meiosis I, or sister chromatids in meiosis II, to separate properly and to move correctly to opposite poles. Meiosis depends on the establishment of specialised interactions between chromosomes along with specific modifications to the mitotic cell cycle regulatory processes. Errors in these processes, which usually occur during meiosis I, can result in defective segregation. Abnormalities arising from this include numerical alteration and structural defects in chromosomes. While chromosomal defects associated with germ cells generally lead to embryonic death, in some instances offspring may survive and exhibit developmental defects. Alterations of chromosome numbers may involve either autosomes or sex chromosomes.

Further reading

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K. and Walter, P. (2002) *Molecular Biology of the Cell*, 4th edn. Garland Science, New York.

Klug, W.S. and Cummins, M.R. (1999) *Essentials of Genetics*. Prentice-Hall, Upper Saddle River, New Jersey.

Levine, E.M. (2004) Cell cycling through development. *Development* **131**, 2241–2246.

Marston, A.L. and Amon, A. (2004) Meiosis: cell-cycle controls shuffle and deal. *Nature Reviews: Molecular and Cell Biology* **5**, 983–997.

2 Gametogenesis

The sequential stages in the differentiation and maturation of primordial germ cells into gametes in male and female animals are referred to as gametogenesis. Primordial germ cells in the endoderm of the yolk sac migrate via the dorsal mesentery to the developing gonads. During migration these cells undergo mitosis, producing large numbers of germ cells which populate the gonads. Germ cells undergo similar sequential development in male and female animals.

Spermatogenesis

Primordial germ cells undergo a series of mitotic divisions producing stem cells which, in association with mesodermal cells, form seminiferous cords in the developing testis. In this location, they remain quiescent until the onset of puberty, when sexual maturation begins. At puberty, these dormant germ cells become activated and, through a series of mitotic divisions, produce clones of cells referred to as type A spermatogonia (Fig. 2.1). Subsequently, some type A cells divide, giving rise to type B spermatogonia, from which primary spermatocytes arise.

The diploid primary spermatocytes undergo the first stage of meiotic division resulting in the formation of haploid secondary spermatocytes. When these haploid secondary spermatocytes undergo the second stage of meiotic division, they form haploid spermatids (Fig. 2.1).

The process whereby a spermatid undergoes metamorphosis into a spermatozoon is termed spermiogenesis (Fig. 2.2). Initially the spermatid has the organelles of a typical mammalian cell including a spherical nucleus, a Golgi complex, mitochondria, paired centrioles and endoplasmic reticulum. Granules, which are synthesised in the Golgi complex, fuse forming a single large acrosomal vesicle. When this vesicle covers the anterior aspect of the condensed nucleus, it is referred to as the acrosome. The centrioles, which migrate to the pole of the nucleus opposite the acrosome, form the axial filament from which the tail of the spermatozoon develops. Mitochondria aggregate in the proximal region of the filament forming the middle piece of the spermatozoon. Excess portions of cytoplasm shed from individual spermatids are collectively referred to as residual bodies. A unique feature of spermatogenesis is that the cytoplasmic divisions of the dividing spermatogonia are incomplete as the spermatocytes remain attached by cytoplasmic bridges. The time required for the production of spermatozoa from type A spermatogonia may range from 40 to 60 days depending on the species.

As spermatogenesis proceeds, the spermatogenic cells develop in close association with Sertoli cells in the seminiferous tubules. The germ cells are almost completely surrounded by the cytoplasm of Sertoli cells which nourish and support them during differentiation. Tight junctions between adjacent Sertoli cells divide seminiferous tubules into basal compartments and adluminal compartments, thereby preventing the entry of cells involved in the generation of immunological responses into the adluminal compartments. These junctions also prevent macromolecules from crossing from the adluminal compartments into the animal's circulation. The structures which isolate the cells on the adluminal side of seminiferous tubules from the testicular vascular supply constitute the blood–testis barrier. At the completion of spermiogenesis, immature spermatozoa are extruded from their intimate association with the Sertoli cells into the lumen of the seminiferous tubules, a process referred to as spermiation. Prior to their release, most of the cytoplasm of the immature spermatozoa is shed and phagocytosed by Sertoli cells. At the time of its release into the lumen of the seminiferous tubule, a small amount of cytoplasm, the protoplasmic droplet, remains attached to the middle piece of the immature spermatozoon. The spermatozoa within the seminiferous tubules are immotile and are carried passively by the tubular fluid to the rete testis. From this location they are conveyed by ten to 20 efferent ductules to the epididymis through the ciliary action of duct epithelium and the contractions of the smooth muscle of the duct wall.

The epididymis, which consists of a long, tightly convoluted tube, is anatomically divided into three regions, head, body and tail. During their passage through the epididymis, spermatozoa undergo a maturational

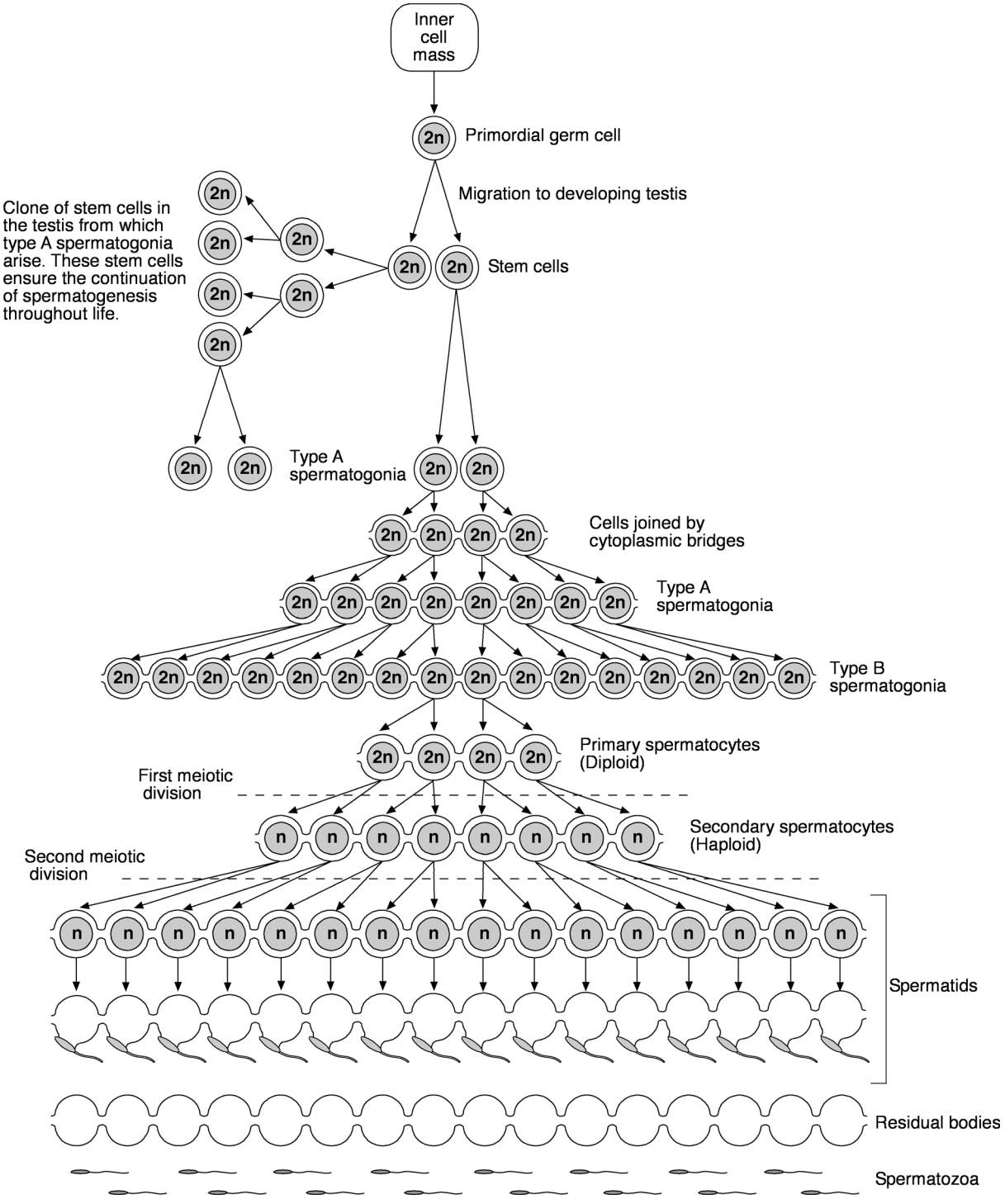

Figure 2.1 Stages in the development of spermatozoa from a primordial germ cell.

process which confers on them the ability to fertilise an ovum. As they mature, spermatozoa undergo a number of morphological and physiological changes. These include alterations in nuclear chromatin, changes in the composition of the plasma membrane and loss of the protoplasmic droplet. In addition, spermatozoa acquire the ability to propel themselves forward. Associated with the maturational process in the epididymis, some seminiferous and efferent duct fluid is absorbed resulting in an increased concentration of spermatozoa in the remaining fluid.

Mature spermatozoa capable of fertilisation are stored in the tail of the epididymis prior to ejaculation. In domestic animals, spermatozoa may remain viable for up to three weeks while in humans they may be stored in

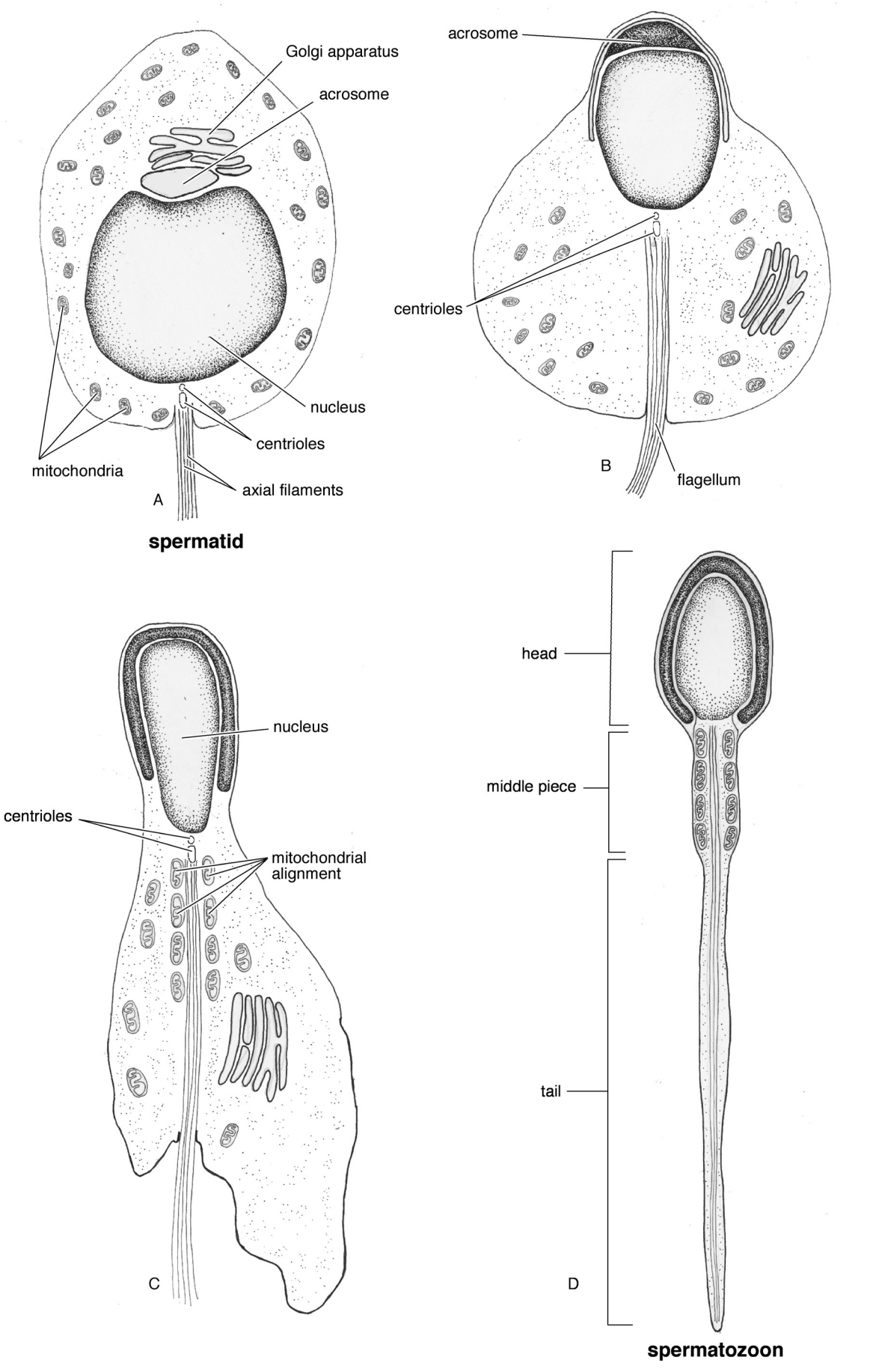

Figure 2.2 The morphological changes whereby a mammalian spermatid is converted into a spermatozoon.

the epididymis for only a few days before losing their viability. Most of the unejaculated spermatozoa are gradually discharged into the urinary system; a small percentage which remain in the epididymis undergo degenerative change and are phagocytosed. The transport of spermatozoa through the epididymis, due to contractions of the smooth muscle of the epididymal duct wall, takes up to 12 days in the bull and ram and up to 14 days in the boar and stallion. With increased frequency of ejaculation, transport time may be reduced.

Oogenesis

Oogonia, which arise from primordial germ cells in the endoderm, undergo repeated mitotic divisions in the foetal ovary. The duration of this period of mitosis varies in individual species. Irrespective of species, the mitotic phase of oogenesis ceases in mammals soon after birth. When they have completed their cycles of mitosis, oogonia enter the prophase of the first of two meiotic divisions and become primary oocytes which are diploid. Such diploid cells are given the designation 2n to indicate that they contain a full complement of chromosomes. All primary oocytes are formed before puberty (Fig. 2.3).

A primary oocyte surrounded by a single layer of squamous epithelial cells is known as a primordial follicle (Fig. 2.4). Primary oocytes do not complete the prophase of the first meiotic division but enter a prolonged resting or dictyate stage until activated by gonadotrophic hormones which induce further development. During both the proliferative and resting phases, a high proportion of primordial follicles undergo atresia. Completion of the initial stage of the first meiotic division follows hormonal stimulation. During puberty, the oocyte increases in size and the surrounding epithelial follicular cells form a stratified layer around the oocyte. This structure is now known as a primary follicle. Glycoproteins, secreted primarily by the oocyte, condense forming a prominent translucent acellular layer, the zona pellucida, located between the vitelline membrane of the oocyte and the follicular cells. As the follicle enlarges, the thickness of the zona pellucida increases. The oocyte and the follicular cells maintain contact by means of microvillous cytoplasmic processes which penetrate the zona. Gap junctions between the oocyte and the cytoplasmic processes of follicular cells allow intercellular communication. As the follicle continues to increase in size, small fluid-filled spaces appear between the follicular cells which gradually coalesce forming a fluid-filled cavity known as the antrum. The squamous follicular cells, which become cuboidal, form stratified layers and are referred to as granulosa cells. The oocyte remains attached to the follicular wall by an accumulation of granulosa cells termed the cumulus oophorus (Fig. 2.4). Those granulosa cells which surround the oocyte in a radial fashion are referred to as the corona radiata. The mature follicle is now referred to as a vesicular or Graafian follicle. The completion of the first meiotic division results in the production of two haploid cells of unequal size. The cell which receives most of the cytoplasm is referred to as the secondary oocyte and the other, which receives a minimal amount of cytoplasm, is the first polar body (Fig. 2.3). Following formation of the first polar body, the secondary oocyte commences the second meiotic division.

Ovulation

Release of the ovum from the follicle is referred to as ovulation (Fig. 2.4). Prior to ovulation, the oocyte and corona radiata detach from the cumulus oophorus and float in the follicular fluid. Rupture of the follicle is attributed to the formation of a blister-like area, the stigma, on the ovarian surface directly above the follicle. While it is accepted that the stigma arises from constriction of blood vessels as a result of hormonal or enzymatic activity, the exact details of follicular rupture are poorly understood.

Although ovulation generally occurs near the end of oestrus, the precise time at which it occurs differs among domestic species (Table 2.1). Ovulation occurs spontaneously in most species (spontaneous ovulation). In cats, rabbits, ferrets and camels, however, ovulation is induced by coitus (induced ovulation). The number of ova released, which is characteristic for a given species, is strongly influenced by genetic factors. In most mammals, ovulation occurs during the metaphase of the second meiotic stage of oogenesis. Exceptions include dogs and foxes, where ovulation usually occurs during the metaphase of the first meiotic division. Completion of the second meiotic division and formation of the second polar body occur after fertilisation.

Transport of ova in the uterine tube

After ovulation, the ovum enters the uterine tube, the site of fertilisation in mammals. Tubal wall contractions aided by the ciliary beat of the epithelium of the tube are responsible for the transportation of ova along the tube. Whether or not they are fertilised, ova normally reach the uterus within three to four days after ovulation. However, in domestic carnivores it may take up to seven days for ova to reach the uterus. Fertilised ova of horses and bats enter the uterus, whereas non-fertilised ova are retained at the isthmus of the uterine tube. In rabbits, opossums and dogs, a mucopolysaccharide coat forms around the zona pellucida while the ovum is in the uterine tube. As the uterus provides a favourable environment for the survival of spermatozoa but not for the blastocyst, it is essential that fertilised ova be transported

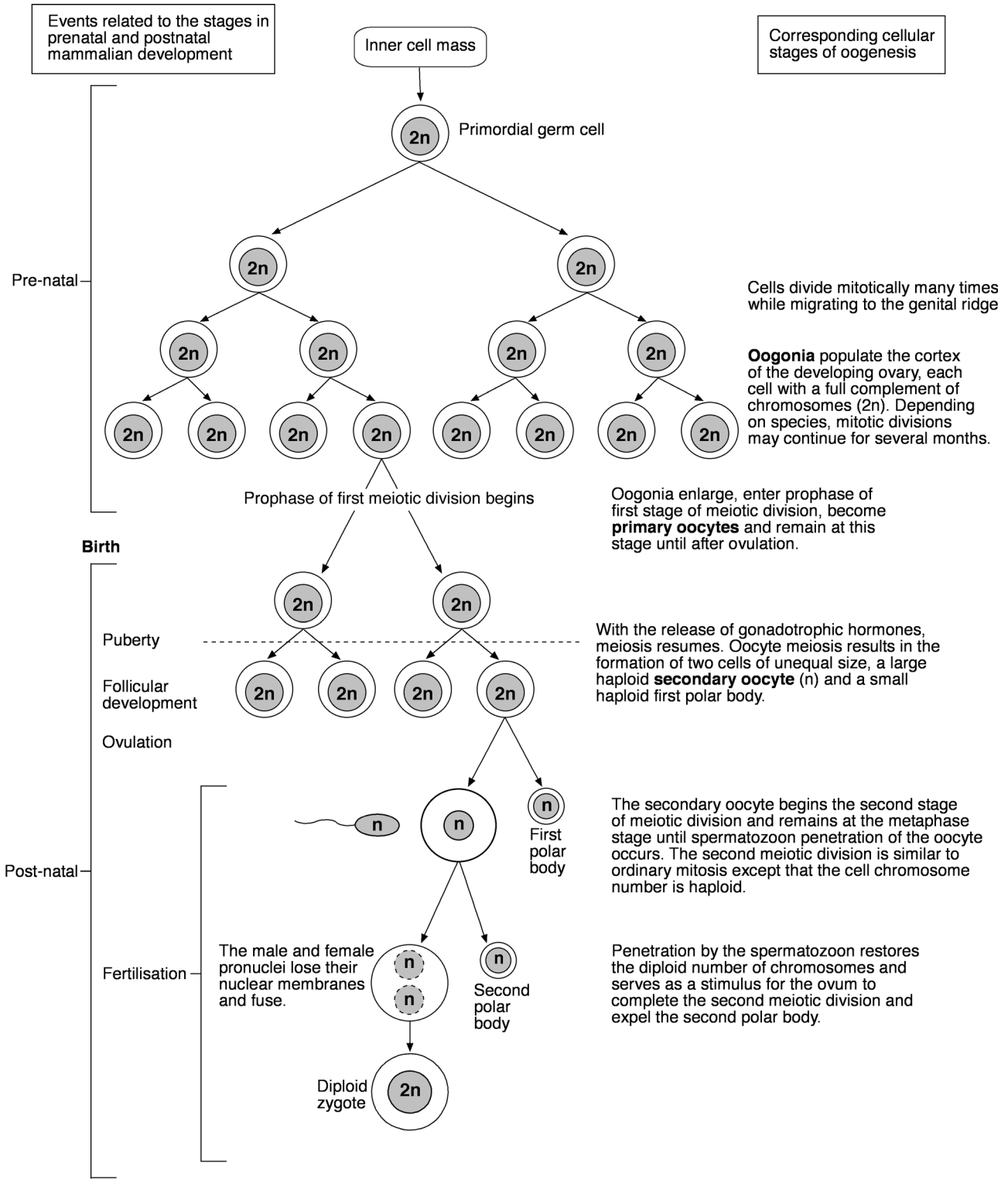

Figure 2.3 Oogenesis, which begins in foetal life, is not completed until animals are sexually mature. Oocytes, gametes produced by female animals, provide the maternal genetic material and nourishment for the developing zygote.

slowly to the uterus. The uterine microenvironment is favourable for the survival of developing embryos during the luteal stage of the oestrous cycle only. In embryo transfer procedures, therefore, it is essential for implantation that the reproductive physiological status of the donor and recipient are synchronised.

In utero *migration of embryos*

Migration of the embryo from one uterine horn to the other occurs in pigs, dogs, cats and horses. Between the 12th and 14th days of pregnancy in the mare, the conceptus (embryo including foetal membranes) moves

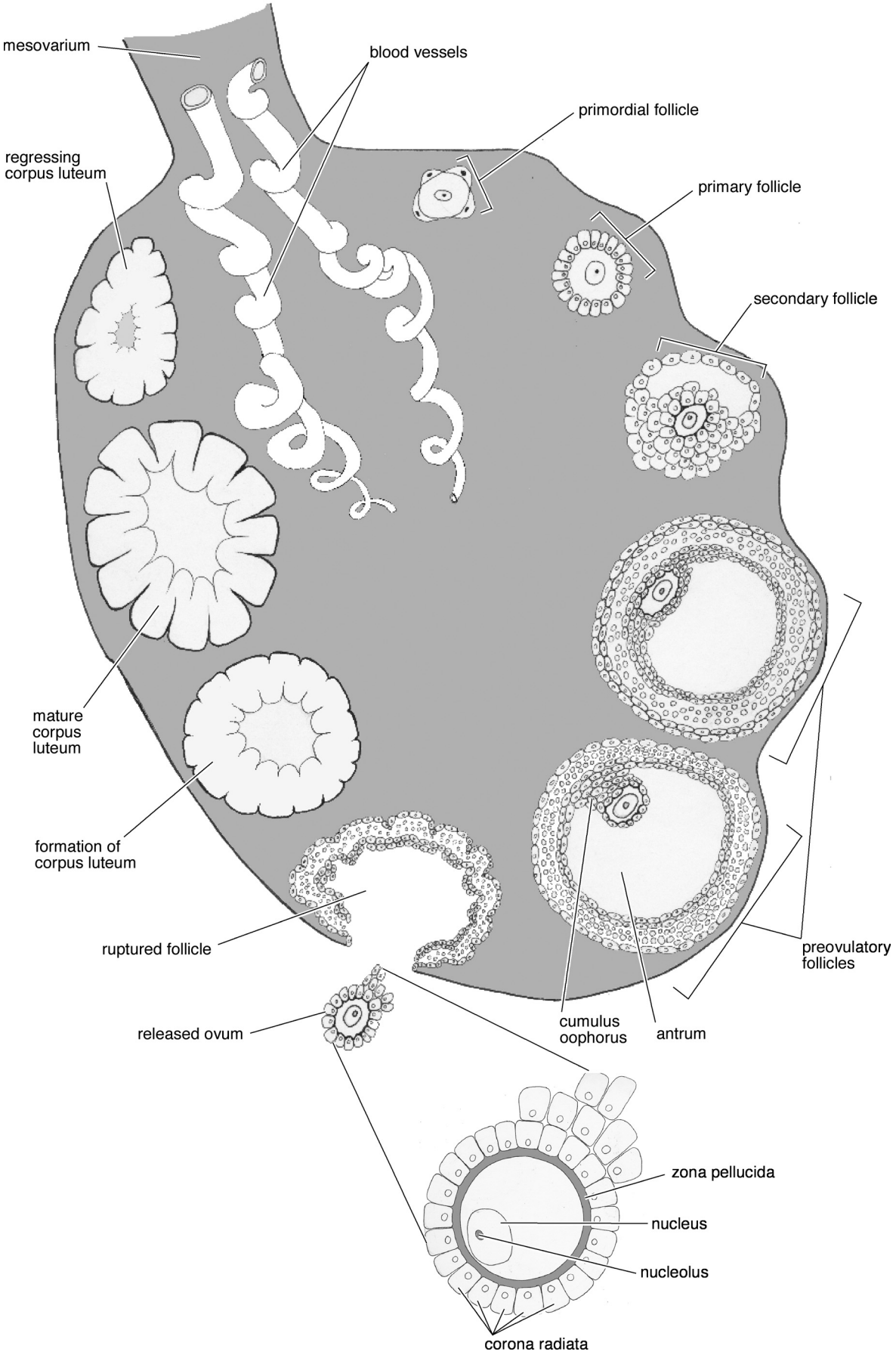

Figure 2.4 Follicular development, ovulation, formation and regression of the corpus luteum in the mammalian ovary. Details of the released ovum and its associated structures are illustrated.

Table 2.1 Features of the oestrous cycle in domestic animals.

Animal	Length of oestrous cycle in days	Duration of oestrus	Number of ova usually released from ovary	Time at which ovulation occurs
Bitch	140	9 days	2 to 10	2 to 3 days after commencement of oestrus
Cow	18 to 24	18 hours	1	14 hours after end of oestrus
Ewe	15 to 17	36 hours	1 to 3	24 to 30 hours after onset of oestrus
Goat	18 to 22	24 to 48 hours	2 to 3	24 to 36 hours after onset of oestrus
Mare	18 to 24	4 to 8 days	1	1 to 2 days before end of oestrus
Queen	17	3 to 6 days	2 to 8	24 hours after coitus
Sow	19 to 22	48 hours	10 to 25	36 to 48 hours after onset of oestrus

from one uterine horn to the other up to 14 times per day. While intrauterine migration can occur in cattle and sheep, the frequency is low in sheep (4%) and rare in cattle (0.3%). Embryo migration and spacing within the uterus appear to be regulated by peristaltic contractions of the myometrium, influenced by hormones released from the conceptus.

Optimal time for fertilisation of the ovum

In individual species, there is a maximum period during which an ovum remains capable of being fertilised. Loss of viability is gradual and although ageing ova may be fertilised, the resulting embryos are usually not viable. Senescence appears to predispose to polyspermy, the entry of more than one spermatozoon into the ovum. Fertilisation involving aged gametes is considered to contribute to the occurrence of some congenital abnormalities, particularly in the human population. Unfertilised ova undergo fragmentation and are phagocytosed in the female reproductive tract.

Retention of fertilising capacity of spermatozoa

In the female reproductive tracts of domestic animals, spermatozoa retain their ability to fertilise ova for at least 24 hours. It has been suggested that there is a correlation between the duration of oestrus and the retention of viability of spermatozoa and their ability to fertilise ova after deposition in the female reproductive tract. Motile spermatozoa have been observed in the reproductive tracts of mares for up to six days after mating, and for up to 11 days in bitches. In domesticated fowl, spermatozoa, which are stored in special sperm nests in the female tract, may remain capable of fertilising ova for up to 21 days. In some species of bats in which coitus takes place in the autumn, spermatozoa remain viable in the female reproductive tract until ovulation occurs in the spring.

Semen used for artificial insemination retains its viability at 4°C for several hours. When stored at –196°C in liquid nitrogen, viability is retained indefinitely.

Further reading

Bracket, B.J. (2004) Male reproduction in mammals. In *Duke's Physiology of Domestic Animals.* Ed. W.O. Reece. Comstock Publishing Association, Cornell University Press, Ithaca, NY, pp. 670–691.

Eddy, E.M. and O'Brien, D.A. (1994) The spermatozoon. In *Physiology of Reproduction*, Vol. 1, 2nd edn. Eds. E. Knobil and J.D. Neill. Raven Press, NY, pp. 29–77.

Hafez, E.S.E. and Hafez, B. (2000) Folliculogenesis, egg maturation, and ovulation. In *Reproduction of Farm Animals*, 7th edn. Eds. E.S.E. Hafez and B. Hafez. Lippincott, Williams, and Wilkins, Philadelphia, pp. 68–82.

Robl, J.M. and Fissore, R.A. (1999) Gametes, an overview. In *Encyclopedia of Reproduction*, Vol. 2. Eds. E. Knobil and J.D. Neill. Academic Press, San Diego, pp. 430–434.

Senger, P.L. (2003) Endocrinology of the male and spermatogenesis. In *Pathways to Pregnancy and Parturition.* Current Conceptions Inc., Pullman, Washington, pp. 214–240.

Thompson, T.N. (2004) Female reproduction in mammals. In *Duke's Physiology of Domestic Animals*, Ed. W.O. Reece. Comstock Publishing Association, Cornell University Press, Ithaca, NY, pp. 692–719.

Wassarman, P.M. and Albertini, D.F. (1994) The mammalian ovum. In *Physiology of Reproduction*, Vol. 1, 2nd edn. Eds. E. Knobil and J.D. Neill. Raven Press, NY, pp. 79–122.