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# Part 1 Fertilisation



## CHAPTER 1

# Principles of development

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## Case

Jamie is a 4-month-old boy presenting with disparity between limb length, trunk length and cranial circumference. His height is under the fourth percentile, his weight is under the fourth percentile and his head circumference is above the 97th percentile. Motor development milestones are delayed. Jamie's mother and father have typical heights (168 cm and 176 cm respectively).

## Learning outcomes

- You should be able to recognise the stages of cell division in mitosis and meiosis.
- You should be able to describe the basic principles of growth and differentiation.

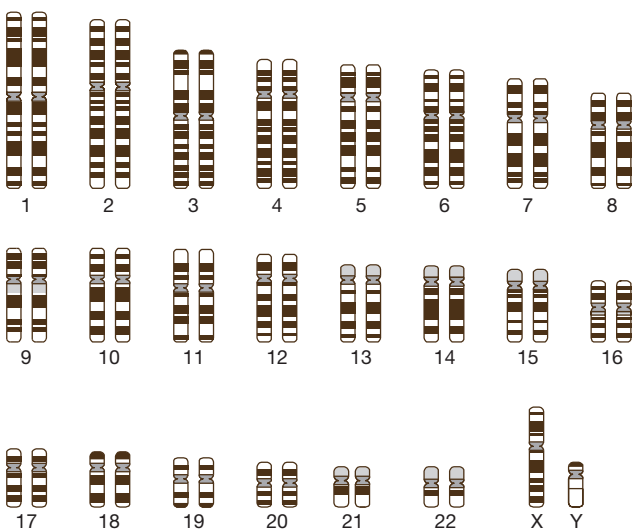
## Chromosomes

As a basis of biology cell theory is a crucial part of understanding development. Complex organisms grow from a single cell. The cell is the fundamental unit of structure in the organism, and new cells are formed from existing cells. All structure, function and organisation relates to the unit of the cell. In development we consider how the cells of the gametes merge to form a cell with a new genetic composition, the division of that cell to form new cells, and how those cells become organised, form shapes and tissues of multiple differentiated cell types.

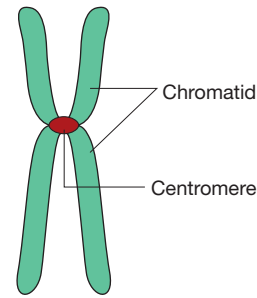
DNA is stored in chromatin form within the nuclei of cells, and RNA is present in the cytoplasm. When cells divide the chromosomes are duplicated and the daughter cells gain exact copies of the DNA of the parent cell (hopefully, if the replication and error checking mechanisms work correctly).

Somatic cells contain 23 pairs of chromosomes including 22 pairs of autosomes and one pair of sex chromosomes (Figure 1.1). Each chromosome is an organised package of DNA.

In a homologous pair of chromosomes the same genes are encoded on each chromosome but the genes may occur as slightly different versions. One chromosome has been inherited from the father, and the other from the mother. For example, the gene for head hair pigment colour will occur on both chromosomes of a homologous pair, but one copy may encode for blonde hair and the other for brown. These copies are alleles, and the dominant pigment allele will be represented in the phenotype of the individual. This is a simplified example, and many hair pigments are at play in determining a person's final hair colour, accounting for the wide variation of natural shades that occurs. The mixing up of alleles across



**Figure 1.1 Human karyotype.** (Source: S. Webster and R. de Wreede (2016) *Embryology at a Glance*, 2nd edn. Reproduced with permission of John Wiley & Sons, Ltd.)



**Figure 1.2 The structure of a chromosome.** (Source: S. Webster and R. de Wreede (2016) *Embryology at a Glance*, 2nd edn. Reproduced with permission of John Wiley & Sons, Ltd.)

homologous chromosomes during cell division is an important part of the genetic diversity advantage given by sexual reproduction over asexual reproduction.

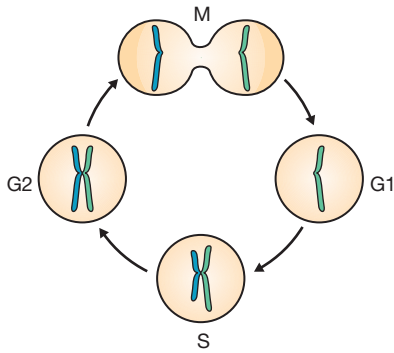
If a cell has two copies of each kind of chromosome (e.g. one copy from the mother and one copy from the father) it is said to be diploid. If it only had one copy it would be haploid.

We can also describe a cell by the number of copies ( $n$ ) of each unique double-stranded length of chromosomal DNA. Chromosomal DNA inherited from the mother is different to chromosomal DNA inherited from the father. In a pair of chromosomes the genes are the same but the alleles are different. A haploid cell has only one copy of each kind of chromosome so it is described as  $1n$ . Somatic cells are normally diploid, and during part of the cell cycle only have one DNA strand for each kind of chromosome so are described as  $2n$ . They have two copies of each kind of chromosome (one from the mother and one from the father). When a cell copies its DNA in preparation for cell division it will have four copies of each kind of chromosome and be described as  $4n$ .

If the DNA strand of a chromosome is duplicated its two duplicates are joined together at the centromere forming the familiar X shape of most chromosomes (Figure 1.2). Each of the two duplicates is a sister chromatid.

## Mitosis

Mitosis is the process by which cells divide and increase in number in eukaryotic organisms. The result of mitosis is two daughter cells that contain the same genetic information. Mitosis is the method by which cells repair tissues, it is one way in which growth can occur, and it is how cells lost through normal processes are replaced. Some cells are very good at proliferating by mitosis, such as epidermal keratinocytes, which are lost daily as flakes of skin, and some cells are very poor at mitotic division, such as neurones of the central nervous system, which are expected to survive for the lifetime of the organism (although it is not yet clearly understood how long neurones live, but they are not naturally replaced after brain



**Figure 1.3 The cell cycle.** (Source: S. Webster and R. de Wreede (2016) *Embryology at a Glance*, 2nd edn. Reproduced with permission of John Wiley & Sons, Ltd.)

damage). Mitosis is a major mechanism of growth in the embryo and fetus.

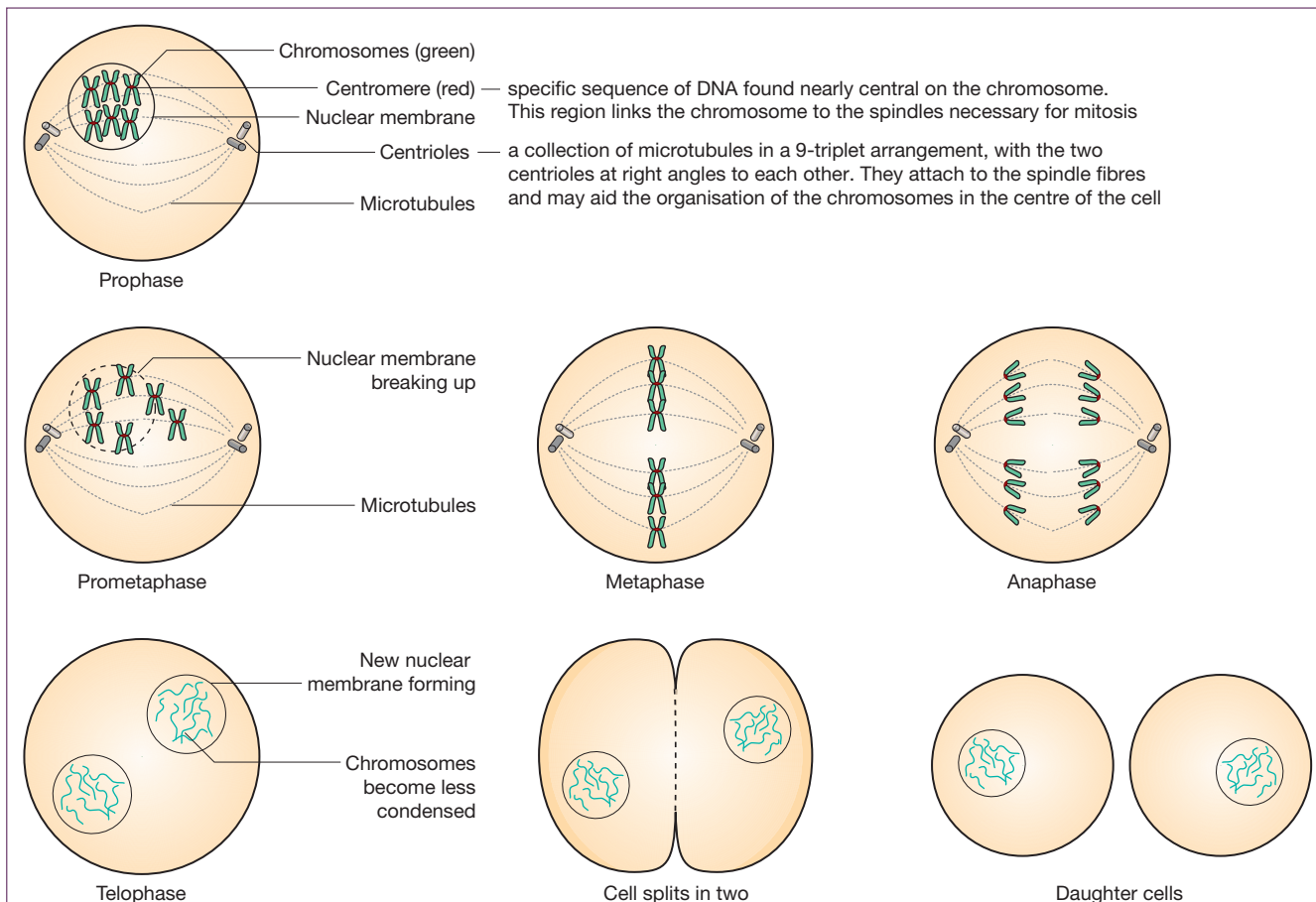
Cell division is a step within the cell cycle (Figure 1.3). The cell cycle describes a series of carefully controlled events in the life of cell that take part in cell division, and cells that

do not divide are considered to have left the cell cycle. The stages of the cell cycle are gap 1 (G1), synthesis (S), gap 2 (G2) and mitosis (M). The stages of G1, S and G2 are also known collectively as interphase. A cell's DNA is duplicated during S phase, adding a sister chromatid to the existing chromatid. A cell that no longer divides can be described as existing within a G0 phase.

When a cell begins mitosis its chromosomes become condensed and form their recognisable X shapes during the first phase of mitosis, called prophase (Figure 1.4). At this stage it is diploid ( $4n$ ). Centrioles are cylindrical structures that have a number of functions within eukaryotic cells, and during mitosis they arrange and separate DNA. During prophase the centrioles move to opposite ends of the cell.

In the next stage, prometaphase, the nuclear membrane breaks down and disappears releasing the DNA into the cytoplasm. Microtubules link the centromeres of the chromosomes to the centrioles, and during metaphase the chromosomes begin to move, pulled by the microtubules to line up along the middle of the cell.

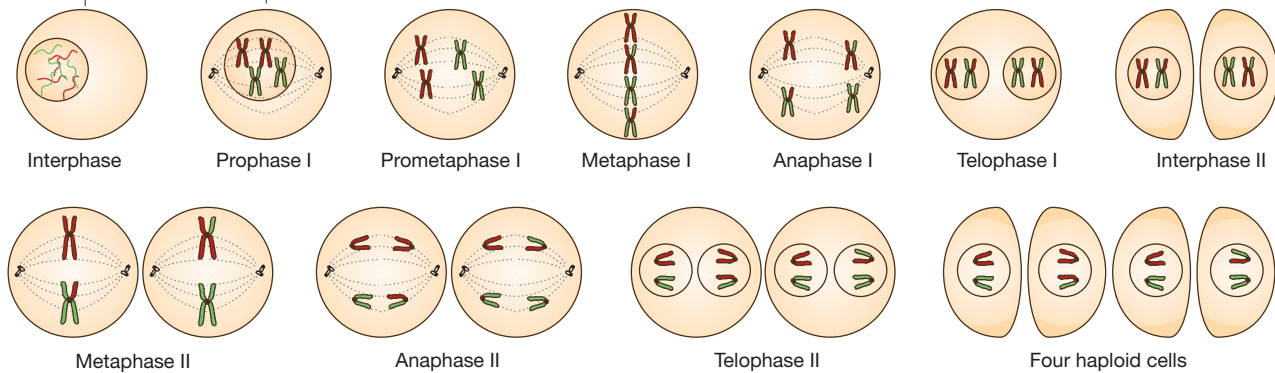
The centromeres are cut in the telophase step, splitting each chromosome into its separate, genetically identical chromatids.



**Figure 1.4 Mitosis.** (Source: S. Webster and R. de Wreede (2016) *Embryology at a Glance*, 2nd edn. Reproduced with permission of John Wiley & Sons, Ltd.)

Red and green strands are pairs of (homologous) chromosomes (a pair has one red and one green chromosome). The red strand signifies the paternal DNA and the green strand the maternal DNA within this cell

Homologous chromosomes begin to swap sections of DNA (alleles)



**Figure 1.5 Meiosis.** (Source: S. Webster and R. de Wreede (2016) *Embryology at a Glance*, 2nd edn. Reproduced with permission of John Wiley & Sons, Ltd.)

One of each pair of chromatids is pulled to opposite ends of the cell by microtubules and the centrioles.

In telophase the chromatids reach the ends of the cell, begin to lengthen again and are no longer visible under a light microscope. Two new nuclear membranes begin to form around the chromatid DNA to create two nuclei. Cytokinesis follows during which a ring of actin filaments appears around the midline of the cell and shrinks, splitting the cell into two. Mitosis is complete, and the two cells return to the G1 phase. During the G1 phase each cell has a full, diploid complement of DNA but only one copy of each chromosome ( $2n$ ).

## Meiosis

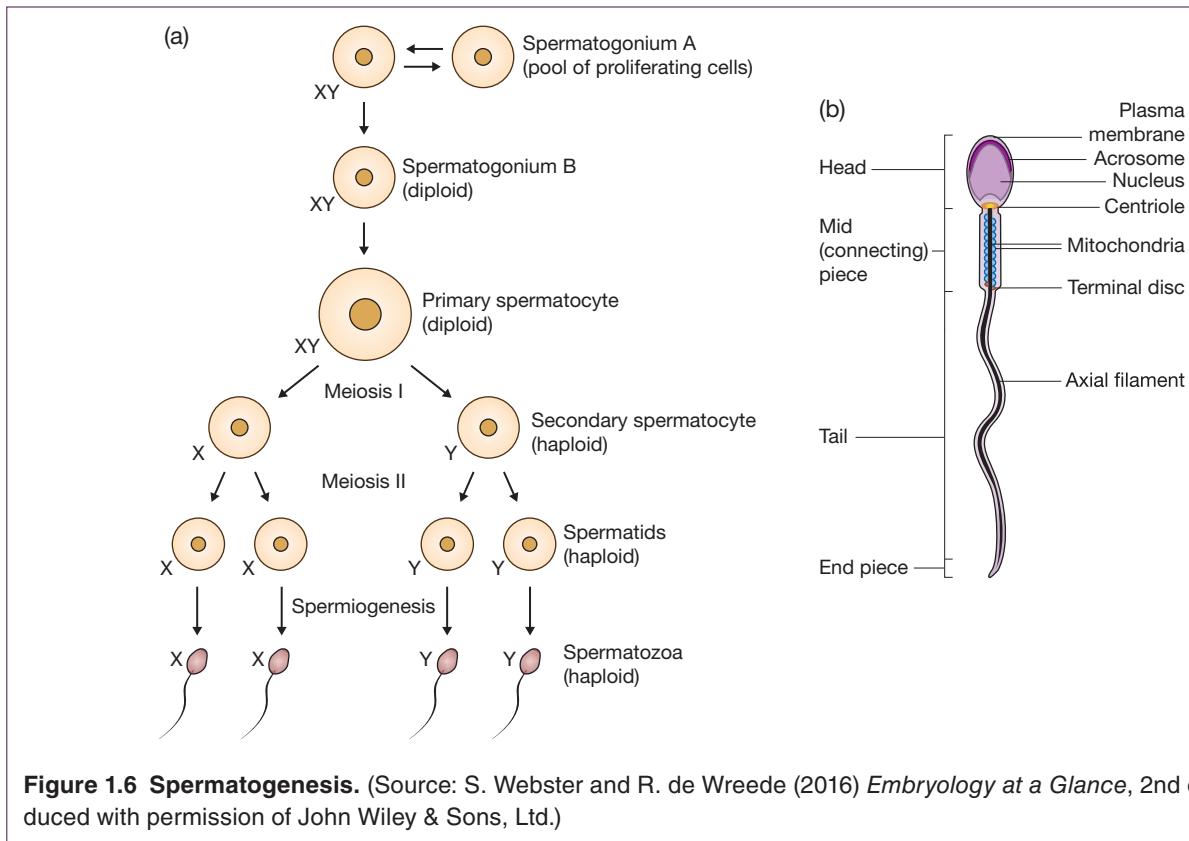
Meiosis is a specialised method of cell division in eukaryotes that produces gamete cells. The primary function of meiosis is to produce cells with a haploid ( $n$ ) complement of chromosomes. Somatic cells have two homologous copies of each chromosome (diploid) and gametes have one copy of each chromosome (haploid,  $n$ ). When the male and female gametes combine during fertilisation the resulting cell has a restored, diploid complement of 23 pairs of chromosomes.

Meiosis is similar to mitosis, but differs in a couple of ways. Cell division occurs twice during a full cycle of meiosis, producing four daughter cells from one cell. Alleles of homologous chromosomes are randomly exchanged between those chromosomes during a process known as homologous recombination. Cells produced as a result of meiosis will have all of the genes of the parent cells (hopefully in the same locations within chromosomes as the parent cells if the process occurs accurately) but with a random allocation of the alleles of those genes. This genetic variability is an important advantage of

sexual reproduction over asexual reproduction. If, for example, the original diploid cell contained the allele for a blue iris on one chromosome and the allele for a green iris on the homologous chromosome, any cell formed as a result of meiosis could contain either allele. Alleles of the genes on the same chromosome may or may not be carried across with the allele for iris colour, as homologous recombination maintains the order of genes but alleles may be swapped around.

During S phase the cell's DNA is duplicated. The two parts of meiosis are described as meiosis I and meiosis II. Prophase I begins with homologous recombination of DNA across homologous chromosomes before the chromosomes shorten, thicken and become condensed (Figure 1.5). The centrioles move to either end of the cell and microtubules are extended, beginning to form the mitotic spindle. The cell at this stage has a diploid ( $4n$ ) complement of DNA. Metaphase I follows, with the chromosomes aligning themselves along the midline of the cell. During anaphase I pairs of homologous chromosomes split up, with one chromosome of each pair pulled to either end of the cell by the mitotic spindle. Each chromosome at this stage is made up of a pair of identical sister chromatids joined together at the centromere. With telophase I, two new nuclear membranes form around the chromosomes that have collected at either end of the cell, forming two nuclei. An actin ring appears around the middle of the cell and constricts, splitting the cell into two daughter cells by cytokinesis. The cells resulting from meiosis I are haploid ( $2n$ ). They have 23 chromosomes and each chromosome has two chromatids. The chromosomes are not paired at this stage.

In the second part of meiosis the cell goes through division again, beginning with prophase II. The cell's DNA is not duplicated between meiosis I and meiosis II, so it enters



the second part with the haploid ( $2n$ ) complement of chromosomes. Again the chromosomes are in a condensed, thickened configuration and the centrioles move to either end of the cell. In prometaphase II the nuclear membranes break down and microtubules link the chromosomes to the centrioles. The chromosomes become aligned along the middle of the cell during metaphase II, and then the chromosomes are split during anaphase II. The chromosomes are divided into their two sister chromatids, which are each pulled towards opposite ends of the cell. In telophase II the chromatids reach the ends of the cell and nuclear membranes begin to form around them, forming two nuclei. Cytokinesis forms an actin ring around the middle of the cell that contracts and splits the cell into two.

At the end of meiosis four cells have been produced from one, and each cell has 23 unpaired chromosomes. Each cell is haploid ( $n$ ).

## Spermatogenesis

Spermatogenesis describes the development of haploid spermatozoa from germ cells in the testes. The germ cells of the seminiferous tubules are diploid spermatogonia (typically  $2n$  before they duplicate their DNA to  $4n$  for cell division) with a full complement of chromosomes, including X and Y

sex chromosomes. Spermatogonia maintain their numbers throughout life by mitotic division.

Spermatogenesis comprises two stages: spermatocytogenesis and spermiogenesis. A type A spermatogonium cell from the pool of proliferating cells will enter the process of maturation, becoming a type B spermatogonium B cell (Figure 1.6). Groups of type B spermatogonia cells begin spermatocytogenesis in synchrony, eventually producing large numbers of mature spermatozoa. Type B spermatogonia cells are linked to one another at this stage by cytoplasmic bridges and divide mitotically, increasing their numbers and becoming primary spermatocytes.

Primary spermatocytes enter the first round of meiotic division (meiosis I). One diploid ( $4n$ ) primary spermatocyte becomes two haploid ( $2n$ ) secondary spermatocytes, and a secondary spermatocyte may contain either an X or a Y sex chromosome as part of their complement of 23 chromosomes. During this first round of meiosis homologous recombination of chromosomes occurs.

Secondary spermatocytes divide again through the stages of meiosis II. The resulting cells are spermatids (haploid,  $n$ ) and four spermatids are derived from one primary spermatocyte. There are 23 unpaired chromosomes within each cell at the end of meiosis II. The spermatid stage marks the end of spermatocytogenesis.



### Clinical notes 1.1 Male fertility

Biological and environmental factors can affect the processes of spermatogenesis, producing abnormal sperm. These factors include smoking, sexually transmitted diseases, toxins, increased testicular temperature and radiation. During semen analysis the spermatozoa are graded by counting the total number of spermatozoa and their concentration, the proportions of motile cells and live cells, and the proportion of abnormal sperm cells. Volume, pH and liquefaction time may also be measured.

The spermatid changes shape, lengthening and forming a rounded head and an elongated tail during the process of spermiogenesis. The tail is packed with mitochondria, the cell loses cytoplasm, and the head contains the nucleus. An acrosome layer of specialised enzymes that will enable penetration of an ovum forms around the head of the cell. With these changes the spermatid becomes a spermatozoon.

These processes of spermatogenesis take around 64 days to produce spermatozoa from spermatogonia A cells, and the spermatozoa remain inactive as they are passed to the epididymis. They continue to mature over a seven-day period within the epididymis, at which point they become motile and ready for fertilisation.

## Oogenesis

Oogenesis describes the development of haploid oocytes, within follicles, from germ cells in the ovaries. Female germ cells are diploid and contain a pair of X sex chromosomes. They divide mitotically to produce a large number of oogonia, which will enter meiosis (Figure 1.7).

Oogonia begin meiosis I during the 12th week of fetal development. The cell at this stage is known as the primary oocyte, and becomes surrounded by a thin layer of squamous epithelial cells to form a primordial follicle. The primary oocyte only passes through meiosis as far as prophase I with homologous recombination and condensation of the chromosomes (diploid,  $4n$ ). The primary oocyte is held, paused in this state. It will only continue its development if it is released from the ovary by ovulation.

Millions of primordial follicles are formed during the first trimester but many degenerate leaving around 400 000 follicles at birth. When puberty begins some of the paused primary oocytes continue their development. Each month a few primordial follicles change. The primary oocyte within becomes larger and the follicular cells become cuboidal. The follicular layer thickens to form a primary follicle. The follicle becomes a secondary follicle when more than one layer of follicle cells has developed. The granulosa cells of the follicle and the oocyte create a layer of glycoproteins on the surface of

the oocyte. This layer is the zona pellucida and has important functions during fertilisation (see also Chapter 4).

Although during any particular monthly cycle a number of follicles begin to develop further only one continues leaving the others to degenerate. It is not clear how one follicle is chosen over the others. In the follicle that survives the number of layers of follicular cells continues to increase, and the follicle becomes an antral follicle when it has more than five layers of cells. An antrum appears as a space between the layers of granulosa cells, and this structure becomes the cumulus oophorus.

The follicle is embedded within an ovary, and has been growing and becoming a more prominent structure. The cells of the ovary around the follicle now respond to the follicle's development by differentiating to build two layers of theca interna and theca externa. The follicle is considered to be a mature vesicular follicle (or Graafian follicle). In response to luteinising hormone (LH), thecal cells produce androgens, which are converted into oestrogen. Oestrogens cause repair and thickening of the endometrial lining of the uterus between days 5 and 14 of the menstrual cycle, preparing the endometrium to receive a blastocyst (see Chapter 4).

Only now, in response to spikes in LH and follicle-stimulating hormone (FSH), does the oocyte resume meiosis I and continue in its stalled processes of cell division. At the end of meiosis I the cell divides into a large secondary oocyte (haploid,  $2n$ ) and a small polar body (haploid,  $2n$ ). The oocyte retains most of its mass and cellular components and the polar body acts as a vessel for the removed chromosomal material. The oocyte is now a haploid cell and the polar body degenerates.

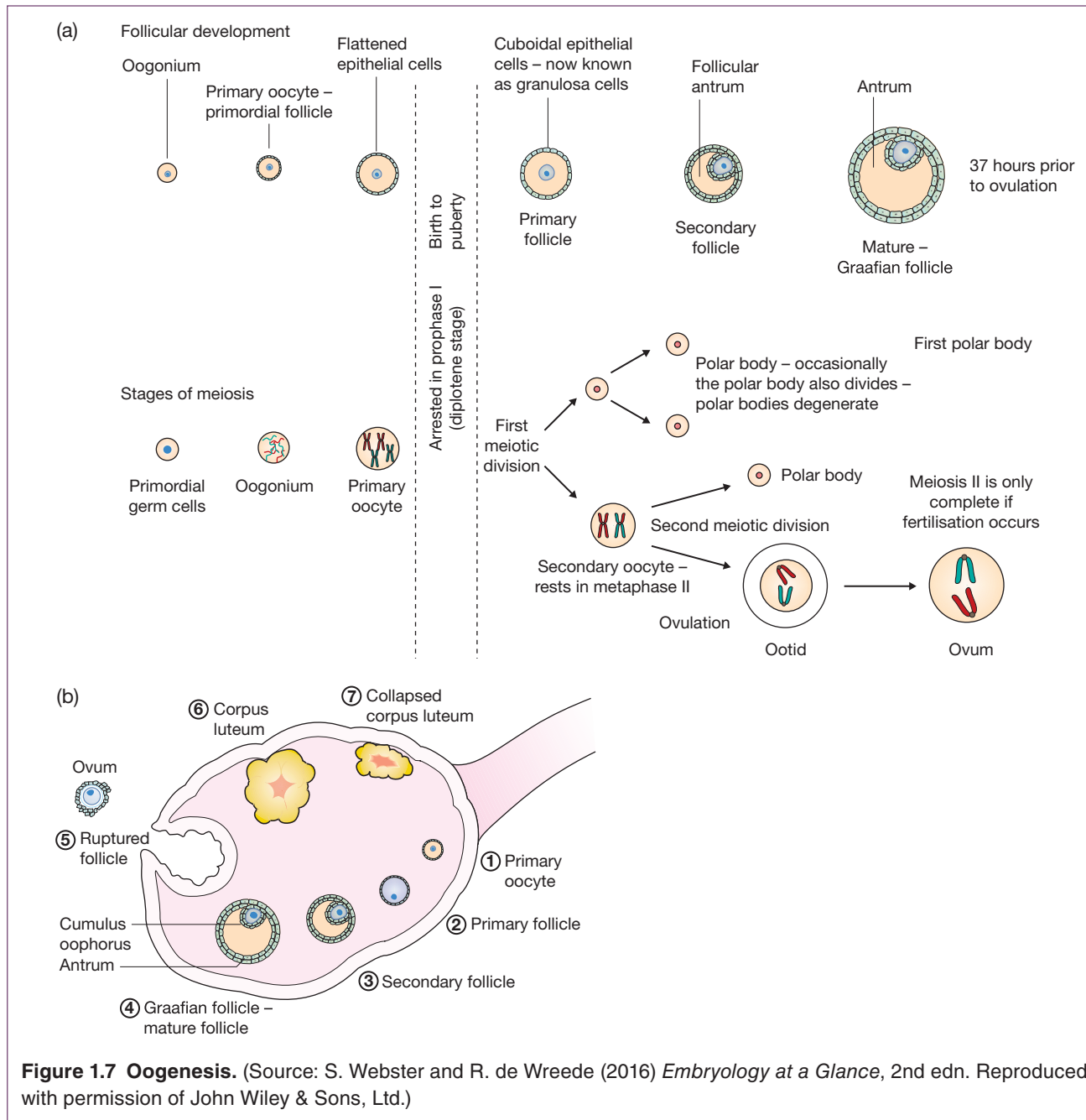
The secondary oocyte enters meiosis II but stalls again, during metaphase II. It will only continue to divide if it is fertilised by a spermatozoon. If this occurs the cell becomes the definitive oocyte (haploid,  $n$ , if considered on its own and ignoring the spermatozoon) and produces a second polar body (haploid,  $n$ ).

If you do the maths and assume that ovulation begins with puberty at around age 11, and ends with menopause at around age 55, at 12 ovulations per year for 44 years only 528 primary oocytes (in this scenario) will continue their development. In truth, only if an oocyte is fertilised by a spermatozoon will it complete meiosis (see Chapter 4), so of the millions of oogonia originally produced only a few are likely to survive.



### Clinical notes 1.2 Female fertility

The arrested development of the primary oocyte in meiosis I may last for 40 or 50 years. Some oocytes will not be stimulated to continue until a menstrual cycle late in reproductive life. The likelihood of DNA fragmentation of a cell increases with time, and DNA fragmentation within these oocytes is more common in older women. This may be the reason for reduced fertility with increasing age.



## Growth

Biological growth may be defined as an increase in the mass or size of a tissue or organism, and is a key process of development. Growth can occur through three mechanisms: an increase in cell number, an increase in cell size, or an increase in extracellular material.

Cellular proliferation, that is, an increase in cell number through mitotic cell division, is the most common method of achieving growth. The cells of many adult tissues are also able to proliferate, often as part of a repair mechanism in response

to injury. Some adult tissues are very good at cellular proliferation, and some are very poor. Stem cells are an important source of cell renewal in tissues that lose cells constantly, such as the epidermis of the skin and the epithelium of the gut.

Cellular hypertrophy describes an increase in cell size, and is a normal part of the endochondral ossification process, for example, in which chondrocytes lay down a cartilaginous precursor that is modified by hypertrophic chondrocytes and replaced by bone. In adult tissues skeletal muscle responds to the repeated loading of weight training with hypertrophy.

Cells of connective tissues secrete and surround themselves with the extracellular matrix that forms much of the tissue. Chondrocytes and osteocytes increase the amount of matrix in response to loading, increasing the size of the tissue by accretion.

## Differentiation

Embryological multipotent cells have the potential to form the wide range of cells needed to build the structures and tissues of the embryo. The process of an embryonic stem cell becoming a specialised, determined, mature cell type is differentiation. The differentiated cell type is stable, meaning that cells formed as a result of mitotic division are typically of the same cell type. Differentiated cells do not normally change cell type, but it is possible to take some mature cells and direct them to dedifferentiate and return to a stem-cell-like state in the laboratory. Adult stem cells also exist and are partly differentiated and able to produce a limited number of cell types, often relevant to the tissue in which they reside.

## Signalling

A signal produced by a cell or group of cells is able to influence another cell or group of cells that have receptors for that signal. This is an important concept in embryological development, and much of contemporary research investigates what signals are involved and how they affect cells during development.

Hormones are an example of a signal in adult physiology, and often act in a system-wide manner by passing through the circulatory system from a local source to cells elsewhere in the body. In the embryo the signals may remain attached to the secretory cell or be released to diffuse through the tissue. The distances involved are very small.

Cells respond to the signals in different ways, by differentiating, migrating, proliferating, changing shape, or entering apoptosis, for example.

## Organisation

The first shape that the embryo forms after the embryoblast ball of cells is a flat sheet. The sheet appears to be a uniform, oval plate of cells, and to the eye it would be difficult to guess which end will be the head or the tail, and which side is left and right, yet the cells by this point are organised and will respond to one another to form the structures, cell types, and tissues appropriate to the region they are within and the phase of development.

Cells are aware of their location within the embryo. One way in which this can occur is by the diffusion of signalling molecules synthesised by one group of cells across the tissue, and a variable response to the concentration of that signal by cells with appropriate receptors. The cells may respond differently depending upon whether the concentration of the molecule is high, low or somewhere in between.

Morphogens acting as signals in this way are a fundamental part of development. If this signalling is interfered with it can have profound effects and may prevent the embryo from continuing to develop or cause a congenital abnormality.

## Morphogenesis

The formation of shape during development is morphogenesis. Cells are able to change their shape, extend processes to pull themselves along and migrate, and a tissue may grow in size. These are all processes that occur during development to cause cells to form shapes and structures. The flat sheets of the germ layers roll up in the very early stages of development, forming tubes and spaces, for example.

### Clinical case

#### Investigations and treatments

Jamie is displaying characteristic features of achondroplasia. He has disproportionate short stature, macrocephaly (large cranium), megalencephaly (large brain), frontal bossing (a prominent forehead), a low nasal bridge, and some facial features are underdeveloped. Jamie has a long trunk and shortened limbs. He has limited elbow extension and forearm supination.

The diagnosis can be further confirmed by radiology and genetic analysis. Radiological investigations will give further insight into specific aspects of Jamie's condition. Jamie will have hypermobile joints and display genu varum (bowed legs). His hearing should be assessed regularly as children with achondroplasia are more likely to develop

middle ear infections. Children with achondroplasia are also more likely to have obstructive sleep apnoea.

#### Case conclusions

There is no cure for achondroplasia, and Jamie may develop a number of issues as he grows. It is typically caused by a mutation in the *FGFR3* gene that encodes a fibroblast growth factor receptor important in bone and brain growth and development. The mutation is inherited in an autosomal dominant pattern, and only one copy of the defective gene will cause achondroplasia. If two copies of the gene are inherited the developmental defects are likely to be severe enough to cause death, as the thoracic cage is too poorly developed for effective respiration.

In most cases of achondroplasia both parents are of normal height and are not carriers of a defective *FGFR3* gene. The cause of achondroplasia in these cases is a spontaneous mutation of the gene.

It is likely that Jamie will develop normal intelligence although developmental milestones will be delayed.

Milestones and growth charts for children with achondroplasia can be used to track Jamie's development and growth. He should have a normal lifespan and live independently when he becomes an adult. He may develop spinal and joint problems, or respiratory and cardiovascular difficulties during his development or later in life.

### Key learning points

- An understanding of spermatogenesis and oogenesis helps explain many causes of subfertility.
- Men are able to produce new gametes throughout life, but a woman's ova are all produced before birth and are suspended partway through meiosis until each is selected during an ovulatory cycle. The decision to have children later in life has effects on fertility and on the risk of occurrence of some congenital genetic conditions.
- The processes of growth are relevant to embryonic development, fetal growth, childhood development and adolescence. In adults processes of repair are similar.



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