CHAPTER 1

1 Sex

The reproduction of mammals involves sex. Sex is defined formally in biology as a process whereby a genetically novel individual is formed as a result of the mixing of genes from two or more individuals. So the essential feature of mammalian *sexual reproduction* is that the new individual receives its chromosomes in two equal portions: half carried in a *male gamete*, the *spermatozoon*, and half carried in a *female gamete*, the *oocyte*. These gametes come together at *fertilization* to form the genetically novel *zygote*. In order to reproduce itself subsequently, the individual must transmit only half its own chromosomes to the new zygotes of the next generation. In sexually reproducing species, therefore, a special population of *germ cells* is set aside. These cells undergo a *reduction division* known as *meiosis*, in which the chromosomal content of the germ cells is *reduced by half* and the genetic composition of each chromosome is modified as a result of the exchange of pieces of homologous chromosomes (Fig. 1.1). The increased genetic diversity that is generated within a sexually reproducing population may offer a richer and more varied source of material on which natural selection can operate. The population would therefore be expected to show greater resilience in the face of environmental challenge.

However, sex is not by any means an essential component of reproductive processes. Thus, *asexual* (or *vegetative*) reproduction occurs continuously within the tissues of our own bodies as individual cells grow, divide *mitotically* (Fig. 1.1) and generate two offspring that are genetically identical to each other and to their single parent. Many unicellular organisms reproduce themselves mitotically just like the individual cells of the body. Among multicellular organisms, including some complex vertebrates such as lizards, several reproduce themselves by setting aside a population of oocytes that can differentiate into embryos in the absence of a fertilizing spermatozoon to generate a complete new organism that is genetically identical or very similar to its parent. This asexual process of reproduction, often called *parthenogenetic* development, is simply not available to mammals. Although it is possible to stimulate a mammalian oocyte (including a human oocyte) in the complete absence of a spermatozoon, such that it undergoes the early processes of development and may even implant in the uterus, these parthenogenetic embryos always fail and die eventually. It seems that a complete set of chromosomes from a father and a complete set from a mother are an absolute requirement for normal and complete development to occur in mammals (see Chapter 9 for discussion as to why this is).

The consequences of obligatory sexual reproduction permeate all aspects of mammalian life. At the core of the process lies the creation and fusion of the two types of gamete. This occurs in mammals in two distinct types of

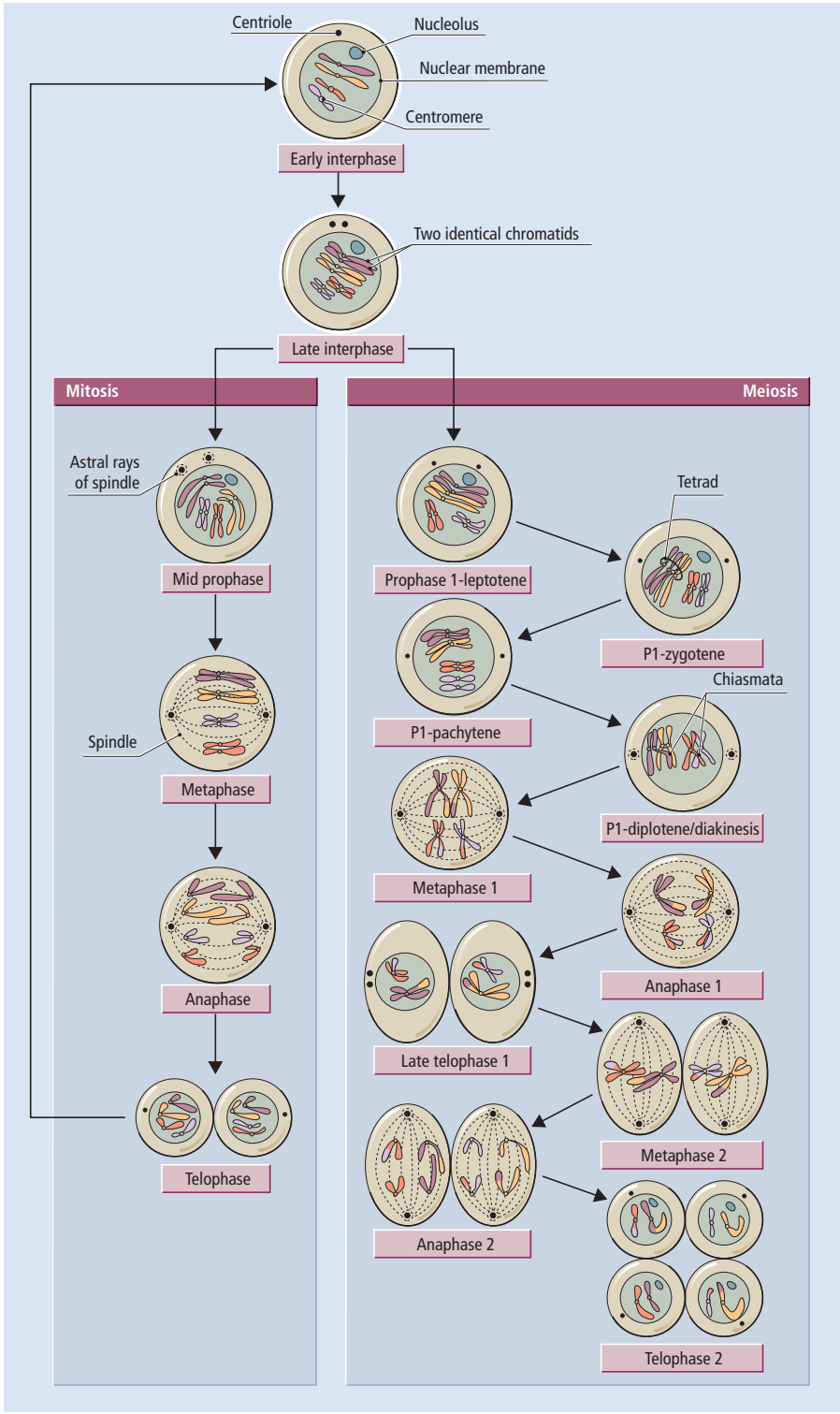
Centriole
Nucleolus
Nuclear membrane
Centromere
Early interphase
Two identical chromatids
Late interphase
Mitosis
Meiosis
Astral rays of spindle
Mid prophase
Spindle
Metaphase
Anaphase
Telophase
Prophase 1-leptotene
Tetrad
P1-zygotene
P1-pachytene
Chiasmata
P1-diplotene/diakinesis
Metaphase 1
Anaphase 1
Late telophase 1
Metaphase 2
Anaphase 2
Telophase 2

Fig. 1.1 Mitosis and meiosis in human cells. Each human cell contains 23 pairs of homologous chromosomes, making 46 chromosomes in total (see Fig. 1.2). Each set of 23 chromosomes is called a *haploid* set. When a cell has two complete sets, it is described as being *diploid*. In this figure, we show at the top a single schematized human cell with just 2 of the 23 homologous pairs of chromosomes illustrated, each being colour coded. Before division, the cell is in *interphase*, during which it grows and duplicates both its *centriole* and the DNA in each of its chromosomes. As a result, each chromosome consists of two identical *chromatids* joined at the *centromere*. Interphase chromosomes are not readily visible, being long, thin and decondensed (but are shown in this figure in a more condensed form for simplicity of representation).

In *mitotic prophase* (left-hand side), the two chromatids become distinctly visible under the light microscope as each shortens and thickens by a spiralling contraction; at the end of prophase the *nucleoli and nuclear membrane* break down. In *mitotic metaphase*, microtubules form a *mitotic spindle* between the two *centrioles* and the chromosomes lie on its *equator*. In *mitotic anaphase*, the centromere of each chromosome splits and the two chromatids in each chromosome each migrate to opposite poles of the spindle (*karyokinesis*). *Mitotic telophase* sees: the reformation of nuclear membranes and nucleoli; division of the cytoplasm into two daughters (known as *cytokinesis*); breakdown of the spindle; and decondensation of chromosomes so that they are no longer visible under the light microscope. Two genetically identical daughter cells now exist where one existed before. Mitosis is a non-sexual or vegetative form of reproduction.

Meiosis involves two sequential divisions (right-hand side). The *first meiotic prophase* (prophase 1) is lengthy and can be divided into several sequential steps: (1) *leptotene* chromosomes are long and thin; (2) during *zygotene*, homologous pairs of chromosomes from each haploid set come to lie side by side along parts of their length; (3) in *pachytene*, chromosomes start to thicken and shorten and become more closely associated in pairs along their entire length at which time *synapsis*, *crossing over* and *chromatid exchange* take place and nucleoli disappear; (4) in *diplotene* and *diakinesis*, chromosomes shorten further and show evidence of being closely linked to their homologue at the *chiasmata* where crossing over and the reciprocal exchange of DNA sequences has occurred, giving a looped or cross-shaped appearance. In *meiotic metaphase 1*, the nuclear membrane breaks down, and homologous pairs of chromosomes align on the equator of the spindle. In *meiotic anaphase 1*, homologous chromosomes move in opposite directions. In *meiotic telophase 1*, cytokinesis occurs; the nuclear membrane may re-form temporarily, although this does not always happen, yielding two daughter cells each with half the number of chromosomes (only one member of each homologous pair), but each chromosome consisting of two genetically unique chromatids (because of the crossing over at chiasmata). In the *second meiotic division*, these chromatids then separate much as in mitosis, to yield a total of four haploid offspring from the original cell, each one containing only one complete set of chromosomes. Due to chromatid exchange and the random segregation of homologous chromosomes, each haploid cell is genetically unique. At fertilization, two haploid cells will come together to yield a new diploid zygote.

individual, known as the two sexes: *male* and *female*. The gametes themselves take distinctive male or female forms (to prevent self-fertilization) and are made in distinctive male and female *gonads*: the *testis* and *ovary*, respectively. In addition, each gonad elaborates a distinctive group of hormones, notably the *sex steroid hormones*, which modify the tissues of the body to generate distinctive male and female somatic phenotypes suited to maturing and transporting their respective gametes. In most mammals, the sex steroids also affect the behaviour and physiology of the individuals of each sex to ensure that mating will only occur between different sexes at times of maximum fecundity. Finally, in mammals, not only do the steroids provide conditions to facilitate the creation of new individuals, they also prepare the female to carry the growing embryo for a prolonged period of *pregnancy* (*viviparity*), and to nurture it after birth through an extended period of *maternal lactation* and *parental care*.

Thus, the genetic mixing inherent in sexual reproduction has ramifying consequences for mammalian biology, shaping not just anatomy and physiology, but also aspects of behaviour and social structure. This ramification of sex throughout a whole range of biological and social aspects of mammalian life is mediated largely through the actions of the gonadal hormones. However, in humans and in other higher primates, social learning also plays an important role in generating sex differences. Children are taught how to behave as women or men, what is *feminine* and what is *masculine*. In this way they acquire a sense of their *gender*. Thus, although studies on mammals in general are relevant to humans, they are not in themselves sufficient. In order fully to understand human reproduction and sexuality, humans must be studied too. In this chapter, we examine how two sexes arise, differentiate and mature physically. In Chapter 2 we examine the related but distinct issues of gender development and sexuality.

The genesis of two sexes depends on genetic differences

The genetic determinant of sex is on the Y chromosome

In mammals, the genesis of two sexes has a genetic basis. Examination of human chromosomes reveals a consistent difference between the sexes in *karyotype* (or pattern of chromosomal morphologies). Thus, the human has 46

chromosomes, 22 pairs of *autosomes* and one pair of *sex chromosomes* (Fig. 1.2). Human females, and indeed all female mammals, are known as the *homogametic sex* because the sex chromosomes are both *X chromosomes* and all the gametes (oocytes) are similar to one another in that they each possess one X chromosome. Conversely, the male is termed the *heterogametic sex*, as his pair of sex chromosomes consists of one X and one Y, so producing two distinct populations of spermatozoa, one bearing an X and the other a Y chromosome (Fig. 1.2). Examination of a range of human patients with chromosomal abnormalities has shown that if a Y chromosome is present then the individual develops the male gonads (testes). If the Y chromosome is absent the female gonads develop (ovaries). The number of X chromosomes or autosomes present does not affect the primary determination of gonadal sex (Table 1.1). Similar studies on a whole range of other mammals show that Y-chromosome activity alone is sufficient to determine gonadal sex. Thus the first step towards sexual dimorphism in mammals is the issuing of an instruction by the Y chromosome saying: 'make a testis'.

The Y chromosome itself is small. Moreover, most of its DNA is *heterochromatic* (that is, very condensed and incapable of synthesizing RNA). Therefore, the many structural genes required to make an organ as complex as the testis cannot be located on the Y chromosome alone. Indeed, these genes are known to lie on other autosomal chromosomes, and some even lie on the X chromosome. What the Y chromosome contains is a 'switching' or controller gene, which then somehow regulates the expression of all these other structural genes by determining whether and when they should become activated. The identity and location on

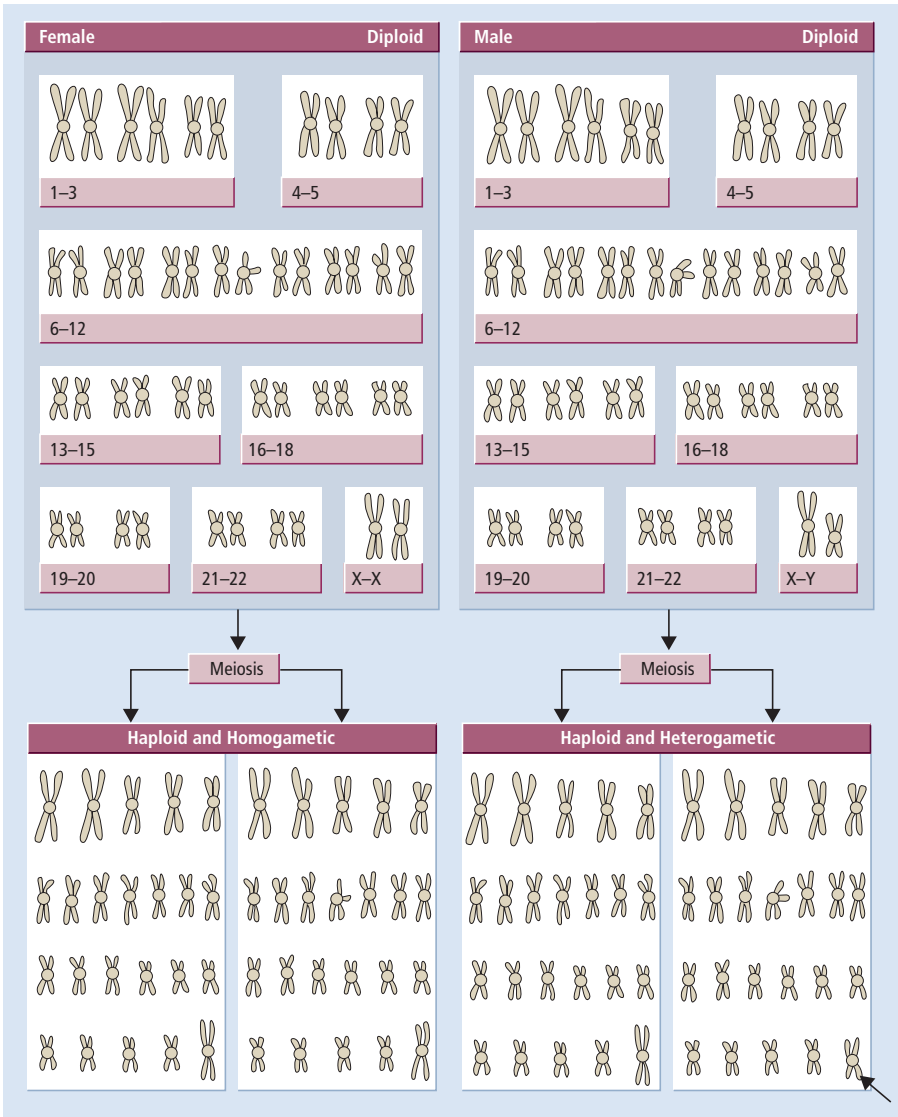

Fig. 1.2 Karyotypes of two mitotic human cells: one male and one female. Each cell was placed in colchicine, a drug that arrested them in mitotic metaphase when the chromosomes were condensed and clearly visible (see Fig. 1.1). The chromosomes were stained and then classified according to the so-called *'Denver' system*. The 44 *autosomes* (22 pairs of homologues) are grossly similar in size in each sex, but the pair of sex chromosomes are distinguishable by size, being XX (both large) in the female and XY (one large, one small) in the male. After meiotic division, all four female cells (only two shown) contain one X chromosome: the *homogametic sex*. In contrast, two of the male cells each contain an X chromosome and two contain a Y chromosome: the *heterogametic sex*. An arrow indicates the position of the *SRY* gene on the short arm of the human Y chromosome.

Table 1.1 Effect of human chromosome constitution on the development of the gonad.

Chromosomal number			
Autosomes	**Sex chromosomes**	**Gonad**	**Syndrome**
44	XO	Ovary	Turner's
44	XX	Ovary	Normal female
44	XXX	Ovary	Super female
44	XY	Testis	Normal male
44	XXY	Testis	Klinefelter's
44	XYY	Testis	Super male
66	XXX	Ovary	Triploids (nonviable)
66	XXY	Testis	
44	XX^{sxr}	Testis	Sex reversed*

*An X^{sxr} chromosome carries a small piece of Y chromosome translocated onto the X: see text.

the Y chromosome of this 'make a testis' gene was discovered initially by the study of some rare and atypical individuals.

Clinicians identified a few men with an XX sex chromosomal constitution and women with an XY chromosomal constitution—a situation called *sex reversal* At first sight, these sex-reversed people appear to contradict all that has been said above (see Table 1.1). However, careful examination of the DNA sequences on the short arm of the Y chromosome of many XY females has revealed either that short pieces of DNA are missing (*chromosomal deletions*) or that there are *mutations* of one or more nucleic acid bases. By comparing the DNA sequences in a large number of such patients, it is possible to find one region of the Y chromosome common to all of them that is affected by deletion or mutation. This region is a likely locus for a testis-determining gene. Supportive evidence comes from many of the XX males, who are found to have translocations of small pieces of the Y chromosome to one of their autosomes or X chromosomes. Again, the critical piece of Y chromosome that must be translocated to yield an XX male seems to come from the same region as is damaged in the XY females. This region contains a gene called *SRY* (in humans), which stands for 'sex-determining region of the Y gene'. The gene is located close to the end of the short arm of the human Y chromosome (see arrow in Fig. 1.2). Genes that code for a common sequence of 88 amino acids (the *Sry* box) have been found in other mammals, and are also associated with the development of a testis. In the mouse the gene is called *Sry* and also lies on the short arm but nearer to the centromere.

The identification of the mouse homologue was important, because it enabled a critical experimental test of the function of this region of the Y chromosome to be performed. Thus, a region of DNA containing only the *Sry* gene was excised from the Y chromosome and injected into the nuclei of one-cell XX mouse embryos. The excised material can integrate into the chromosomal material of the XX recipient mouse, which now has an extra piece of DNA. If this piece of DNA is functional in issuing the instruction 'make a testis', the XX mouse should develop as a male. This is what happened, strongly supporting the idea that the region containing the controller gene had been identified. This gene encodes a protein that binds DNA and localizes to the nucleus (Box 1.1). These features might be expected in a controller gene that influences other downstream genes. But when and where does *SRY* act to cause a testis to be generated?

The two gonads develop from a bipotential precursor plus three waves of ingressing cells

The early development of the gonad is indistinguishable in males and females. In both sexes the gonads are derived from common *somatic mesenchymal tissue* precursors called the *genital ridge primordia.* These primordia develop at about 3.5–4.5 weeks in human embryos, on either side of the central dorsal aorta, on the posterior wall of the lower thoracic and upper lumbar region (Fig. 1.3b,c). These two knots of mesenchyme form the basic matrices of the two gonads. Three waves of ingressing cells expand this matrix and do so in sex-specific ways to give the final forms of the ovary and testis.

One wave of migration consists of the gamete precursors called the *primordial germ cells* (PGCs). These are first identifiable in the human embryo at about 3 weeks in the epithelium of the yolk sac near the base of the developing allantois (Fig. 1.3a). By the 13–20-somite stage, the PGC population, expanded by mitosis, can be observed migrating to the connective tissue of the hind gut and from there into the gut mesentery (Fig. 1.3b). From about the 25-somite stage onwards, 30 days or so after fertilization, the majority of cells have passed into the region of the developing kidneys, and thence into the adjacent genital ridge primordia. This migration of PGCs is completed by 6 weeks and occurs primarily by amoeboid movement. The genital ridges may produce a chemotactic substance to attract the PGCs, as PGCs co-cultured in a dish with a genital ridge move towards it. Moreover, gonad primordial tissue grafted into abnormal sites within the embryo attracts germ cells to colonize it.

At about the same time as the PGCs are entering the genital ridges, a second group of cells also migrates in. These cells are derived from the columnar *coelomic* (or *germinal*) *epithelium* that overlies the genital ridge mesenchyme. They migrate in as columns called the *primitive sex cords* (Fig.

BOX 1.1 The molecular biology of SRY* action

There is uncertainty as to how SRY protein acts

In some mammals it binds DNA and localizes in the nucleus, which may suggest an action as a *conventional transcription factor* by binding to target gene promoter sites. However, few genes have been identified that it activates or represses directly in this way. It also has the property of opening up or remodelling chromatin (so-called *DNA bending*), thereby making genes accessible to conventional transcription factors, which has suggested a possible action as an *'architectural' transcription factor*. There is also some evidence that it can affect RNA stability and/or pre-RNA splicing.

***Sry* may not be quite the master gene that we first thought**

Studies of naturally occurring or induced mutations in humans and mice have implicated a number of other genes in the 'make a testis' instruction. These include genes called *Sox9*, *Dax1* and *Wnt4*. Deletions or mutations of *Sox9* lead to XY human and mouse females, while deletions or mutations of *Dax1* and *Wnt4* lead to XX males. These findings have led to the suggestion that *Sox9* enhances and *Dax1* and *Wnt4* oppose Sry activity. In support of this idea, *Sox9* expression rises in males shortly after *Sry*, while *Dax1* and *Wnt4* expression decline in males over the same period of embryogenesis.

Interestingly, over-expression of *Sox9* in XX embryos leads to XX males and over-expression of *Dax1* and *Wnt4* in XY embryos to XY females. These dosage effects suggest that it may not be the absolute amount of Sry protein that is important for the instruction 'make a testis' so much as the ratio of Sry and/or Sox9 to Dax1 and/or Wnt4. In normal development, perhaps *Sry* expression promotes *Sox9* and depresses *Dax1* and *Wnt4* expression, but disturbances in the expression levels of these down-stream genes can override the original *Sry* push to 'make a testis'.

Finally, downstream of all these 'make a testis' genes there seem to be at least two '*confirm a testis*' genes. One of these, encoding fibroblast growth factor 9 (*Fgf9*) is discussed in the main text; in embryos genetically lacking *Fgf9* genes, mesonephric cell invasion fails, myoid cells do not develop, the emergent seminiferous cords collapse and the gonad reorganizes as an XY ovary. The second gene is *prostaglandin D synthase* (*Ptgds*), which is produced by both pre-Sertoli and primordial germ cells and catalyses synthesis of prostaglandin D (PGD). Exogenous PGD can convert female gonads at least partially to XX male gonads, and endogenous PGD is thought to have a testicular reinforcement role in the developing testis.

Overall, the developing testis seems to use multiple genes in a 'belt and braces' approach triggered by *Sry* expression (the belt). However, this approach leaves testis development vulnerable to rare genetic mutations in the downstream 'braces' genes, which, helpfully, are also facilitating elucidation of the molecular web of male testis formation.

Advanced reading

Adams IR, McLaren A (2002) Sexually dimorphic development of mouse primordial germ cells: switching from oogenesis to spermatogenesis. *Development* **129**, 1155–1164 (prostaglandin D synthase).

Chaboissier M-C *et al*. (2004) Functional analysis of *Sox8* and *Sox9* during sex determination in the mouse. *Development* **131**, 1891–1901 (Sox 9).

Colvin JS *et al*. (2001).Male-to-female sex reversal in mice lacking fibroblast growth factor 9. *Cell* **104**, 875–889 (*Fgf9* mutants).

Grosschedl R *et al*. (1994) HMG domain proteins: architectural elements in the assembly of nucleoprotein structures. *Trends in Genetics* **10**, 94–100. (DNA bending properties of the Sry box).

Ohe K *et al*. (2002) A direct role of SRY and SOX proteins in pre-mRNA splicing. *Proceedings of the National Academy of Sciences of the USA* **99**, 1146–1151 (evidence about the molecular mechanism of action of Sry).

Swain A *et al*. (1998) *Dax1* antagonizes *Sry* action in mammalian sex determination. *Nature* **391**, 761–767.

Vainio S *et al*. (1999). Female development in mammals is regulated by *Wnt-4* signalling. *Nature* **397**, 405–409.

Vidal VPI *et al*. (2001) *Sox9* induces testis development in XX transgenic mice. *Nature Genetics* **28**, 216–217 (*Sox9* dosage effect).

***Gene/Protein notations**

Throughout the book uses the following gene/protein notation (Sry as example):

Human genes/mRNAs – *SRY*; Human protein SRY.

Mouse genes/mRNAs – *Sry*; mouse protein Sry.

Where a generic statement about mammals is made, the mouse notation is used.

1.3d). The further development of these cells depends on whether the *Sry* gene is expressed or not. In the developing males, *Sry* expression is *restricted to the cells of the sex cords*. These cells proliferate vigorously and penetrate deep into the medullary mesenchyme, surrounding most of the PGCs to form *testis cords* (Fig. 1.4a). They will eventually become *Sertoli cells*, the main supporting cell for spermatogenesis. Because *Sry* expression is limited to the precursor Sertoli cells (*pre-Sertolic cells*), it has been suggested that the *Sry* gene actually issues the instruction: '*make a Sertoli cell*'. Now enclosed within the cords, the PGCs are known as *prospermatogonia* and will later give rise to spermatozoa.

In contrast, females lack *Sry* expression, and their sex cords are ill-defined and do not penetrate deeply into the ridge. Instead, the cells condense cortically as small clusters around the PGCs, now called *oogonia*. This clustering initiates formation of the *primordial ovarian follicles* (Fig. 1.4c,d). In these follicles the condensing cord cells will give rise to the *granulosa cells* of the primordial follicle, while the oogonia will give rise to *oocytes* (see Chapter 5).

The third wave of migratory cells comes from the mesonephric primordia, which lie just lateral to the genital ridges (Fig. 1.3c), and, like the sex cords, they show major sex differences. In the male, mesonephric cells are thought

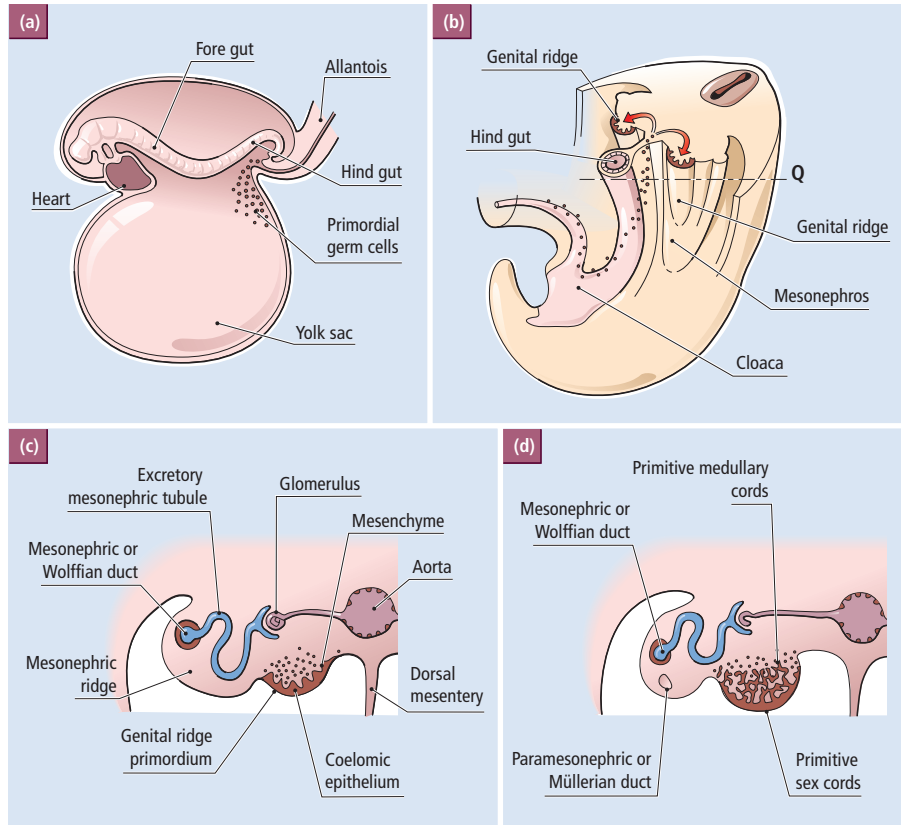

Fig. 1.3 A 3-week human embryo showing: (a) the origin of the primordial germ cells; and (b) the route of their migration. Section Q is the plane of transverse section through the lumbar region shown at 4 weeks in (c). In (d) the same plane of section is shown at 5 weeks of development: the 'indifferent gonad' stage.

to contribute at least three major cell types to the testis. Some cells contribute the vasculature tissue of the testis. Other cells synthesize steroid hormones and cluster between the cords to form *Leydig cells*—the main source of androgens. The third group of mesonephric cells condenses on the developing testis cords and stimulates formation of a basement membrane on which they then sit as *myoid cells*, thereby forming the *seminiferous cords*, the forerunners of the adult *seminiferous tubules* (Fig. 1.4b). The inward migration of this latter group of mesonephric cells giving rise to myoblasts is attributable to the chemotactic action of a growth factor called *fibroblast growth factor 9 (Fgf9)*, which is produced by the developing Sertoli cells. Should this migration fail (for example, in mice lacking *Fgf9* genes), the testis cords regress, emphasizing the important role of myoid cells in testis formation. The mesonephric tissue also forms the *rete blastema* or *rete testis cords*, later becoming the *rete testis*, which forms part of the male sperm-exporting duct system (Fig. 1.4a,b). In the female, no myoid cells migrate and the rete blastema is vestigial and transient, leaving only a vestigial *rete ovarii* in the adult (Fig. 1.4c,d). However, the mesonephric vascular and Leydig cell precursors in males may be paralleled in females by equivalent cells that will eventually form respectively blood vessels and condensations around the developing follicles called *thecal cells*.

With these three waves of inward migration completed, the basic patterns of testis and ovary are established. However, although the initial decision as to whether to make an ovary or a testis depends on the presence or absence of the *SRY* activity in developing Sertoli cells, subsequent development of the gonad, particularly of the ovary and its follicles, is dependent on the presence of a population of normal germ cells. For example, women suffering from *Turner's syndrome* (see Table 1.1), who have a normal autosomal complement but only one X chromosome, develop an ovary. Subsequently, however, normal oocyte growth requires the activity of both X chromosomes, and the activity of only one X in individuals with Turner's syndrome leads to death of the oocyte. Secondary loss of the follicle cells follows, leading to *ovarian dysgenesis* (abnormal development), and a highly regressed or *streak* ovary. Conversely, men with *Klinefelter's syndrome* (see Table 1.1) have a normal autosomal complement of chromosomes but three sex chromosomes, two X and one Y. Testes form normally in these individuals as a result of the expression of *SRY*. However, most of the germ cells die much later in life when they enter meiosis and their death is the result of the activity of two X chromosomes rather than one. These syndromes provide us with two important pieces of clinical evidence. First, *initiation* of gonad formation can occur when sex *cord cells* have only *one* Y (testis) or *one* X (ovary)

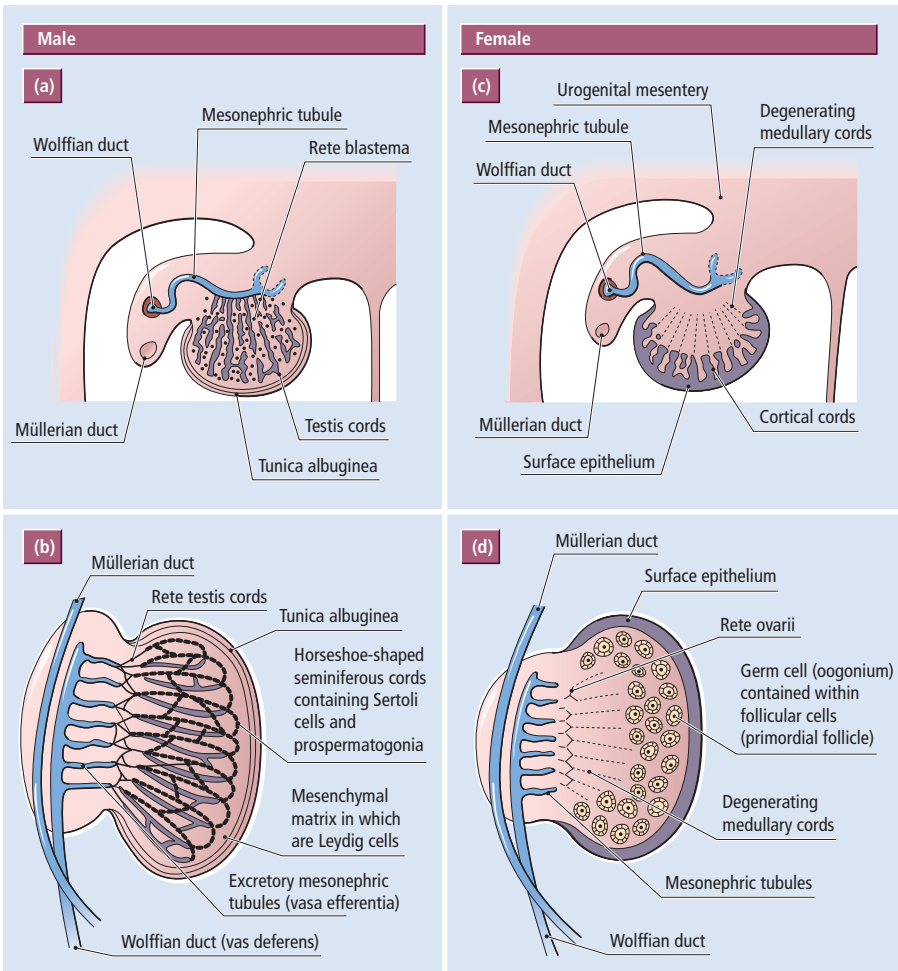

Fig. 1.4 Testicular development during (a) week 8 and (b) weeks 16–20 of human embryo-fetal life. (a) The *primitive sex cords* proliferate into the medulla, establish contact with the *mesonephric medullary cords* of the rete testis blastema and become separated from the coelomic epithelium by the *tunica albuginea* (fibrous connective tissue), which eventually forms the *testicular capsule*. (b) Note the horseshoe shape of the *seminiferous cords* and their continuity with the *rete testis cords*. The *vasa efferentia*, derived from the mesonephric tubules, connect the seminiferous cords with the *Wolffian duct*.

Comparable diagrams of ovarian development around (c) week 7 and (d) the weeks 20–24 of development. (c) The primitive sex cords are less well organized and cortical, while medullary mesonephric cords are absent or degenerate. The cortical coelomic epithelial cells condense around the arriving primordial germ cells to yield *primordial follicles* shown in (d). In the absence of medullary cords and a true persistent *rete ovarii*, no communication is established with the mesonephric tubules. Hence, in the adult, oocytes are shed from the surface of the ovary, and are not transported by tubules to the oviduct (compare with the male, see Chapters 4 & 5).

chromosome. Second, *completion* of normal gonad development requires that the *germ* cells have *two X* chromosomes in an *ovary* but *do not have more than one X* chromosome in a *testis*.

Primary hermaphrodites have both ovarian and testicular tissues

We have established that *Sry* activity on the Y chromosome converts an indifferent gonad into a testis, whereas the absence of its activity results in an ovary. *Genetic maleness* leads to *gonadal maleness*. This primary step in sexual differentiation is remarkably efficient, and only rarely are individuals found to have both testicular *and* ovarian tissue. Such individuals are called *primary* (or *true*) *hermaphrodites* and arise in many cases because of the presence of a mixture of XY and XX (or XO) cells.

The main role of the *Sry* gene in sexual determination is completed with the establishment of the fetal gonad, and the gene is no longer expressed in the fetus. From this point onwards, the gonads themselves assume the pivotal role in directing sexual differentiation both pre- and postnatally.

Again, it is the male gonad, like the Y chromosome before it, which plays the most active role, taking over the 'baton of masculinity' in this sexual relay.

The differentiation of two sexes depends on the endocrine activity of the fetal testis

Endocrine activity in the ovaries is *not* essential for sexual differentiation during fetal life. In contrast, the testes actively secrete two *essential* hormones. The interstitial cells of Leydig secrete steroid hormones, the *androgens*, and the Sertoli cells within the seminiferous cords secrete a dimeric glycoprotein hormone called *Müllerian inhibiting hormone* (MIH; also called MIS for Müllerian inhibiting substance and AMH for anti-Müllerian hormone). These hormones, which are discussed in more detail in Chapter 3, are the messengers of male sexual differentiation sent out by the testis. In their absence, female sexual differentiation occurs. Thus, sexual differentiation must be actively diverted along the male line, whereas differentiation along the female line again seems to reflect an inherent trend requiring no active intervention.

The male and female internal genitalia develop from different unipotential precursors through the actions of androgens and MIH

Examination of the primordia of the male and female *internal genitalia* (see Figs 1.4 & 1.5) shows that instead of one indifferent but bipotential primordium, as was the case for the gonad, there are two separate sets of primordia, each of which is *unipotential*. These are both located in the mesonephros adjacent to the developing gonad, and are called the *Wolffian* or *mesonephric* (male) and *Müllerian* or *paramesonephric* (female) ducts. In the female, the Wolffian ducts regress spontaneously and the Müllerian ducts persist and develop to give rise to the *oviducts, uterus* and *cervix* and *upper vagina* (Fig. 1.5). If a female fetus is *castrated* (its gonads removed), internal genitalia develop in a typical female pattern. This observation demonstrates that ovarian activity is not required for development of the female tract.

In the male, the two testicular hormones prevent this spontaneous development of female genitalia. Thus androgens, secreted in considerable amounts by the testis, actively maintain the Wolffian ducts, which develop into the *epididymis, vas deferens* and *seminal vesicles*. If androgen secretion by the testes should fail, or be blocked experimentally, then the Wolffian duct system regresses and these organs fail to develop. Conversely, exposure of female fetuses to androgens causes the development of male internal genitalia.

Testicular androgens have no influence on the Müllerian duct system, however, and its regression in males is under the control of the second testicular hormone, MIH. Thus, *in vitro* incubation of the primitive internal genitalia of female embryos with MIH provokes abnormal regression of the Müllerian ducts.

The male and female external genitalia develop from a single bipotential precursor through the actions of androgens

The primordia of the external genitalia, unlike those of the internal genitalia, are bipotential (Fig. 1.6). In the female, the *urethral folds* and *genital swellings* remain separate, thus forming the *labia minora* and *majora*, while the *genital tubercle* forms the *clitoris* (Fig. 1.6). If the ovary is removed, these changes still occur, indicating their independence of ovarian endocrine activity. In contrast, androgens secreted from the testes in the male cause the urethral folds to fuse (so enclosing the *urethral tube* and contributing, together with cells from the genital swelling, to the *shaft of the penis*); the genital swellings to fuse in the midline (so forming the *scrotum*); and the genital tubercle to expand (so forming the *glans penis*) (Fig. 1.6). Exposure of female fetuses to androgens will 'masculinize' their external genitalia, while castration, or suppression of endogenous androgens, in the male results in 'feminized' external genitalia.

Secondary hermaphrodites have genitalia that are not of the sex expected from their gonads

Failure of proper endocrine communication between the gonads and the internal and external genital primordia can lead to a dissociation of gonadal and genital sex. Such individuals are called *secondary* (or *pseudo*) *hermaphrodites*. For example, in the genetic syndrome of *androgen insensitivity syndrome* (*AIS*; also called *testicular feminization* or *Tfm*) the genotype is XY (male), and testes develop normally and secrete androgens and MIH. However, the fetal genitalia are genetically insensitive to the action of androgens (see detailed discussion in Chapter 3), which results in complete regression of the androgen-dependent Wolffian ducts and in the development of female external genitalia. Meanwhile, the MIH secreted from the testes exerts its action fully on the Müllerian ducts, which regress. Thus, this genetically male individual, bearing testes and having androgens circulating, nonetheless appears female with labia, a clitoris and a vagina, but totally lacks other components of the internal genitalia (Fig. 1.7a).

A naturally occurring counterpart to testicular feminization is the genetically based *adrenogenital syndrome (AGS*; also called *congenital adrenal hyperplasia* or *CAH)* in female fetuses, in which the XX female develops ovaries as usual. However, as a result of genetic defects in the corticosteroid synthesizing enzymes, the fetal adrenal glands become

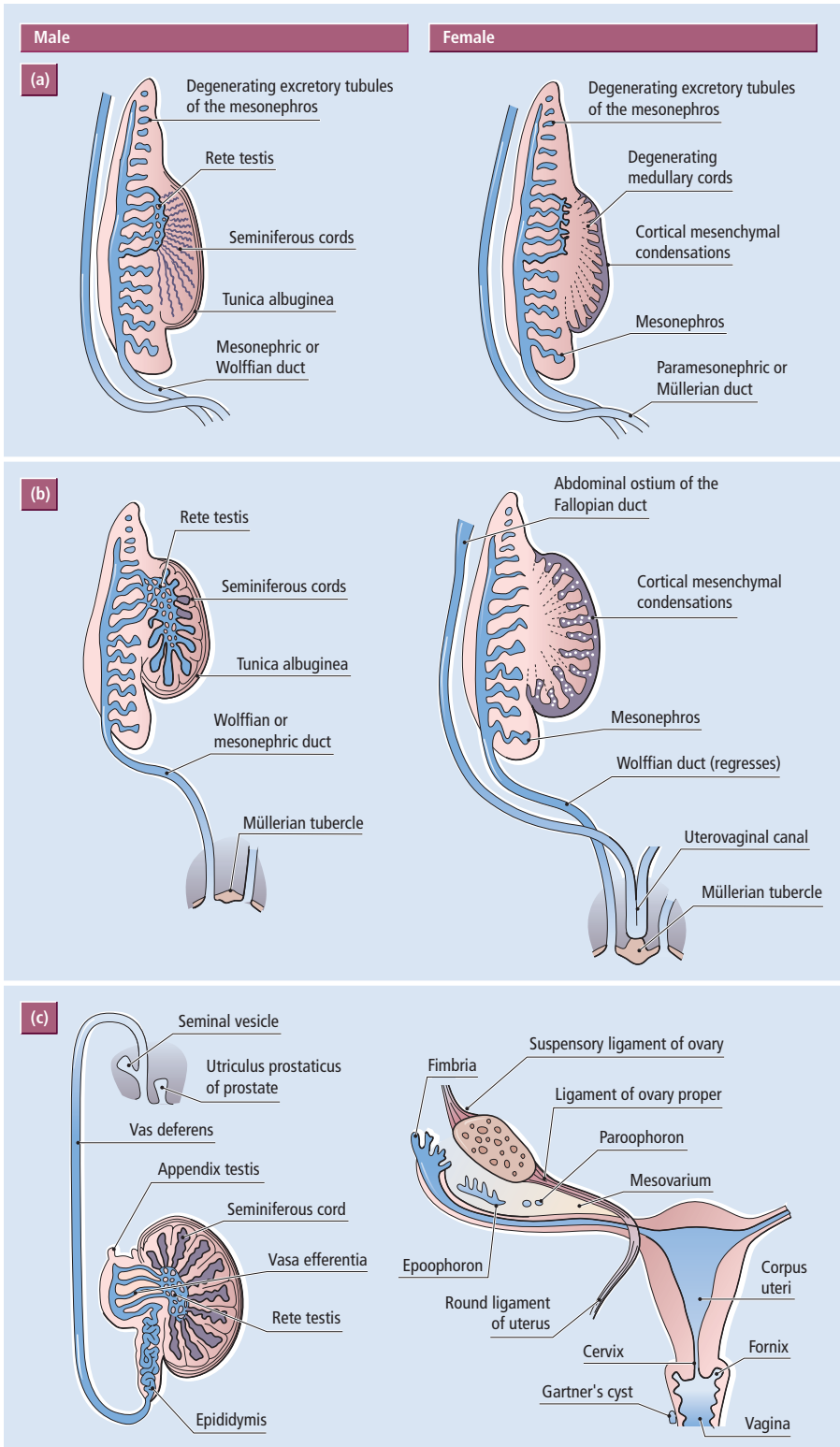

Fig. 1.5 Differentiation of the internal genitalia in the human male (left) and female (right) at: (a) week 6; (b) the fourth month; and (c) the time of descent of the testis and ovary. Note the *paramesonephric Müllerian* and *mesonephric Wolffian* ducts are present in both sexes early on, the former eventually regressing in the male and persisting in the female, and vice versa. The *appendix testis* and *utriculus prostaticus* in the male, and *epoophoron*, *paroophoron* and *Gartner's cyst* in the female are thought to be remnants of the degenerated Müllerian and Wolffian ducts, respectively.

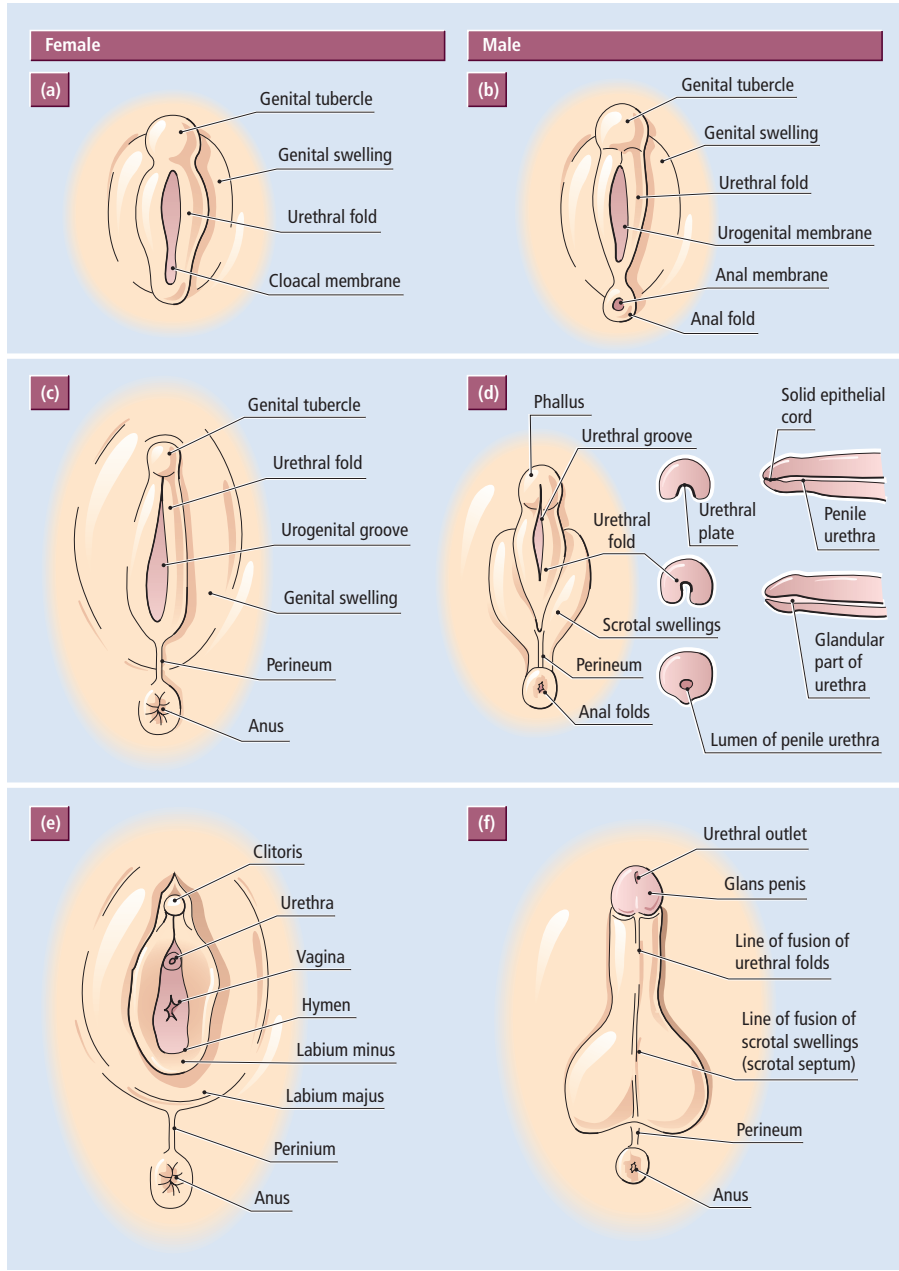

Fig. 1.6 Differentiation of the external genitalia in the human female (left) and male (right) from common primordia shown at: (a) 4 weeks; and (b) 6 weeks. (c) In the female, the *labia minora* form from the *urethral folds* and the *genital tubercle* elongates to form the *clitoris*. (d) Subsequent changes by the fifth month are more pronounced in the male, with enlargement of the genital tubercle to form the *glans penis* and fusion of the urethral folds to enclose the *urethral tube* and form the *shaft of the penis* (the genital swellings probably also contribute cells to the shaft). (e) The definitive external genitalia of the female at birth. (f) The definitive external genitalia of the male at birth.

hyperactive in an attempt to overcome the lack of corticosteroids, and secrete large quantities of precursor steroids, some with strong androgenic activity (see Chapter 3 for details of steroid biosynthetic pathways). These androgens stimulate development of the Wolffian ducts, and also cause the external genitalia to develop along the male pattern. The Müllerian system remains, as no MIH has been secreted. Thus, the individual appears partially or even wholly masculinized with a penis and scrotum, but is genetically and gonadally *female* and possesses the internal genitalia of *both* sexes (Fig. 1.7b).

Individuals with *persistent Müllerian duct syndrome* present as genetic males in whom either MIH production, or responsiveness to it, is inadequate. They therefore have testicular androgens that stimulate external genitalia and Wolffian ducts, but *retain* Müllerian duct structures. These men are thus genetically and gonadally *male* but possess the internal genitalia of *both* sexes.

Apart from the problems of immediate clinical management raised by diagnosis of these syndromes, abnormalities of development of the external genitalia may have important long-term consequences. The single, most

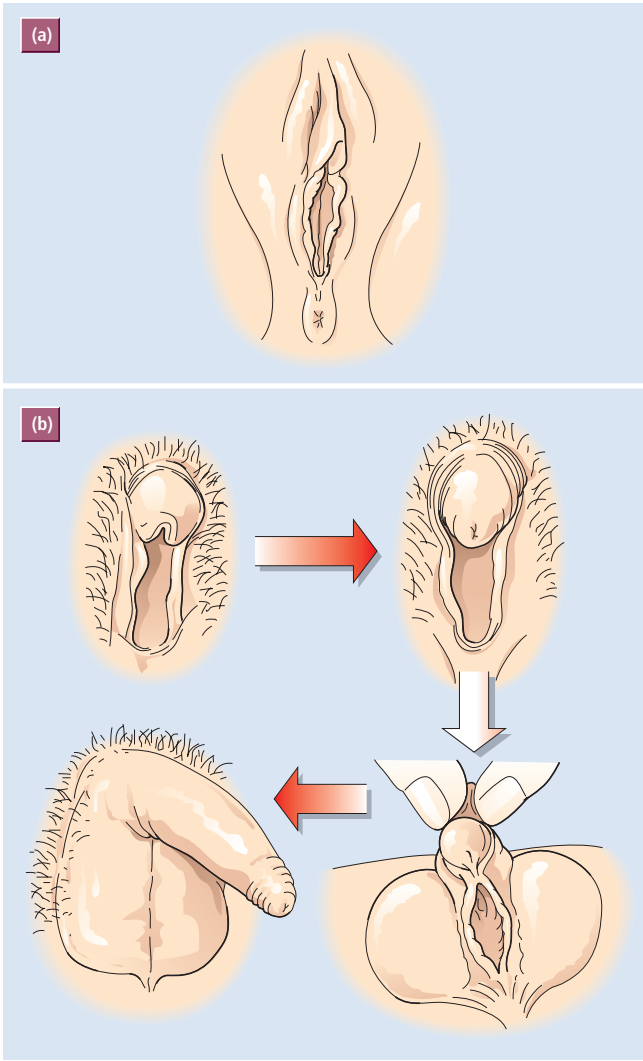

Fig. 1.7 (a) External genitalia of an XY adult with complete *androgen insensitivity syndrome (testicular feminization)*. Although an androgen-secreting testis is present internally, the external genitalia are indistinguishable from those of a female, and at birth the child would be classified as a girl (see Chapters 2 & 3 for more details). (b) The external genitalia from XX girls with *adrenogenital syndrome* show varying degrees of masculinization, from an enlarged clitoris to development of a small penis and (empty) scrotum. Ovaries are present internally. The adrenal cortex has inappropriately secreted androgens at the expense of glucocorticoids during fetal life and directed development of the genitalia along the male line. Clearly, the more severe cases could lead to sex assignment as a boy, or to indecision (see Chapter 2).

important event in the identification of sex of the newborn human is examination of the external genitalia. These may be unambiguously male or female, regardless of whether the genetic and gonadal constitutions correspond. They may also be ambiguous as a result of partial masculinization during fetal life. Sex assignment at birth is one important step that contributes to the development of an individual's *gender identity*, so uncertainty or error at this early stage can have major consequences for an individual's self-perception later in life as a man or a woman. This issue is discussed in more detail in Chapter 2.

Pre- and postnatal growth of the gonads is slow until puberty

We have seen how phenotypic features of the male and female develop. The female path of development is taken unless there is intervention via genetic (*SRY*) and then endocrine (androgens and MIH) activities, when a male develops. During the remainder of prenatal life and during postnatal life up to puberty, further sexual divergence of physical phenotypes occurs only at a very slow pace and both internal and external genitalia remain immature, growing slowly in line with general body growth. In the male (but not the female) this process is dependent on low and variable levels of gonadal hormones. Despite the relative quiescence, some important reproductive changes do occur over this period.

The testes migrate to a scrotal position

The gonad develops in the upper lumbar region of the embryo, yet by adulthood in most mammalian species, including humans, the testes have *descended* through the

abdominal cavity, and over the pelvic brim through an inguinal canal to arrive in the scrotum (Fig. 1.8). Evidence of this extraordinary migration is found in the nerve and blood supplies to the testis, which retain their lumbar origins and pass on an extended course through the abdomen to reach their target organ. The transabdominal descent of the testis towards the inguinal canal involves two ligamentous structures: at the superior pole of the testis is the *suspensory ligament* while inferiorly is the *gubernaculum*, which attaches the testis to the posterior abdominal wall (Fig. 1.8). As the fetal male body grows, the suspensory ligament elongates but the gubernaculum does not, and thus in the male the relative position of the testis becomes increasingly caudal or pelvic. Two hormones, both secreted by the developing Leydig cells, are responsible for these male-specific effects. Androgens act on the suspensory ligament, allowing its elongation, while *insulin-like growth factor 3* (Insl3) acts on the gubernaculum to mature and stabilize it.

Testicular migration in humans may be arrested developmentally at some point on the migratory route resulting

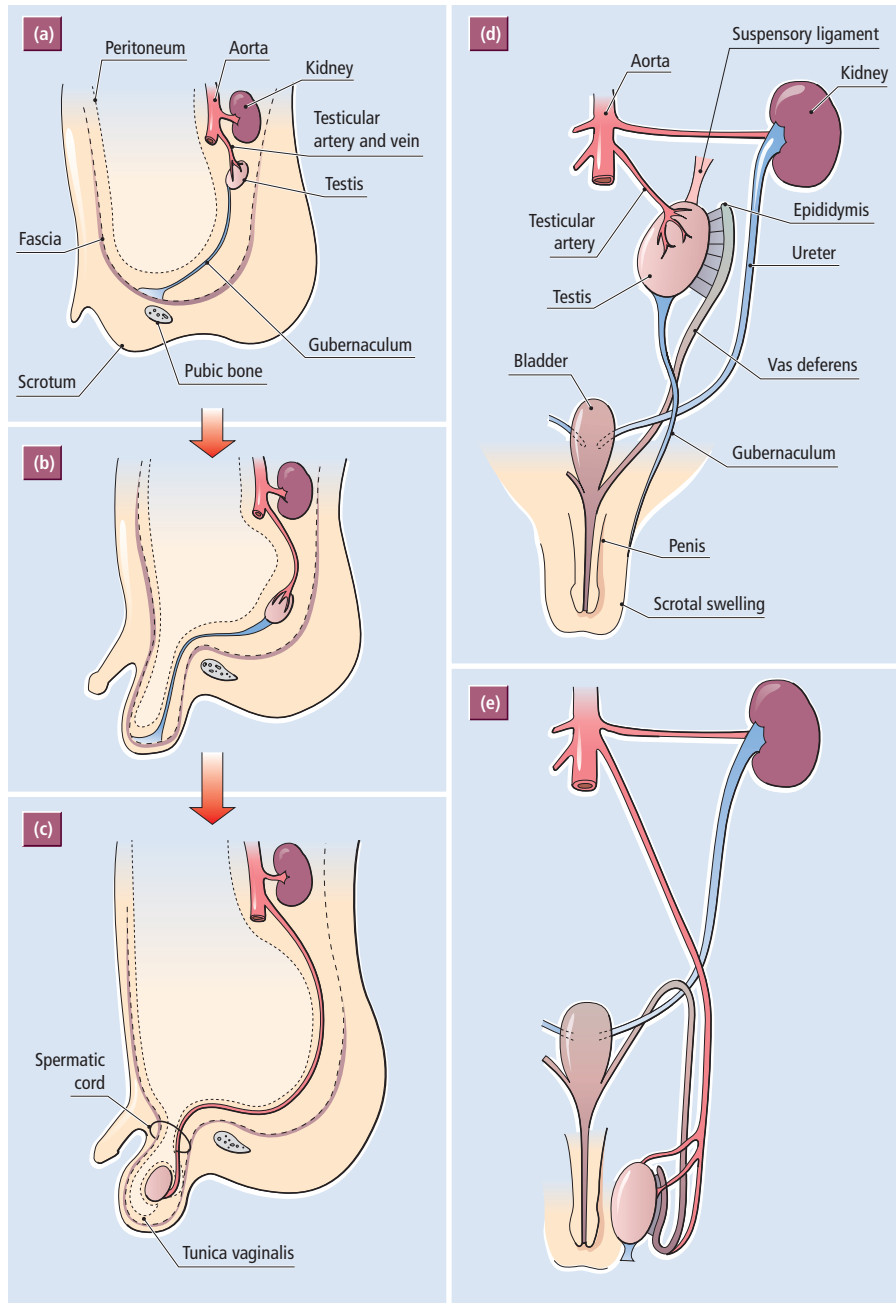

Fig. 1.8 (a–c) Parasagittal sections through a developing male abdomen. The initial retroperitoneal, abdominal position of the testis shifts pelvically between 10 and 15 weeks, extending the blood supply (and Wolffian duct derivatives, not shown) as the *gubernaculum* shortens and the *suspensory ligament* (d) (connecting the testis to the posterior abdominal wall) lengthens and regresses. A musculofascial layer evaginates into the scrotal swelling accompanied by peritoneal membrane, which forms the *processus vaginalis*. Between weeks 25 and 28 of pregnancy in the human, the testis migrates over the pubic bone behind the processus vaginalis (which wraps around it forming a double-layered sac), reaching the scrotum by weeks 35–40. The fascia and peritoneum become closely apposed above the testis, obliterating the peritoneal cavity leaving only a *tunica vaginalis* around the testis below. The fascial layers, obliterated stem of the processus vaginalis, vas deferens and testicular vessels and nerves form the *spermatic cord*. (d, e) Front view of the migration, showing the extended course ultimately taken by the testicular vessels and vas deferens.

in one or both testes being non-scrotal, a condition known as *cryptorchidism* (hidden gonad). The consequences of cryptorchidism in men demonstrate that a scrotal position is essential for normal testicular function. Although adult endocrine activity is not affected in any major way, spermatogenesis is arrested, testicular metabolism is abnormal and the risk of testicular tumours increases. These effects can be simulated by prolonged warming of the scrotal testis experimentally or by wearing thick tight underwear. The normal scrotal testis functions best at temperatures 4–7 °C lower than abdominal 'core' temperature. Cooling of the testis is improved by *copious sweat glands in the scrotal skin* and by the blood circulatory arrangements in the scrotum. The *internal spermatic arterial* supply is coiled (or even forms a rete in marsupials) and passes through the *spermatic cord* in close association with the draining venous *pampiniform plexus*, which carries peripherally cooled venous blood (Fig. 1.9). Therefore, a heat exchange is possible, cooling the arterial and warming the venous blood (see also Box 1.2).

Testicular growth and activity are important for male development

By weeks 16–20 of human fetal life, the testis consists of an outer *fibrous tunica albuginea* enclosing vascularized stromal tissue, which contains condensed Leydig cells and solid seminiferous cords comprised of a basement membrane, Sertoli cells and prospermatogonial germ cells. These germ cells are quiescent and mitotic divisions are rarely observed.

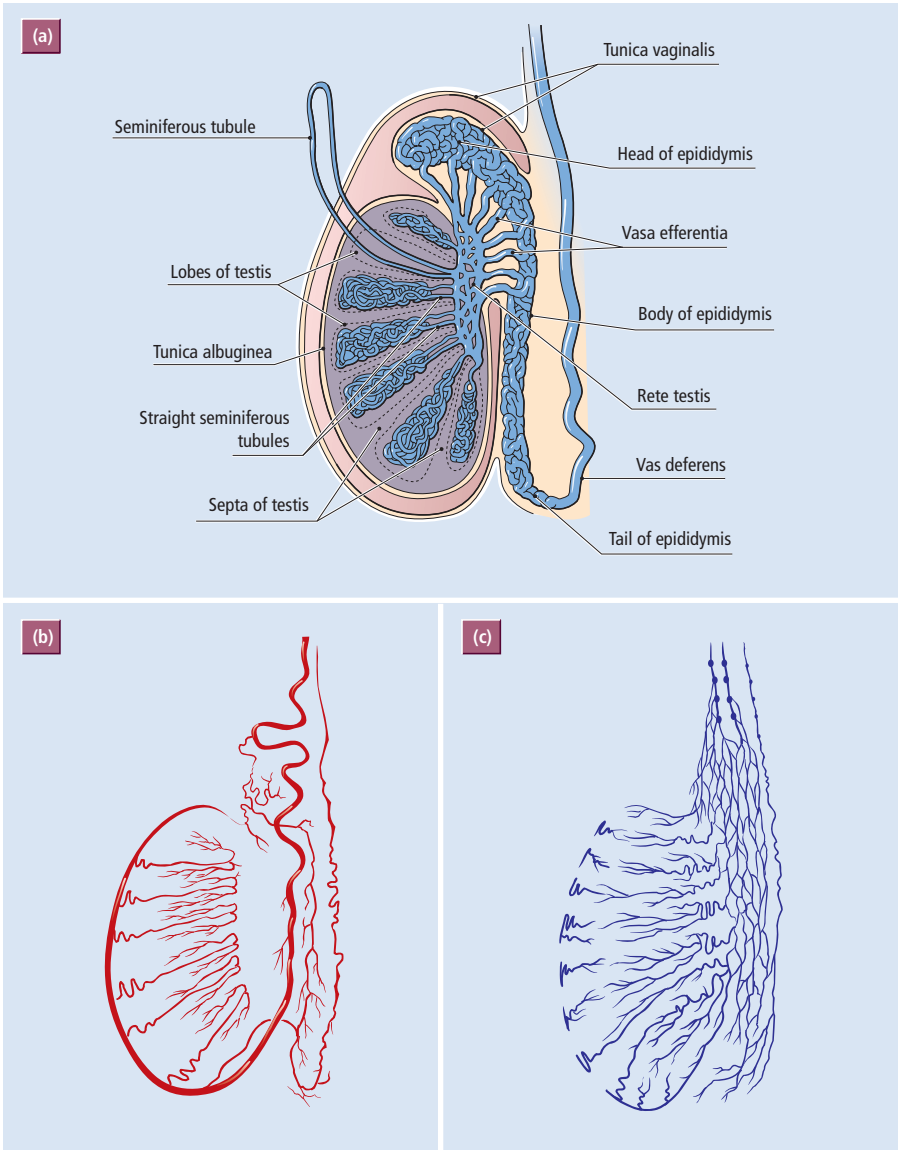

Fig. 1.9 Section through an adult human testis to show: (a) general structure; (b) arterial supply; and (c) venous drainage.

BOX 1.2 Evolutionary evidence of testis migration

Comparative biological study of the male testis provides evidence of 'evolutionary cryptorchidism'. Thus:

- in elephants, hyraxes and the monotremes (platypus and echidna), the testes normally do not descend at all from the lumbar site
- in armadillos, whales and dolphins, the testes migrate only part of the route to the rear of the lower abdomen
- in hedgehogs, moles and some seals, they lodge in the inguinal canal
- in most rodents and wild ungulates, they retain mobility in the adult, migrating in and out of the scrotum to and from inguinal or abdominal retreats.

In animals, such as humans, that have scrotal testes, a null mutation of the *Insl3* gene results in no Insl3 expression and failure of gubernacular maturation and testicular descent. It would be interesting to look at *Insl3* gene expression in the above non-scrotal species!

The human scrotal testis clearly *requires* a lower ambient temperature for normal function, but this requirement may be a secondary *consequence* of its scrotal position rather than the original evolutionary *cause* of its migration. Thus, those species in which testes remain in the abdomen survive, flourish and reproduce despite the high testicular temperature. It remains unclear as to why testes are scrotal in so many species, given their greater physical vulnerability!

Advanced reading

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The seminiferous cords connect to the cords of the *rete testis*, the *vasa efferentia* and thereby to the *epididymis*.

The Leydig cells in the human testis actively secrete testosterone from at least weeks 8–10 of fetal life onwards, with blood levels peaking at 2 ng/ml at around weeks 13–15. Thereafter, blood levels decline and plateau by 5–6 months at a level of 0.8 ng/ml. This transient *prenatal peak* of blood testosterone is a feature of many species, although in some, for example the rat and sheep, the peak may approach, or span, the period of parturition, and only begin its decline postnatally. The males of some primate species, including humans, show a *postnatal peak* in plasma testosterone, concentrations reaching 2–3 ng/ml by 3 months postpartum, but declining to around 0.5 ng/ml by 3–4 months. A second modest infantile rise in androgens occurs at 1 year and extends to puberty, when a prepubertal peak in androgen output occurs, reaching levels of about 9 ng/ml. The capacity to secrete testosterone is, as we have seen, essential for establishment of the male phenotype. It is also important for the continuing development of the male phenotype and, in many if not all species, can also influence the development of masculine behaviour patterns (see Chapter 2). The Sertoli cells continue to produce MIH throughout fetal life up until puberty, when levels drop sharply.

Throughout fetal and early postnatal life, testis size increases slowly but steadily. The prospermatogonial germ cells undergo only limited mitotic proliferation and contribute little to this growth. At puberty there is a sudden increase in testicular size to which all parts of the testis contribute: the solid seminiferous cords canalize to give rise to tubules; the intratubular Sertoli cells increase in size and activity; the germ cells resume mitotic activity and begin the process of spermatozoal formation; and endocrine secretion by the intertubular Leydig cells increases sharply. These changes herald the onset of sexual maturity and the development of fertility. The causes and consequences of this sudden growth at puberty will be discussed in detail in Chapter 7. The details of how the mature testis functions are described in Chapter 4.

Most ovarian germ cells die before puberty and all of them enter meiosis

The ovary, unlike the testis, retains its position within the abdominal cavity, shifting slightly in some species, such as the human, to assume a pelvic location. It is attached to the posterior abdominal wall by the *ovarian mesentery* or *mesovarium*. The ovary, like the testis, grows slowly but steadily in size during early life. As in the testis, little of this growth is due to the germ cells themselves. However, quite unlike the situation in the testis, the ovarian germ cells undergo three major changes.

- First, whereas in the male the prospermatogonial germ cells remain in a mitotic cell cycle, albeit rarely dividing, in the female *all the oogonial germ cells cease dividing mitotically* either before birth (human, cow, sheep, goat, mouse), or shortly thereafter (rat, pig, cat, rabbit, hamster), to *enter into their first meiotic division*, thereby becoming *primary oocytes*. The termination of mitosis and entry into meiosis seems to be programmed into all PGCs, since even XY-bearing PGCs enter meiosis spontaneously when cultured in female genital ridges or within the extragonadal parts of the male embryo itself should they have gone astray and not reached the male genital ridge. It seems that the Sry-driven enclosure of the XY germ cells within the seminiferous cords *suppresses meiotic onset* and maintains the PGCs as mitotic cells. There is a major consequence for women of this early termination of mitosis in that by the time of birth *a woman has all the oocytes within her ovaries that she will ever have*. If

these oocytes are lost, for example by exposure to X-irradiation, they cannot be replaced from stem cells and the woman will be infertile. This situation is distinctly different from that in the male in which the mitotic proliferation of spermatogonial stem cells continues throughout adult reproductive life (see Chapter 4).

• Second, having entered meiosis so prematurely, the germ cells, as described earlier, form primordial follicles as a result of the condensation of surrounding granulosa cells derived from invading sex cords (Fig. 1.4c). The formation of the primordial follicles precipitates the second major change. The oocytes abruptly arrest their progress through first meiotic prophase at *diplotene*, their chromosomes still enclosed within a nuclear membrane called the *germinal vesicle* (Fig. 1.10b; see also Fig. 1.1 for details of meiosis). The oocyte halted at this point in meiosis is said to be at the *dictyate stage* (also called *dictyotene*). The primordial follicle may stay in this arrested meiotic state for up to 50 years in women, with the oocyte metabolically ticking over and waiting for a signal to resume development. The reason for storing oocytes in this extraordinary protracted meiotic prophase is unknown. Although a few follicles may resume development sporadically and incompletely during fetal and neonatal life, regular recruitment of primordial follicles into a pool of growing follicles occurs first at puberty.

• The third remarkable feature occurring over this prepubertal period is the death of most of the meiotically arrested oocytes at or around the time of birth, depending on the species (Fig. 1.11). The cause of death is unknown and the reasons for it are unclear. The consequence of it is that

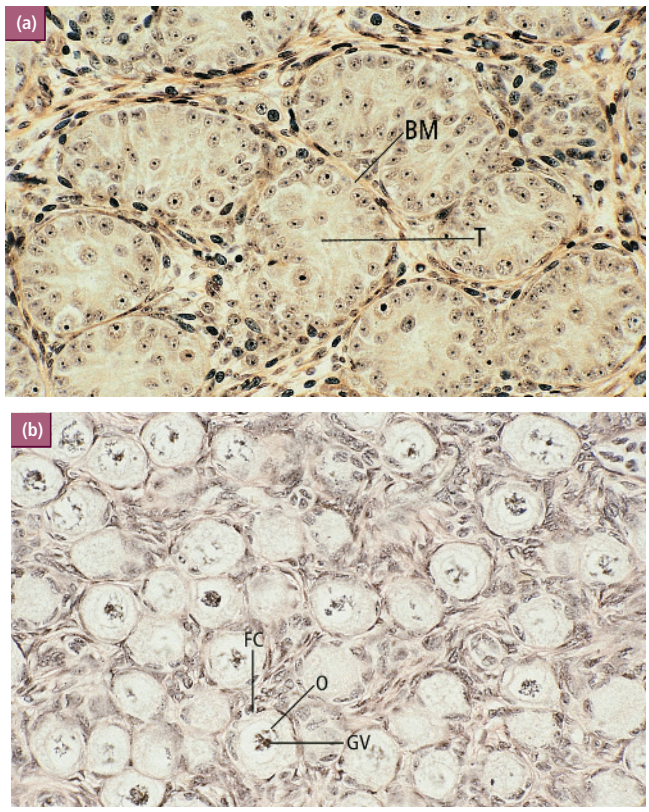

Fig. 1.10 Sections through immature (a) testis and (b) ovary, both at the same magnification. Note in (a) that each tubule is surrounded by a basement membrane (BM) and within the tubule there is no lumen (T) and a relatively homogeneous-looking set of cells comprising a very few spermatogonial stem cells and mostly Sertoli cells as seen here. Note in (b) that each oocyte (O) is relatively large and contains within it a distinctive nucleus (the germinal vesicle, GV). The ooctye is surrounded by a thin layer of follicle granulosa cells (FC) to form the primordial follicle.

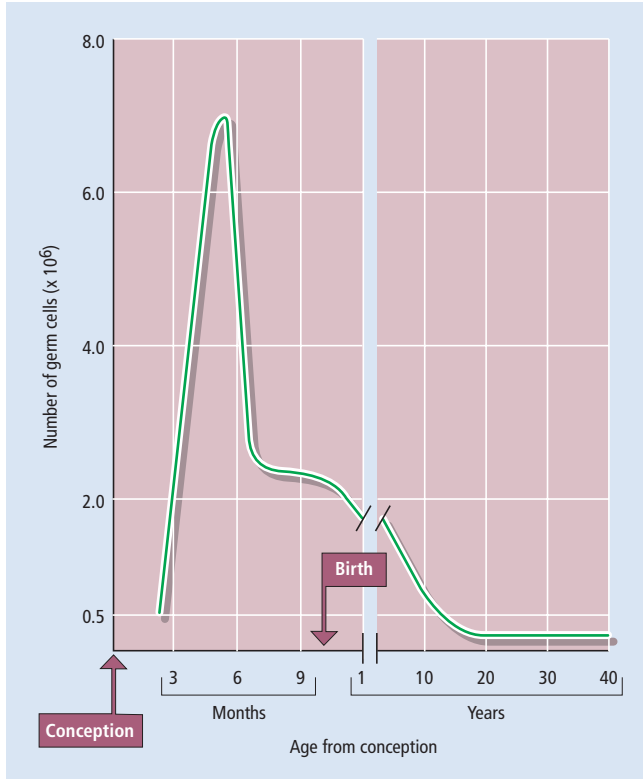

Fig. 1.11 Numbers of ovarian germ cells during the life of a human female. After an initial period of migration, mitotic proliferation commences at around days 25–30. The first meiotic prophases can be detected at around days 50–60, and the first diplotene-stage chromosomes at around day 100. All germ cells are at the *dictyate stage* by birth. *Atresia* of oocytes in the meiotic prophase is first obvious by about days 100, and continues throughout fetal and neonatal life. The period from entry of first germ cells into meiosis to full attainment of the dictyate stage by all germ cells varies between species, being in days after conception (date of birth in brackets): sow 40–150 (114); ewe 52–110 (150); cow 80–170 (280); rat 17.5–27 (22).

the stock of female germ cells available for use in adult life is reduced even further.

The ovary is not essential for prepubertal development

Over the prepubertal period the output of steroids by the ovary is minimal and, indeed, removal of the ovary does not affect prepubertal development. In some species, including humans, there may be a transitory stimulation of ovarian endocrine activity spanning the period of birth, but this does not appear to be important for female development. However, at puberty marked changes in both the structure and endocrine activity of the ovary occur, and for the first time the ovary becomes an essential and positive feminizing influence on the developing individual. How it does so is discussed further in Chapters 5, 7 and 8.

Summary

In this chapter we have seen that sexual differentiation is an enduring process of divergence, which begins with the expression of a genetic message that establishes the structure and nature of the fetal gonad, and then extends from the gonad via its hormonal secretions to many tissues of the body. Thus, sex may be defined at several levels and by several parameters. Concordance at all levels may be incomplete, and the medical, social and legal consequences of this 'blurring' of a clear, discrete sexual boundary may pose problems. However, in this chapter we have been able to define, by a broad set of criteria, how the two sexes are established. In the next chapter, we examine the issues of gender and sexuality and how they are related to the establishment of the two sexes.

KEY LEARNING POINTS

- Sexual reproduction in mammals involves the creation of a genetically novel individual by the contribution of equal numbers of chromosomes from two parents of different sexes.
- Sexual reproduction requires the production of male and female gametes (spermatozoa and oocytes) by the process of meiosis during which the chromosome number of somatic cells is halved and genetic recombination occurs.
- The male and female gametes are made in male and female gonads (testis and ovary) in male and female individuals (men and women).
- Males and females are distinguished simply by the presence or absence of a Y chromosome.
- A single gene called *Sry* on the Y chromosome acts by issuing the instruction 'make a testis'.
- The developing gonad arises from a unipotential genital primordium of mesenchymal tissue and three invading cell populations: the primordial germ cells, the germinal epithelial cells, and mesonephric cells.
- Sertoli cell precursors in the testis derive from the male germinal epithelial cells and are the sole site of *Sry* expression: so 'make a testis' may be expressed as 'make a Sertoli cell'.
- The granulosa cells of the follicle derive from the female germinal epithelial cells.
- Mesonephric-derived myoid cells migrate into the male genital ridge under the influence of fibroblastic growth factor 9 and are essential for stabilizing testis tubule development.
- Leydig cells in the male and thecal cells in the female are also derived from ingressing mesonephric cells, as are the vascular cells of the gonads.
- The embryonic testis makes two main hormones (androgens in Leydig cells and Müllerian inhibiting hormone, MIH, in Sertoli cells).
- The androgens stimulate the Wolffian ducts to make the epididymis, vas deferens and prostate.
- MIH causes the Müllerian ducts to regress.
- In the absence of MIH, the Müllerian duct becomes the oviduct, uterus, cervix and upper vagina.
- Common bipotential precursors of the external genitalia are stimulated to become the scrotum and penis by the testicular androgens, but become the labia and clitoris in the absence of androgens.
- The ovary is not required for the development of the prepubertal female, but the testis is required for the development of the prepubertal male.
- A sex reversed individual has an XX testis or an XY ovary.
- A primary hermaphrodite has both ovarian and testicular tissue.
- A secondary hermaphrodite has internal and/or external genitalia at variance with the sex of their gonads.
- In the fetal/neonatal ovary, the germ cells enter meiosis and then arrest in prophase of first meiosis at the germinal vesicle stage.
- Most female germ cells die around the time of birth.
- The testes migrate caudally under the combined influence of androgens and insulin-like growth factor 3 (Insl3), and, in most male mammals, assume a scrotal position.

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