
Chapter 1: Basics of vulval embryology, anatomy and physiology

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Embryology

A basic knowledge of the events in the embryogenesis and organogenesis of the female reproductive tract is important in order to understand the congenital abnormalities that may arise. The female genital tract is closely linked to the development of both the urinary and terminal part of the gastrointestinal tracts, explaining why some congenital abnormalities of the female reproductive tract may be found in association with anomalies of the urinary and gastrointestinal systems.

Sexual determination and differentiation

The term 'determination' describes events that commit cells to a certain course of development, and 'differentiation' describes the processes whereby these cells achieve this development. The differentiating processes are regulated by at least 30 specific genes located on sex chromosomes or autosomes that act through a variety of mechanisms (Grumbach & Conte 1992). Since the genetic sex of an individual is established at fertilization this may be regarded as the point of determination with all that follows being processes of embryonic differentiation. Although the genetic sex is determined at fertilization, the gonads and external genitalia remain sexually indeterminate for the first 6 weeks. A female phenotype develops in the absence of the androgens testosterone, dihydrotestosterone (DHT), anti-Mullerian hormone (AMH) and Mullerian-inhibiting substance (MIS) hormone. However incomplete masculinization can occur when testosterone fails to convert to DHT or when DHT fails to act within the cytoplasm or nucleus of the cells of the external genitalia and urogenital sinus. This can happen despite the presence of testes.

The presence or absence of the Y chromosome determines the sex of the indifferent gonad. Earlier investigation of patients with abnormalities of the sex chromosomes

had shown the Y chromosome to be extremely potent in inducing testicular differentiation (Ford *et al.* 1959, Jacobs & Strong 1959). The testis-determining factor (TDF) is a 35 kilobase pair (kbp) sequence on the 11.3 sub-band of the Y chromosome, in an area termed the sex-determining region of the Y chromosome (SRY). When this region is absent or altered, the indifferent gonad develops into an ovary. The SRY gene has been found in some cases of Turner's syndrome where there is no detectable Y chromosome in the karyotype. This finding demonstrates that the presence of a single dominant Y chromosomal gene alone is not enough to determine testicular differentiation (Mittwoch 1992). In addition to the Y chromosome, genes on other chromosomes also play a part in testicular development, including Wilm's tumour suppressor (*WT1*) gene, which regulates SRY expression, *DAX1* on the X chromosome, *SFI* on chromosome 9, *SOX9* on chromosome 17 and *AMH* on chromosome 19 (Mittwoch & Burgess 1991).

Hormones also have an important influence on sexual differentiation. The development of the internal ducts is a result of a paracrine effect from the ipsilateral gonad. A female phenotype develops in the absence of testicular tissue owing to the lack of testosterone, MIS or AMH. The level of local testosterone necessary for mesonephric (Wolffian) duct differentiation needs to be high. This is shown as maternal ingestion of androgens does not result in male internal differentiation in a female fetus, nor does this differentiation occur in females with congenital adrenal hyperplasia (CAH). Conversely, high levels of oestrogens can sometimes reduce MIS action, resulting in some paramesonephric (Mullerian) duct development. Two functional X chromosomes are normally required for ovarian development, but female differentiation of the internal and external sexual organs can still occur in the absence of a testis whether or not ovaries are present.

In summary, the genetic sex determines gonadal sex, which then determines the differentiation/regression of the internal ducts (i.e. Mullerian and Wolffian ducts) and the ultimate phenotypic sex. However, the final sexual identity of an individual depends not only on the phenotypic

appearance but also on the brain's prenatal and postnatal development.

Early female embryogenesis

In the first 8 weeks of development after ovulation, a system known as Carnegie staging is used to denote the maturity of the embryo. There are 23 Carnegie stages and each stage is based on internal and external physical features of the embryo (O'Rahilly & Muller 1987). The crown-rump length is also included.

Carnegie stage 1: post-ovulatory day 1; approx. size 0.1–0.2 mm

This is the point of fertilization in which the human zygote, with its XX sex chromosome constitution, is conceived in the distal third of the uterine tube. An acellular envelope, the zona pellucida encases the zygote.

Carnegie stage 2: days 2–3; approx. size 0.1–0.2 mm

This stage starts with the first cleavage division, which occurs 24–30 hours after fertilization. The 2 cell zygote increases to 8–16 blastomeres.

Carnegie stage 3: days 4–5; approx. size 0.1–0.2 mm

This is the period of development during which a blastocyst with its fluid-filled cavity forms. There are 16–32 blastomeres, which start to form an inner cell mass (embryonic pole) and outer cell mass (mural and polar trophoblast). The blastocyst eventually comes to lie free within the reproductive tract as the surrounding zona pellucida degenerates (Fig. 1.1).

Carnegie stages 4 and 5: days 6–31; approx. size 0.1–0.2 mm

The blastocyst penetrates and embeds in the uterine endometrium. During this period, the outer envelope of cytotrophoblast, forming the wall of the blastocyst, generates

syncytiotrophoblast on its external surface (Enders 1965, Tao & Hertig 1965) and extraembryonic mesoderm on its internal surface (Hertig & Rock 1949). This structure is termed the chorion (Fig. 1.2a).

The primitive amniotic cavity develops at approximately 7–9 days after ovulation (Blechs Schmidt 1968, Luckett 1973) and its floor forms the primary ectoderm (Fig. 1.2b). The primary endoderm is probably formed from cells that originate from the ectoderm that migrate around the blastocoelic cavity (Heuser & Streeter 1941) and enclose the yolk sac. The ectoderm covering the floor of the amniotic cavity and the endoderm forming the roof of the yolk sac, together in apposition, establish the bilaminar embryonic disc (Fig. 1.2c). A projection of the yolk sac endoderm into the extraembryonic mesoderm forms the allantoic diverticulum, which identifies the caudal end of the bilaminar embryonic disc and the site of the body stalk (Fig 1.2d).

Carnegie stage 6: days 13–15; approx. size 0.2 mm

The primitive streak (Fig. 1.3a) is formed and lies caudally in the midline of the embryonic disc (Heuser & Streeter 1941). The primitive streak subsequently generates intra-embryonic mesoderm, which migrates through the bilaminar embryonic disc, in the plane between ectoderm and endoderm (Fig. 1.3b), converting it into a trilaminar disc. The disc remains bilaminar at the caudal and rostral ends. The caudal end forms the cloacal membrane.

Carnegie stage 8: approx. days 17–19; approx. length 1.0–1.5 mm

The primordial germ cells, which are the antecedents of the male and female gametes, are present in the endoderm around the allantoic diverticulum (now a ventral outpouching of the hindgut) and are usually seen in the 17–20 day embryo (Jirasek 1977), although Hertig *et al.* (1958) identified possible primordial germ cells in a younger 13 day embryo. The primordial germ cells are ectodermal in origin,

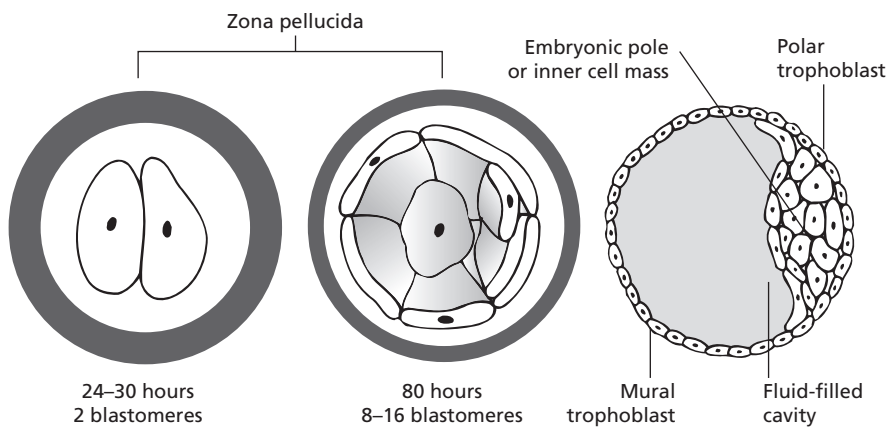


Fig. 1.1 The conceptus is enclosed within an acellular envelope, the zona pellucida. After the formation of the blastocyst and dissolution of the zona pellucida, the characteristic fluid-filled cavity, the embryonic pole or inner cell mass and the mural trophoblast can be identified.

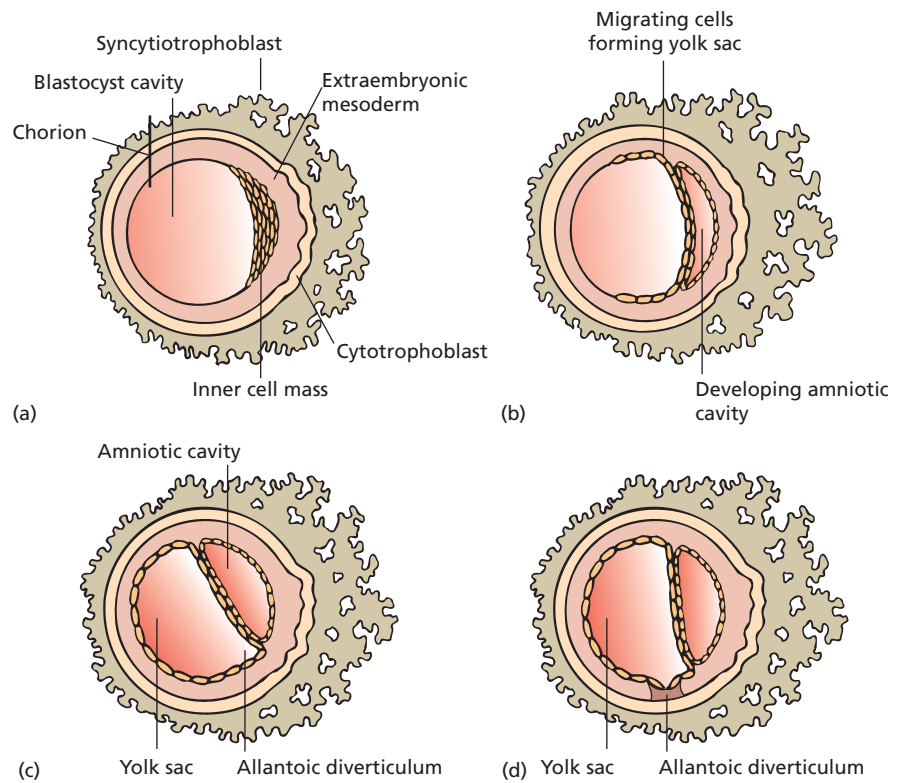


Fig. 1.2 The conceptus continues to differentiate, forming (a) the chorion, (b) the amniotic cavity and (c) the yolk sac. The area of contact between the amniotic cavity and yolk sac is the bilaminar embryonic disc. (d) Projection of yolk sac endoderm into the mesoderm to form the allantoic diverticulum.

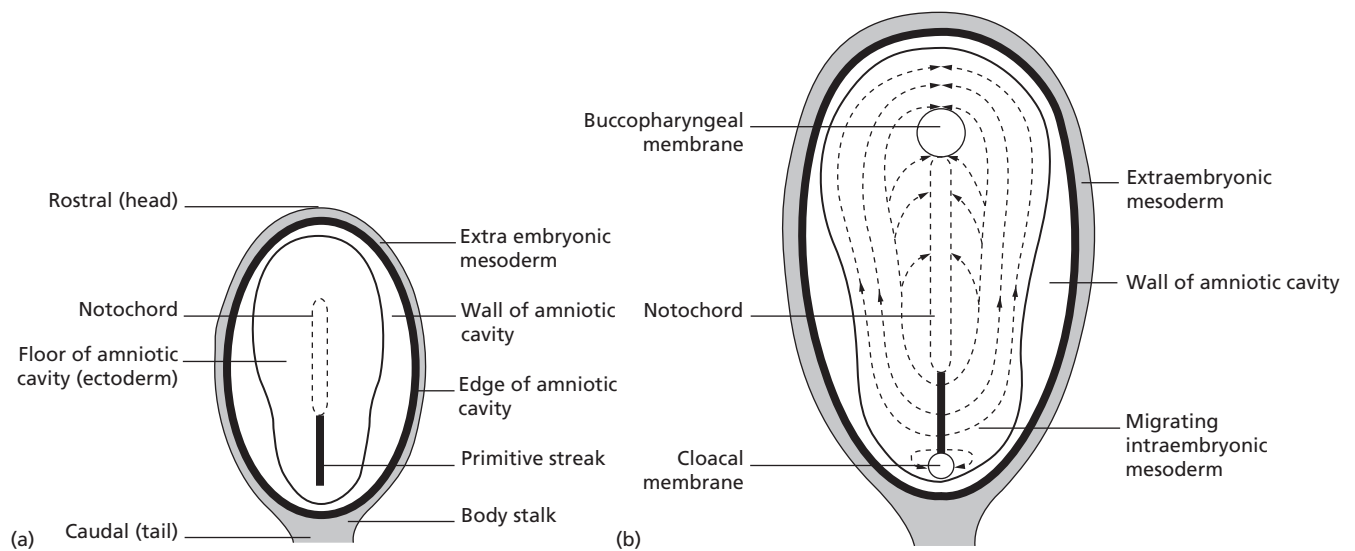


Fig. 1.3 (a) The floor of the amniotic cavity, the dorsal surface of the bilaminar embryonic disc, revealing the primitive streak and notochord. (b) Intraembryonic mesoderm, generated by the primitive streak and interposed between the floor of the amniotic cavity and roof of the yolk sac, converts the bilaminar embryonic disc into a trilaminar disc. The buccopharyngeal and cloacal membranes remain bilaminar.

having migrated to the allantoic diverticulum from the epiblast, and they retain two functional X chromosomes in contrast to the somatic cells, which possess only one functional X chromosome (Lyon 1974). From here, they migrate through the mesoderm surrounding the hindgut

and into the dorsal mesentery (Hardisty 1978). Their final destination is the medial aspect of the intermediate mesoderm adjacent to the mesonephros, the gonadal ridge, and they begin to reach this area in the human embryo at 35 days (Jirasek 1971) (Fig. 1.4). The primordial germ cells

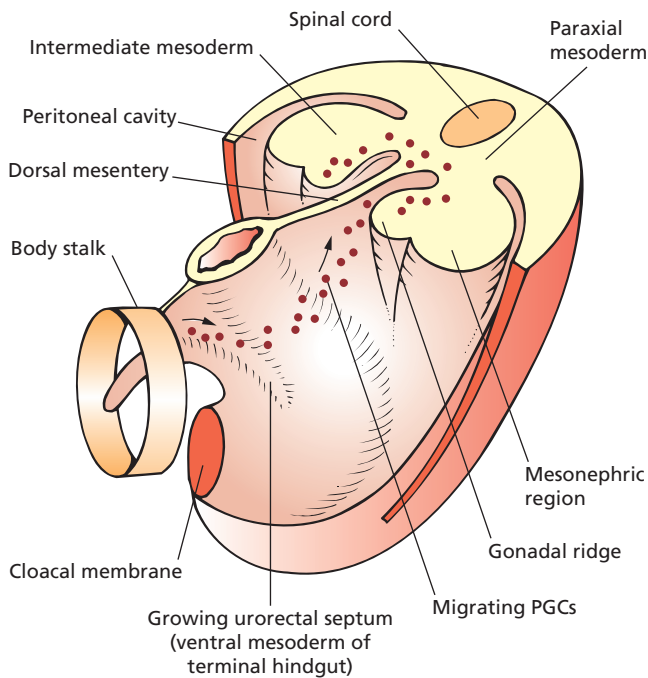


Fig. 1.4 Migration of primordial germ cells (PGCs) into the genital ridge from the body stalk.

migrate to an area known as the indifferent gonad until gonadal sex is established.

Carnegie stage 9: days 19–21; approx. length

1.5–2.0 mm

The neural plate and the longitudinally running neural ridges develop. The embryo undergoes a process of flexion to accommodate the neural tube (Fig. 1.5a–c), and in so doing reorients the primitive embryonic tissues and their relationship to each other. The endoderm of the dorsal part of the yolk sac is drawn into the ventral concavity of the embryo and is subdivided into foregut, midgut and hindgut (Fig. 1.6a,b). The hindgut appears about the 20th post-ovulatory day and is enclosed within the tail fold of the embryo. In this situation, the hindgut lies caudal to the rostral limit of the allantoic diverticulum and dorsal and rostral to the cloacal membrane. The mesoderm in the mid-embryo region is divided into paraxial, lateral and intermediate mesoderm. The paraxial mesoderm surrounds the neural tube and the intermediate mesoderm lies ventrally and lateral to the paraxial mesoderm. The intermediate mesoderm differentiates medially into the gonadal ridge and laterally into the mesonephric region (Fig. 1.7). The intermediate mesoderm at the rostral limit of the allantoic diverticulum extends dorsally then caudally, in line with the curvature of the tail fold, dividing the hindgut into ventral and dorsal parts. As this division proceeds, the two parts of the hindgut remain in continuity with each other caudal

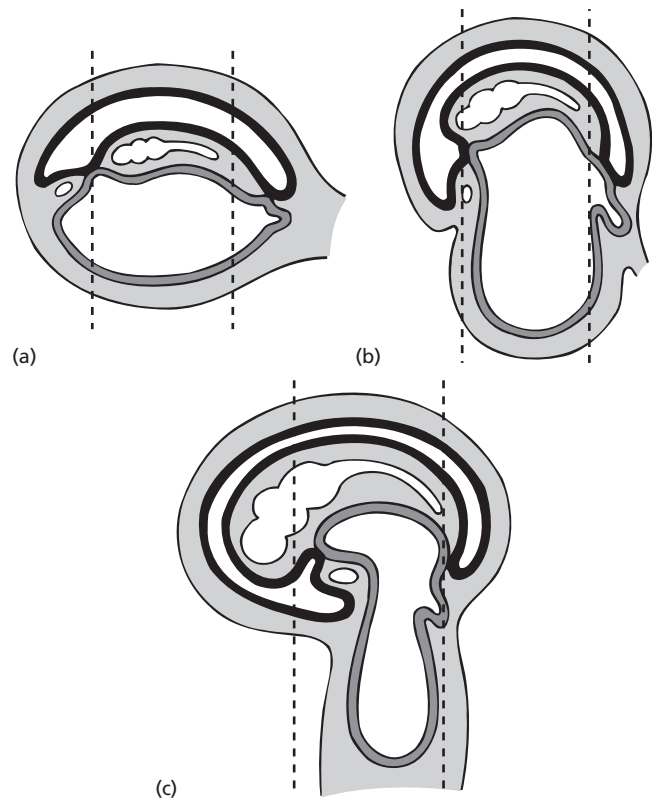


Fig. 1.5 (a) As the neural tube is enclosed within the intraembryonic mesoderm it lengthens, and expands rostrally, causing a dorsal convexity and ventral concavity. (b) Further growth of the neural tube increases this curvature in the longitudinal plane, (c) with the eventual formation of the head and tail folds.

to the advancing mesoderm of the urorectal septum. The caudal end of the hindgut is lined with endoderm and is known as the cloaca. On the ventral aspect of the cloaca there is a membrane which separates the endoderm from the surface ectoderm, the *cloacal membrane*. As development continues a mesenchymal septum, the *urogenital septum*, migrates caudally (Fig. 1.8a–c).

Carnegie stage 11: days 23–25; approx. length

2.5–3.0 mm

At day 24, when the embryo flexion has been completed, the anterior limit of the extensive cloacal membrane abuts on the base of the umbilical cord. On either side of the cloaca are the paired primordia of the genital tubercle (Fig. 1.9a).

Over the next few days the cloaca retracts from the umbilical cord to form an anterior wall and the two primordia of the genital tubercle fuse. Posterior to the tubercle and running laterally by the sides of the cloacal membrane are the cloacal folds, and lateral to these are the genital swellings (Fig. 1.9b).

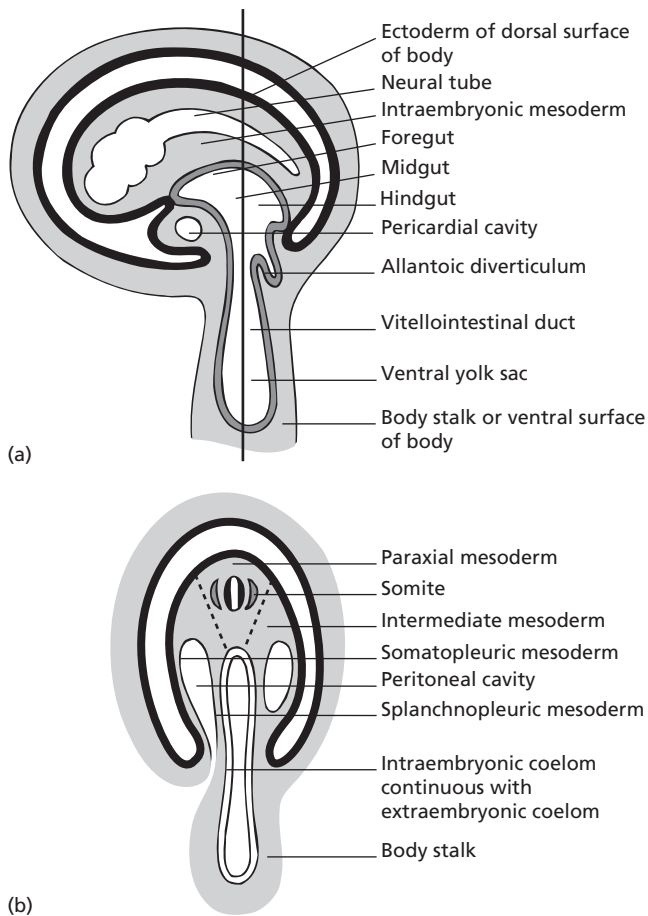


Fig. 1.6 (a) Midline section of the embryo after formation of the head and tail folds. (b) A transverse section of the mid-embryo region after formation of the lateral folds.

Carnegie stages 13 and 14: days 28–35; approx. length 4–7 mm

The urogenital septum reaches the cloacal membrane at 30–32 days (O’Rahilly 1977) and fuses with the cloacal membrane, dividing the embryonic hindgut into the ventral (anterior) urogenital sinus and dorsal (posterior) rectum. Failure of the urogenital septum to reach the cloacal membrane leaves the urethra, vagina and rectum converging into a single channel with a solitary opening on to the perineum.

The cloacal membrane is also divided, the ventral part forming the urogenital membrane and the dorsal part forming the anal membrane. The cloacal membrane will ultimately rupture to form the urogenital and anal orifices. The genital folds develop from the anterior part of the cloacal folds and the anal folds form the posterior component (Fig. 1.9c).

At the same time as the cloaca divides, the two primitive gonadal streaks are proliferating. At the ventral tip of the cloacal membrane, each forms a genital tubercle. The two genital tubercles proliferate further and need to reach a

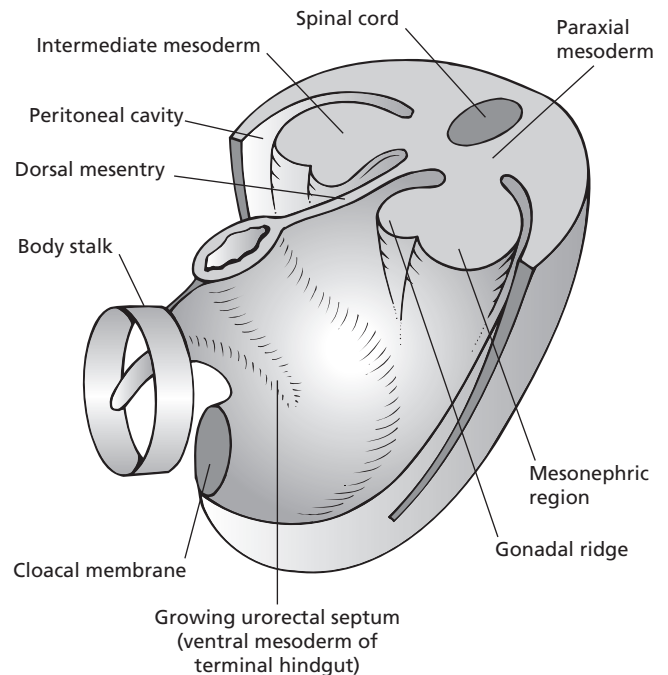


Fig. 1.7 The caudal half of the embryo showing the gonadal ridge and mesonephric region of the intermediate mesoderm, which, at its caudal limit, is continuous with the mesoderm investing the hindgut.

critical mass otherwise only rudimentary structures will be formed. The two tubercles then fuse to form the glans of the clitoris. Labioscrotal swellings and urogenital folds develop on each side of the cloacal membrane. As the urogenital septum reaches the cloacal membrane the labioscrotal folds fuse posteriorly, forming the perineal body which separates the urogenital membrane from the anal membrane. The sexual differentiation at this stage is still indeterminate and remains so until 63–77 days.

Carnegie stages 15 and 16: days 35–42; approx. length 7–11 mm

At 30–32 days cells from cephalic mesonephric vesicles invade the coelomic epithelium on the medial aspect of the adjacent intermediate mesoderm to induce the formation of the indifferent gonad (Wartenberg 1982). At 35 days the indifferent gonad begins to develop on the medial aspect of the mesonephros by the invasion of three other cell types: the primordial germ cells, cells from the overlying coelomic epithelium and the cells from the adjacent mesonephros. All cell types are probably essential to the proper differentiation of the gonad (Byskov 1981).

The paramesonephric ducts (Mullerian ducts) appear at about 40 days. The precursor of each paramesonephric duct extends caudally as a solid rod of cells in the intermediate mesoderm, in close association with, and initially lateral to, the mesonephric (Wolffian) duct. The mesonephric

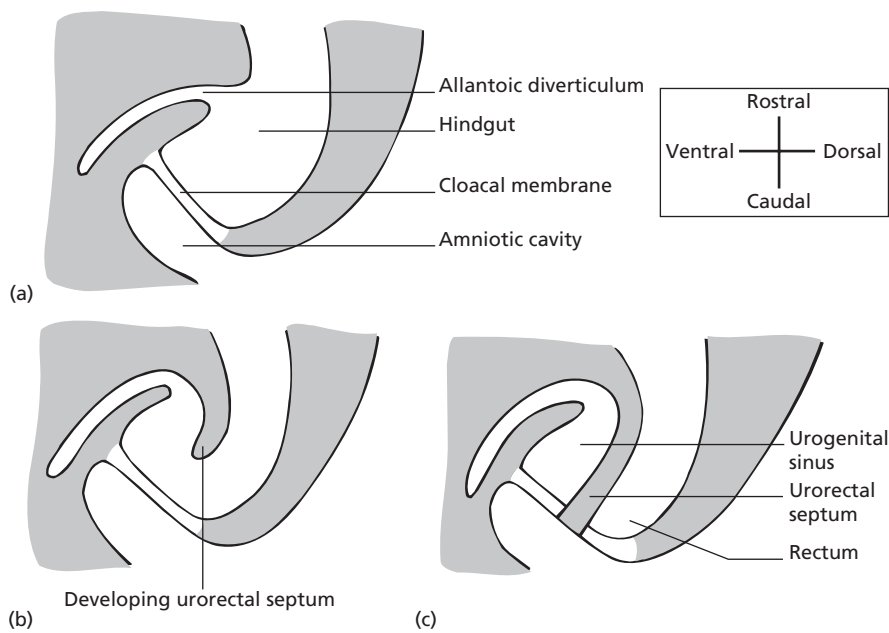


Fig. 1.8 (a) The primitive hindgut is enclosed within the embryonic tail fold. (b) The developing urorectal septum grows dorsally and caudally from the rostral limit of the allantoic diverticulum. (c) The fusion of the urorectal septum with the cloacal membrane divides the hindgut into urogenital sinus and rectum.

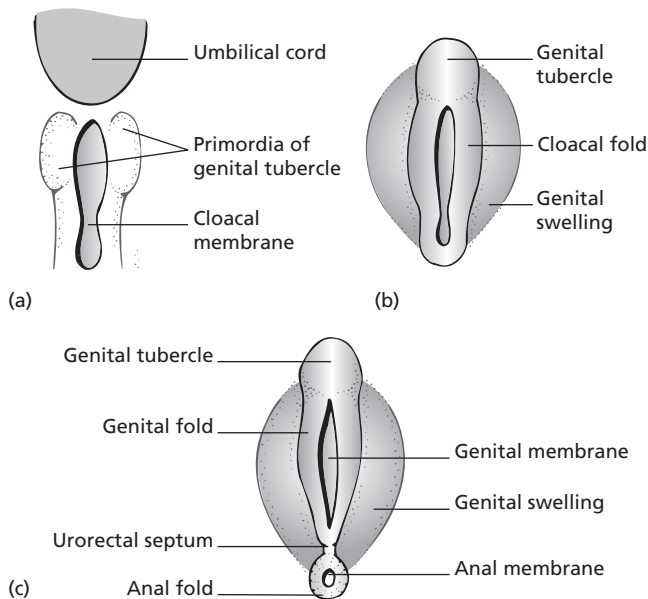


Fig. 1.9 (a) The paired primordia of the genital tubercle lie immediately caudal to the umbilical cord. (b) Migration of tissue towards the midline from both sides separates the umbilical cord and cloacal membrane, causes fusion of the primordia to form a midline genital tubercle and establishes bilateral cloacal folds and genital swellings. (c) Fusion of the urorectal septum with the cloacal membrane separates an anterior genital region from a posterior anal region.

duct has been shown experimentally both to induce the paramesonephric duct (Didier 1973a,b) and to guide its descent (Gruenwald 1941). The growing caudal tip of the paramesonephric duct lies within the basement membrane of the mesonephric duct (Frutiger 1969). As they descend, the paramesonephric ducts pass ventral to the mesonephric ducts and, coming into close association with one another, reach the posterior aspect of the urogenital sinus within the urorectal septum (Fig. 1.10). The two paramesonephric ducts begin to fuse even before their growing ends reach the urogenital sinus (Koff 1933). As the urorectal septum reaches the cloacal membrane at 30–32 days the caudal end of the mesonephric duct, having already opened into the urogenital sinus, gives origin to the ureteric bud and begins to be incorporated into the posterior wall of the urogenital sinus (Keith 1948). The portion of each duct incorporated into the urogenital sinus subsequently forms the trigone of the bladder and the posterior wall of the urethra (Fig. 1.11a–d). At 42 days post ovulation there are 300–1300 primordial germ cells within the indifferent gonads destined to become either spermatogonia or oogonia. The close association between the gonad and adrenal at this early stage of development can result in adrenal cells being sequestered in the gonad and maintaining their function in the mature ovary or testis (Grumbach & Conte 1992).

Carnegie stages 17 and 18: days 43–48; approx. length 13–17 mm

The transformation of the indifferent gonad into an embryonic testis occurs in the 43–49 day embryo.

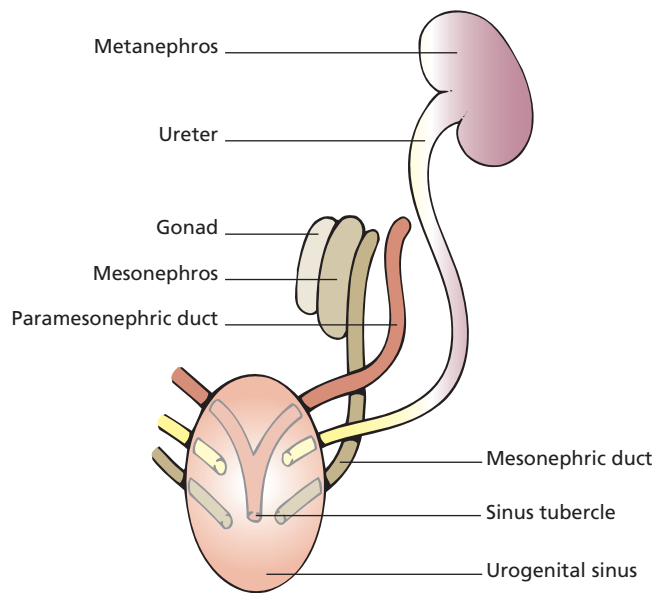


Fig. 1.10 The indifferent human embryo possesses mesonephric and paramesonephric ducts. The terminal paramesonephric ducts fuse within the urorectal septum and reach the urogenital sinus at the sinus tubercle situated between the openings of the two mesonephric ducts.

Carnegie stage 19: days 48–51; approx. length 16–18 mm

The mesonephric ducts terminate in the urogenital sinus on either side of the sinus tubercle at 49 days. At the rostral end of the sinus tubercle, the urogenital sinus is referred to as the vesicourethral canal and from it arise the bladder and the whole of the female urethra (Jirasek 1977). The portion

of the urogenital sinus caudal to the sinus tubercle continues to be referred to as the urogenital sinus and is subdivided into pelvic and phallic portions.

Carnegie stage 20: days 51–53; approx. length 18–22 mm

The development of the indifferent gonad into an embryonic ovary occurs gradually in the 45–55 day embryo (Jirasek 1977).

Carnegie stage 23: days 56–60; approx. length 27–31 mm

At this stage the external genital primordium has developed, which is still indeterminate.

End of the female embryonic period

At the end of the embryonic period, the fetus has gonads that are recognizable as ovaries, but still has indifferent external genitalia and both mesonephric and paramesonephric duct systems are still present. Subsequent sexual differentiation of these ducts in the female develops because of a lack of anti-Mullerian hormone. The mesonephric ducts (Wolffian ducts) degenerate but occasionally remnants may be left behind. A remnant of the cephalic mesonephric duct and adjacent vesicles is a constant finding associated with the ovary (Duthie 1925). A more caudal portion of the mesonephros may be encountered in the broad ligament as the paroophoron, while remnants of the terminal mesonephric duct may persist lateral to the uterus and vagina or are incorporated into the cervix (O’Rahilly 1977, Buntine 1979). Remnants of this duct found adjacent to the lower genital tract are referred to as Gartner’s ducts.

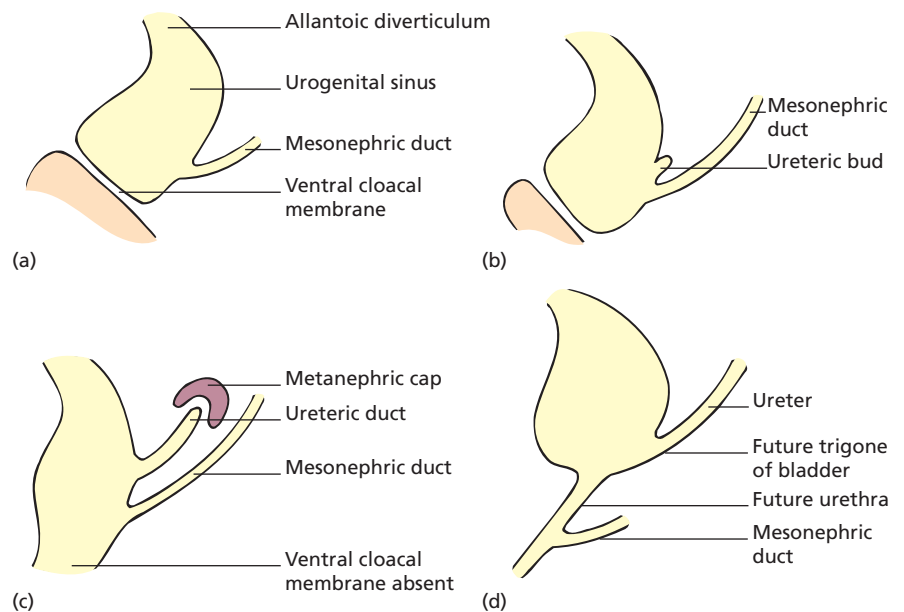


Fig. 1.11 (a) The mesonephric duct, within the urorectal septum, opens into the urogenital sinus. (b) The caudal limit of the mesonephric duct gives origin to the ureteric bud. (c) The metanephric cap forms at the growing end of the ureteric bud or duct. (d) The mesonephric duct gives origin to the ureter and forms the trigone of the bladder and the posterior wall of the urethra.

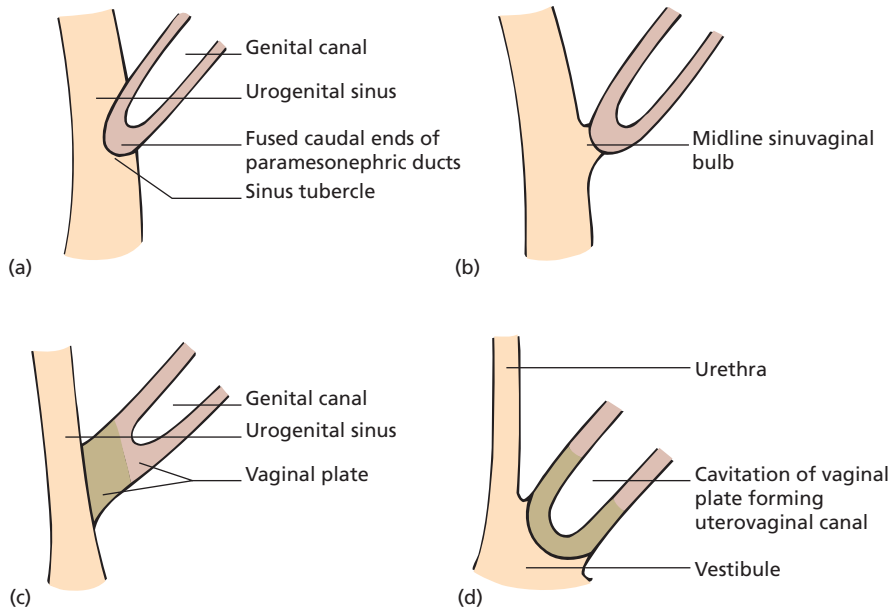


Fig. 1.12 (a) The fused paramesonephric ducts form the genital canal, the solid caudal end of which abuts on the posterior wall of the urogenital sinus at the sinus tubercle. (b) Cellular proliferation of the sinus epithelium generates the sinuvaginal bulbs, which displace the genital canal dorsally. (c) Further cellular proliferation converts the sinuvaginal bulbs into solid tissue projections, which participate in the formation of the vaginal plate. (d) Extensive caudal growth of the vaginal plate brings its lower surface into the primitive vestibule.

The fallopian tubes develop from upper unfused portions of the paramesonephric ducts and the uterus and vagina from the lower fused portion (Fig. 1.12a). The arrival of the caudal end of the genital canal on the urogenital sinus stimulates cellular proliferation of the sinus epithelium to form three projections (sinuvaginal bulbs) that displace the genital canal dorsally (Fig. 1.12b). Failure of these bulbs to develop results in vaginal agenesis. These sinuvaginal bulbs become solid and together with the solid end of the genital canal form the vaginal plate, which is complete at 19 weeks (Fig. 1.12c). The sinuvaginal bulbs later fuse, but eventually undergo apoptosis to form a lumen. Some time between 14 weeks (Terruhn 1980) and 20 weeks (O’Rahilly 1977) the vagina opens into the pelvic portion of the urogenital sinus converting it into the vaginal vestibule (Fig. 1.12d).

Further feminization of the external genitalia begins be-

tween 63 and 77 days, when the genital tubercle lengthens to form the phallus. This then bends caudally to form the glans. During this period the anogenital distance remains unchanged, there is no fusion of the genital folds and the urogenital sinus remains open. The urethral and vaginal openings separate later. The phallus becomes the clitoris, being incorporated within the fused anterior ends of the genital folds, which develop into the labia minora. The genital swellings, lateral to the labia minora, become the labia majora and are continuous with the future mons pubis. The labia minora develop from the genital folds and divide anteriorly into the prepuce and frenulum of the clitoris (Fig. 1.13).

Development of the epithelia

The epidermis of the vulval skin and its appendages – hair

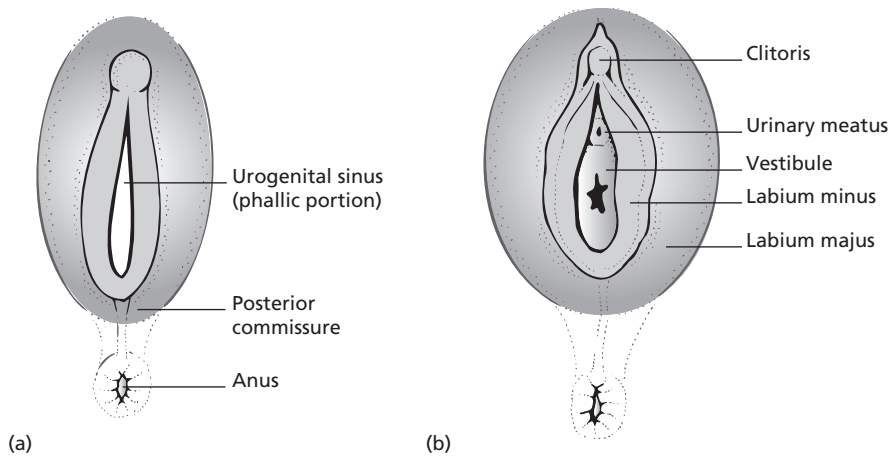


Fig. 1.13 (a) The genital and anal membranes rupture. (b) In the female fetus the urogenital sinus remains open as caudal growth of the vaginal plate brings the urethral and vaginal openings into this region converting it into the vestibule.

and sebaceous and sudoriferous glands – is developed from ectoderm. The dermis is developed from mesoderm.

The primitive epidermis is established about the eighth day when ectoderm differentiates within the developing embryo. At this stage the epidermis is a single layer of cells, but over the ensuing 3 weeks specific features develop that set it apart from other epithelia in the body. A second outer layer develops – the periderm – beneath which the primitive epidermis begins the process of stratification. Keratinization occurs at the end of the sixth month, and the periderm is sloughed into the amniotic fluid (Lind *et al.* 1969). Cells that are shed from the stratum corneum combine with sebaceous secretions to form the vernix, which makes the embryo impervious to the amniotic fluid and persists until birth (O’Rahilly & Muller 1992).

Three cell types invade the developing epidermis during the first 6 months of intrauterine life. Melanocytes, derived from neural crests (Niebauer 1968), and Langerhans cells, derived from mesoderm (Breathnach & Wyllie 1965), are present at the end of the third month, while Merkel cells, the origin of which is uncertain, are present by the sixth month (Breathnach 1971).

The dermal–epidermal junction is flat in all parts of the body until hair and glandular primordia reach the dermis. Primary vellus hair follicles begin to form during the third month of gestation and the process proceeds in a cephalocaudal manner. Secondary follicles form in close association with the primary follicles and it is thought that the full complement of hair follicles is present at birth.

Sebaceous glands arise as buds mostly from the hair follicles (O’Rahilly & Muller 1992). They begin to appear during the fourth month and differentiate into sebum-producing cells rapidly. The development and function of sebaceous glands before birth and in the neonatal period is thought to be regulated by maternal androgens and endogenous fetal steroids. At birth the glands are large and well developed over the entire body and display the same regional variation in size as is seen in the adult. Postnatally they involute and remain quiescent until puberty.

Eccrine sweat glands appear during the third month of prenatal life and their ducts are open to the skin surface by the sixth month. The premature infant usually shows an absent or limited sweating response (Sinclair 1972), even though the glands are innervated as soon as they develop. The number of sweat glands, like hair follicles, seems to be complete at birth.

Apocrine development occurs at 6 months of intrauterine life and it has been suggested that their primordia develop in association with each hair follicle but regress in all areas except the areola, axilla, scalp, eyelids, external auditory meatus, umbilicus and anogenital region (Serri *et al.* 1962, Hashimoto 1970). The glandular activity begins during the

last trimester but ceases soon after birth, and begins again at puberty.

The dermis originates from the mesoderm in the second month of embryonic life. The mesodermal cells form fibroblasts, macrophages, melanoblasts and mast cells and the matrix is composed of collagen and elastin. The organization of the dermis is progressive throughout gestation and is not complete until some months after birth.

Ambiguous external genitalia

If a female fetus is exposed to significant androgen levels before 84–98 days, complete external virilization will occur at the end of this period of development. Lower levels of testosterone or exposure later will produce variations of incomplete virilization (Grumbach and Ducharme 1960). The sex of the newborn is ascertained from the appearance of the external genitalia but occasionally this may not be possible as the phenotypical appearance may not be characteristic of either male or female.

Classification of these abnormalities has been based on anatomical and aetiological mechanisms. The classification used in this chapter is one based on aetiological mechanisms and clinical syndromes. It is adapted from Grumbach and Conte (1992) and Simpson (1982):

- 1 disorders of gonadal differentiation
- 2 female pseudohermaphrodite
- 3 male pseudohermaphrodite.

Almost all of the affected infants have some degree of phallic enlargement with a small opening for voiding urine on the ventral surface, at its base or on the perineum. A second opening or depression may be identified more posteriorly, while on either side of the midline there is some virilization, ranging from rugose labia majora-like structures to scrotal sacs (Dewhurst 1980).

It is now recognised that there is a need for a reappraisal of these classifications as advances are made in molecular genetics. There are also ethical issues to be considered as well as patient opinion. (Dreger *et al.* 2005, Hughes 2006). Terms such as intersex and hermaphrodite are unacceptable to the patients and can be confusing for the clinician. The new nomenclature proposed would use Disorders of Sex Development in place of intersex and the term hermaphrodite would be dropped completely.

Disorders of gonadal differentiation

Ovarian dysgenesis

Approximately 50% of all patients with ovarian dysgenesis have a chromosomal complement of 45X; a further 25% have sex chromosomal mosaicism without a structural chromosomal abnormality (45X/46XX; 45X/46XY) while

the remainder have either a structurally abnormal X or Y chromosome or no detectable chromosomal abnormality (Simpson 1982).

45X Turner's syndrome

Only a small number of 45X embryos survive intrauterine life (Boue *et al.* 1975, Cockwell *et al.* 1991). Individuals with this karyotype have germ cells which rarely survive meiosis, follicular formation usually fails and the resulting streak gonads are sterile and devoid of endocrine activity (Carr *et al.* 1968). At birth, the genital ducts and external genitalia are entirely female, although clitoral enlargement may occasionally be present (Grumbach & Conte 1992). The ovaries, located in their normal anatomical positions, consist mainly of fibrous stroma and are termed streak gonads. However, almost 25% of girls with Turner's syndrome show some secondary sexual development, 2–5% have spontaneous menses owing to some residual ovarian function (Saenger 1996), and, rarely, they bear children (King *et al.* 1978, Kohn *et al.* 1980). Patients with Turner's syndrome are of short stature and exhibit a range of somatic abnormalities including webbing of the neck, coarctation of the aorta and renal anomalies. Also associated with the condition is a predisposition to develop diabetes mellitus (Engel & Forbes 1965) and other autoimmune diseases, particularly those affecting the thyroid (Elsheikh *et al.* 2001).

45X/46XX mosaicism and X chromosome abnormality

This form of mosaicism is the most common cause of ovarian dysgenesis after Turner's syndrome. One gonad may be of the streak type and the contralateral gonad a normal or hypoplastic ovary; alternatively, both ovaries may be either normal or hypoplastic (Grumbach & Conte 1992). There are fewer of the somatic abnormalities associated with Turner's syndrome, the phenotype is invariably female and some may menstruate and even be fertile.

45X/46XY mosaicism and Y chromosome abnormality

A highly diverse phenotype is encountered in 45X/46XY mosaicism since the presence of a Y-bearing cell line may induce some testicular differentiation. Such individuals may appear typically male or female or may possess ambiguous external genitalia with varied genital duct development. In a series of 60 patients with 45X/46XY mosaicism, two-thirds were reared as females (Zah *et al.* 1975). Several cases of structural abnormality of the Y chromosome have been reported (Davis 1981); the affected individuals are phenotypically female with bilateral streak gonads.

46XX, 46XY

Gonadal dysgenesis may occur in association with apparently normal 46XX or 46XY karyotypes. These individuals

are phenotypically female with streak gonads and remain sexually immature. The 46XX form of gonadal dysgenesis appears to be inherited as an autosomal recessive condition and may occur in association with neurosensory deafness, which may also affect otherwise normal male siblings (Pallister & Opitz 1979). The 46XY form of gonadal dysgenesis is again a genetically heterogeneous syndrome, being associated with deletions and/or mutations involving the Y chromosome (Blagowidow *et al.* 1989) and/or the X chromosome (Scherer *et al.* 1989). Clitoral enlargement is not uncommon, but the most important aspect of this condition is the increased incidence of gonadal neoplasms (Simpson 1982). Bilateral gonadectomy is therefore indicated as a prophylactic measure (Dewhurst 1980, Grumbach & Conte 1992). In all forms of ovarian dysgenesis oestrogen replacement therapy is recommended at 12–13 years of age, eventually to be cycled monthly with progesterone (Grumbach & Conte 1992).

Pure gonadal dysgenesis

These patients have a 46XY karyotype but do not possess any gonadal tissue. The condition, variously termed the 'XY gonadal agenesis syndrome' (Sarto & Opitz 1973) or the 'testicular regression syndrome' (Coulam 1979), is characterized by the presence of ambiguous genitalia in association with hypoplastic Mullerian and Wolffian derivatives. A small phallus, ill-developed labia majora and fusion of the labioscrotal folds are features of the external genital appearance (Sarto & Opitz 1973).

True hermaphroditism

True hermaphrodites possess both ovarian and testicular tissue, with an ovary on one side and a testis on the other or, more commonly, with ovotestes situated bilaterally or unilaterally (Grumbach & Conte 1992). The differentiation of the genital tract, the appearance of the external genitalia and the development of secondary sexual characteristics are all variable. The external genitalia are often ambiguous and three-quarters of reported cases have been reared as males because of the size of the phallus (van Niekerk 1976). The majority of the gonads present have oocytes but not spermatozoa, a uterus is almost invariably present and 60% of them have a 46XX karyotype. Four 46XX true hermaphrodites have become pregnant (Tegenkamp *et al.* 1979). In one review (van Niekerk 1976), the remaining 40% of true hermaphrodites were equally distributed between 46XY karyotypes, 46XX/46XY chimeras and sex-chromosome mosaics.

Female pseudohermaphroditism

The chromosomal makeup is 46XX with the ovaries and Mullerian duct derivatives as normal. The external genitalia are abnormal. The clitoris is enlarged, variable degrees of

labial fusion are seen and the urethral opening may not be distinct from the vagina. The external genitalia of the male fetus are completely masculinized by 84–98 days (Jirasek 1977). If a female fetus is exposed to significant androgen levels, in the presence of 5 α -reductase, before the end of this period of development, complete virilization will occur (Grumbach & Ducharme 1960). Lower levels or later exposure will produce various forms of incomplete virilization. The source of the virilizing influence may be fetal, maternal or exogenous.

Fetal androgens

Since the adrenal glands begin to function during the third month of intrauterine life, excessive production of adrenal androgens will virilize the fetal female external genitalia. CAH accounts for most of the cases of female pseudohermaphroditism and approximately half of all patients with ambiguous external genitalia. There are several types of CAH and all are transmitted as an autosomal recessive trait (Laue & Rennert 1995). The first, caused by 21-hydroxylase deficiency or 11 β -hydroxylase deficiency, limits cortisol production and leads to adrenal overproduction of androgens and their precursors. The second, caused by 3 β -oldehydrogenase deficiency or 17 α -hydroxylase deficiency, reduces cortisol production and also impairs the synthesis of sex steroids by the gonads and adrenals. With 3 β -oldehydrogenase deficiency the only androgen synthesized is dehydroepiandrosterone (DHEA), which is relatively weak, and females with this deficiency are less virilized than females with 21- or 11 β -hydroxylase deficiencies. In 17 α -hydroxylase deficiency the female external genitalia are normal at birth but no secondary sexual development occurs at puberty (Simpson 1982).

Maternal androgens

In rare instances, virilization of the female fetus may occur if the mother has certain ovarian or adrenal tumours or if she has unrecognized CAH. The absence of virilization in the mother does not exclude a maternal source of androgens since the level of androgen required to virilize the external genitalia of the early female fetus is much less than would be required to have a virilizing effect on the adult female (Kai *et al.* 1979).

Exogenous androgens

Virilization of the external genitalia of female infants has been frequently observed following maternal ingestion of testosterone or synthetic progestational agents during the first trimester of pregnancy (Grumbach & Ducharme 1960, Wilkins 1960, Dewhurst & Gordon 1984, Reschini *et al.* 1985). Administration of such agents between the eighth and 12th week of gestation causes marked virilization, while later in pregnancy their use causes clitoral enlargement.

These agents, as well as stilboestrol, were often prescribed in the past for women with habitual or threatened abortion. Exposure to stilboestrol during intrauterine life is known to cause malformations in the reproductive tracts of both sexes and an increased incidence of cervical and vaginal neoplasia (Herbst *et al.* 1972, 1974), and it has also been shown to cause female pseudohermaphroditism (Bongiovani *et al.* 1959). Intrauterine exposure to danazol, used in the treatment of endometriosis, has also been associated with the occurrence of female pseudohermaphroditism (Rosa 1984, Shaw & Farquhar 1984).

Maternal cocaine use during pregnancy has been associated with ambiguous genitalia in both male and female infants, as well as other congenital malformations (Chasnoff *et al.* 1988).

Structural defects

Lower reproductive tract

Vaginal agenesis

This is the absence of a vagina and occurs in female pseudohermaphroditism and in some cases of 46XX females. Total vaginal agenesis is usually found in association with tubal and uterine agenesis, but occasionally the upper reproductive tract is normal because there has been only a partial Mullerian defect or the vaginal plate has failed to form or cavitate. In a survey of 167 women with total vaginal agenesis, one-third had associated renal tract defects while others had skeletal abnormalities (Evans *et al.* 1981). The Rokitansky–Kuster–Hauser syndrome (Simpson 1982) describes cases of vaginal agenesis in which there is a very shallow vagina associated with a rudimentary upper reproductive tract.

Vaginal atresia

In this condition the urogenital sinus fails to form the inferior portion of the vagina. The lower vagina is replaced by fibrous tissue above which there is a normal reproductive tract (Simpson 1976).

Vaginal septa

Transverse vaginal septa are said to be located at the junction of the upper one-third and lower two-thirds of the vagina (Simpson 1976). The probable cause of transverse vaginal septa formation is the failure of either the Mullerian or urogenital sinus contributions to the vagina to cavitate completely so the septa can occur at any level. In the Amish community this abnormality is inherited as an autosomal recessive trait (McKusick *et al.* 1964). Patients with transverse vaginal septa may present at puberty with retained menstrual products or alternatively with a continuous vaginal discharge.

A longitudinal vaginal septum may present in the midline as the result of a fusion defect in the Mullerian system and will be associated with abnormalities of the upper reproductive tract.

Imperforate hymen

This is commonly caused by the failure of the central epithelial cells of the hymenal membrane to degenerate. However, this condition may arise as the result of an inflammatory reaction in the hymen after birth. Presentation is usually at puberty with a haematocolpos.

Vaginal cysts

In the neonatal period vaginal cysts may be found in the anterior or lateral walls of the vagina at the introitus and usually rupture spontaneously (Warkany 1971). Occasionally, one or more of these may enlarge and obstruct the urethra. They are thought to be inclusions from the urogenital sinus epithelium and may persist asymptotically into adulthood (Robboy *et al.* 1978). Mucous cysts are found in the same location, interior to the labia minora and external to the hymen, in about 3% of adults attending a vulval clinic (Friedrich & Wilkinson 1973). In addition, the Wolffian ducts, which degenerate in the female, leave caudal remnants in the lateral walls of the vagina. These remnants may undergo cystic degeneration, when they are termed Gartner's cysts.

External genitalia

As detailed above, various abnormalities of the vulva are caused by disturbances of sexual differentiation, which lead to an ambiguous appearance of the external genitalia. Other vulval defects, such as duplication, occur in association with abnormalities of the upper reproductive tract and urinary system. Congenital anomalies of the vulva that occur in isolation involve the clitoris and the labia.

Clitoris

The clitoris may be absent (Falk & Hyman 1971), probably as a result of the genital tubercles remaining hypoplastic or failing to fuse. Enlargement occurs in the rare genetically determined condition of lipoatrophic diabetes (Lawrence-Seip syndrome) (Burton & Cunliffe 1992).

Labia minora

Hypertrophy and/or marked asymmetry of the labia minora may occur without any underlying problem but in rare instances it may be a manifestation of neurofibromatosis (Friedrich & Wilkinson 1985). True hypoplasia of the labia minora occurs infrequently, and may be a sign of defective steroidogenesis. Fusion of the labia minora may occur in association with defective sexual differentiation. This should

not be confused with the superficial labial adhesions seen in the neonatal period or in infancy as a result of an inflammatory condition (see Chapter 5).

Vulval and urinary system abnormalities

Kidney

Bilateral renal agenesis is a lethal congenital malformation (Potter 1946), and in the female is frequently associated with anomalies of the external genitalia, absence of the uterus and vagina and abnormalities of other systems (Potter 1965). Unilateral renal agenesis may be associated with malformation of the external genitalia. The incidence of genital anomalies in unilateral renal agenesis is about 40% in females and 12% in males (Warkany 1971).

Ureter

The ureteric bud arises from the Wolffian (mesonephric) duct and separates off when the duct is incorporated into the urogenital sinus to form the trigone of the bladder and urethra. Failure of dissociation between the ureteric bud and Wolffian duct in the female will allow the ureteric orifice to be located at any site along the caudal remnant of the Wolffian duct (Gartner's duct). Secondary rupture of Gartner's duct into the vagina (Weiss *et al.* 1984) allows for vaginal drainage of urine from the ectopic ureter.

Bladder

Exstrophy of the bladder is caused by a failure of the subumbilical portion of the anterior abdominal wall to meet in the midline above the genital tubercles. The genital tubercles remain as paired primordia and the anterior wall of the bladder is either partially or totally absent. This condition may therefore exist as incomplete or complete bladder exstrophy and is always associated with epispadias and other abnormalities of the external genitalia. A more severe form of this structural defect is cloacal exstrophy, in which the urorectal septum fails to divide the hindgut, and the abdominal wall deficit gives access not only to the bladder but also to the terminal gastrointestinal tract (Diamond & Jeffs 1985). Repairing the abdominal defect is possible but the refashioning of the external genitalia must take into account the chromosomal and gonadal sex of the infant (Dewhurst 1980).

Urethra

Congenital abnormalities of the urethra have a much lower incidence in females than in males. Duplication of the urethra is a cause of urinary incontinence in the female. The accessory urethra usually arises from the trigone of the bladder and opens onto the anterior wall of the vagina. Mild forms of epispadias may occur in the female giving rise

to disturbance of bladder control and urinary incontinence. In this condition the urethral opening lies deep to the mons between two clitoral elements. Hypospadias in the female occurs in association with female pseudohermaphroditism. In both epispadias and hypospadias, the female urethra is congenitally short (Burbige & Hensle 1985). Meatal stenosis is uncommon in the female but may simulate bladder neck obstruction (Warkany 1971). Prolapse of urethral mucosa occurs only in the female (Capraro *et al.* 1970). Urethral cysts may develop in the Skene's glands which open at the termination of the urethra and may be the cause of recurrent urinary symptoms. Finally, an ectopic ureter may open into the urethra. Any of these urethral abnormalities may present with urinary incontinence, which may cause an irritant contact vulval dermatitis.

Vulval and intestinal abnormalities

In the female an imperforate anus or anal stenosis may be associated with a variety of abnormalities of the genital tract and vulva (Hall *et al.* 1985). An ectopic opening of the lower gastrointestinal tract may be found in the vagina or elsewhere in the perineum. When a rectovaginal fistula is formed there are often urinary tract abnormalities present as well.

Vulval mammary tissue

Originally it was believed that mammary tissue found in the vulva was a result of incomplete atresia of the mammary ridges. It is now thought that the mammary tissue is in fact mammary-like anogenital glands (van der Putte 1994, van der Putte & van Group 1995). However there are rare reports of ectopic mammary glands with associated nipples occurring in the vulva (Green 1936).

Anatomy

The vulva is situated within the perineum, which is the outer area inferior to the sheet of muscle forming the pelvic floor. The perineum is an embryological junctional zone derived from the body wall ectoderm, hindgut endoderm and the intervening mesoderm that surrounded the original cloacal membrane. The perineum furthermore is divided into an anterior urogenital triangle and posterior anal triangle. The vulva lies mainly within the anterior urogenital triangle but extends anteriorly to the pubic symphysis. The anal canal and ischiorectal fossa occupy the posterior anal triangle. The perineal body is a fibromuscular mass which in the female lies between the upper half of the anterior anal wall and the entire posterior portion of the vagina. It is thicker in the female than in the male.

The vulva consists of the mons pubis, the labia majora and minora, the vestibule of the vagina, the hymen, the clitoris and the external urethral orifice (Fig. 1.14).

Mons pubis

The mons pubis (mons) in the adult female is a prominent pad of hair-bearing skin and subcutaneous fat overlying the pubic symphysis. The character of pubic hair varies with ethnic background and age, but its distribution rarely extends more than 2 cm beyond the upper limit of the genitofemoral folds (Lunde 1984). This pattern of distribution produces the horizontal upper margin of female pubic hair.

<i>Arterial supply</i>	Superficial external pudendal artery which is a branch of the femoral artery.
<i>Venous drainage</i>	Via the pudendal veins to the long saphenous.

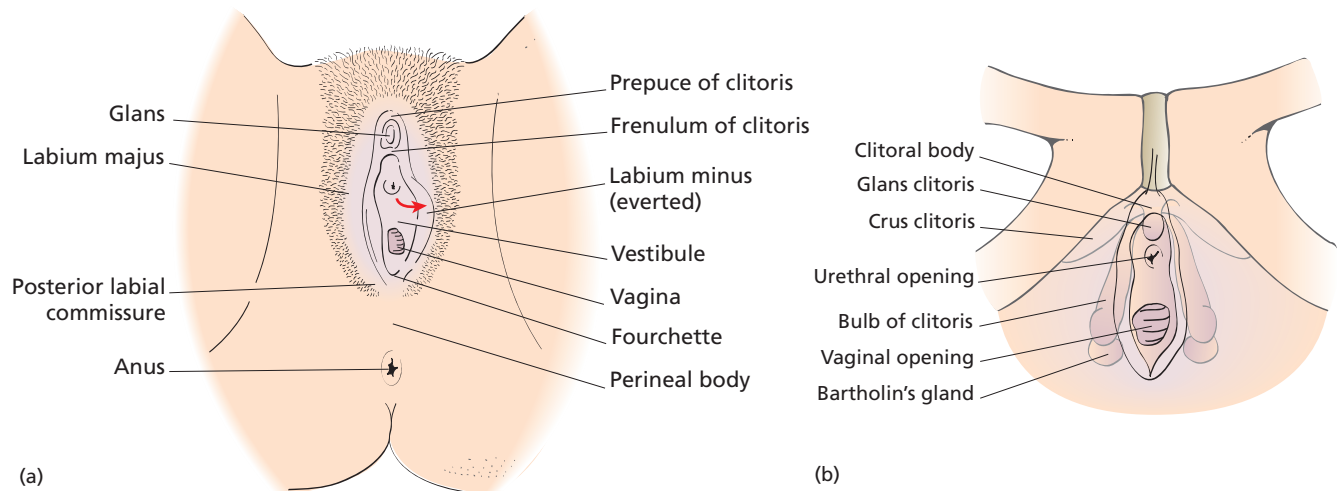


Fig. 1.14 (a) The vulva. (b) The clitoris.

Nerve supply Branches of the perineal nerve.
Lymph drainage To superficial inguinal nodes and then to deep femoral nodes and pelvic nodes.

Labia majora

The labia majora are two cutaneous folds that form the lateral boundaries of the pudendal cleft. They originate from the mons pubis anteriorly and merge with the perineal body posteriorly (the posterior labial commissure). After puberty there is an increase in pigmentation and hair as well as the deposition of subcutaneous fat. The deposition of fat mainly occurs in the medial aspects and the labia tend to flatten out as they reach the perineal body. The increased pigmentation and hair extends to the perianal area. The lateral surfaces of the labia majora are adjacent to the medial surfaces of the thighs and are separated from them by a deep groove, the genitocrural fold. The medial surfaces may be in contact with each other but may be separated by the labia minora if they are long.

The labia majora have their full complement of adnexal structures, but the inner aspects lack hair in the pilosebaceous unit. An unusual mammary-like gland, the anogenital sweat gland, may also be found at this site (see below).

Arterial supply Labial branches of the internal pudendal artery.
Venous drainage Tributaries to the superficial external pudendal vein which then go to the great saphenous vein.
Nerve supply Labial branches of the perineal nerve.
Lymphatic drainage Superficial inguinal nodes and the inferior aspect to the rectal lymphatic plexus.

Labia minora

The labia minora are two thin folds of cornified skin, again devoid of hair but still possessing sebaceous and eccrine glands. They lack a layer of subcutaneous fat, and lie medial to the labia majora and lateral to the vestibule. The labia minora are separated from the labia majora by interlabial furrows in which the normal secretions from the adjacent skin surfaces may accumulate. Anteriorly, the labia minora divide into lateral and medial parts. The lateral parts unite anterior to the clitoris, in a fold of skin overhanging the glans to form the prepuce of the clitoris. The medial parts unite on the undersurface of the clitoris to form its frenulum. This anterior division of the labia minora in relation to the clitoris is variable. Posteriorly, the labia minora fuse to form a transverse fold behind the vaginal opening, the fourchette.

There is great variation in the size and morphology of the labia minora. Hypertrophy of the labia minora may be

associated with local irritation and discomfort in walking and sitting or may interfere with sexual intercourse. Surgical removal of excess labial tissue, leaving the clitoris and fourchette intact, is recommended in such patients (Baruchin & Cipollini 1986). There are two surgical techniques for the simple reduction of the labia minora (see Chapter 12).

The skin of the labia minora is smooth or mildly rugose and pigmented, particularly at the tips. The dermis of the labia minora is composed mainly of elastic fibres and blood vessels and possesses a rich innervation. The arrangement of blood vessels within the labia minora forms erectile tissue similar to that in the penile corpus spongiosus, their embryological counterpart in the male. During sexual excitation the blood supply to the labia minora is increased and causes not only a change in colour but also significant enlargement, sufficient to induce a minimal degree of traction on the clitoris.

Arterial supply Labial branches of the internal pudendal artery.
Venous drainage Tributaries to the superficial external pudendal vein which then go to the great saphenous vein.
Nerve supply Labial branches of the perineal nerve.
Lymph drainage Superficial inguinal nodes.

The clitoris

The clitoris is a specialized structure covered with a stratified squamous epithelium that is thinly cornified. There are no sebaceous, apocrine or sweat glands present. There has been poor one-dimensional presentation of clitoral anatomy in textbooks (O'Connell *et al.* 1998). Magnetic resonance imaging (MRI) has been used to investigate clitoral function and has complemented the results of anatomical dissection studies (O'Connell & Delancey 2005, Yang *et al.* 2006).

The clitoris has five component erectile tissue parts, most of which lie beneath the skin: the *glans* and *body*, two *crura* and two *clitoral bulbs*. The clitoris is not a flat structure, and therefore not easily represented in a planar diagram. It has a wishbone-like structure; the two arms are the paired crura, which extend forward as the corpora cavernosa and meet in the midline to form the body of the clitoris. The crura are attached to the pubic rami and are covered by the ischiocavernosus muscle. The clitoral body is attached to the pubic symphysis by a suspensory ligament. The tip of the body bends anteriorly away from the pubis forming the glans clitoris, which is the only visible part of the clitoris. The glans is covered by the clitoral hood, which is formed by the labia minora. The clitoral bulbs lie between each clitoral crus and the vaginal opening. The clitoral bulbs were previously termed the vestibular bulbs, but are now more accurately named as anatomically they form

part of the clitoris (O'Connell *et al.* 1998, Puppo 2006). They are covered by the bulbospongiosus muscles, which extend from the perineal body, around the vagina and urethra, to the glans clitoris. The whole of the clitoris is composed of similar erectile tissue with the exception of the glans (Yang *et al.* 2006) and is the homologue of the penis.

Arterial supply Superficial and deep terminal branches of the internal pudendal artery.

Venous drainage Deep dorsal vein to vesicle plexus, deep external pudendal veins to femoral vein and internal pudendal veins to internal iliac vein.

Nerves Dorsal nerve of the clitoris, a branch of the pudendal nerve.

Lymph drainage To deep inguinal and internal iliac nodes.

The vestibule

The vestibule extends anteroposteriorly from the frenulum of the clitoris to the fourchette and laterally from the hymenal ring to a variable position on the inner aspect of each labium minus. The line of demarcation of the vestibule and the labium minus is known as Hart's line. The vulval vestibule is covered by non-cornified stratified squamous epithelium, i.e. a mucosa, and is devoid of any part of the pilosebaceous unit or other adnexal structures. The openings of the vagina, urethra, the ducts of Bartholin's glands and the minor vestibular glands are all localized within the vestibule. That part of the vestibule between the vaginal orifice and the frenulum of the labia minora forms a shallow depression termed the vestibular fossa or fossa navicularis. The clitoral bulbs lie in the superficial perineal pouch adjacent to the lateral wall of the vagina. They are attached to the inferior surface of the urogenital diaphragm by the overlying bulbospongiosus muscle. Thus, the bulbar erectile tissue embraces the vaginal opening and, during sexual arousal, its engorgement narrows the vaginal introitus.

Surrounding the edge of the vestibule and the opening of the vagina is a thin membrane of connective tissue, the *hymen*. The hymen is frequently incomplete but usually ruptures with exercise, the use of tampons or sexual intercourse. The rupture of the hymen will leave an irregular ragged edge around the vaginal opening termed the *hymenal caruncle* or *carunculae myrtiformes*. Occasionally, it may be a more rigid structure which does not rupture spontaneously and this may lead to haematocolpos. In some cases there is a partial division, e.g. a septate hymen, and this will lead to difficulties with tampon use or intercourse.

Arterial supply Branches of the internal pudendal artery.

Venous drainage Tributaries to the external pudendal vein.

Nerve supply Perineal branch of the pudendal nerve.

Lymph drainage To superficial inguinal nodes.

Major and minor vestibular glands

The openings of the greater and minor vestibular glands can be seen with the naked eye on the lateral part of the vestibule. *Bartholin's glands*, also known as the greater vestibular glands, are situated deeply within the posterior parts of the labia majora. Each gland lies just inferior and lateral to the bulbocavernosus muscle and is normally not palpable. The glandular secretion is clear, mucoid and alkaline and is increased during sexual arousal. Krantz (1977) maintains that these glands 'undergo involution, shrink in size and become atrophic after the thirtieth year of life'. Nevertheless, they may be the site of infection or cyst formation at any age. Bartholin's glands are lobulated and contain multiple acini grouped around the termination of each of the many branching ducts. The acini are lined with cuboidal epithelium and the ducts with stratified transitional epithelium. The main duct of each Bartholin's gland passes deep to the labium minus to open at the lateral margin of the vagina at 5 and 7 o'clock (Fig. 1.15). Argentaffin cells have been described in the epithelial lining of the Bartholin duct system predominantly in the transitional epithelium of the main excretory duct and are similarly found in the paraurethral glands (Fetissov *et al.* 1985). The *minor vestibular glands* are similar in structure to those of the greater



Fig. 1.15 Opening of Bartholin's ducts.

vestibular glands and in postmortem studies vary in number from one to more than 100 (Robboy *et al.* 1978).

The external urethral meatus and urethra

The external urethral orifice lies in the vestibule between the vagina and the clitoris. It is positioned in the midline but its exact location is variable. The orifice is easily seen and on occasions there may be bright red projections of prolapsed mucosa herniating out. The female urethra is 4 cm long and runs from the bladder downwards and forwards, embedded in the anterior wall of the vagina behind the symphysis pubis. After passing through the pelvic floor and perineal membrane it ends at the external urethral orifice. The urethra is fixed at its origin by the pubovesical ligaments and throughout its length by the anterior wall of the vagina. As it enters the perineum it is fixed by the perineal membrane, also known as the urogenital diaphragm or triangular ligament. This is a fibrous membrane that is attached to the pubic rami (Fig. 1.16) and separates the urogenital triangle into superficial and deep perineal pouches. The perineal membrane has three midline breaks, one at the apex of the triangle just below the pubic symphysis where the clitoral vessels and nerves pass from the deep to the superficial perineal pouch and two more posteriorly for the entrances of the urethra and vagina. The pelvic urethra has the same blood and nerve supply as well as lymphatic drainage as the bladder neck. The perineal urethra is supplied by the pudendal vessels and nerves, and the voluntary muscle of the external urethral sphincter is supplied by the perineal branch of the pudendal nerve. The lymphatic drainage of the perineal urethra is to the inguinal nodes.

Arterial supply Vesical and vaginal arteries which are branches of the anterior internal iliac artery.

Venous drainage Via plexus around the urethra to vesicle plexus around the bladder neck and into the pudendal veins.

Nerve supply Pudendal nerve.

Lymph drainage Urethral lymphatics drain into the internal and external iliac nodes.

The vagina

The vagina is a fibromuscular tube that extends some 7–10 cm from its opening on the vulval vestibule upwards and backwards, to be attached around the periphery of the uterine cervix at some distance above its lower margin. As the long axis of the vagina forms a right angle with the long axis of the normal anteverted uterus, the cervix projects downwards and backwards into the upper vagina. The circumferential vaginal attachment is achieved by the posterior wall of the vagina being some 2 cm longer than the anterior wall. The vaginal fold around the periphery of the cervix is divided into anterior, posterior and lateral fornices. The deep posterior fornix is continuous, via the lateral fornices on either side of the cervix, with the shallow anterior fornix. The anterior and posterior walls of the undistended vagina are in contact with each other throughout most of their length, giving the vagina a crescentic or H-shaped appearance in cross-section.

The vagina is related anteriorly to the base of the bladder and to the urethra, which is embedded in its anterior wall. Posteriorly, the upper part of the vaginal wall is covered with peritoneum. Below the rectouterine pouch the posterior vaginal wall is directly related to the ampulla of the rectum, while in the perineum the fibromuscular perineal body separates it from the anal canal (Fig. 1.17). The upper vagina gives attachment to the uterosacral ligaments posteriorly, the cardinal or transverse ligaments laterally, and the base of the bladder anteriorly, which itself is supported by the pubovesical ligaments. As the vagina passes through the pelvic floor the most medial fibres of the pubococcygeus blend with its walls to form a supporting muscular sling. Below the pelvic floor the vagina is supported by the urogenital diaphragm, the perineal body and the perineal musculature. Thus, the vagina has three compartments:

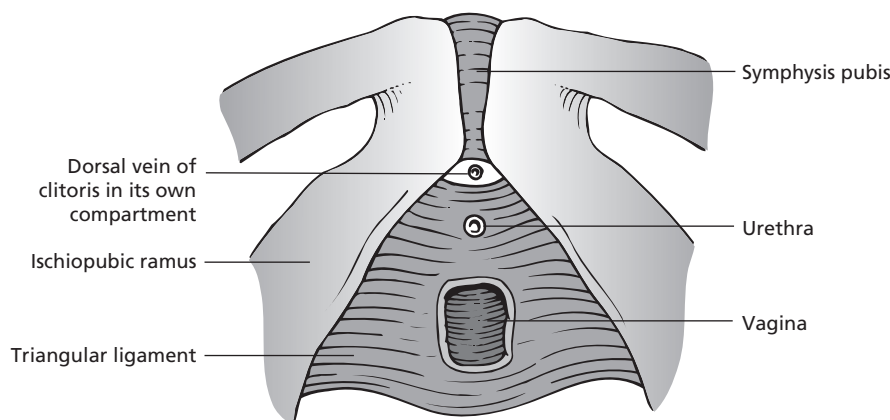


Fig. 1.16 The urogenital diaphragm.

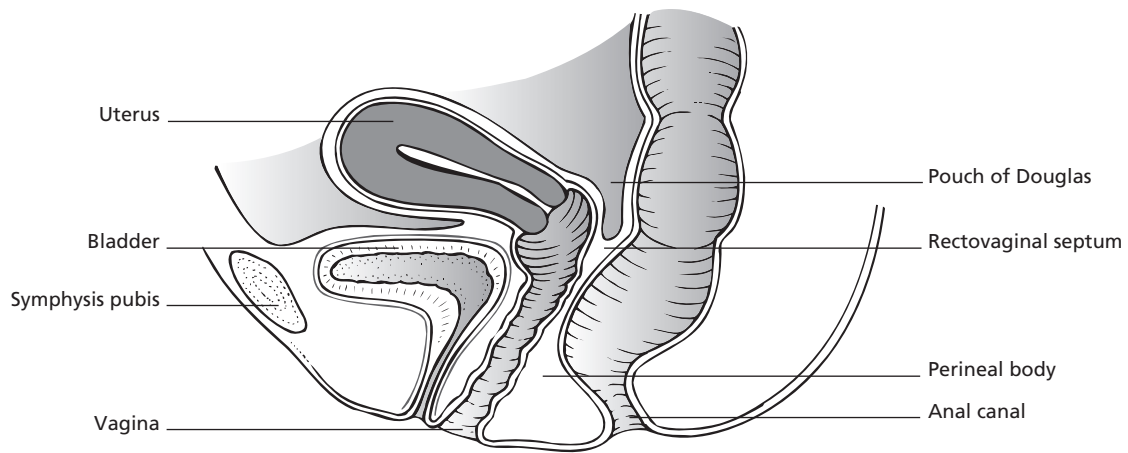


Fig. 1.17 A midline section through the pelvis and perineum.

an upper, which is above the pelvic floor and related to the rectum; a middle, which traverses the pelvic floor and urogenital diaphragm; and a lower, which is in the perineum (Blaustein 1982).

The vagina has an outer adventitial coat of fibroelastic tissue by which it is bound to the urethra and anchored to the pelvic walls by the pelvic ligaments. The intermediate coat of circular and longitudinal smooth muscle is intermingled with striated muscle from the pelvic floor. Between the muscular and inner epithelial layers is a layer of loose fibroelastic tissue in which there is an extensive network of venous channels. This venous network, with distension, changes the vaginal walls into erectile tissue and is the probable source of vaginal secretion during sexual intercourse (Smith & Wilson 1991). The inner aspect of the vagina is lined with non-cornifying stratified squamous epithelium, the cells of which are heavily glycogenated. The vaginal surface of the cervix is covered with stratified squamous epithelium while the cervical canal is lined by columnar epithelium in which there are numerous mucus-secreting cells. The squamocolumnar junction may occur at the external os but more often there is a transformation zone of variable extent situated around the external os, the nature and development of which is described by Singer and Chow (2003).

The outer wall of the vagina accommodates its vascular, lymphatic and nerve supply.

Arteries The vaginal artery may arise from the internal iliac artery or one of its branches, most commonly the internal pudendal artery (Hollinshead 1971). The uterine artery supplies a descending branch to the upper vagina and there is frequently a vaginal branch from the middle rectal artery. The lower vagina is supplied by branches of the

internal pudendal artery. These vessels anastomose with each other in or on the vaginal walls. Vessels from the right and left sides anastomose to form unpaired, midline, anterior and posterior azygos arteries.

Veins

The veins of the vagina drain to the uterovaginal plexus, which itself communicates with the uterine, vesical and rectal venous plexuses, all of which drain mainly to the internal iliac veins.

Nerves

Nerve fibres accompany the vessels as they penetrate the walls of the body of the uterus, cervix and vagina. There is general agreement that almost all of the motor fibres to uterine muscle are sympathetic while afferent innervation of the body is sympathetic and of the cervix is parasympathetic (Swash 1991).

Lymphatics

The upper two-thirds is with the cervix to the internal and external iliac nodes. The lower vessel third drains with the rest of the perineum to the superficial inguinal nodes.

The perineum

The perineum is a diamond-shaped area bounded by the symphysis pubis anteriorly, the ischial tuberosities laterally and the coccyx posteriorly. It is divided into two triangles – the urogenital triangle and the anal triangle. The perineal body is the fibromuscular mass that lies between the vestibule and the anus. It is the central point where muscles attach to the ischial tuberosities. It is supplied by the perineal artery and venous drainage is through the internal pudendal vein. The lymph drainage is to the superficial inguinal glands.

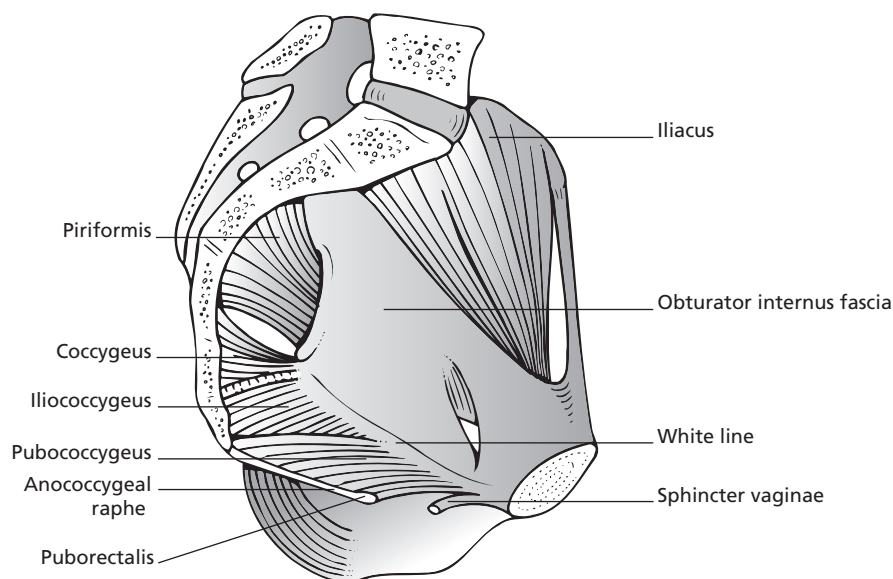


Fig. 1.18 The muscles of the pelvic walls and pelvic floor.

The urogenital triangle and diaphragm

The urogenital triangle is contained within the subpubic arch and is divided into superficial and deep perineal pouches by the tough fibrous membrane attached to the pubic rami, the urogenital diaphragm (also known as the perineal membrane or triangular ligament) (Fig. 1.16). This membrane is attached to the structures of the external genitalia. The deep perineal pouch is bounded above by the pelvic floor and pubovesical ligaments and below by the urogenital diaphragm. On either side lie the pubic rami, while posteriorly it is continuous with the ischioanal fossae. Passing through the deep perineal pouch, in the midline, are the urethra and vagina. The vessels and nerves pass forwards on either side of the urethra and vagina. The clitoral branches leave the deep perineal pouch through the apical opening in the urogenital diaphragm. The deep pouch also contains voluntary muscle fibres, some of which surround the urethra and vagina while others run transversely into the perineal body behind the vagina.

Anal triangle

The anal triangle is the triangular area bounded by the ischial tuberosities and the coccyx. The anal canal lies within this area. The ischioanal fossae lie laterally to the anal canal. These are pyramid-shaped areas bounded by ischium and obturator internus laterally, levator ani medially and the perianal skin inferiorly. The fossae contain fat and connective tissue and the nerves to the anus, perineum and external genitalia traverse this space. The anal canal is the terminal 2.5–3.5 cm of the large intestine and passes through the levator ani. It has an internal involuntary sphincter derived from the smooth muscle of the rectum around its upper two-thirds and an external voluntary

sphincter muscle around the lower two-thirds. This sphincter muscle blends with the puborectalis muscle (Fig. 1.18).

Muscles of the pelvic floor

The pelvic floor, or pelvic diaphragm, is composed of sheets of muscles arranged around the midline urethra, vagina and anal canal. This sheet of muscle is made up of the ischiococcygeus, iliococcygeus and pubococcygeus, and should be regarded as one morphological entity. Their linear origin, from the white line overlying the obturator fascia on the side wall of the pelvis, extends from the ischial spine posteriorly to the pubic bone anteriorly. From this bilateral linear origin the muscles reach their midline insertion into the sacrum, coccyx, anococcygeal raphe and perineal body, forming a gutter-shaped pelvic floor, which slopes downwards and forwards (Fig. 1.19).

The ischiococcygeus muscle arises from the ischial spine and is inserted into the fifth sacral vertebra and the coccyx. The iliococcygeus and pubococcygeus arise in linear continuity from the ischial spine to the body of the pubis. The iliococcygeus arises from the posterior half of the fibrous linear origin and, overlying the pelvic surface of the ischiococcygeus, it is inserted into the coccyx and anococcygeal raphe. This raphe is the interdigitation of muscle fibres from the right and left sides and it extends from the tip of the coccyx to the anorectal junction.

The pubococcygeus arises from the anterior half of the fibrous linear origin and from the posterior surface of the body of the pubis. The muscle fibres arising from the fibrous linear origin sweep backwards on the pelvic surface of iliococcygeus to be inserted into the anococcygeal raphe. Those fibres arising from the pubic bone form a muscle sling around the anorectal junction, which produces a forward

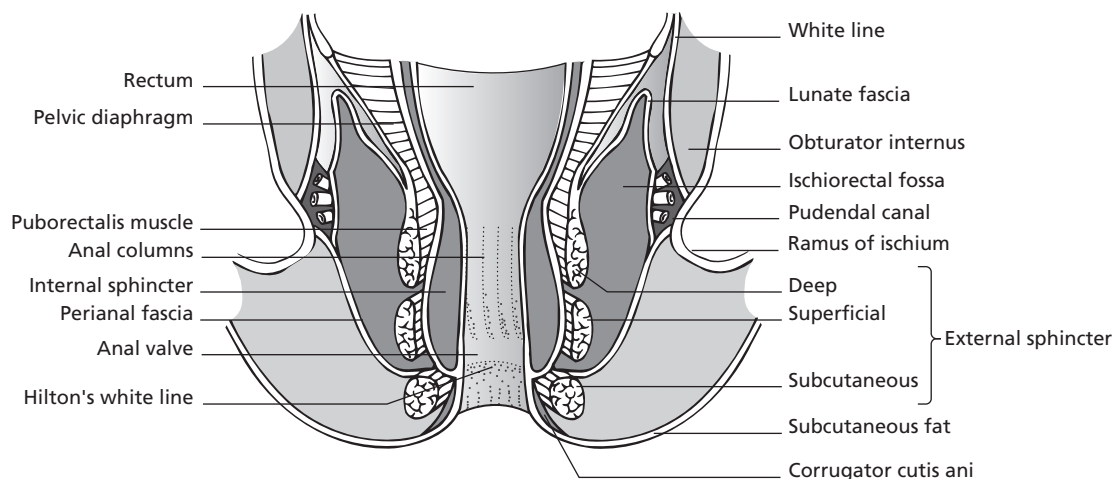


Fig. 1.19 A coronal section through the anal canal and ischiorectal fossa.

angulation of the junction. This part of the pubococcygeus is referred to as puborectalis and it lies beneath the anococcygeal raphe and intermingles with the deep part of the external anal sphincter. The most medial fibres arising from the pubis form a muscle sling around the vagina. This part of the pubococcygeus is the sphincter vaginae and behind the vagina its fibres intermingle with the fibromuscular tissue of the perineal body. The midline gap between the medial edges of the sphincter vaginae is occupied by the pubovesical ligaments and the deep dorsal vein of the clitoris.

The main functions of the pelvic diaphragm are to support the pelvic viscera and to assist in the maintenance of continence when intra-abdominal pressure is raised during episodes of coughing, sneezing and muscular effort. The posterior midline portion of the pelvic diaphragm is an important component of the post-anal plate (Smith & Wilson 1991) upon which the terminal rectum rests.

The nerve supply of the pelvic diaphragm is from the lumbosacral plexus (S2–S4).

The superficial perineal pouch

This lies below the urogenital triangle. The paired clitoral cruri are attached to the lateral margins of the undersurface of the urogenital diaphragm and to the ischiopubic rami. Each is covered by the ischiocavernosus muscle. These cruri extend forward as the corpora cavernosa, which fuse at the subpubic angle to form the body of the clitoris. The clitoral body is attached to the pubic symphysis by a suspensory ligament. Between each clitoral crus and the vaginal opening lies the clitoral bulb (vestibular bulb), which is also attached to the urogenital diaphragm. These bulbs extend forwards beyond the urethra where they fuse to form a slender band of erectile tissue, which is situated on the ventral surface of the clitoris. These bulbs are covered by the bulbospongiosus

muscles, which extend from the perineal body, around the vagina and urethra, to the clitoris. Just behind the vestibular bulbs, also lying on the urogenital diaphragm, are the greater vestibular glands of Bartholin, which open into the vestibule on its lateral aspect. Lying transversely across the base of the urogenital triangle at the posterior margin of the superficial perineal pouch are the superficial transverse perineal muscles (Fig. 1.20). The superficial perineal pouch therefore contains the structural elements of the female external genitalia, which, with their skin covering, exhibit the external appearance characteristic of the vulva.

The inguinofemoral region

The femoral triangle is a gutter-shaped depression below the groin, with its apex situated medially and inferiorly. Its base is formed by the inguinal ligament, the lower free aponeurotic margin of the external oblique muscle of the anterior abdominal wall. The inguinal ligament extends from the anterior superior spine of the iliac bone laterally to the tubercle on the body of the pubic bone medially. Inferiorly, the inguinal ligament gives attachment to the fascia lata, the deep fascia of the thigh. Midway between the pubic symphysis and the anterior iliac spine the external iliac artery becomes the femoral artery as it enters the femoral triangle deep to the inguinal ligament and the fascia lata.

As the external iliac vessels enter the femoral triangle they create a short downward extension of the abdominal fascia, the femoral sheath, which encloses the femoral vessels, with the femoral artery lying lateral to the vein. Medial to the femoral vein is that portion of the femoral sheath termed the femoral canal. The long saphenous vein ascends the leg in the superficial fascia and at the medial end of the inguinal ligament passes through the saphenous opening in the fascia lata to enter the femoral vein.

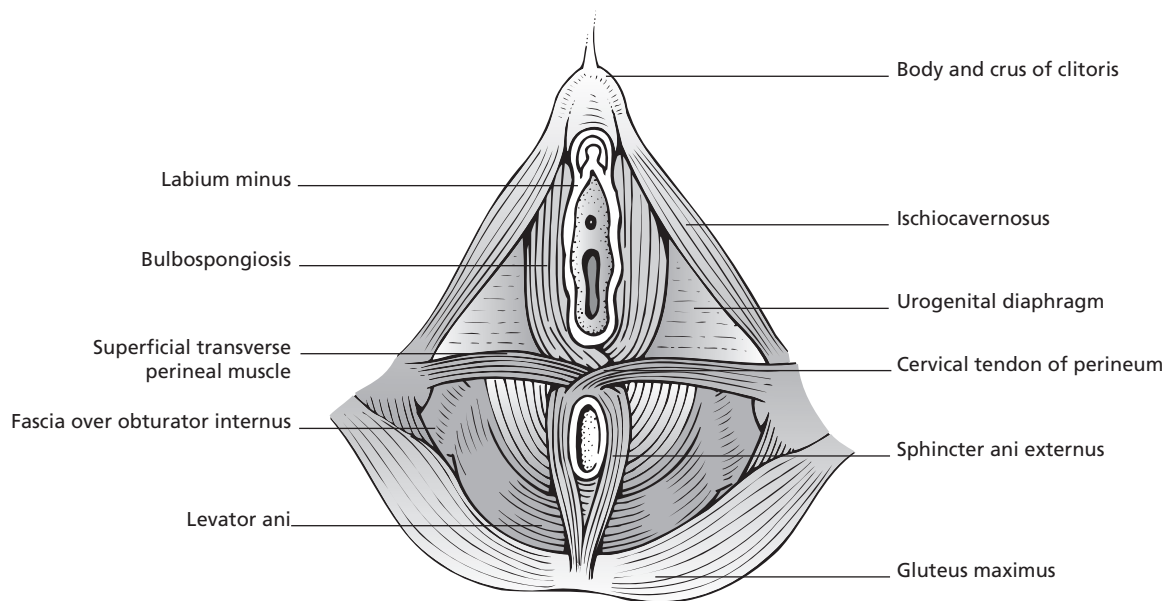


Fig 1.20 The superficial structures of the perineum.

Blood supply

Branches of the internal iliac and femoral artery supply the perineum.

The internal pudendal artery, a branch of the internal iliac artery, leaves the pelvis through the greater sciatic notch below the piriformis muscle. Lying on the tip of the ischial spine it turns forwards through the lesser sciatic foramen to enter the anal triangle posteriorly. Within this triangle it runs forwards on the side wall of the ischioanal fossa enclosed by the fascia of the pudendal canal. During its course through the ischioanal fossa it gives off the inferior rectal artery, which arches over the fascial roof of the fossa to reach and supply the anococcygeal raphe, anal canal and perineal body. Entering the urogenital triangle the internal pudendal artery gives off the perineal branch to the perineal body and the structures situated more posteriorly in the superficial perineal pouch. The parent artery enters the deep perineal pouch and supplies the erectile tissue lying in the vestibule, by perforating branches into the superficial perineal pouch, and the clitoris, by way of its deep and superficial terminal branches. The latter vessel reaches the body of the clitoris by entering the superficial perineal pouch through the apical deficit in the urogenital diaphragm.

Within the femoral triangle the femoral artery gives off the superficial and deep external pudendal arteries. The superficial external pudendal artery pierces the deep fascia of the thigh anteriorly, to overlie the round ligament of the uterus. It runs medially to supply the mons pubis and labia of the vulva. The deep external pudendal artery pierces the

deep fascia of the thigh medially to enter the labia of the vulva. Within the superficial perineal pouch the terminal branches of the internal and external pudendal arteries anastomose with one another.

The venous drainage of the perineum is similarly arranged and eventually reaches the femoral and internal iliac veins. The internal iliac veins drain a rich venous plexus in the pelvic floor, which, at least in part, drains all the pelvic viscera. Thus, the venous drainage of the terminal gastrointestinal tract is partially to the pelvic plexus but principally to the portal system via the superior rectal and thence the inferior mesenteric vein. The pelvic venous plexus therefore provides a portal systemic anastomosis and portal hypertension predisposes to distension and even thrombosis of the pelvic, rectal, vaginal and vulval veins. Vulvovaginal varices, however, are most common during pregnancy, although they may occur in patients with endometriosis, pelvic inflammatory disease or pelvic tumours. Since many women develop vulval varices during the first trimester of pregnancy, the underlying cause is probably not obstructive but hormonal, and indeed progesterone is known to cause increased venous distensibility (Gallagher 1986). In the non-pregnant patient, particularly those using anovulant contraceptives, vulval varices may undergo cyclic change during the menstrual cycle (Gallagher 1986).

Lymphatic drainage

Lymphatic capillaries arise in the extracellular tissue spaces and form larger channels, which drain to the regional

lymph nodes. Efferent vessels leave these regional lymph nodes and the lymph passes through a series of intermediate lymph nodes before returning to the thoracic duct. The regional lymph nodes of the perineum are situated in the groin at the base of the femoral triangle. These superficial lymph nodes subsequently drain to deep nodes in the pelvis and ultimately to para-aortic nodes on the posterior abdominal wall. Any midline structure, and especially an anatomical region as well defined as the perineum, has bilateral lymphatic drainage. Thus, the lymphatic drainage of either labium minus is to both the ipsilateral and contralateral superficial lymph nodes (Iversen & Aas 1983).

The regional lymph nodes of the perineum are arranged in two groups at the base of the femoral triangle. A variable number of lymph nodes lie transversely in the superficial fascia of the thigh, immediately below the medial two-thirds of the inguinal ligament. The superficial femoral or subinguinal lymph nodes, numbering 3–20, lie on both the medial and lateral aspects of the long saphenous vein. Those on the lateral side send efferent lymphatics, through the saphenous opening, to the external iliac group of deep lymph nodes. The superficial lymph nodes of the femoral triangle communicate freely with one another and drain the whole of the perineum, including the lower thirds of the urethra, vagina and anal canal.

The external iliac lymph nodes are described in relationship to the external iliac vessels. The medial group of 3–6 nodes lies on the medial side of the origin of the external iliac vein. Up to three of these nodes may be found in the femoral triangle medial to the femoral vein, and in this situation they are referred to as the deep femoral nodes. If all three are present, the lower one is situated just below the junction of the great saphenous and femoral veins, the medial node in the femoral canal and the uppermost node is known as the node of Cloquet or Rosenmuller. However, this last node is frequently missing (Borgno *et al.* 1990). The anterior group is variable and when present comprises no more than three nodes lying in the sulcus between the external iliac artery and vein. The lateral group of 2–5 nodes lies on the lateral side of the external iliac artery. The nodes of the external iliac group communicate freely with one another and with the obturator node. This large constant node, so named because of its proximity to the obturator nerve, lies below the external iliac vessels on the side wall of the pelvis and probably belongs to the external iliac group.

The efferent lymphatics from the external iliac group drain to the common iliac nodes situated on the lateral side of the common iliac artery. The external and common iliac nodes drain, either directly or indirectly, the lower limb, the lower anterior abdominal wall, the perineum and some of the pelvic viscera. Many small nodes lie close to each pelvic viscus and these drain into the numerous nodes embedded

in the extraperitoneal tissue on the walls of the pelvis. These pelvic nodes are situated alongside the branches of the internal iliac artery and many groups are named according to the vessels with which they are associated. All lymphatics from the pelvis eventually drain to the para-aortic nodes.

Innervation

The perineum has both somatic and autonomic innervation and in each there are sensory and motor components. The perineum having arisen from the most caudal part of the developing embryo derives its somatic innervation from the most caudal segments, S₁–S₄. The nerve supply of the perineal area anteriorly is supplemented by input from the upper lumbar segments, L₁ and L₂. The autonomic or visceral innervation of the perineum is entirely from the most caudal elements of both the sympathetic and parasympathetic systems. The sympathetic outflow from and input to the central nervous system are restricted to the region between the first thoracic and second lumbar levels of the spinal cord. The sympathetic innervation of the perineum is located therefore at L₁ and L₂. It reaches the perineum via postganglionic grey rami communicantes, arising from the first two lumbar and all four sacral ganglia of the sympathetic trunks. These fibres are distributed with the first and second lumbar segmental nerves and the first, second, third and fourth sacral segmental nerves. In addition, other sympathetic fibres from L₁ and L₂ leave the sympathetic trunk as the hypogastric nerves (lumbar splanchnics, presacral nerves) and descend into the pelvis to be associated with the autonomic pelvic plexuses, which are distributed with the blood vessels. The parasympathetic outflow from and input to the central nervous system consists of cranial and caudal portions. The cranial portion is associated with four of the cranial nerves whereas the caudal portion is associated with the second and third, or third and fourth, sacral segments of the spinal cord as the nervi erigentes. These nerves together with the hypogastric sympathetic nerves form the autonomic pelvic plexuses.

The cutaneous innervation of the perineum conveys all modalities of common sensation – touch, pain, itch, warmth and cold – as well as complex sensations such as wetness. In addition, these cutaneous nerves carry postganglionic sympathetic nerves that are motor to sweat glands, pilomotor units and the adventitia of the microvasculature. No parasympathetic fibres participate in this cutaneous innervation, which is provided by the terminal or perineal branches of several nerves. The anterior part of the perineum is supplied by two nerves that emerge from the superficial inguinal ring just above the body of the pubic bone. These are the ilioinguinal nerve (L₁) and the genital branch (L₂) of the genitofemoral nerve (L₁ and L₂). The lateral aspect of the perineum, more posteriorly, is supplied by the

perineal branch (S_1) of the posterior cutaneous nerve of the thigh (S_1 – S_3). The remainder of the cutaneous innervation of the perineum is supplied by the pudendal nerve (S_2 – S_4) and the perineal branch of the fourth sacral nerve, which also supplies the skin of the anal margin. The pudendal nerve enters the ischiorectal fossa, close to the tip of the ischial spine on the medial side of the pudendal artery. Running anteriorly on the lateral wall of the ischiorectal fossa it gives rise to the inferior haemorrhoidal nerve, which arches over the roof of the fossa to reach the midline, where it supplies the terminal part of the anal canal and the perianal skin. The pudendal nerve then divides into the perineal branch, which supplies the rest of the perineal skin, and the dorsal nerve of the clitoris, which supplies the anterior labia minora and the glans of the clitoris.

These sacral spinal nerves also supply motor innervation to the muscles of the perineum. The pudendal nerve, through its inferior haemorrhoidal branch, supplies the deep and subcutaneous parts of the external anal sphincter and through its perineal branch the muscles of both deep and superficial perineal pouches, as well as the anterior part of the levator ani muscle and the sphincter urethra. The remainder of the levator ani muscle and the superficial part of the external anal sphincter are supplied by the perineal branch of the fourth sacral nerve. Damage to the pudendal nerves may cause loss of muscle tone in the pelvic floor and be associated with problems of incontinence.

The sensory components of the parasympathetic innervation of the perineum mediate the sensation of distension from the anal canal and vagina while its motor component is responsible for the vascular engorgement of vaginal erectile tissue.

Epithelia of the vulva and vagina

The vulva is covered in epithelia that gradually change from normal skin type epithelium on the outer aspects to a mucosa in the vestibule. The vagina is also lined with mucosal epithelium.

Typically cornified stratified squamous epithelium is made up of four layers histologically:

- 1 a basal layer, or stratum germinativum, the lower border of which rests on the basal lamina
- 2 a spinous or prickle-cell layer, which forms the bulk of the epidermis
- 3 a granular layer
- 4 a horny layer or stratum corneum.

Differentiation is the process that occurs as the keratinocytes move upwards through the spinous layers to form the tough, protective, flexible outer surface of the skin. The keratinocytes flatten and lose their nuclei as they progress upwards, ending up as flattened structureless squames at the surface (Fig. 1.21).

The epidermis of the labia majora, labia minora and the frenulum of the clitoris is cornified stratified squamous epithelium. The epithelium on the inner aspects of the labia minora is still cornified, but towards the lower part it merges with the vestibule, which is covered in non-cornified stratified squamous epithelium, i.e. a mucous epithelium. Hart's line may be clearly seen with the naked eye in some patients, marking this transition from cornified to non-cornified epithelium (see Chapter 2).

The mons pubis, the lateral and exposed aspects of the labia majora and the perianal area are covered with hair-bearing skin (Fig. 1.22). The pilosebaceous unit comprises

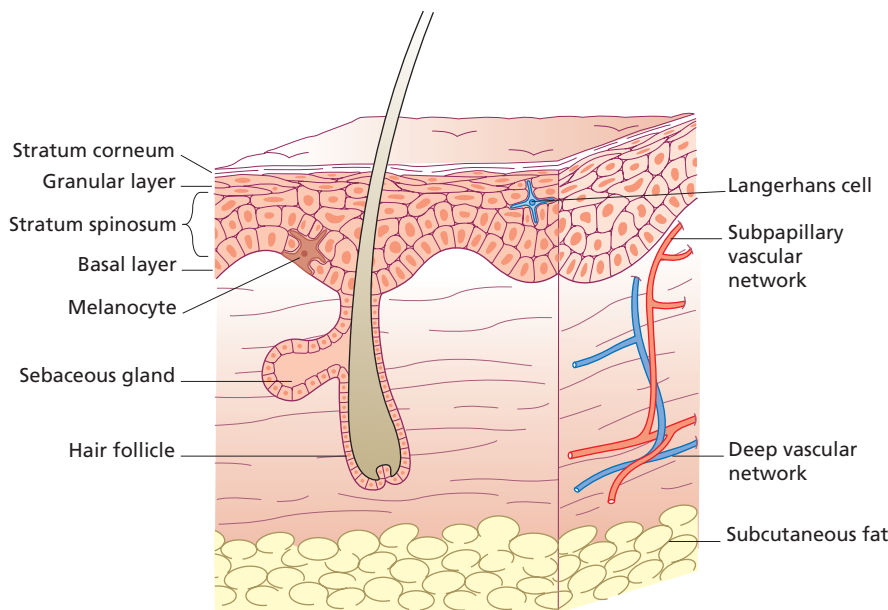


Fig. 1.21 Section (diagrammatic) of skin.

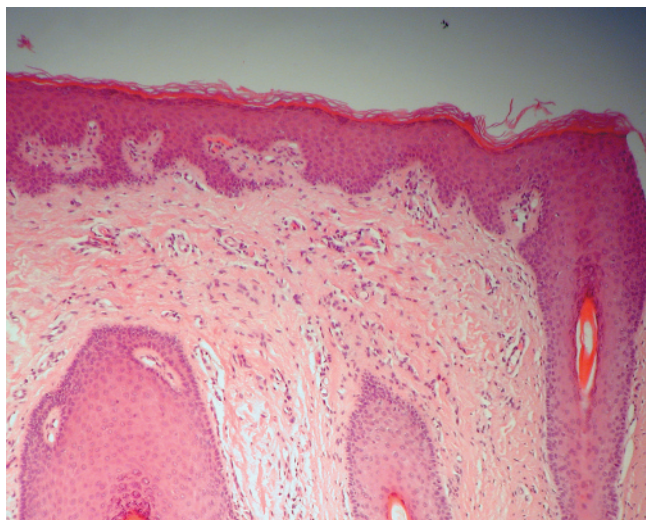


Fig. 1.22 Histological section of skin from labium majus showing hair follicles.

the hair follicle, the hair, the sebaceous gland and the arrectores pilorum muscle and some are associated with an apocrine gland. Eccrine glands are also present.

The inner aspects of the labia majora, the inner and outer aspects of the labia minora and the frenulum and prepuce of the clitoris are covered with non-hair-bearing skin but the sebaceous glands are still present and they open directly onto the skin. These areas are devoid of apocrine glands and eccrine glands are rarely seen.

A previously undescribed gland – the *anogenital ‘sweat’ gland* – has been discovered (van der Putte 1991). These glands are found predominantly in the interlabial sulci of the vulva and have distinctive morphological, histological and histochemical features. The glands cannot be categorized as eccrine, apocrine or mammary glands, although they may share some of their morphological features. Interestingly, similar glands have been described in association with hidradenoma papilliferum and on the labia minora of postmortem specimens (Woodworth *et al.* 1971).

The morphology of the glands ranges from a simple wide duct with a few small diverticula to a complicated, convoluted tubular system (Fig. 1.23). Most glands, however, consist of a coiled duct with diverticular extensions at irregular intervals along their length. Some of the diverticula are short and acinar whereas others are more duct-like and have their own acini. The glands all have a straight excretory duct that opens directly onto the skin surface. Occasionally, some of the glands reach a complexity that gives them a lobular appearance reminiscent of the mammary glands of the breast. The anogenital glands extend deeper into the dermis than the eccrine or apocrine glands. They are lined by simple columnar epithelium resting on a layer of myoepithelium. The columnar epithelium extends

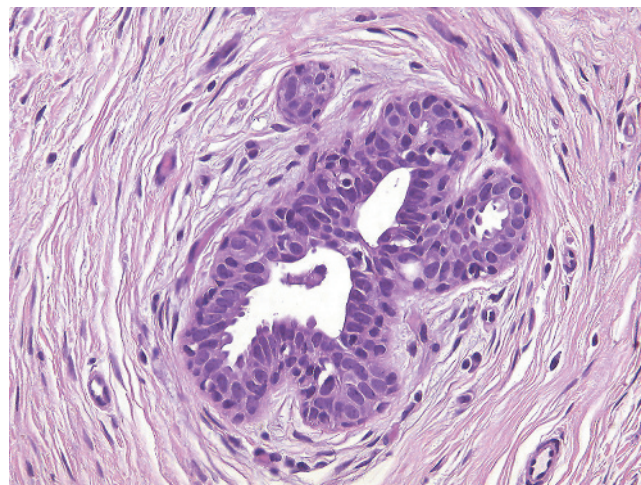


Fig. 1.23 Histological section through lobulated anomammary gland lined with compact columnar epithelium (courtesy of Dr James Scurry).

into the straight excretory duct until a short distance from the surface and then gives way to non-cornifying, stratified squamous epithelium without a myoepithelial layer. The columnar cells have oval nuclei and a small to moderate amount of cytoplasm with a cytoplasmic ‘snout’ at the luminal side. Ultrastructurally, these glands are unique with features not seen in other tubular cutaneous glands (van der Putte 1993).

In addition to keratinocytes there are three other cell types in the epidermis: melanocytes, Langerhans cells and Merkel cells.

Melanocytes

Melanocytes are cells of neural crest origin that specialize in the production of melanin pigments, and they are situated in the basal layer of the epidermis. There is a regional variation in the ratio of melanocytes to basal keratinocytes of between 1:10 and 1:5. They appear as rounded cells with clear cytoplasm. Melanocytes convert the amino acid tyrosine to melanin pigments within the melanosomes, which are membrane-bound organelles. These melanosomes are transferred to keratinocytes through the melanocytes’ dendritic extensions (Jimbow *et al.* 1993). It is generally assumed that the main purpose of this transfer within the ‘epidermal melanin unit’ is the protection of keratinocyte DNA from damage by ultraviolet radiation and certain toxins (Slominski *et al.* 1993).

It is now recognized that melanocytes have a considerably wider range of secretory activity than that involved in melanin transfer. Human melanocytes are capable of secreting a number of signal molecules targeting keratinocytes, lymphocytes, fibroblasts, Langerhans cells, mast cells and endothelial cells, all of which express receptors (Slominski

et al. 1993). Hormones profoundly influence human melanocyte activity, although their precise action at the cellular level is obscure. There is also a marked regional variation in the sensitivity of melanocytes to specific hormones. During pregnancy oestrogens and progestogens stimulate increased melanogenesis in the areola, nipples and perineum, and to a lesser extent in the face and midline of the anterior abdominal wall. The facial hyperpigmentation associated with anovulant contraceptives is accentuated by exposure to ultraviolet light and may not resolve completely after they are discontinued.

Langerhans cells

Langerhans cells are found in the epidermis and are bone marrow-derived dendritic cells, present in all layers of the epidermis. They lack pigment and their unique identifying feature is the presence of Birbeck granules (Birbeck *et al.* 1961) in the cytoplasm best seen using transmission electron microscopy. They represent 1–2% of the epidermal cell population and are located mainly in the suprabasal area. Each cell possesses 5–9 dendritic processes, which extend out in the same horizontal plane, covering 25% of the surface area of the skin (Yu *et al.* 1992). An analysis of the distribution of Langerhans cells in healthy tissue of the genital tract showed there were 19 per 100 basal squamous cells in the vulva, six per 100 basal squamous cells in the vagina, and 13 per 100 basal squamous cells in the cervix (Edwards & Morris 1985).

Langerhans cells play an important immunological role in the skin (Rowden *et al.* 1977), being one of the most potent antigen-presenting cells in the body. They are involved in the sensitization and activation of contact allergic dermatitis as well as participating in the immune surveillance of the skin. These immunological functions are particularly important in the epithelia covering the lower female genital tract as these sites are exposed to a wide range of bacteria, viruses and fungi as well as the antigens of the ejaculate. The oncogenic human papillomaviruses are known to induce changes in the Langerhans cell population in the cervix (Morris *et al.* 1983, Tay *et al.* 1987a,b) and skin (Gatter *et al.* 1984).

Merkel cells

These cells are found throughout the skin, situated singly or in clusters in the basal layer of the epidermis. The dendritic cytoplasmic processes surround adjacent keratinocytes (Winkelman 1977) and their cell bodies are intimately associated with contiguous nerve fibres (Winkelman & Breathnach 1973). It had been thought that they were derived from the neural crest and had migrated along the peripheral fibre to the epidermis (Pearse 1969). However, observations on human fetal skin transplanted onto nude mice indicate that Merkel cells arise *in situ* and are derived from epithelial

cells (Moll *et al.* 1990). Immunohistochemical studies have shown that Merkel cells in humans, as well as in many other species, contain vasoactive intestinal polypeptide (VIP) (Gould *et al.* 1985). The precise role of Merkel cells in the skin is unknown. Malignant neoplasms of epidermal Merkel cells may occur (see Chapter 8).

The dermis

The dermis lies below the epidermis and is bounded inferiorly by the subcutaneous adipose layer. It has two layers – the papillary dermis and the reticular dermis. The papillary dermis projects upwards into the rete ridges and is composed of fine collagen fibres, running at right angles to the surface, together with reticular and elastic fibres. This arrangement supports vascular and lymphatic channels as well as nerve terminals. The reticular dermis lies below the papillary dermis and is composed of coarse collagen fibres lying parallel with the surface. Accompanying the collagen fibres are thicker elastic fibres that prevent the dermal collagen from being overstretched, and in the perineal area stress-orientated smooth muscle fibres similar to those seen nipple, penis and scrotum are also present. The vascular and lymphatic plexuses that drain the papillary dermis lie within the reticular dermis, which also contains the nerve fibres associated with the papillary nerve terminals.

Cutaneous vascular system

The dermal microvasculature consists of a deep arterial plexus, the *facial* network. The vessels from this region extend upwards to the border of the subcutaneous fat and the corium and there form a *cutaneous* network. This gives off branches to the appendages and ascending arterioles to a subpapillary plexus, which in turn forms capillary loops in the papillary layer between the dermoepidermal ridges. Blood is drained from these capillaries by venules which drain down to intermediate plexuses.

Cutaneous lymphatic system

The lymphatic system transports fluids such as leaked protein from the extravascular compartment of the dermis. The interconnecting lymphatic spaces arise from the terminal bulbs lying within the papillary dermis. These form the lymphatic network which ultimately drains into the lymph nodes. The vessels have a wide lumen and are lined with a single layer of endothelial cells.

Cutaneous nerve supply

A complex network of neural crest-derived somatic sensory and autonomic nerves supply the skin. The autonomic nerve supply controls the vasculature, adnexae and arrector pili muscles of the hair unit. The cutaneous nerves contain

axons with the cell bodies lying in the dorsal root ganglia. The main nerve trunks enter the subcutaneous fat and divide into smaller bundles that form a horizontal network with fibres ascending alongside blood vessels to form a plexus of interlacing nerves in the superficial dermis. A few reach the basement membrane but do not extend far into the epidermis. The hair follicle has a complex nerve network, being an important cutaneous receptor.

Physiology

The epithelia of the vulva differ in their permeability, barrier function and immune responsiveness as they change from skin type epithelium to a mucosa. There is also the variable response to the hormonal changes throughout the different stages of life. During the reproductive years additional cyclic changes occur in the female reproductive tract as a result of sequential alterations in ovarian hormone secretion. These changes will be affected by pregnancy, which creates its own unique hormonal environment, or by the use of anovulatory drugs or hormone replacement therapy.

The principal hormones involved in these changes are oestrogens, progestogens and small amounts of androgens. Immunohistochemical studies have identified receptors for these hormones at different sites within human skin (Pelletier & Ren 2004). Further studies in the female genital skin have mapped out their distribution in the vulva and vagina, showing variation with different stages of the menstrual cycle (Hodgins *et al.* 1998), with oral contraceptive use (Johannesson *et al.* 2007) and at the menopause (Schmidt *et al.* 1990). In one study, the distribution of the oestrogen receptors was lowest in the mons pubis and labia majora but increased in the labia minora and vagina (Schmidt *et al.* 1990). Androgen receptors on the other hand were greatest in the keratinocytes of the labia majora as well as the adnexal structures and fibroblasts. Progesterone receptors were found only on vaginal epithelium and the distribution of the receptors did not seem to be influenced by the menopause (Hodgins *et al.* 1998).

Barrier function

The barrier function of skin is dependent on the degree of hydration, the presence of a stratum corneum and an intact surface. Transepidermal water loss (TEWL) is an indicator of the skin's barrier function and varies with the number of cell layers in the epidermis, which in turn vary at different body sites. This number is lowest in the genital area (Ya-Xian *et al.* 1999). Studies assessing TEWL have shown that water diffuses faster across the stratum corneum of the labium majus than the forearm (Britz & Maibach 1979, Elsner *et al.* 1990). There is no information, however, on

the variation with the inner aspects of the vulva where the stratum corneum becomes thinner and the area more occluded. In the vestibule there is no stratum corneum as it is a mucosa, and therefore will have physical properties similar to the vagina and mouth. Mechanical friction in the vulva is increased and influenced by occlusion, obesity, immobility and use of sanitary wear. This friction contributes to impairment of the surface integrity with maceration and chafing. All these factors will influence the effect and absorption of topically applied preparations (Britz *et al.* 1980). In cases of suspected contact irritancy and allergy, routine patch testing may not be sufficient (Wakashin 2007) and additional tests may be needed to allow for the effects of friction and epidermal morphology (Farage & Maibach 2004).

Birth to puberty

During the first few weeks of life the reproductive tract of the female infant is responsive to the sex steroids which she has received transplacentally from her mother. The effects of these hormones are entirely physiological and may be evident for about 4 weeks (Dewhurst 1980). During this period the infant's vagina will be lined with a stratified squamous epithelium rich in glycogen as a direct effect of the maternal oestrogen, and lactobacilli will therefore make up part of the normal flora. There will often be an obvious vaginal discharge, which in some cases will be bloodstained as the result of the infant's endometrium breaking down as oestrogen levels begin to fall. The vaginal epithelium then becomes thinner and the epidermal cells less glycogenated. During the prepubertal years the absence of glycogen from the vaginal epithelium restricts the action of lactobacilli and acidification of the vaginal environment. The resultant neutrality or alkalinity of the vaginal secretions renders all young girls vulnerable to vulvovaginitis and the possibility of lower urinary tract infections.

The physical changes associated with puberty are breast development, hair growth in the axillae, mons pubis and labia majora, and the onset of the menses. The development and growth of pubic hair is described in five stages (Tanner 1962), although during stage 1 there is no pubic hair. In stage 2 sparse hair appears on the labia majora and on the mons pubis in the midline. There is an increase in quantity and coarseness of the hair, particularly on the mons pubis during stage 3. Further increase occurs during stage 4 such that only the upper lateral corners of the usual triangular distribution are deficient. Stage 5 describes the normal adult pubic hair pattern with its extension from the labia on to the medial aspects of the thighs. The adult distribution of pubic hair is usually attained between 12 and 17 years of age. Axillary hair growth is described in three stages: from

a stage at which it is absent, through an intermediate stage to full development.

Breast development has been described in five stages (Tanner 1962):

- Stage 1* the infantile state, which persists from the time that the effects of maternal oestrogen have regressed until the changes of puberty begin.
- Stage 2* the 'bud' stage, during which the breast tissue appears as a small mound beneath an enlarged areola. This is the first sign of pubertal change in the breast.
- Stage 3* establishes a small adult breast with a continuous rounded contour.
- Stage 4* is associated with further enlargement of the nipple and areola to produce a secondary projection above the contour of the remainder of the breast.
- Stage 5* is the typical adult breast with smooth, rounded contour, the secondary projection present in the preceding stage having disappeared.

The first signs of breast development may occur at any age from 8 years onwards, and it is unusual for it not to have begun by 13 years of age. Some girls never show a typical stage 4, passing directly from stage 3 to stage 5, while others persist in stage 4 until the first pregnancy or beyond. Premature breast development seems to occur more often in Afro-Caribbean girls than in any other ethnic group. The enlargement may occur as early as 4–5 years of age but is not accompanied by other evidence of puberty or signs of endocrine disease (Black 1985).

The apocrine glands of the axilla and vulva begin to function at about the time that axillary and pubic hair appears, and the sebaceous glands at all body sites become more active. The growth of any individual is dictated by a number of factors, and Marshall and Tanner (1969) have found that most adolescent girls achieve maximum growth rate between their 10th and 14th birthdays.

The average age of the menarche is 13.0 years in the UK, with a standard deviation of approximately 1 year. Thus, 95% of the adolescent female population have their menarche between their 11th and 15th birthdays. It is unusual for a girl to menstruate before her breasts have reached stage 3 of development, but 25% have done so at this stage. The majority of girls, however, begin to menstruate while they are in stage 4 of breast development, but, in about 10% of girls, menarche is delayed until their breasts have reached stage 5.

During the 2 years preceding the menarche the ovaries increase in size. There is an increase in the number of enlarging follicles, although they subsequently regress. This

follicular development is associated with increasing levels of oestrogen production, which is responsible for the thickening of the vaginal epithelium and the glycogenation of the cells. The vagina and cervical canal both begin to lengthen and the cervical glands become active. The vaginal secretions increase in quantity and become more acidic, with a pH of between 4 and 5. Fat deposition increases the size of the labia majora and the prominence of the mons pubis. The labial skin becomes rugose, the clitoris increases in size and the urethral orifice becomes more obvious. Coincidentally, the Bartholin glands become active and the hymenal orifice increases in diameter.

The reproductive years

In addition to ovarian steroids and pituitary gonadotrophins, it is now known that hypothalamic releasing factors, other ovarian hormones and numerous growth factors regulate intraovarian events and the female reproductive system. Ovulation, however, is the significant event of the ovarian cycle and occurs approximately midway between two successive episodes of menstruation. During the pre-ovulatory or follicular phase of the cycle, oestrogen secretion increases to reach a peak before ovulation. Oocyte release probably occurs as a result of coincident surges in the secretion of luteinizing hormone and follicle-stimulating hormone. During the postovulatory or luteal phase of the cycle, progesterone is the predominant hormone, while oestrogen secretion is maintained at a level below that of the preovulatory peak. These cyclic changes in ovarian hormone secretion influence the female reproductive tract so as to create the appropriate environment for internal fertilization and implantation of the embryo.

Cervix

The cervical mucus secretion varies daily from 600 mg at midcycle to 20–60 mg during other phases of the menstrual cycle (Elstein & Chantler 1991). At midcycle the main function of cervical mucus is to facilitate the entry of spermatozoa into the upper reproductive tract, whereas at other times its function is to act as a barrier between the vagina and the upper reproductive tract. The physical character of the cervical mucus together with its specific immunoglobulins provide its barrier function. Any increase in cervical mucus may lead to an irritant vulval eczema.

Vagina

The stratified squamous epithelium of the vagina is extremely responsive to the influence of ovarian steroids. In the absence of oestrogen, the vaginal epithelium is thin and poorly differentiated. Oestrogen causes a thickening of the epithelium and its differentiation into the well-recognized

basal, intermediate and superficial layers characteristic of the reproductive years. The percentage of superficial cells present in a vaginal smear is an indicator of the amount of oestrogenic activity. Progesterone produces a relative decrease in the number of superficial cells while increasing the number of intermediate cells.

The normal vaginal flora is mixed, but lactobacilli and corynebacteria, which predominate, utilize the epithelial glycogen to produce lactic acid and therefore lower the vaginal pH. This discourages the overgrowth of *Candida* species, which may be a normal commensal in the vagina. Antibiotics, by inhibiting the growth of lactobacilli and corynebacteria, disturb the equilibrium and allow candidal overgrowth. The acidity of the vaginal environment may be reduced by the alkaline secretions of the cervical glands, particularly in the presence of a large cervical erosion, by the alkaline menstrual flow and by alkaline ejaculate. A more subtle change takes place in the vagina when the effects of oestrogen are moderated by a relative dominance of progesterone, such as occurs during pregnancy and with the use of some oral contraceptives.

Urethra and vulva

The epithelial lining of the urethra is also influenced by the ovarian hormones and the character of exfoliated urethral epithelial cells changes with the phase of the ovarian cycle. Properly stained smears of epithelial cells in fresh urinary sediment reflects cyclic alterations in oestrogen and progesterone levels in sexually mature women. These cells are more accessible than vaginal epithelial cells in young girls and may be examined for diagnostic purposes when excessive oestrogen production is suspected. Changes corresponding to those in the vaginal epithelium may be observed in the epithelium lining the inner aspects of the labia minora (Tozzini *et al.* 1971).

The physiological vulval change that occurs during a woman's reproductive years is the subjective sensation of wetness, which is present about the time of ovulation. This vulval symptom is a consequence of the changes in the quantity and quality of the cervical mucus during the menstrual cycle. During the preovulatory phase of the cycle, the effect of oestrogen on the endometrium is to stimulate mitotic activity and to replace the endometrial lining shed during the previous menstrual flow. The mucus secretion increases and becomes transparent, more viscous and elastic. These changes are most marked just before ovulation when oestrogen secretion is maximal (Ross & Van de Wiele 1981, Elstein & Chantler 1991). After ovulation the endometrium becomes more vascular under the influence of progesterone, which reduces the quantity of mucus produced. Women interpret these changes as a sensation of vulval 'wetness' (Etchepareborda *et al.* 1983), and studies on the enzymatic content of cervical mucus indicate significant

changes in the concentration of a number of enzymes (Blackwell 1984, Elstein & Chantler 1991).

Changes related to coitus and pregnancy

Sexual arousal is the end result of physiological changes brought about by neurobiological changes in the central nervous system. Arousal can be divided into three components: *central nervous system arousal*, *non-genital arousal* (nipple erection, increase in blood pressure and heart rate) and *genital arousal* (Graziottin 2004). In genital arousal there is an increase in vaginal lubrication, lengthening and dilatation of the lumen of the distal vagina, uterine elevation from the posterior vaginal wall (vaginal tenting), increase in clitoral length and diameter and engorgement of the labia and clitoral bulbs (Levin 2003).

Release by the efferent nerve fibres of nitric oxide and VIP controls the relaxation of the vascular smooth muscle allowing for the increase in the blood flow to the cavernosal tissues. The role of the epithelium in arousal is through its sensory function of touch, which stimulates release of neurotransmitters (Martin-Alguacil *et al.* 2006). These trigger central nervous system arousal via the pudendal nerve.

There are many conflicting studies on sexual desire and arousal and their relationship to the menstrual cycle. Riley and Trimmer (1991) maintain that in the most reliable studies peaks of sexual interest have been consistently shown to occur in the midfollicular and late luteal phases and that the physiological response is significantly lower during the periovulatory phase than in the immediate post-menstrual and midluteal phases. The difficulties inherent in such studies are that vulval and related problems may make sexual intercourse painful, thus influencing the willingness to engage in sexual activity. In normal circumstances, a minority of women (35–42%) would appear to have very little interest in sexual intercourse (Reader 1991). Low-oestrogen oral contraceptives have also been shown to have an adverse affect on sexual desire and arousal (Caruso *et al.* 2004).

In pregnancy there is a change in the hormonal environment created by the steroid and protein hormones produced by the placenta (Casey *et al.* 1992). Blood flow through the pelvic circulation is increased fivefold during the first 2 months of pregnancy and doubles again during the third month. Progesterone causes an increase of venous distensibility (Gallagher 1986) and in the progesterone-dominant state of pregnancy predisposes to vulval varicosities. In addition, the diminished availability of glycogen from the vaginal epithelium renders the vagina more likely to candidal overgrowth, which is reported to be 10–20 times more common in pregnant than in non-pregnant women (Wallenburg & Wladimiroff 1976). The immune system is downregulated in pregnancy (Toder & Shomer 1990), perhaps to ensure the survival of the fetal allograft, and

these alterations appear to make the pregnant woman more susceptible to primary infection, reinfection and reactivated infection (Brabin 1985). Pregnancy may cause hyperpigmentation of the labia majora.

Injury to the genital tract and vulva may occur during delivery, and rupture of vessels outside the wall of the genital tract may lead to paragenital haematoma formation. In these cases, it is important to ascertain whether the haematoma lies above and below the levator ani muscle (Beazley 1981). Infrlevator haematomas may occur as the result of an inadequate episiotomy repair, which allows continual ooze into the surrounding tissues. In this way a great quantity of blood can escape into the ischioanal fossae or paravaginal tissues. Injuries to the vaginal wall and vulva that affect the perineum and may occur spontaneously or as a result of episiotomy are important as they may be associated with functional disturbances later.

The menopause and old age

The menopause is defined by the World Health Organization as the permanent cessation of menstruation resulting from the loss of follicular activity. The term perimenopause is the period beginning with the first menopausal symptoms and menopause is not reached until a full year after the final menstrual period (Burger 1996). Thereafter, oestrogen and progesterone levels remain low while gonadotrophin levels increase and may remain elevated for perhaps 20–30 years (Davey 1981). The median age for the menopause is 50 years for women in Western industrialized societies, but somewhat earlier in non-European women (Ginsberg 1991). The current life expectancy for Western women is now about 80 years, so the menopause will constitute a third of a woman's life.

The postmenopausal changes in the genital and urinary tracts are a result of the fall in oestrogen levels. The vagina becomes less rugose, narrower and drier and the epithelium more fragile and easily damaged (Schaffer & Fantl 1996). Microscopically the epithelial layers are reduced in number as is the intracellular glycogen, and the stroma is infiltrated with lymphocytes and plasma cells. As a consequence of these changes the vaginal environment becomes alkaline (Davey 1981, Utian 1987) and the number of lactobacilli are reduced (Hillier & Lau 1997).

The vulval changes include loss of hair on the labia majora and central part of the mons pubis (Bologna 1995) owing to a loss in the number of hair follicles. The labia majora become less prominent and slack due to loss of the subcutaneous fat and the introitus may become patulous. Loss of muscle tone contributes to vaginal and uterine prolapse. Urinary incontinence is present in about 12% of women over 65 years of age and in as many as 9% of

younger women (Thomas *et al.* 1980), and this will have significant clinical effects upon the skin of the perineum.

Changes similar to those in the vaginal epithelium also occur in the vulval vestibule, transitional epithelium of the urethra and bladder with the consequent increased risk of recurrent urethritis and cystitis. These changes may be modified, to an undefined extent, by hormone replacement therapy.

Sexual desire and arousal are also reduced (Berman 1999). A decrease in skin sensitivity to touch and pressure occurs with low oestrogen levels (Romanzi *et al.* 2001), which may also be a contributory factor. The clitoral erectile tissue also diminishes (O'Connell *et al.* 1998).

Premature ovarian failure affects approximately 1% of all women under the age of 40 years (Baber *et al.* 1991). Although premature ovarian failure may be caused by some genetic defect, an autoimmune disorder or following treatment for malignancy, no definite cause can be established in the majority of affected women. It has also been proposed that oestrogen deficiency may follow tubal ligation in some women (Cattanach 1985).

Immune responsiveness

Skin is an important site for antigen presentation and both epidermal Langerhans cells and dermal dendritic cells participate in T-cell-mediated immune responses initiated in the skin (Williams & Kupper 1996). An intact immune surveillance of skin is important in the defence against bacterial, fungal and viral infections. Langerhans cells are important in the immune responses of squamous epithelia and it is interesting that there is a variation in their distribution throughout the genital tract. The vulva has the highest density and the vagina the lowest (Edwards & Morris 1985). There is no change of the Langerhans cell density during the different phases of the menstrual cycle in the vagina and little change in the epithelial thickness (Patton *et al.* 2000).

An important component of the genital immune system is the cervical mucus, which contains antibodies, in particular secretory immunoglobulin A (IgA). This locally produced antibody is bactericidal in the presence of lysozyme and complement and can agglutinate bacteria and opsonize them for phagocytosis. In addition, secretory IgA can inactivate antigens by forming non-absorbable complexes with them, and can diminish an organism's adhesiveness to mucosa. It also has the capacity to neutralize viruses.

Large numbers of allogeneic spermatozoa enter the female reproductive tract during coitus and some penetrate the tissues of the female host (Zamboni 1971, Hafez 1976). The invading spermatozoa are destroyed by an immune response that generates cytolytic T lymphocytes specifically

effective against the alloantigens expressed by the spermatozoa (McLean *et al.* 1980). This coital immune response is limited, by the immunosuppressive function of seminal fluid, to the immediate postcoital period (Thomas & McLean 1984) so that the conceptus, which expresses paternal alloantigens, is not subject to immune attack during implantation. Protection against viral infection also requires an effective cytolytic T-lymphocyte response, and any limitation of this response will increase the possibility of an oncogenic virus in the ejaculate escaping destruction. Seminal fluid, by virtue of its physiological role in reproduction, may limit the antiviral response and thereby predispose the female genital tract to viral infection. An additional factor that may render sexually active young women more vulnerable to such infection is the reduced immune responsiveness associated with anovulant contraceptives (Gerretsen *et al.* 1980). It is also known that pregnancy reduces immunity. Organ transplant recipients are also vulnerable as their therapeutic immunosuppression renders them more susceptible to malignant neoplasia. This has been noted in young women developing vulval squamous cell carcinomas (Caterston *et al.* 1984).

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