# PART 1

# *Basic structure and function of the cervix*

CHAPTER 1

# *Morphogenesis and differentiation of the cervicovaginal epithelium*

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## INTRODUCTION

Although the study of prenatal life dates back to antiquity, the first attempt to describe human prenatal development, utilising only human material, was the *Manual of Human Embryology* published in two volumes by Kiebel and Mall in 1910 and 1912. The Carnegie collection, established by Mall in 1915 with 813 human embryos, is now, with its several thousand specimens, the most important and most thoroughly studied collection of human embryos in the world.

As neonates of placental mammals must be capable of sustaining extra-uterine life, all essential organs are developed and functional at birth. This prerequisite for survival is achieved during markedly different periods of gestation. An interval of 14–16 days between fertilisation and parturition is sufficient for the hamster, 20–23 days suffice for mice and rats while the comparable period for elephants is 22 months and for humans is approximately 38 weeks. The first 8 weeks of prenatal human life constitute the embryonic phase and the remaining 30 weeks the fetal phase. During embryogenesis, all essential organ systems develop and, with the exception of the lungs, are functional while the recognisable form of the human infant is established. The majority of congenital anomalies arise as a result of defective morphogenesis during the later part of embryogenesis and early part of the fetal period.

Because development is a continuous process, various means have been used to identify and tabulate the progression of events during normal human embryogenesis. The founder of the Carnegie collection, Mall, was the first to introduce staging into human embryology. Mall's observations, together with those of his successor, Streeter, form the basis upon which the first 8 weeks of human development are described in 23 Carnegie stages. Using the Carnegie collection and other human embryos, including some fertilised *in vitro*, O'Rahilly (1973) produced the first comprehensive and authoritative account of the prenatal period from fertilisation to the end of the third week of gestation (Carnegie stages 1–9). In 1987, with Muller as co-author, the 1973 publication was revised and extended to cover the whole of embryogenesis (Carnegie stages 1–23).

Generally, the crown–rump length of larger embryos and all fetuses should be stated in preference to, or at least in addition to, the supposed age (O'Rahilly and Muller, 1987) but, in this account, the reference point will be the postovulatory age, i.e. the length of time since the last ovulation, related, when appropriate, to the Carnegie stage. As ovulation and fertilisation are closely related in time, the postovulatory interval is an adequate measure of embryonic age. Embryonic age, length and stage are all interrelated. Age, however, conveys an immediate meaning as it is a familiar yardstick, but it must be recognised that prenatal ages are only as useful as postnatal ages, because they are reference points for the usual pattern or range of developmental events.

Much of the available information on human embryogenesis is derived from traditional embryological studies. However, with the advent of assisted conception and the establishment of the Human Fertilisation and Embryology Authority, direct observation of the human embryo *in vitro* has provided an additional source of information on the preimplantation phase of human embryogenesis. Furthermore, the use of sophisticated computer technology in association with specifically designed three-dimensional transvaginal probes allows more detailed *in vivo* three-dimensional ultrasound reconstructions of embryos and early fetuses. The results obtained using these techniques correspond well to those obtained from classical human embryology (Blaas *et al.*, 1998).

## EARLY EMBRYOGENESIS

The embryo, at fertilisation, initiates, sustains, directs and controls its own development. Embryogenesis begins with fertilisation, Carnegie stage 1, and is followed by a phase of preimplantation development, Carnegie stages 2 and 3. Implantation begins on day 6 and is completed on day 12, Carnegie stages 4 and 5. During these few days, the embryo enters into an intimate vascular relationship with the mother and becomes dependent on her for continued existence. Significant events are also taking place within the embryo during this period: continuous cell division and differentiation; the formation of the blastocyst within which is the embryonic

pole; the appearance of the amniotic cavity within the embryonic pole on day 7 and, with it, the formation of two basic cell types, ectoderm and endoderm, establishing the bilaminar embryo; the appearance of the primitive umbilical cord on day 11; and, during Carnegie stage 6, days 13–15, the formation of the primitive streak and the conversion of the embryo into a trilaminar structure of ectoderm, intraembryonic mesoderm and endoderm.

The central nervous system and the skin, together with its appendages, arise from ectoderm; endoderm gives origin to the gastrointestinal tract, the respiratory tract and the urogenital sinus, while the remaining body structures are formed from intraembryonic mesoderm, within which the pericardial, pleural and peritoneal cavities also develop. The flat trilaminar early embryo, however, remains bilaminar at two sites, the buccopharyngeal membrane at the rostral end of the embryo and the cloacal membrane at its caudal end.

## FLEXION

The neural tube, derived from ectoderm, is enclosed within the intraembryonic mesoderm during Carnegie stages 9–13, days 19–28. The rapid growth of the neural tube causes the flat trilaminar embryo to fold along its longitudinal and transverse axes, creating a marked dorsal convexity and ventral concavity and forming head, tail and lateral folds (Fig. 1.1a and b).

The endoderm is drawn into the ventral concavity of the embryo and is subdivided into foregut, midgut and hindgut. The hindgut is caudal to the rostral limit of the allantoic diverticulum and also dorsal and rostral to the cloacal membrane (Fig. 1.1a). The intraembryonic mesoderm in the midembryo region (Fig. 1.1b) is subdivided into paraxial mesoderm, lateral mesoderm and intermediate mesoderm. The paraxial mesoderm surrounds the neural tube and is the site of somite formation. The lateral mesoderm, so named because of its location in the flat trilaminar embryo, is carried ventrally by the formation of the lateral folds. The lateral mesoderm accommodates the primitive peritoneal cavity, which divides it into splanchnopleuric mesoderm, associated with endoderm and destined to form the visceral muscle of the gut tube and bladder, and somatopleuric mesoderm, associated with ectoderm and destined to participate in the formation of the body wall.

The intermediate mesoderm extends the length of the body cavity. It is lateral and ventral to the paraxial mesoderm and adjacent to the midline, dorsal mesentery of the gut tube. At the caudal end of the primitive peritoneal cavity, the intermediate mesoderm is in continuity with the mesoderm investing the terminal portion of the hindgut and, ventral to the hindgut, with the urorectal septum (Fig. 1.2). Within the intermediate mesoderm, structures involved in the morphogenesis of the urinary and reproductive systems develop.

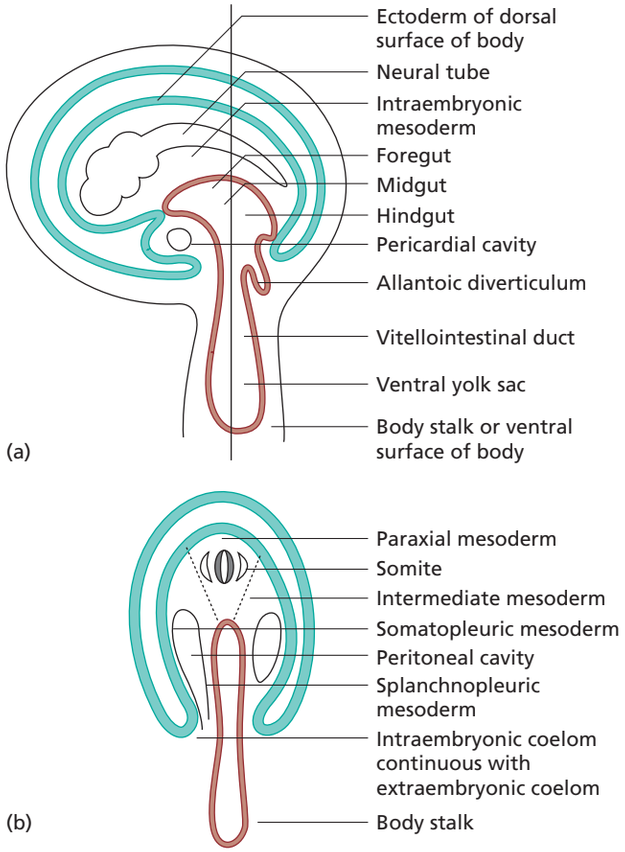



*Fig. 1.1* (a) A midline section of the embryo after formation of the head and tail folds. (b) A transverse section of the midembryo region after formation of the lateral folds.

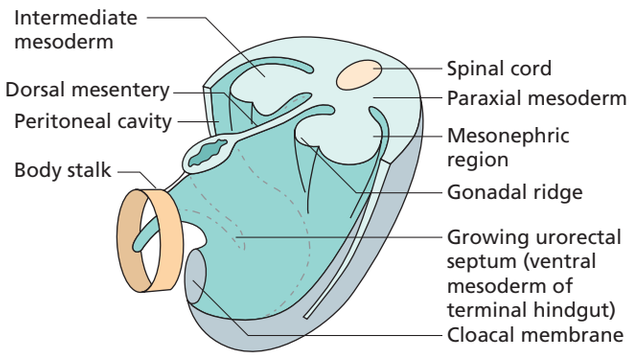
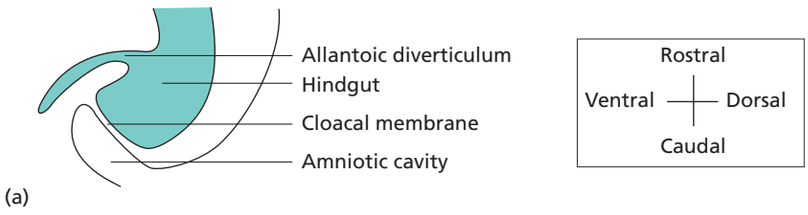



*Fig. 1.2* The caudal half of the embryo showing the gonadal ridge and mesonephric region of the intermediate mesoderm which, at its caudal limit, is continuous with the mesoderm investing the hindgut.

### The urorectal septum

The hindgut, established during the process of flexion, is the endoderm enclosed within the tail fold of the embryo. It lies caudal to the rostral limit of the allantoic diverticulum and



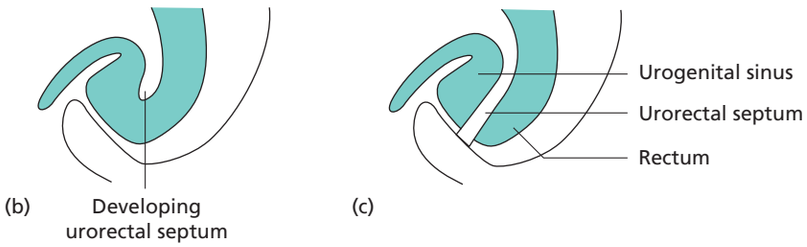




*Fig. 1.3* (a) The primitive hindgut is enclosed within the embryonic tail fold. (b) The developing urorectal septum grows dorsally and caudally from the rostral limit of the allantoic diverticulum. (c) The fusion of the urorectal septum with the cloacal membrane divides the hindgut into urogenital sinus and rectum.

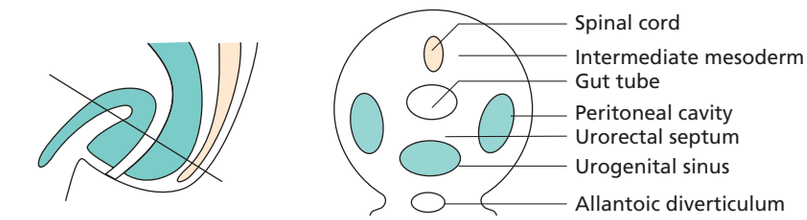



*Fig. 1.4* A transverse section through the embryonic pelvis demonstrates the continuity of the intermediate mesoderm with the mesoderm of the urorectal septum.

dorsal and rostral to the cloacal membrane (Fig. 1.3a). The mesoderm at the rostral limit of the allantoic diverticulum extends dorsally and caudally in the line of the tail fold curvature, as the urorectal septum, dividing the hindgut into ventral and dorsal parts. As the division proceeds, the two parts of the hindgut remain in continuity with each other caudal to the advancing mesoderm of the urorectal septum (Fig. 1.3b). The mesoderm reaches the cloacal membrane at 30–32 days as the Carnegie stage moves from 13 to 14 (O'Rahilly and Muller, 1987). As the urorectal septum fuses with the cloacal membrane, the embryonic hindgut is completely divided into the ventral urogenital sinus and dorsal rectum (Fig. 1.3c).

The urorectal septum, interposed between the dorsal gut tube and the ventral urogenital sinus, is in direct continuity across the wall of the gut tube with the intermediate mesoderm (Fig. 1.4).

## The mesonephric ducts

The first indication of the urinary system in the human embryo appears at 21 days when mesonephric vesicles develop within the intermediate mesoderm (O'Rahilly and Muecke, 1972). These vesicles associate medially with branches of the dorsal aorta and laterally with a solid rod of cells developing within the lateral part of the intermediate mesoderm at 24 days. This solid rod of cells acquires a lumen at 26 days and forms the mesonephric duct. The mesonephric vesicles open into the mesonephric duct as it extends caudally through the intermediate mesoderm. Skirting the gastrointestinal hindgut, the mesonephric duct enters the urorectal septum to reach the posterior (dorsal) surface of the urogenital sinus into which it opens at 28 days during Carnegie stage 13 (O'Rahilly, 1977). At 30–32 days (O'Rahilly and Muller, 1987), the urorectal septum completes the separation of the urogenital sinus from the rectum. The functioning mesonephros produces an increase in pressure in the closed urogenital sinus and ruptures the ventral part of the cloacal membrane, allowing urogenital sinus endoderm to come into apposition with body wall ectoderm and the urogenital sinus to communicate with the amniotic cavity (Ludwig, 1965).

In 1759, Caspar Friedrich Wolff described a symmetrical pair of paravertebral swellings in the chick embryo as the precursors of the kidneys. The adjective Wolffian is therefore often used to describe the duct and vesicles of the mesonephros (Stephens, 1982). The mesonephros has only a transient renal function in the human embryo, but the mesonephric duct is crucial to the subsequent morphogenesis of the kidney and female reproductive tract. As the urorectal septum reaches the cloacal membrane at 30–32 days, the caudal end of each 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; Davies, 2001). The portion of the mesonephric duct incorporated into the urogenital sinus subsequently forms the

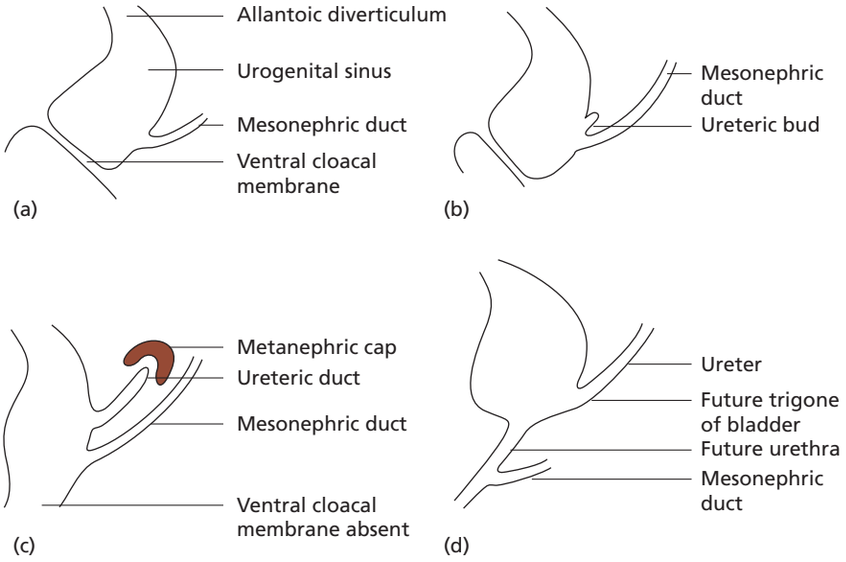



*Fig. 1.5* (a) The mesonephric duct, within the urorectal septum, opens into the urogenital sinus. (b) The caudal limit of the mesonephric duct gives origin to the ureteric bud. (c) The metanephric cap forms at the growing end of the ureteric bud or duct. (d) Tissue of mesonephric origin is incorporated into the posterior aspect of the urogenital sinus between the ureter and the mesonephric duct.

trigone of the bladder and the posterior wall of the urethra (Fig. 1.5). The ureteric bud, arising from the mesonephric duct, ascends the duct's path of descent and, acquiring a lumen, eventually forms the collecting system and ureter of the ipsilateral kidney (Davies, 2001) with the metanephric cap forming renal tissue.

## The paramesonephric ducts

Johannes Müller (1830), describing genital development, identified another cord of cells on the outer aspect of the mesonephric or Wolffian cord. He concluded that, although the two cords were either attached or adjacent to each other, they were 'two quite different things'. These Müllerian ducts, now termed paramesonephric ducts, appear in the human embryo between Carnegie stages 16 and 17 at 37–41 days (O'Rahilly and Muller, 1987). Each duct appears as an invagination of the peritoneal epithelium on the lateral aspect of the intermediate mesoderm at the cephalic end of the mesonephros (Felix, 1912; Faulconer, 1951). Initially, each paramesonephric duct extends caudally as a cord of cells in the intermediate mesoderm, in close association with, and initially lateral to, the mesonephric duct (Fig. 1.6). The mesonephric duct has been shown experimentally both to induce the paramesonephric duct (Didier, 1973a,b) and to guide its descent (Gruenwald, 1941); indeed, the growing caudal tip of the paramesonephric duct lies within the basement membrane of the mesonephric duct (Frutiger, 1969). As the paramesonephric cord of cells continues its descent, a lumen appears in its cranial portion and extends caudally behind the growing tip of the paramesonephric cord, converting it into a duct. During descent, the paramesonephric duct passes ventral to the mesonephric duct and completes its journey to

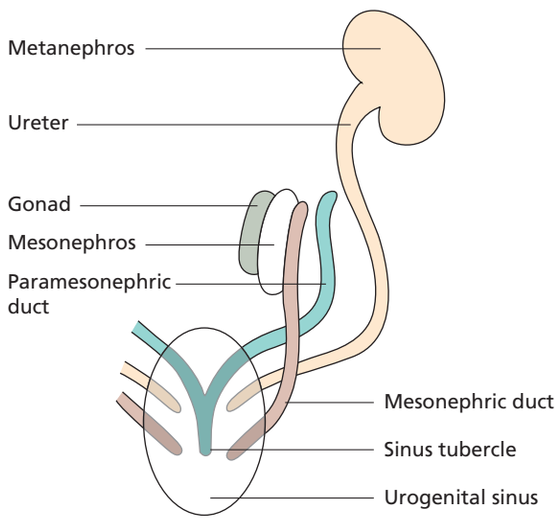



*Fig. 1.6* The indifferent human embryo possesses mesonephric and paramesonephric ducts. The terminal paramesonephric ducts fuse within the urorectal septum and reach the urogenital sinus at the sinus tubercle situated between the openings of the two mesonephric ducts.

the posterior aspect of the urogenital sinus within the urorectal septum on the medial aspect of the mesonephric duct and in close apposition to the contralateral paramesonephric duct (Fig. 1.6). Indeed, as soon as the paramesonephric ducts come into close contact with each other, they begin to fuse even before their growing ends reach the urogenital sinus (Koff, 1933). The external surfaces of the medial walls, initially in apposition, begin to fuse and eventually the duct lumina are separated only by a median septum (O'Rahilly, 1977).

At 49 days, before the paramesonephric ducts reach the urogenital sinus, a tubercle appears on the internal aspect of its

posterior wall, between the openings of the mesonephric ducts. This tubercle is not formed by the paramesonephric ducts but identifies the site at which the common paramesonephric duct fuses with the posterior wall of the urogenital sinus at 56 days (Glenister, 1962; Josso, 1981).

### Sexual determination and differentiation

The sex of an individual is determined at fertilisation with sexual dimorphism being brought about by subsequent differentiation. The genetic or chromosomal sex of the zygote determines the gonadal sex of the embryo, which itself regulates the differentiation of the internal and external genital apparatus and hence the sexual phenotype of the individual. At puberty, the development of secondary sexual characteristics reinforces the phenotypic manifestations of sexual dimorphism, which achieves its biological fulfilment in successful procreation.

Both male and female embryos possess the same indifferent gonadal and genital primordia. The indifferent gonad differentiates as a testis under the influence of 'a battery of genes on the Y chromosome . . . and certain genes on other chromosomes' (Mittwoch and Burgess, 1991; Slaney *et al.*, 1998). In placental mammals, sexual dimorphism is mediated by the testis and its secretions and takes place in an environment of high estrogen and progestogen concentrations. However, it has long been recognised that these differentiating processes are regulated by numerous specific genes, located on sex chromosomes and autosomes, that act through a variety of mechanisms, including organising factors, sex steroid and peptide secretions and specific tissue receptors (Grumbach and Conte, 1981). More recently, 79 genes have been identified as being specifically expressed in the developing gonad and sex ducts, and 21 of the gonad-specific genes showed sexual dimorphic expression, suggesting a role in sex determination and/or gonad differentiation (Wertz and Herrmann, 2000).

## GENITAL DUCT DIFFERENTIATION

During Carnegie stage 14, at 32 days, the gonadal ridge begins to form on the medial aspect of the mesonephros (O'Rahilly and Muller, 1987). Until approximately 42 days, Carnegie stage 17, this ridge forms the indifferent gonad, and male and female embryos are morphologically indistinguishable (Fig. 1.7). The transformation of the indifferent gonad into an embryonic testis or ovary takes place over the following 14 days, during Carnegie stages 18–23.

At the end of the embryonic period, with the completion of Carnegie stage 23, the fetus has either testes or ovaries, but possesses both mesonephric and paramesonephric duct systems (Fig. 1.7). Subsequent sexual differentiation of these ducts is governed by fetal testicular hormones (Jost, 1947), which cause regression of the paramesonephric ducts and

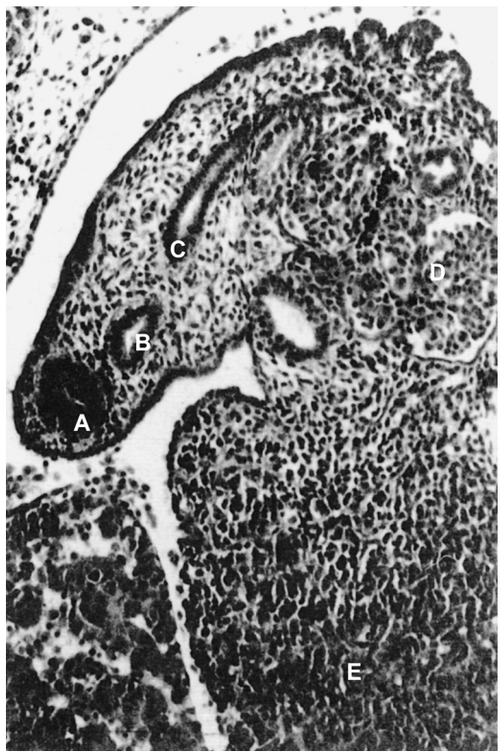



*Fig. 1.7* A transverse section of the right side of the upper abdomen of a 42-day embryo showing: A, the paramesonephric duct with a barely discernible lumen; B, the mesonephric duct; C, a mesonephric tubule; D, the mesonephros; E, the gonad. H&E × 325.

stabilisation of the mesonephric ducts. In the female fetus, the absence of testicular hormones allows regression of the mesonephric ducts and stabilisation of the paramesonephric ducts.

### Male differentiation

#### *Paramesonephric duct*

In the male paramesonephric duct, regression begins at 56–60 days (Jirasek, 1977). Once initiated, regression extends caudally and cranially and is complete at 70 days (Jirasek, 1971). Remnants survive bilaterally: the closed cranial portion is associated with the testis as its appendix (hydatid of Morgagni), and the caudal ends participate in the formation of the prostatic utricle.

Regression of the male paramesonephric ducts is caused by anti-Müllerian hormone (AMH), a glycoprotein produced by Sertoli cells (Blanchard and Josso, 1974; Price, 1979), first identified in the fetal testes at 60 days (Jirasek, 1977). AMH is capable of causing paramesonephric duct regression for a limited time during intrauterine life and, unless its action is initiated early in the fetal period and rapidly completed, the

paramesonephric ducts become resistant to its inhibitory effect (Josso *et al.*, 1977). Persistence of paramesonephric ducts has been observed in otherwise normal human males and in some animals (Jost, 1965; Josso, 1979). This abnormality is X-linked (Sloan and Walsh, 1976) and may be due to a defect in AMH production or a block to its action. The gene for human AMH has been localised to the short arm of chromosome 19 (Cohen-Haguenauer *et al.*, 1987).

The morphological evidence of AMH's action on the paramesonephric ducts indicates dissolution of the basal membrane and mesodermal condensation around the duct (Josso and Picard, 1986). Recent work has confirmed these observations. In transgenic mice of both sexes, overexpression of the human AMH gene is associated with increased apoptosis in the Müllerian epithelium, while in AMH-deficient male mice, there is decreased apoptosis (Allard *et al.*, 2000). Although apoptosis is the decisive event in Müllerian duct regression, the epitheliomesenchymal interaction is required for its completion (Allard *et al.*, 2000). The fetal and postnatal testis continues to produce AMH, and its serum concentration begins to decline only with the onset of puberty (Hudson *et al.*, 1990). Its role during this period is unknown, but it has been suggested that it is involved in the process of testicular descent and the suppression of meiotic maturation of male germ cells (Josso and Picard, 1986). Postnatally, granulosa cells also secrete AMH (Ueno *et al.*, 1989), resulting in serum concentrations in pubertal girls and women that are similar to those in men (Gustafson *et al.*, 1992). These authors observed increased concentrations of AMH in a patient with an ovarian sex cord tumour. A more recent immunohistochemical study of AMH expression in human prenatal and postnatal gonadal tissue confirmed its restriction to Sertoli and granulosa cells and provided evidence of ovarian expression just before birth (Rajpert-DeMetys *et al.*, 1999).

### *Mesonephric duct*

The second aspect of male differentiation is the integration of the mesonephric duct into the genital system when the metanephric kidney begins to function at 50 days (Potter and Osathanondh, 1966). The cranial mesonephric tubules establish continuity with the rete testis to become the vasa efferentia, the mesonephric duct forms the epididymis and vas deferens, while its closed cranial end persists as the appendix of the epididymis.

The persistence of the mesonephric duct and its incorporation into the genital system of the male fetus is testosterone dependent. Testosterone production by Leydig cells of the testis begins at 56 days (Siiteri and Wilson, 1974) under the control of maternal chorionic gonadotrophin (Hudson and Burger, 1979). Stabilisation of the mesonephric ducts occurs between 56 and 70 days in synchrony with the degeneration of the paramesonephric ducts (Price *et al.*, 1975). Mesonephric ducts in young female embryos can be stabilised by exposure to testosterone before the end of the 'critical period' for sex differentiation. In man, this critical period embraces the end of embryogenesis and the beginning of early fetal life. Thereafter, exposure of the female fetus to testosterone does not prevent the degeneration of the mesonephric ducts (Josso, 1981) but will cause varying degrees of virilisation of the external genitalia (Grumbach and Ducharme, 1960).

## Female differentiation

As male differentiation of the paramesonephric and mesonephric ducts occurs in the XY fetus, the comparable structures in the XX fetus are already irreversibly committed to female organogenesis (Josso *et al.*, 1977). Female organogenesis involves regression of the mesonephric ducts and stabilisation of the paramesonephric ducts.

### *Mesonephric duct*

The mesonephric duct has been shown experimentally to induce the paramesonephric duct (Didier, 1973a,b) and to guide its descent (Gruenwald, 1941). The caudal end of each mesonephric duct gives origin to the ureteric bud and is incorporated into the urogenital sinus to form the trigone of the bladder and the posterior wall of the urethra (Fig. 1.5). As the metanephric kidney begins to function at 50 days (Potter and Osathanondh, 1966), the female mesonephric system becomes redundant, although it has been suggested that the mesonephric ducts contribute to the formation of the uterus (Witschi, 1970) and the vagina (Forsberg, 1965).

Towards the end of embryogenesis, the mesonephric vesicles begin to degenerate together with the mesonephric ducts. The lumen of the mesonephric duct is obliterated at 75 days and only remnants persist at 105 days (Josso, 1981). A number of mesonephric derivatives may be located in the adult female. A constant finding is the epoophoron associated with the ovary and derived from the cephalic mesonephric duct and adjacent vesicles (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 incorporated into the cervix (O'Rahilly, 1977; Buntine, 1979). Adjacent to the lower genital tract, such remnants are referred to as Gartner's ducts.

### *Paramesonephric duct*

#### *Uterine tube*

The upper segment of each paramesonephric duct develops fimbriae at its cephalic end and subsequently forms the uterine tube. The transverse lie of the uterine tube is established by the descent of the ipsilateral ovary into the pelvis. The uterotubal junction is demarcated by an abrupt increase in the diameter of the uterine segment.

*Uterus*

The morphogenesis of the uterus begins as the paramesonephric ducts come into apposition within the urorectal septum and begin to fuse. At the end of the embryonic period, the caudal segment of the common paramesonephric duct reaches the posterior wall of the urogenital sinus and fuses with the sinus tubercle (Fig. 1.8a), situated between the openings of the two mesonephric ducts (Glenister, 1962; O'Rahilly, 1977; Josso, 1981). At 63 days, the body and the cervix are distinguishable by the presence of a constriction between them (Hunter, 1930). Linear extension of the cavity occurs by further fusion of the paramesonephric ducts rostrally and by their continued growth caudally (O'Rahilly, 1977). This growing caudal end in contact with the posterior wall of the urogenital sinus induces additional cellular proliferation, essential to the development of the vagina.

*Sinuvaginal bulb*

After the common paramesonephric duct fuses with the sinus tubercle, in the posterior wall of the urogenital sinus, two dorsal projections from the sinus are identified which unite to form the midline sinuvaginal bulb (Fig. 1.8b). As the sinuvaginal bulb arises following the fusion of the common paramesonephric duct with the urogenital sinus, at a site between the openings of the two mesonephric ducts, it has been variously suggested that the vagina, through the contribution made to it by the sinuvaginal bulb, arises from the paramesonephric ducts (Felix, 1912), the mesonephric ducts (Forsberg, 1973), the urogenital sinus (Bulmer, 1957; Fluhmann, 1960) or from a combination of paramesonephric and mesonephric tissue (Witschi, 1970), paramesonephric and sinus tissue (Koff, 1933; Agogue, 1965), or mesonephric and sinus tissue (Forsberg 1973), with the relative contributions of each tissue an additional matter for controversy (O'Rahilly, 1977).

*Vaginal plate*

Subsequent proliferation of the lining epithelium of the sinuvaginal bulb converts it into a midline solid tissue projection, displacing the genital canal, at the caudal limit of the common paramesonephric duct, in a dorsal direction (Fig. 1.8c). The solid sinuvaginal outgrowth and the solid caudal end of the genital canal together form the vaginal plate identified in the fetus at 87 days (Fig. 1.8c). The formation of the vaginal plate is followed immediately by its caudal extension towards the cloacal vestibule. As this caudal extension occurs, desquamation of cells from the vaginal plate establishes the uterovaginal canal (Fig. 1.8d), opening into the cloacal vestibule at 14 weeks (Terruhn, 1980).

*Uterine cervix and vagina*

The cervix, which forms the distal two-thirds of the fetal uterus (Pryse-Davies and Dewhurst, 1971), is of paramesonephric origin (Koff, 1933; Forsberg, 1965; Witschi, 1970) with its epithelium probably derived from the urogenital sinus (Fluhmann, 1960). The solid sinovaginal bulb and the caudal portion of the common paramesonephric duct together form the vaginal plate, which establishes the vagina after canalisation.

For many years, the conflicting opinions expressed concerning the morphogenesis and differentiation of the cervicovaginal epithelium were of little more than academic interest.

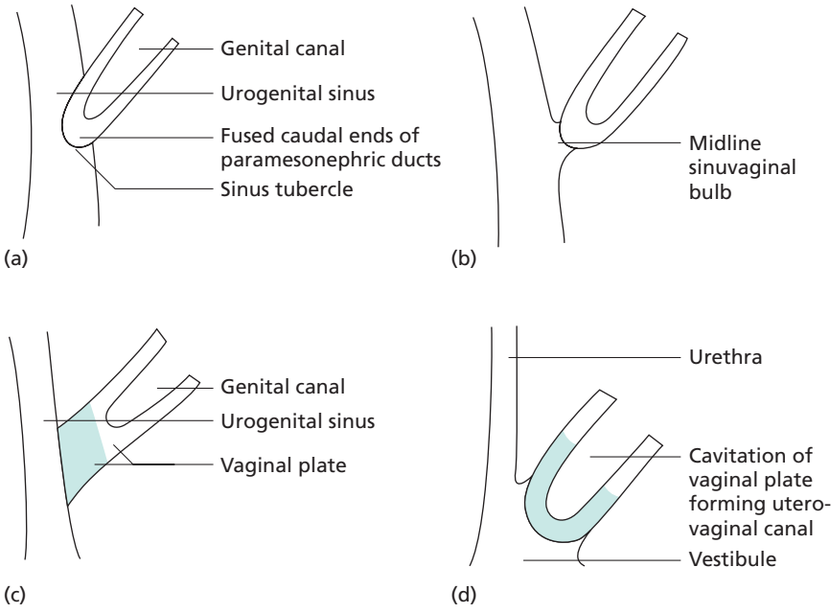



*Fig. 1.8* (a) The fused paramesonephric ducts form the genital canal, the solid caudal end of which abuts the posterior wall of the urogenital sinus at the sinus tubercle. (b) Cellular proliferation of the sinus epithelium generates the sinuvaginal bulbs, which fuse and displace the genital canal dorsally. (c) Further cellular proliferation converts the sinuvaginal bulb into a solid tissue projection which participates in the formation of the vaginal plate. (d) Extensive caudal growth of the vaginal plate brings its lower surface into the primitive vestibule.

However, since the early 1970s, many publications have associated the occurrence of cervical and vaginal ridges, vaginal adenosis, ectropion and clear cell carcinoma of the vagina in young adult females with prenatal exposure to diethylstilbestrol (Greenwald *et al.*, 1971; Herbst *et al.*, 1971, 1972, 1974; Fetherston *et al.*, 1972; Hill, 1973; Pomerance, 1973; Barber and Sommers, 1974). The drug was considered to have had a teratogenic effect on the developing lower genital tract. Uterine synechiae and hypoplasia in 60% of exposed females indicate that the teratogen also affects the upper genital tract (Kaufmann *et al.*, 1977). In addition, some 20% of exposed males demonstrate some abnormality of their reproductive tracts such as epididymal cysts, hypoplastic testes, cryptorchidism and spermatozoal deficiencies (Gill *et al.*, 1976).

Differences of opinion concerning the interpretation of the morphological evidence of vaginal development may indicate a variation in the sequence of events involved in its morphogenesis. Nevertheless, it is reasonable to assume that the sinuvaginal bulb, arising from the urogenital sinus (Koff, 1933; Bulmer, 1957), forms the lower part of the vagina. The occurrence of certain congenital anomalies strongly supports this interpretation; otherwise, the presence of a rectovaginal fistula would be difficult to explain, as would the opening of a misplaced ureter into the vagina (Bremer, 1957) and the foreshortening of the vagina in the congenital absence of paramesonephric ducts.

When the vaginal opening reaches the vestibule at 14 weeks (Terruhn, 1980), the sinus and paramesonephric elements both contribute to the vaginal canal, with the sinus portion exhibiting stratified squamous epithelium and the paramesonephric portion pseudostratified columnar epithelium. At this stage, the genital canal has a cervical dilatation which marks the region of the vaginal fornices (Koff, 1933), while at 17 weeks the future os is identified as the site of the squamocolumnar junction (Bulmer, 1957). During this interval, the pseudostratified columnar epithelium of the paramesonephric component of the vagina has been transformed into stratified squamous epithelium (Bulmer, 1957; Davies and Kusama, 1962). It has been variously reported that, at 22 weeks, the cervical canal is lined with stratified squamous epithelium with an entropion present (Eida, 1961), while from 22 weeks to term the squamocolumnar junction is said to be situated some distance external to the os, producing the congenital ectropion (Davies and Kusama, 1962).

A possible interpretation of the evidence is that the cervix and the upper segment of the vagina are initially lined by paramesonephric tissue, which subsequently undergoes apoptosis to be replaced by sinus tissue. In explanation of the teratogenic effect of diethylstilbestrol, it has been suggested that the drug delays or limits this apoptosis and replacement and adversely affects the persisting paramesonephric tissue (Ulfelder and Robboy, 1976). Certainly, the occurrence of upper genital tract abnormalities associated with prenatal exposure to diethylstilbestrol (Kaufmann *et al.*, 1977) supports these suggestions.

In the newborn, the stratified squamous epithelium of the vagina shows evidence of a marked estrogen response. The site of the junction between the cervical and vaginal epithelia is variable and there is a range of normal appearances.

## Role of p63

Apoptosis, programmed cell death, is an integral part of all biological systems and occurs in response to cascades of signals that are highly conserved throughout the animal kingdom. The protein p53, first described in 1979, is the gene product of *TP53*, which maps to chromosome 17p12. p53 has several functions in the cell and is often described as 'the guardian of the genome' because of its role in preventing replication of cells with damaged DNA. Loss or mutation of *TP53* is probably the commonest single genetic change in cancer (Strachan and Read, 1999).

p63 is a recently characterised p53 homologue (Reis-Filho and Schmitt, 2002). p63 protein, the gene for which maps to chromosome 3q27, is required for cutaneous development and is expressed in immature squamous epithelium and reserve cells of the cervix (Ince *et al.*, 2002). As humans with p63 mutations exhibit defects in genitourinary development (Ince *et al.*, 2002), its role in female genital tract development has been investigated in mice. In mice, the transformation of vaginal paramesonephric pseudostratified columnar epithelium to stratified squamous epithelium takes place during the first week of neonatal life (Forsberg, 1963).

In the reproductive tract of the adult female mouse, p63 is highly expressed in basal cells of the vaginal and cervical epithelium but not in uterine epithelium (Kurita and Cunha, 2001). These investigators, although unable to detect p63 in embryonic paramesonephric epithelium, demonstrated its expression in the differentiated vaginal epithelium during the first week of postnatal life. They further demonstrated that neonatal exposure to diethylstilbestrol induced irregularities in p63 expression and epithelial differentiation during the first week of postnatal life. They concluded that such exposure disturbs uterine and vaginal epithelial differentiation by perturbing epithelial expression of p63 during development.

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