EMBRYOLOGY OF THE REPRODUCTIVE SYSTEM

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LEARNING OBJECTIVES:

- Name the structures in kidney development that contribute to the development of the reproductive organs.
- Predict how the presence or absence of the Y chromosome and the expression of the SRY gene would influence the development of the gonads.
- Predict how the presence or absence of testosterone, dihydrotestosterone, and anit-Mullerian hormone would influence the development of the genital ducts and indifferent primordia of the external genitalia.

I. Introduction

In general, the function of the genital (reproductive) system in males and females is the **formation, nurture, and transport of germ cells**. In females, an additional function is to provide the **proper milieu for the fetal development** after conception.

Like the urinary system, the genital system derives from **intermediate mesoderm**. The development of these two systems is tightly interwoven as structures that develop as parts of the urinary system gain function in the genital system.

In the adult, the sexual organs differ between males and females. The early genital system, however, is similar in both sexes, and the sexual differentiation of this **initially indifferent, bipotential system** starts only in the seventh week of embryonic development. The details on how sexual differentiation is determined will be discussed below, but it is worth mentioning here that irregularities in this process result in **disorders of sexual differentiation (DSDs). DSDs** occur in approximately 1 in 4,500 live births and will be discussed in a separate lecture.

II. Development of the Kidneys, Mesonephric Duct, and Paramesonephric Duct.

We will briefly review the development of the kidneys because structures that result from kidney development eventually function as genital ducts in the male reproductive system and persist in the form of remnants in the female reproductive system.

The bilateral kidneys develop from intermediate mesoderm. Their development occurs in three phases; in each phase, one pair of kidneys develops. The first two pairs regress; only the last pair, the hindkidney or metanephroi (sing. metanephros), persists in the adult. There is also a spatial aspect to the development of the three pairs: They develop in a cranio-caudal manner.

A. *Pronephroi*

The pronephroi appear early in week 4 in the cervical region. While the pronephroi degenerate quickly, their ducts, the **pronephric ducts**, which connect the pronephroi with the cloaca, persist.

B. *Mesonephroi*

The mesonephroi appear late in week 4 in the upper to mid thorax. The mesonephroi are composed of glomeruli and corresponding **mesonephric tubules**. These mesonephric tubules are connected to the ventral portion of the cloaca through the bilateral **mesonephric ducts**, which derive from the pronephric ducts. Like the pronephroi, the mesonephroi also degenerate, but in males some of the **mesonephric tubules persist as efferent ductules**, which connect the testis with the epididymis. The **mesonephric ducts (Wolffian ducts)** form several derivatives in the male reproductive system (see below).

C. *Metanephroi*

The metanephroi and their metanephric ducts start to form in the fifth week in the abdominal region and become the permanent kidneys. They begin to excrete urine by week 9.

D. Formation of the paramesonephric ducts

On each side, parallel to the mesonephric duct, an additional duct, **the paramesonephric duct (Mullerian duct)** forms by invagination. These ducts open cranially into the peritoneal cavity and are the origin of many female reproductive organs. Initially, both pairs of genital ducts, the mesonephric and paramesonephric ducts, are formed in male and female embryos. It is only during the phase of sexual differentiation that one set of ducts develops further while the other regresses (Fig. 1).

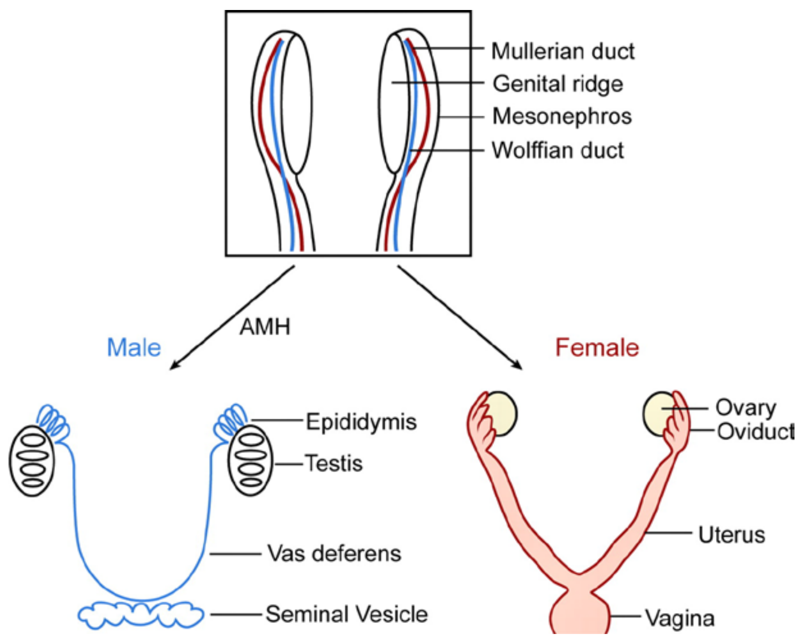

Fig 1. Development of the gonads and genital ducts. Initially, a pair of indifferent gonads (here shown in the stage of the genital ridge) and two sets of ducts, the mesonephric (Woffian) and paramesonephric (Mullerian) duct develop in male and female embryos. During sexual differentiation, in males, driven by the SRY gene, the indifferent gonads develop into testes. Under the influence of hormones produced by the testes, the mesonephric ducts develop into the duct system; the paramesonephric ducts regress. In females, in the absence of the SRY gene, ovaries develop and the paramesonephric ducts develops into the ovarian tubes, uterus, and parts of the vagina; the mesonephric ducts regress. © Wilhelm D. et al., Physiol. Rev 87, 2007

III. Development of Indifferent Gonads

A. *Contributing tissues*

The gonads, the organs that produce germ cells (sperms in males, oocytes in females), derive from three sources:

1. **Mesothelium**
   This is epithelium of mesodermal origin, which lines the posterior abdominal wall.
2. **Underlying mesenchyme**
   This is embryonic connective tissue, which, like the mesothelium on its surface, derives from intermediate mesoderm.
3. **Primordial germ cells**
   These are the earliest, undifferentiated sex cells. They are large, spherical cells, which are initially not located in the developing gonads but **in the secondary yolk sac**.

B. *Development*

Starting at week five, medial to the mesonephros, the mesothelium and its underlying mesoderm start to proliferate and thereby form the **gonadal ridge** (also called genital ridge, Fig. 1). The mesothelium forms finger-like cords, **gonadal cords**, which project into the underlying mesenchyme. The outer layer of mesothelium forms the **cortex** and the mesenchyme interspersed with the gonadal cords forms the **medulla** of the indifferent gonads.

When the embryo starts to fold, and consequently the secondary yolk sac is incorporated into the embryo, the primordial germ cells start to migrate along the dorsal mesentery of the hindgut toward the gonadal ridge. By week six, they enter the medulla of the gonads and are incorporated into the gonadal cords. The primordial germ cells proliferate, both, during their migration and after they have been incorporated into the gonadal tissue.

IV. Sex Determination

A. Role of the Y chromosome in the development of genitalia

The genetic sex of an individual is determined at fertilization: While the oocyte always carries an X chromosome, the sperm can carry a Y or X chromosome and therefore fertilization can result in a female (XX) or male (XY) zygote. A gene on the Y chromosome, the **sex-determining region of the Y chromosome (SRY) gene,** has been proven to be critical in sexual differentiation. Only if the cells carry a Y chromosome and express the SRY gene, will the indifferent gonads develop into testes and subsequently will the other reproductive organs develop a male phenotype. In the absence of the SRY gene, the individual will develop ovaries and a female phenotype.

The SRY gene encodes for a transcription factor that causes the indifferent gonads to develop into testes (see below for details). The hormones produced by the testes then direct the genital ducts and the indifferent external genitalia (see below) to develop into the male phenotype. In the absence of SRY, the indifferent gonads develop into ovaries and overall female genitalia form.

B. Other factors that influence sexual differentiation

Note that for a long time SRY was considered to be the only determining factor of sexual differentiation. Newer research, however, indicates that the effects of SRY on the testes is mediated by **Sox9** and further regulated by other transcription factors, (e.g., steroidogenic factor 1 [SF1] and Wilms' tumor suppressor gene 1 [WT1]). Furthermore, in females, it seems not to be simply the absence of SRY but also the expression of other, ovary-promoting factors that induces the development of female genitalia.

V. Development of Internal Genitalia

A. *Male*

1. **Testes**
   a. **Sertoli cells**
   Starting at week 8, under the influence of SRY, the somatic cells of the indifferent gonads develop into Sertoli cells. Sertoli cells have various functions in the developing testes as well as in the adult testes:
      i. They surround and thereby protect the primordial germ cells. In the fully developed testes they support spermatogenesis.
      ii. They induce the differentiation of Leydig cells (see below).
      iii. They produce **anti-Mullerian hormone (AMH)**. AMH suppresses the development of the paramesonephric (Mullerian) ducts into female genitalia.

   b. **Leydig cells**
   During weeks 8-9, Leydig cells start to develop under the influence of Sertoli cells. They produce the hormone testosterone, which in turn induces the development of the mesonephric ducts into the definite male genital ducts (see below).

   c. Starting at weeks 7-8, the **seminiferous tubules** and a network of connecting tubules, the rete testes, is formed.

   d. A fibrous capsule, the **tunica albuginea** develops around the organ.

   e. The primordial germ cells cease proliferating during fetal development, but resume mitosis shortly after birth.

2. **Genital ducts and glands**
   In males, under the influence of testosterone (produced by the Leydig cells), the mesonephric tubules and mesonephric ducts develop into the male genital ducts and glands. Tissue around the urogenital sinus converts testosterone into **dihydrotestosterone (DHT)**, which also regulates some of the developments described below. The paramesonephric ducts regress under the influence of AMH; the only remnants are the prostatic utricle (a small outpouching of the prostate gland) and the appendix testis.

   In detail, the following structures form in the male.

   a. The persisting mesonephric tubules become the **efferent ductules**. They connect the rete testis, a network of ducts in the testis, with the mesonephric duct, and later with its derivative, the duct of the epididymis.

   b. The proximal part of the mesonephric duct develops into the convoluted **duct of the epididymis**, into which the efferent ductules open.

   c. More distal, the mesonephric duct develops into the **ductus deferens**.

   d. A lateral outgrowth at the caudal end of the mesonesphric duct develop into the **seminal vesicle**.

e. The continuation of the ductus deferens, into which the duct of the seminal vesicle merges, is the **ejaculatory duct**. The ejaculatory ducts from both sides drain into the prostatic urethra.
f. The **prostate** and the **bulbourethral glands** are outgrowths from the urethra, which itself is of endodermal origin.

B. ***Female***

1. **Ovaries**
   a. The primordial germ cells continue to proliferate and the cells get pushed toward the periphery of the developing ovaries. Cells closer to the center enter meiosis starting around week 20, whereas more peripheral primordial germ cells continue to proliferate. The cells that have entered meiosis (primary oocytes) go in meiotic arrest in meiosis I; they resume meiosis only in puberty, when one oocyte at a time continues its meiotic division.
   b. Beginning at week 21, somatic cells, **granulosa cells**, start to surround individual oocytes and thereby form **primordial follicles**. Formation of primordial follicles continues until week 28. After that period oocytes continue to grow and the size and number of surrounding granulosa cells increases.
   c. Midgestation, **theca cells**, which are capable of synthesizing steroid hormones, start to form.
   d. After birth, a fibrous capsule, the **tunica albuginea**, forms around the ovary.

2. **Genital ducts and glands**
   Testosterone, DHT, and AMH are virtually absent in the developing female genitalia. This hormonal milieu induces the development of the paramesonepric duct and the regress of the mesonephric duct, which persists in the female only in the form of a few, non-functional remnants (i.e., appendix vesiculosa, epoophoron, paroopheron, Gartner duct cysts).
   In detail, the following structures develop from the paramesonephric duct:
   a. On each side, a **uterine tube** develops from the cranial portion of the paramesonephric duct.
   b. The caudal portions of the paramesonephric ducts from both sides fuse and form the **uterovaginal primordium**, which develops into the **uterus** and the superior part of the **vagina**.
   c. The **urethral and paraurethral glands** are outgrowth from the urethra; the **greater vestibular glands** are outgrowths from the urogenital sinus.
   d. A bicorneate uterus forms when the fusion of the caudal portions of the paramesonephric ducts is incomplete.
3. The inferior portion of the **vagina** develops from the urogenital sinus.

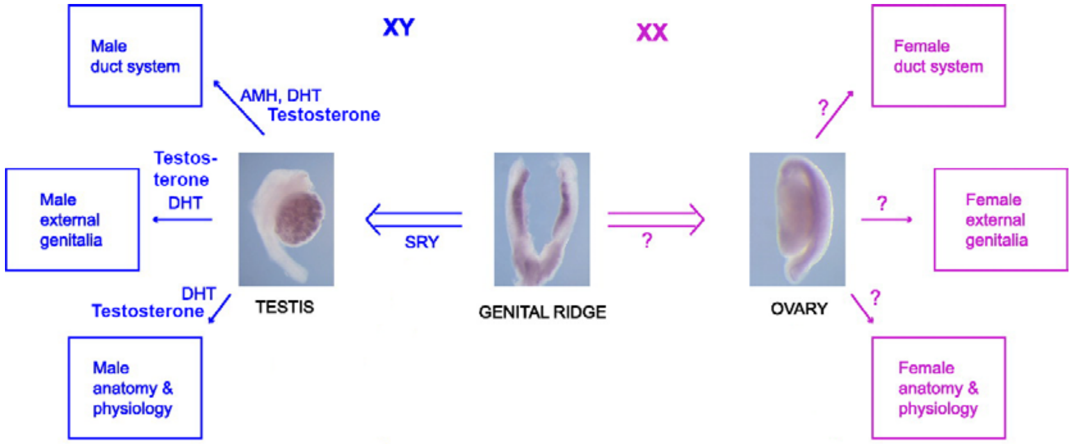

Fig 2. Development of the Sexual phenotype in individuals with XY and XX genotype. Modified from Wilhelm D. et al., Physiol. Rev 87, 2007

VI. Development of External Genitalia (Fig. 3)

A. *Indifferent primordia*

Initially, the external genitalia are also indifferent. In week 4, a **genital tubercle** is formed cranially to the cloacal membrane. On both sides of the cloacal membrane, **urogenital folds** can be found; lateral to those **genital swellings** (scrotolabila swellings) are located. In week 8, the cloacal membrane breaks down and results in the opening of the urogenital sinus through the urogenital orifice. The genital tubercle grows into the primordial phallus. Their sex differentiation starts only in gestational week 9 and is regulated by sex hormones.

B. *Male*

Male external genitalia develop under the influence of testosterone and DHT.

1. The primordial phallus enlarges and elongates to form the *glans penis*.
2. The urogenital folds fuse and thereby form the **spongy urethra** and the **body of the penis**. At the same time, a canal forms in the glans penis that connects the spongy urethra with the tip of the glans penis; its opening is the **external urethral orifice**. *Failure of the urethral folds to completely fuse results in* ***hypospadias****, which is characterized by an ectopic urethral opening on the ventral surface of the penis.*
3. The genital swellings develop into the scrotum. The testes, which develop in the abdominal cavity descend through the inguinal canal into the scrotum around the time of birth. *In 3-5% of boys born at full term, the testes have not completely descended, causing* ***cryptorchidism****. If the condition does not resolve spontaneously until the first year of life, surgical correction is required, because undescended testes tend to develop germ cell tumors and/or become atrophic.*

C. *Female*

In the absence of male sex hormones, the indifferent primordia develop into female external genitalia.

1. The primordial phallus grows relatively little and develops into the **clitoris**.
2. The genital folds only fuse posteriorly; there they form the frenulum of the labia minora. The unfused parts develop into the **labia minora**.
3. The genital swellings fuse anteriorly and develop into the anterior labial commissure and the **mons pubis**. Posterior they fuse and form the posterior labial commissure. For the largest part, they remain unfused and form the **labia majora**.

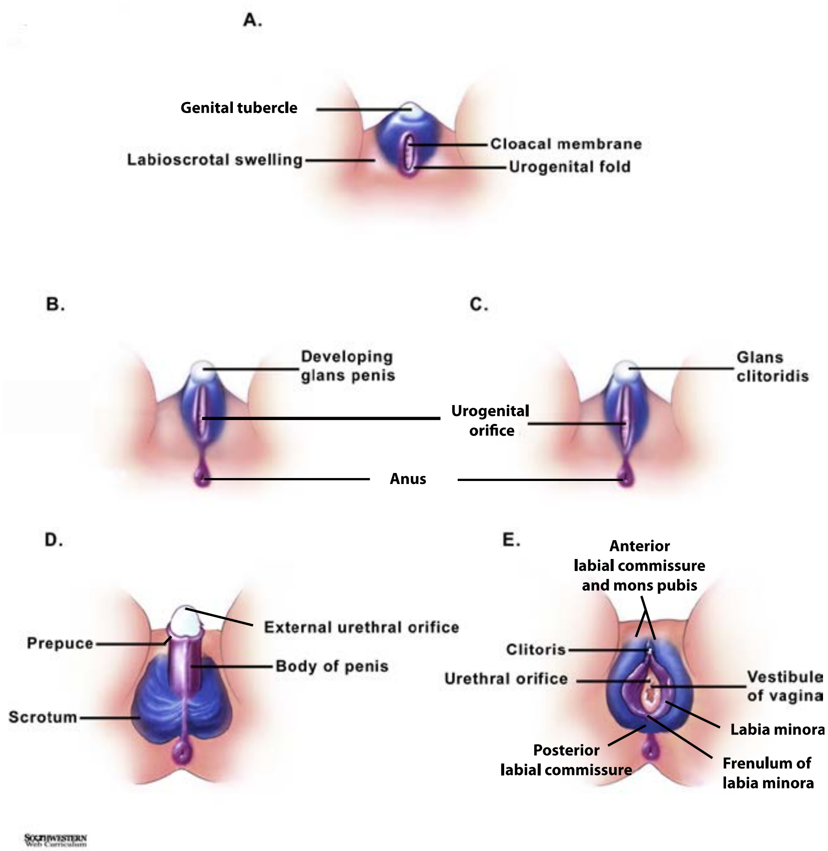

Fig 3. Development of the external genitalia. Starting from indifferent primordia (A), male (B+D) or female (C+E) external genital develop.

Questions:

1. Which statement best describes the function of a hormone that is produced in Leydig cells?
A. It induces the development of the uterus.
B. It suppresses the development of the paramesonephric duct.
C. It supports the differentiation of the indifferent gonads into testes.
D. It induce the development of Sertoli cells.
E. It supports the differentiation of the mesonephric duct.

What is most likely to happen in the absence of AMH?
A. The cranial portions of the paramesonephric ducts from both sides will develop into the uterus.
B. The mesonephric tubules will become involved in the transportation on gametes.
C. A caudal portion of the paramesonephric ducts will contribute to the formation of the vagina.
D. Leydig cells will start to produce testosterone.
E. The formation of the ductus deference will be induced.

Which is a correct pairing between a structure of the indifferent external genital primordia and its derivative?
A. Cloacal membrane – frenulum of labia minora
B. Genital tubercle – labia majora
C. Genital swelling – labia minora
D. Genital swelling – scrotum
E. Urogenital fold – glans of penis

Answers:
1-E
2-C
3-D