Types of Sex and Normal Sexual Differentiation

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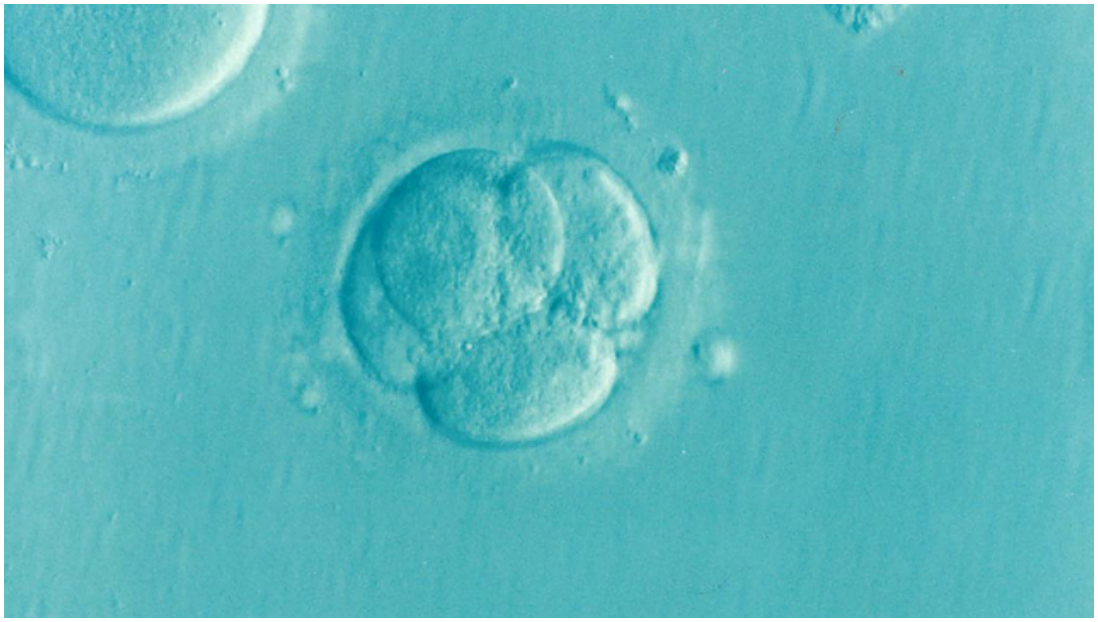
This article deals with the normal sexual development in humans. It defines the various terms used to describe gender and sex, from genetic to phenotypic sex. The genetics behind sex determination is shortly dealt with, as well as the development of the internal and external genitalia in embryonic and fetal life. The differentiation of the mesonephric and paramesonephric ducts is briefly described, and we end with a short summary of sex determination in the male and the female.



Definition

Gender identity is determined by the genetic, gonadal, and phenotypic sex. Genetic or chromosomal sex is defined by the sex chromosomes, typically XX or XY. **Chromosomal or genetic sex determines the differentiation of the gonads** into testes or ovaries – gonadal sex.

95% of the Y chromosome has multiple copies of unique genes expressed in the testis, with one copy of the SRY (Sex-determining Region on Y) gene on Yp11.3. Gonadal sex determines the type of fetal hormones produced and thereby the differentiation of the external genitalia into male or female form. In addition, the production of male or female hormones from the gonads at puberty leads to the appearance of secondary sexual characteristics.

Phenotypic sex is determined by the external genital appearance and by the development of the secondary sexual characteristics at puberty. The sex hormones act

on the brain to create sexual differentiation which, together with the rearing and sex assignment by the caregivers, and secondary sexual development, gives rise to gender identity.

Gender identity refers to the individual's behavior in terms of sexuality, whether in reference to the gestures, turns of speech, hobbies and recreations, and dreams.

The following definitions are relevant to the understanding of normal sex development, both somatic and psychosexual:

- Sex determination—the transformation of the indifferent gonad into a testis or an ovary.
- Sex differentiation—development of the phenotype (somatotype) as an expression of hormones produced by the gonad (in reality, testis only during fetal development).
- Gender (sex) assignment—allocation of male or female at birth, usually instantaneous.
- Gender identity—the sense of self as being male or female.
- Gender role—sex-typical behaviors and preferences in which males and females differ (e.g. toy preferences, aggression).
- Sexual orientation—refers to the target of sexual arousal.
- Gender attribution—assigning as male or female on the first encounter with an individual.

Genetic Sex Determination

SRY expression upregulates Sox9 and Fgf9 (both operating in a positive feedback loop), but downregulates Wnt4, Rspo1, Dax1 and Fox12 to produce the Sertoli cells of the testes. Fgf9 inhibits Rspo1.

The XX genotype lacks SRY. Female-specific activation of Wnt4, Rspo1, and other genes lead to Sox9 repression to produce the granulosa cells of the ovaries. If these **genes are deleted, an ovotestis is formed containing testicular cords** and Leydig cells.

Wnt4 activates Rspo1 which regulates ovarian differentiation by:

- Repressing mesonephric cell migration via Wnt4.
- B-catenin mediated loss of cell adhesion between germ cells, thus enabling their meiosis.
- Downregulating Sox9.

Thus, the developmental fate of the bipotential embryonic gonad is decided by the presence or absence of the functional SRY, which tips the balance towards testis or ovary development, respectively.

The fetal testis then synthesizes anti-mullerian hormone (AMH) at about 7 weeks as well as testosterone, both of which determine the male phenotype, while the **absence of these hormones causes the female phenotype** to develop.

Embryonic Gonadal Development

Week 5

- Genital or gonadal ridge arises over the mesonephric duct.
- Primordial germ cells (PGC) migrate into it and proliferate.

- In mice, this requires surface receptor tyrosine kinase c-KIT, and stem cell factor from surrounding cells.

This is the indifferent or **bipotential stage of gonadal development**. If germ cells are absent, testicular cords may develop but not ovarian tissue (leading to streak ovaries). Germ cell differentiation depends on the chromosomal sex of the somatic cells in the gonads rather than their own.

Week 6–7

- Testis begins to develop.
- Somatic cells express SRY and, in turn, Sox9, to form Sertoli cells which become testicular cords surrounding the PGC.
- Sertoli cells are the first to differentiate in fetal testis.
- PGC give rise to the fetal spermatogonia.
- Cells are surrounded by Sertoli cells and by myoid cells which later promote sperm transport.
- Spermatogonia become part of the testicular cords and are arrested in mitosis as prospermatogonia.
- Begin to multiply again after birth.

Sertoli cells produce:

- Androgen-binding protein (ABP).
- Inhibin.
- Fgf9 which stimulates proliferation of neighboring cells and increases Sox9 expression.
- Prostaglandin D which promotes non-SRY cells to express Sox9 and differentiate into Sertoli cells.

At a certain level, Sox9 inhibits further SRY expression.

Week 8

- Interstitial cells become fetal Leydig cells and endothelial cells under SRY control.
- Leydig cells produce testosterone, which peaks at weeks 15–18.
- After they start to regress until only a few cells remain at birth.
- Fetal human chorionic gonadotropin levels regulate and are mirrored by fetal Leydig cells (peaking at about 10 weeks and lowest at 20 weeks).
- Leydig cells also respond to ACTH.
- **Endothelial cells form the testicular interstitial network.**

ACTH and hCG initiate sexual differentiation, which is later maintained by fetal pituitary LH levels. The **fetal Leydig cells multiply and produce more testosterone in response to high gonadotropins** (positive feedback loop). Later, they are replaced by adult Leydig cells, which are activated at puberty, and show a negative feedback loop to hCG.

Week 8–9

- Ovarian differentiation is 2 weeks later in the absence of the Y.
- Requires the presence of somatic cells, and germ cells.

Oogenesis begins at 6–8 weeks, increases between 12 and 16 weeks, and peaks around 16–20 weeks at about 5–7 million.

At 9 weeks, germ cells undergo mitosis forming oogonia.

11–12 weeks

- Approximately, 5 million begin meiosis to become primary oocytes.
- Proceed throughout pregnancy to be arrested in diplotene of meiosis I by full-term by granulosa cell secretions.
- Begin to form primordial follicles at about 18 weeks.
- Primordial follicle comprises an oocyte arrested in prophase I surrounded by one layer of pregranulosa cells and a basement membrane.

20 weeks

- Ovarian cortex and stroma are present, with the rete ovarii consisting of vestigial tubules and Leydig cells.
- Activins and BMP promote primordial follicle development, but **inhibins and AMF inhibit development.**

Oocytes and granulosa cells are linked by gap junctions with proteins called connexins. These junctions allow ions, pyruvate or nucleic acids, amino acids, cholesterol and second messengers. **Oocytes depend on granulosa cells for nutrition**.

At birth, about 1–2 million oocytes are left, while the others undergo apoptosis. About **20.000 oocytes are left by puberty, and no oogonia**.

- FSH induces resumption of meiosis through the gap junctions.
- Meiosis I is completed just before ovulation.
- Meiosis II coincides with fertilization.
- One polar body is extruded each time.
- Gonadotropins stimulate oocytes only when enclosed in granulosa-cumulosa cells.
- The follicular fluid contains sterols which stimulate oocytes meiosis and maturation.

Causes for germ cell loss after 20 weeks:

- Follicular growth and atresia.
- Regression of oocytes in great numbers during meiosis.
- Degeneration of oogonia not enclosed by granulosa cells.
- Migration to the surface to become part of the ovarian surface or pass out into the peritoneum.

Germ cell loss after birth is due to follicular growth and atresia.

Chromosomal anomalies such as **Turner syndrome (45XO) accelerate germ cell loss after the mitosis stage leading to streak ovaries in 80–90% of patients**. Spontaneous menstrual cycles occur only due to mosaic Turner’s syndrome (45 XX, 45 XO).

Paramesonephros (Mullerian) Differentiation

The **Mullerian ducts arise as a cleft lined by the coelomic epithelium**, between the gonadal and mesonephric parts of the urogenital ridge. Wolffian and Mullerian ducts co-exist up to 8 weeks of life. Jost established that female differentiation is constitutive but male differentiation requires active factors.

Sertoli cells secrete AMH or MIF (Mullerian inhibiting factor), of the Transforming Growth

factor-beta family (chromosome 19), leading to ipsilateral Mullerian regression.

Mullerian structures develop if there is no gonad or a normal ovary. This depends on the absence of a Y chromosome and of a functional testis (absence of AMH). Mullerian duct development follows that of the Wolffian duct. Wolffian duct (renal) abnormalities, therefore, accompany Mullerian anomalies.

Week 10

- Mullerian ducts fuse in the midline forming a Y-shaped structure which contacts the sinusal tubercle in the urogenital sinus.
- The fused lower part becomes the uterus and upper 2/3 of the vagina.
- The unfused upper part becomes the fallopian tubes.

Week 8–11

- Lower 1/3 of the vagina is formed from the sinovaginal bulbs on the posterior wall of the urogenital sinus.

Week 12

- Clitoris and vaginal vestibule are formed.

Week 20

- Differentiation of uterine endometrium.

Week 22

- Canalization to form uterine, cervical and vaginal lumen.
- Paraurethral or Skene glands are outgrowths from the urethra.
- Bartholin’s glands or greater vestibular glands arise from the phallic or definitive urogenital sinus.
- Vestigial Mullerian structure in males: prostatic utricle.

Wolffian Differentiation

The paired Wolffian ducts arise from the urogenital ridge of the intermediate mesoderm, laterally to somites 8–13, and end in the cloaca.

Week 9–13

- Cranial part develops into the epididymal head.
- Efferent ducts connect it to the testis.

In the presence of high local levels of testosterone from the adjacent testis and in response to growth factors such as epidermal growth factor EGF and basic fibroblast factor (bFGF), **Wolffian differentiation gives rise to the**:

- Epididymis.
- Vas deferens.
- Seminal vesicles.

The **Wolffian ducts elongate to 6 meters (in adult life), and coil by proliferation**.

- Distal straight end forms the vas deferens.
- Regulated by the HOX genes.
- Vas deferens connects the epididymis to the ejaculatory duct.
- **Surrounding smooth muscle helps move sperm into the ejaculatory**

duct and the urethra.

- Posterior Wolffian ducts give rise to the seminal vesicle.
- Urogenital sinus becomes the prostate.
- Testosterone is also converted to dihydrotestosterone which promotes the differentiation of the urogenital sinus and tubercle.
- They **become male external genitalia, the urethra and the prostate.**
- Ureteric buds arise from the distal mesonephros, branch and grow into the metanephric blastema.
- Form the collecting system of the kidneys, while the metanephric blastema forms the nephrons proper.
- Cloaca is divided by the urorectal septum into the anterior urogenital sinus and posterior rectum.
- Superior urogenital sinus forms the bladder and urethra.
- Attached mesonephric ends and ureteric buds form the bladder trigone.
- Inferior urogenital sinus forms the phallic or definitive urogenital sinus.
- **In the absence of testosterone, Wolffian regression occurs.**
- Vestigial Wolffian structures in the female: epoophoron and Gartner's cyst.

Importance of the Wolffian ducts:

- First opening of the embryo to the outside environment via the cloaca.
- Precursor of the metanephros.
- Induction of paramesonephric duct development.
- Forms male internal genitalia.

Phenotypic Sex: Sexually Indifferent Stage

Week 5-6

- Folds form on either side of the cloaca, and meet anteriorly in the mid-line to form the genital tubercle.
- Anal membrane lies posteriorly.
- On either side are labioscrotal swellings.
- Urorectal septum divides the cloaca into anterior urogenital and posterior rectal compartments.
- Cloacal folds become urogenital and anal folds, respectively.

Phenotypic sex: female

Week 10

- Genital tubercle becomes clitoris.
- Urogenital folds become labia minora.
- Labioscrotal swellings become labia majora in the absence of DHT.
- Definitive urogenital sinus becomes vaginal vestibule with the openings of the urethra, vagina, and Bartholin's glands.

Phenotypic sex: male

Week 10

- **Male development occurs in response to DHT and the presence of abundant androgen receptors** and 5-alpha reductase activity.
- Genital tubercle becomes the penis.

- Urogenital folds enclose the penile urethra giving rise to the penile raphe.
- Labioscrotal swellings become the scrotal sac with the fused portion becoming the scrotal raphe.

Week 10–12

- Process is complete.

Summary of Sex Determination in the Fetus: Male

Week 1:

Primordial germ cells (PGC) migrate through primitive streak.

Week 5:

- Primordial indifferent gonads develop.
- Wolffian ducts grow.
- PGC differentiate.

Week 6:

- PGC reach gonadal ridge.
- Müllerian ducts differentiate.

Week 6–7:

Testicular or seminiferous cords develop.

Week 8–9:

- AMH secretion begins.
- Leydig cells form.
- Cranial regression of Müllerian ducts.

Week 9:

- Leydig cells produce testosterone.
- Wolffian ducts differentiate.
- Urogenital sinus and external genitalia begin to assume male phenotype.
- Testes begin to descend.

Week 10:

- Mullerian ducts disappear.
- Prostatic buds appear.

Week 12:

- Seminal vesicles develop.
- Testis reach internal inguinal ring as cranial suspensory ligament dissolves.

Week 14:

Male urethra completed.

Week 20:

- Testosterone levels reduced.
- Prostatic utricle forms.

Week 24:

Penis begins to grow.

Week 27–30:

Inguinoscrotal descent of testis.

Summary of Sex Determination in the Fetus: Female

Week 1:

Primordial germ cells (PGC) migrate through primitive streak.

Week 5:

- Primordial indifferent gonads develop.
- Wolffian ducts grow.
- PGC differentiate.

Week 6:

- PGC reach gonadal ridge.
- Müllerian ducts differentiate.

Week 10:

- Medullary oocytes enter meiosis I.
- Wolffian ducts start to degenerate.

Week 12:

- Vaginal cord forms.
- Primordial follicles appear.

Week 14:

Primary follicles appear.

Week 22:

Vaginal development reaches perineum.

Week 24:

Graafian follicles appear.

Week 36:

AMH secreted by primary and secondary follicles.

Medical conditions

There are cases when the fetuses does not undergo the above describe processes. There are several factors that cause the difference in the development process; this includes a hormone imbalance and environmental factors among others. There are three hormonal conditions that changes the way the reproductive organs develop in both genders.

Androgen Insensitivity Syndrome (AIS)

This condition occurs in both sexes, but is most common in males than females. AIS is when an individual is genetically male, with one X and one Y chromosome, but the person is resistant to the male hormones. Since this condition is a genetic fault, it means that the body can't respond to testosterone properly. The testes secrete anti-Müllerian hormone and the Wolffian ducts do not receive the proper signals to develop. Both duct systems degenerate.

Congenital Adrenal Hyperplasia (CAH)

This condition is characterized by females having overactive adrenal glands. The condition encompasses a group of autosomal recessive disorders, each of which involves a deficiency of an enzyme involved in the synthesis of cortisol, aldosterone or both. Absence of 21-hydroxylase results to the deletions of CYP21A, which is the most common form of this condition.

5 α–Reductase Deficiency:

Males with 5 α–reductase deficiency (5-ARD) do not undergo the same prenatal sexual differentiation as other males. The enzyme 5 α–reductase helps to regulate proportions of male sex hormones in the body before birth and during puberty. When males have a deficiency of 5-ARD, they will have typical development of the testes, but their external genitalia will resemble that of a female until puberty. During puberty, males will have levels of testosterone that are elevated enough to experience the same changes as males without this condition. After puberty, their genitalia do not look fully masculinized, although many are reported to live perfectly normal male lives.

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Notes