

# Reproduction – sexual differentiation

## Recommended textbook for reproductive biology

M.H. Johnson & B.J. Everitt, *Essential Reproduction*, Blackwell. Third edition (1988) or later.

### Topics to think about

Why do many organisms have sex? Why are there two sexes (and not one, or three)? How does the difference between gametes contribute to the different reproductive strategies of male and female?

Are the mother and fetus working towards the same goals? When might their goals differ? How might such differences lead to problems, and to sex-linked phenotypic differences?

*The Red Queen*, by Matt Ridley (1994), is a fascinating look at these questions. Highly recommended, though has little direct bearing on your course.

### Genetic determinants of sex

- Humans have 46 chromosomes: 22 pairs of *autosomes* and one pair of *sex chromosomes*.
- The male has one X and one Y sex chromosome (the *heterogametic sex*). The female has two X chromosomes (*homogametic*).
- The **Y chromosome** is small and carries few (2) genes<sup>1</sup> – far too few to make a testis, for example. Its function is to confer maleness on the embryo, and it does so by altering the expression of genes on other chromosomes. The critical gene is on a region of the short arm of the Y chromosome and is called **testis-determining factor (TDF)**. If this is present, the embryo will be gonadally male; if it is absent *the default is for the embryo to develop as a female*.
- The precise mechanism by which the TDF gene causes initiates testicular development is unknown.
- The **X chromosome** is large and carries many (>50) genes. The presence of two active X chromosomes in the female would be problematic, so *all but one* X chromosome is inactivated in somatic cells, a process called *lyonization*. The inactive X chromosome is visible as a *Barr body*. The inactivation occurs in somatic cells early in embryonic life, is random (i.e. either the maternal or paternal X chromosome is inactivated), is nearly complete (virtually all of the chromosome is switched off), is permanent and is clonally propagated – i.e. all progeny of a cell with an inactivated paternal X will have their paternal X switched off. As a result, the female is a **mosaic** of cells, each functionally *hemizygous* for one or other X chromosome. Obviously, in germ cells the inactivation is reversible!

### Abnormalities of the sex chromosomes

Remember that a normal karyotype is written 46,XY or 46,XX – the number is the total number of chromosomes.

#### Gonadal males:

- **Klinefelter's syndrome – 47,XXY**. Occurs in 1/1000 male live births. Patients are phenotypically male, as you would expect, but there is postpubertal testicular failure resulting in small testes, infertility due to azoospermia and variable signs of hypogonadism. Occasionally gynaecomastia occurs. Average IQ is slightly reduced – but if the man has more than one X chromosome, the likelihood of mental retardation increases.
- **XXY syndrome**. Can only result from meiotic nondisjunction in the father. Occurs in ~1/1000 male live births; found in 4–20/1000 inmates of mental or penal institutions in the UK... causal? Only phenotype uniformly present is tall stature. Also called “super male”.
- **Sex reversed – 46,XX<sup>sxr</sup>**. The X<sup>sxr</sup> chromosome is the result of a translocation that places the TDF gene on the X chromosome, resulting in an XX male. Incidence ~1/24,000.

#### Gonadal females:

- **Turner's syndrome – usually 45,X**. Incidence at birth is 3/1000. Phenotypically female with gonadal dysgenesis and sexual immaturity. Characterized by primary amenorrhoea and infertility; also short stature and a host of somatic abnormalities including webbing of the neck, increased carrying angle at the elbow and cardiovascular and renal abnormalities. More than half are 45,X. About 15% are 46,XX but have abnormalities (usually deletions) of an X chromosome. The rest are mosaic, with one cell line being 45,X and the other being something else (46,XX; 46,X and abnormal X; 47,XXX etc.). Mosaicism implies that nondisjunction was *mitotic* and followed fertilization, rather than arising from parental meiotic nondisjunction.
- **“Triple X” – 47,XXX**. Also called “super female”.

#### Non-viable:

- **Triploidy**, be it 66,XXX or 66,XXY. The gonad is determined by the presence/absence of a Y chromosome but a triploid fetus is not viable.

<sup>1</sup> Gelehrter & Collins (1990), *Principles of Medical Genetics*, p176.

## Gonadal development

- **Early development of the gonad is identical in males and females.** The gonads are derived from two distinct tissues: *somatic mesenchyme*, which forms the matrix of the gonad, and the *primordial germ cells (PGCs)* which migrate into this matrix to form the gametes.
- The *genital ridge primordia* form at ~4 weeks in humans on either side of the aorta in the lower thoracic and upper lumbar regions. They overlie the developing *mesonephric tissue*. Columns of cells from the mesonephros and the coelomic epithelium form the *primitive sex cords*. PGCs migrate into the genital ridges.
- Thus far we have an *indifferent gonad*. At 6 weeks, gonadal development diverges.
- **Male.** The cortical mesenchyme forms the tough, fibrous *tunica albuginea*. The sex cords deep in the *medullary* region of the gonad develop into seminiferous cords, which will be the *seminiferous tubules* in the adult. The mesodermal cord cells will give rise to *Sertoli cells*. The PGCs will give rise to *spermatozoa*. The loose mesenchyme between the cords will condense to form stromal tissue in which are the *interstitial glands of Leydig*.
- **Female.** The medullary sex cords regress, while the *cortical* coelomic epithelium condenses to surround PGCs and make the *primordial follicles* that are characteristic of the ovary. The mesenchymal cells of the follicles will give rise to the *granulosa cells*, while the PGCs give rise to *oocytes*. Clusters of *interstitial gland* cells form around the primordial follicles.

## Abnormalities of gonadal development

- Initiation of gonadal development does not require functional germ cells – it merely depends on the presence or absence of the Y chromosome. However, subsequent development requires functional germ cells. Women with Turner’s syndrome develop an ovary, but the oocytes subsequently die, leading to secondary loss of the follicle cells and *ovarian dysgenesis* culminating in a regressed or “streak ovary”. Men with Klinefelter’s syndrome form testes normally, but the presence of two X chromosomes leads to death of germ cells as they enter meiosis in later life.
- “Genetic maleness leads to gonadal maleness.” Exceptions to this rule are *extremely rare*, but occasionally individuals are found with both testicular and ovarian tissue – they are called *true hermaphrodites*. In most, if not all cases, this is because of the presence of a mixture of XY and XX (or XO)<sup>2</sup> cells – in other words, *chimaerism*.<sup>3</sup>

## Internal genitalia

Once the gonads have formed, **genetic sex is unimportant** and the gonads assume control of sexual differentiation.

- Ovarian hormone are *not* essential to fetal sexual differentiation. However, the testes secrete two essential hormones. The mesenchymal interstitial cells of Leydig secrete **androgens** (steroid hormones) and the Sertoli cells of the seminiferous cords secrete **Müllerian inhibiting hormone (MIH)**, a glycoprotein. *In their absence, feminine sexual differentiation occurs.*
- **Primordia.** In the case of the gonads, there was one indifferent primordium. For the internal genitalia, there are two sets of primordia, each of which is *unipotential*. They are the *Wolffian* or *mesonephric duct* (male) and the *Müllerian* or *paramesonephric duct* (female).
- **Female.** In normal females, or a castrated fetus of either sex,<sup>4</sup> female development ensues. The Wolffian ducts regress and the Müllerian ducts persist and develop to give rise to the oviducts (Fallopian tubes), uterus, cervix and possibly the upper vagina.
- **Male.** Androgens actively induce the Wolffian ducts to develop to form the epididymis, vas deferens and seminal vesicles. MIH causes the Müllerian ducts to regress.

## External genitalia

- **Primordia.** Like the gonads but unlike the internal genitalia, the primordia of the external genitalia are *bipotential*.
- **Female.** The urethral folds and genital swellings remain separate and form the labia minora and majora. The genital tubercle forms the clitoris. These changes occur even in the absence of ovaries – again, the female path is the default.
- **Male.** Androgens cause the urethral folds to fuse, enclosing the urethral tube and contributing to the shaft of the penis together with cells from the genital swelling. The genital swellings fuse in the midline, forming the scrotum, and the genital tubercle expands to form the glans penis.

<sup>2</sup> XO is another way of writing X – the “O” means “absence”.

<sup>3</sup> A *chimera* is a mixture of cells of different genotypes. It may be produced naturally or artificially by the mixing of early embryonic cells from two distinct conceptuses. It is similar to *mosaicism*, which refers to an individual with two or more genetically distinct cell lines derived from a single zygote, but differing because of mutation or nondisjunction.

<sup>4</sup> Castration means removal of the gonad (ovary or testis).

## Abnormalities of IG and EG development

- **Internal genitalia.** Failure or blockade of androgen production by the testis will cause regression of the Wolffian duct and failure of development of male internal genitalia. Conversely, exposure of female fetuses to androgens will stimulate Wolffian duct development and cause the development of male internal genitalia. Artificial exposure of female fetuses to MIH provokes abnormal regression of the Müllerian ducts.
- **External genitalia.** Exposure of female fetuses to androgens will ‘masculinize’ their external genitalia, whilst castration or suppression of endogenous androgens in the male results in ‘feminized’ external genitalia.
- **Pseudohermaphroditism** refers to a dissociation between gonadal and phenotypic sex. Two examples are:
  - **Testicular feminization (Tfm).** The genotype is XY; testes develop and secrete androgens and MIH. However, the fetal genitalia are insensitive to androgens. Therefore the Wolffian ducts regress and female external genitalia develop. However, MIH is still being secreted so the Müllerian ducts regress as well. The individual is genetically male and has testes, appears female with labia, clitoris and a vagina, yet lacks other components of the internal genitalia.
  - **Androgenital syndrome (AGS).** The individual is XX female and develops ovaries. However, the fetal adrenal cortex is hyperactive and secretes large quantities of steroids, some of which are androgenic.<sup>5</sup> These androgens stimulate Wolffian duct development and cause the development of male external genitalia. However, no MIH is produced so the Müllerian ducts also develop. The individual appears male with penis and scrotum, but is genetically and gonadally female and has the internal genitalia of *both* sexes.

## Behavioural dimorphism

- Sexually dimorphic behaviour may develop as a result of *endocrine* and *social* factors.
- In rats and other non-primate mammals, for example, exposure to androgens in the first 5 days of life (a *critical period*) enhances their capacity to display masculine patterns of sexual behaviour (*masculinization*) and reduces their capacity to display feminine patterns (*defeminization*). Androgens elicit permanent structural changes in the **medial preoptic area** of the hypothalamus. In the absence of any steroids during the critical period, female behaviour patterns develop.
- In non-human primate (rhesus monkeys), the distinction is less clear – exposing female fetuses to androgens leads to masculinization but not complete defeminization. (However, it is unclear that there is a genuine difference from rats: the primate critical period is longer and earlier, and high doses of exogenous androgens *in utero* are not only toxic but have other effects such as masculinizing the genitalia, so we cannot be certain of the effect on the brain.)
- In humans, the picture is even more blurred. Early androgen exposure in females (such as with AGS) results in childhood behaviour that is more ‘masculine’ (*tomboyism* – increased energy expenditure during play, decreased “parental rehearsal” patterns, decreased interest in ‘feminine’ aspects of personal appearance) but has little if any effects on adult behaviour (normal sexual orientation etc.). However, *social rearing* is of crucial importance. If girls with severe AGS are raised as boys, their adult gender identity is masculine, and if boys with complete Tfm are raised as girls they have a feminine gender identity. This social process is thought to be completed by 2–3 years of age.
- Conversely, a bizarre syndrome from the Dominican Republic provides evidence *against* a major social effect. **Guevodoces** (“penis-at-twelves”) are genetic males born with external genitalia small enough to be classified as female. They have a deficiency of 5 $\alpha$ -reductase, so cannot convert the weak androgen testosterone to the powerful androgen 5 $\alpha$ -dihydrotestosterone. However, at puberty their androgen levels rise to the extent that testosterone is capable of completing masculinization. Despite being raised as girls, they can function behaviourally as men.

## Further prepubertal gonadal development

- **Testis.** The testis migrates from the genital ridge, in the upper lumbar region of the embryo, through the abdominal cavity and over the pelvic brim to the scrotum. Failure of migration leads to **cryptorchidism**. Both androgens and MIH are required (the latter probably to remove Müllerian duct structures that otherwise get in the way). The testis may not migrate so much as stay where it is as the rest of the body grows – it is attached caudally by the fibrous *gubernaculum*, which does not grow.
- In mammals with scrotal testes (e.g. *not* elephants) cryptorchidism impairs spermatogenesis and testicular metabolism. The human testis functions best at 4–7°C below abdominal temperatures and has a “heat exchange” blood supply that helps maintain its low temperature – but this temperature requirement may be a *consequence* of its scrotal position rather than the evolutionary *cause* of its migration. Elephants are perfectly fertile.
- **Ovary.** The ovary remains in the abdominal cavity, though in man it moves slightly to assume a pelvic position. Like the testis, it grows during early life, but its steroid output is minimal and not essential for prepubertal development.

## Secondary sexual characteristics

We will consider this later when we discuss puberty in the two sexes.

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<sup>5</sup> This is also called *congenital adrenal hyperplasia*. It is caused by any of a number of adrenal enzyme defects resulting in deficient cortisol secretion. This in turn causes increased pituitary ACTH secretion and consequent overproduction of adrenal androgens. The precise clinical picture depends on which enzyme is deficient.