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Sexual dimorphism of gonadal development

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Sexual dimorphism is a term describing morphological differences between the sexes, but is often extended to include all differences observed between females and males. Sex differentiation in vertebrates is by definition sexually dimorphic and starts at the level of the sex chromosomes. In this review the sexual dimorphism of gonadal differentiation is discussed, with a focus on human development. In the embryo, the indifferent gonadal anlagen harbours four different cell lineages with bipotential fates dependent on the sex of the individual. The different paths taken by these cell lineages in male and female development are reviewed, along with other sexually dimorphic features of gonadal development. These include sex-determining genes, timing of events, dependence on germ cells, spatial organization of stromal cells, steroidogenic cells types, and other aspects.

Key words: testis; ovary; Sertoli cells; Leydig cells; peritubular cells; granulosa cells; theca cells; primordial germ cells; gonocytes.

Sex differentiation is by definition a sexually dimorphic process which is evident at the chromosomal, gonadal, hormonal, somatic and behavioural levels in adults. No morphological sex differences can be observed in the developing gonads until 42 days post conception (dpc) in humans. This period is therefore referred to as the indifferent stage of gonadal development. Gonadal determination is a critical event in sex differentiation as it establishes the hormonal dimorphism which in turn has a decisive impact on several later events of the male and female paths. This overview will deal with sexual dimorphism of gonadal differentiation, with a focus on human embryonic development whenever possible. Sexually dimorphic aspects of the indifferent gonad, germ-line and precursor-cell lineages, sex determination, sex steroid synthesis, testicular descent, and prenatal events in gametogenesis will be discussed.

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FORMATION OF THE PRIMITIVE GONAD

The gonadal anlagen appear by 32 dpc of human embryonic development. These paired bipotential structures are located at the ventromedial surface of the mesonephros and arise from the mesoderm by contributions from somatic mesenchymal cells from the mesonephros and epithelial cells migrating from the coelomic surface of the gonadal ridge. At this stage no sexual dimorphism can be distinguished morphologically, and germ-cell precursors (gonocytes) are not yet present.

The mesonephros also constitutes the primordium of the adrenal glands and the urinary system. Disruption of genital ridge development by gene targeting of any of several transcription factors in mice invariably leads to severely affected phenotypes with multiple malformations of the urogenital tract, adrenals and other structures.[1–6] Interestingly, several peptide growth factors have also been implicated in gonadal development from the indifferent gonadal anlagen, most notably the insulin-like growth factor superfamily.[7]

Two important transcriptional regulators involved in formation of the urogenital ridge are the tumour suppressor gene Wilms' tumour-associated gene-1 (WT1) and the orphan nuclear receptor steroidogenic factor-1 (SF-1). Disruption of WT1 in mice leads to lack of formation of kidneys, gonads and adrenals. In humans distinct but not identical phenotypes arise after WT1 loss-of-function (LOF) mutation, resulting in urogenital and other malformations in boys with WAGR, Deny–Drash or Frasier syndromes.[1,8] SF1 deletion in mice results in failure of gonadal and adrenal development, whereas the corresponding LOF mutation in humans has a milder gonadal phenotype and adrenal insufficiency.[1,9,10]

COLONIZATION OF GONADAL ANLAGEN BY PRIMORDIAL GERM CELLS

By the end of the 5th week pc of human embryonic development the gonadal anlagen are composed of somatic cell types of three different lineages with a bipotential fate, dependent on their future paths (see below). At this stage the indifferent anlagen are colonized by immigrating primordial germ cells (PGCs), which are termed gonocytes when permanently resident in the gonad. The PGCs differentiate from epiblast-derived stem cells in the yolk sac and can be distinguished by their expression of stem-cell markers such as alkaline phosphatase, oct4 and c-kit. Guided by extracellular matrix proteins expressed along the dorsal mesentery of the hindgut, the PGCs migrate to the gonadal ridges. During this process PGCs show active mitotic proliferation, and have expanded in numbers on their journey to the gonadal anlagen.[11,12]

In both sexes it is obvious that lack of germ cells will result in infertility. However, there is a sexually dimorphic influence of germ cells on the fate of the developing gonad. In males, lack of germ cells still allows differentiation of somatic cells, including Leydig cells, with steroidogenic activity. Affected males will undergo pubertal development but are infertile due to the Sertoli-cell-only syndrome.

In the female, the presence of gonocytes is mandatory for further differentiation of the gonadal anlagen along the female path. In the absence of gonocytes, follicular cells degenerate resulting in non-functional streak gonads.[13]

The cell-division path taken by the gonocytes is another sexually dimorphic event in the gonadal anlagen. In males, gonocytes will continue mitotic proliferation and then become mitotically quiescent. They will not be recruited into meiosis until much later.

In female embryos, however, gonocytes are recruited into meiosis soon after arrival into the gonadal anlagen where they will be blocked at an early stage until further differentiation is initiated.

The decision to enter or not to enter into meiosis is thought to be governed by the somatic cells in the male gonad, since both XX and XY PGCs residing in an ovary or outside any gonad will develop along the oocyte path and enter meiosis.[14] However, there are also indications that this is regulated by mechanisms intrinsic to the germ cells.[15]

COMMITTED CELL LINEAGES IN THE BIPOTENTIAL GONAD

At the end of the 6th week pc of human embryonic development the indifferent gonad consists of four different cells lineages with predefined maturational paths dependent on sex (Table 1).

Supporting lineage

Sertoli cells in testis

The supporting cell lineage is critical for sex determination and further gonadal differentiation. In the testis it gives rise to Sertoli cells which are nurse cells for spermatogenesis, supplying the developing germs cells with nutrients and growth factors. In the adult testis sperm output is proportional to the number of Sertoli cells, which is reflected in some lower species by the finding that one single Sertoli cell gives support to one clone of developing germ cells. Thus, control of Sertoli-cell proliferation in the developing testis is of major importance for future production of male germ cells.[16] Follicle-stimulating hormone (FSH) from the pituitary is an important growth factor for Sertoli cells during development. However, during the early phase of Sertoli cell differentiation and proliferation the fetal hypothalamic–pituitary–gonadal (HPG) axis is not yet operative and FSH is not available. Thus, other growth factors are implicated. Indeed, many such growth factors have been described, although the precise control of the early phase of Sertoli-cell proliferation is as yet unknown.[16–18] Sertoli-cell differentiation and proliferation are critical first steps of male sex determination and are vulnerable targets of disruptive actions of endogenous factors and xenobiotics such as inflammatory mediators and endocrine disrupting chemicals (EDCs).[16,18,19] Pre-Sertoli cells are first defined as cells of the supporting lineage expressing SRY and later Sox9, an event defining male sex determination. This is rapidly followed by morphological changes. Sertoli cells constitute a paracrine early source of growth and differentiation factors for other cells in the testis, most notably for differentiating Leydig cells. Production of anti-Müllerian hormone (AMH) starts at an early stage of Sertoli-cell maturation and is required for regression of the anlagen for female internal genitalia. In addition,

Table 1. Cell lineages in the indifferent gonad and their fates after sex differentiation.

Indifferent gonad	Testis	Ovary
Supporting	Sertoli	Granulosa
Steroidogenic	Leydig	Theca
Stromal	Peritubular	Stromal
Gonocytes	Spermatogenesis	Oogenesis
Unknown	Macrophages	Not present

AMH is an intratesticular differentiation factor, and is also produced by the ovary but at a much later stage.

Granulosa cells in ovary

For ovarian histogenesis, the supporting cell lineage gives rise to granulosa cells. They interact physically with ovarian gonocytes to progressively organize into joint structures (start of folliculogenesis), thus mimicking Sertoli cells in this respect. It is therefore logical to propose granulosa cells as candidates to harbour the first expression of the putative ovarian determining factor(s) assumed to be mandatory for ovarian development. However, despite major search efforts such ovary-defining early markers have yet to be discovered. Furthermore, in contrast to testicular development, germ cells are obligatory for ovarian differentiation, as in their absence granulosa cells will not develop along the female path. Instead, they may show transdifferentiation into Sertoli-like cells, and streak gonads will eventually follow in affected XX individuals. The signals exerted by gonocytes to support granulosa-cell differentiation are not known in detail, but close physical intimacy seems to be required, and paracrine growth factors produced by the germ cells have been implicated.[20–22] Unlike Sertoli cells, granulosa cells are also steroidogenic cells of major importance for female reproduction (*vide infra*).

Recently, a winged helix/forkhead transcription factor gene, FOXL2, has been found to be selectively expressed by the developing ovary, more specifically in granulosa cells. Initially this gene was an ovary-determining gene candidate, but this hypothesis was later abandoned. In mice, FOXL2 LOF mutation results in disrupted granulosa-cell differentiation and ovarian failure, and this gene also seems to be required for activation of aromatase expression by granulosa cells. In humans, FOXL2 LOF mutation results in a complex phenotype known as the blepharophimosis–ptosis–epicanthus inversus syndrome (BPES), also including premature ovarian failure. Due to its selective expression pattern, FOXL2 is often used as an ovarian maker in experimental studies.[23–25]

Steroidogenic cells

Leydig cells in testis

In the male, Leydig cells develop from steroidogenic precursor cells immigrating from the coelomic epithelium and the mesonephric mesenchyme to contribute to the indifferent gonad.[26–28] Their differentiation starts in the 7th week pc of human development and is dependent on signals from Sertoli cells. Desert hedgehog (DHH) and fibroblast growth factor-9 (FGF9) are Sertoli-cell-derived factors mandatory for the proliferation and differentiation of functional Leydig cells.[29,30] Human Leydig cells may be classified into three different types according their morphological and maturational stage of development. The fetal-type Leydig cell is the first to appear after testis determination. It shows very different steroidogenic pattern and regulation as compared with the immature-type and adult-type Leydig cells, (see Table 2, describing rat Leydig cells) which appear postnatally before puberty and in adults after completed pubertal development, respectively.[28,31] Fetal-type Leydig cells start to produce androgen during the 8th week of human gestation and are at first regulated by the placental human chorionic gonadotropin (hCG). On Leydig cells, this hormone shares a receptor with pituitary luteinizing hormone (LH), which appears much later in development when the HPG axis is established in the fetus at the beginning of the second trimester of human pregnancy. Leydig cells are located in the interstitial tissue of the testis and

Table 2. Comparative morphological and functional features of fetal and adult-type rat Leydig cells.

Leydig cell type	Morphology SER/Lipid droplets	Predominant steroidogenic enzymes	Predominant steroids
Fetal	++++/++	P450scc, 3βHSD, P450c17, 17βHSD	Testosterone
Progenitor	+/–	3βHSD, 3αHSD	Androsterone
Immature	+++/++++	3βHSD, 3αHSD, 5α-reductase	3α, 5α-Androstanediol
Adult	++++/++	P450scc, 3βHSD, P450c17, 17βHSD	Testosterone

SER, smooth endoplasmic reticulum.

proliferate rapidly after they first appear, reaching up to 40% of the testicular cell mass before mid-gestation. Testicular histogenesis is not dependent on androgen action, which is evident in XY patients with complete androgen insensitivity syndrome.[32]

In addition to androgen, which is crucial for male differentiation of external and internal genitalia, Leydig cells also produce SF1 required for steroidogenesis[9] and insulin-like factor-3 (INSL3). INSL3 and its receptor LGR8 are needed for the first transabdominal phase of testicular descent[33], occurring at weeks 8–16 of fetal age in humans. LOF mutations affecting INSL3 or LGR8 result in cryptorchidism, but are a rare cause of this common malformation in boys.[33,34] In addition to its role in testicular descent, INSL3 seems to have other functions as a paracrine mediator in the testis. It may also serve as a useful differentiation marker of Leydig cells in clinical practice.[34] Further, INSL3 expression is not restricted to the testis as it is also produced by the ovary, where it has been implicated in hyperandrogenism.[35]

Interestingly, adrenocortical and gonadal steroidogenic cells seem to share an embryonic origin in the coelomic epithelium and may exist as one lineage before divergence into the gonadal and adrenocortical paths.[36] In line with this, adrenocorticotropic hormone (ACTH) has been implicated as a regulatory factor for fetal Leydig cells expressing ACTH receptors in the early phase of gonadal differentiation.[28] The idea of a common origin is also supported by the testicular adrenal rest tumours that are often found in male patients with congenital adrenal hyperplasia. These benign tumours are thought to be due to ACTH-driven expansion of adrenocortical or steroidogenic common precursor cells present in the testis.[37] Although much rarer, adrenal rest tumours have also been found in the ovary[38], also supporting the concept of a common origin of the steroidogenic cells.

Leydig cells constitute an obvious target of disruptive actions of xenobiotics and EDCs, but have a large regenerative capacity, at least in adult animals. In adult rats (but not mice) the toxicant ethylene dimethane sulphonate (EDS) kills all Leydig cells within 24 hours after a single dose, and androgen levels fall accordingly. However, within a few days after EDS dosing a regenerative activity starts and after 3 weeks the Leydig-cell population is reconstituted.[39] The resident Leydig precursor cells contributing to this regeneration have not yet been clearly defined, although it has been suggested that peritubular testis cells constitute a reserve pool of steroidogenic cells (*vide infra*). Several growth factors have been implicated in Leydig-cell regeneration and survival. [40,41]

Steroidogenic cells in the ovary

In the ovary, the cellular contribution to steroidogenesis is very different from that of the testis. Steroidogenic precursor cells develop outside the follicles into stromal theca cells which are ovarian counterparts of the Leydig cells. The theca cells synthesize androgen in response to hCG and LH, but are not capable of producing oestrogen

since they lack expression of CYP19 aromatase, an enzyme converting androgen to oestrogen. This enzyme is expressed by granulosa cells, and these cells can produce oestrogen and progesterone in response to LH and FSH stimulation. Thus, both theca cells and granulosa cells are required for oestrogen synthesis by the ovary, and both gonadotropins (LH, FSH) are needed. These joint actions form the basis of the two-cell, two-gonadotropin hypothesis for biosynthesis of oestrogen.[42] This is much more complex than the straightforward situation in the testis, where Leydig cells produce androgen in response to LH (or hCG).

Connective tissue cells or stromal cells

Peritubular cells in the testis

Connective-tissue or stromal-cell lineage cells are important constituents of the testis as they are needed for early histogenesis of the seminiferous cords. These cells are termed peritubular cells (PTCs) or myoid cells in the testis. They form a basal layer surrounding the seminiferous cords where they give support to the Sertoli cells (Figure 1). In the postpubertal testis they may add contractile forces thought to be required for tubular fluid and cell flow. Pre-PTCs migrate directly from the adjacent mesonephros on direct orders from Sertoli cells via chemotactic signals. Also cells contributing to the vasculature of the testis migrate via the same paths.[43] This migration process is an important step in sex determination and is SRY-dependent. PTCs are highly proliferative cells, a feature that seems important for male gonadal development.[44,45] In the adult testis PTCs are metabolically active and show abundant expression of the androgen receptor (AR). Their precise role in adult testicular function is not known in detail, but some data indicate that they have a role as a reserve or stem-cell pool[46], and may be involved in regeneration of Leydig cell numbers after a disruptive injury (*vide supra*).

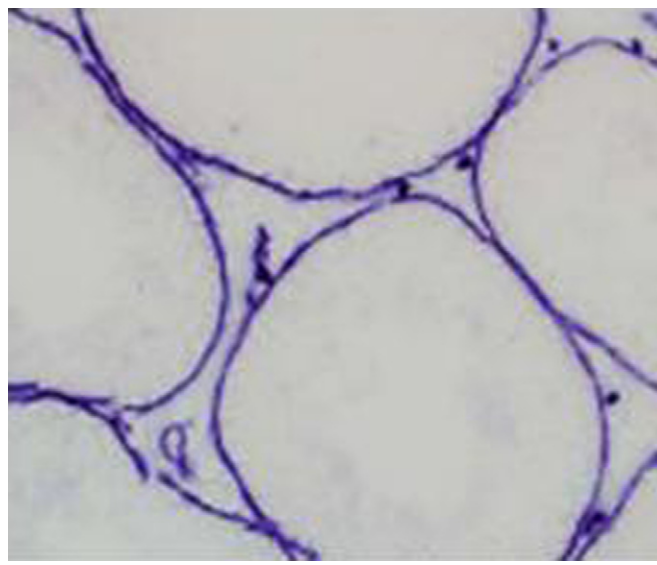

Figure 1. Peritubular cells in adult rat testis stained for alkaline phosphatase. A distinct layer of cells surrounding the seminiferous tubules is apparent.

Macrophages in testis

The testicular interstitium of adult individuals also contains a population of resident macrophages, constituting up to 20% of the interstitial cells. These macrophages can be identified by specific markers (Figure 2). Their physiological role is not fully understood but has been suggested to be associated with testis immune functions and the role of the testis as an immune sanctuary.[47] The embryonic history of the testicular macrophages is obscure. It is also not clear whether or not the testicular macrophages should be regarded as members of the mononuclear phagocyte system (MPS) and thus have a bone-marrow origin and shared functions with MPS cells in other tissues. Some data indicate that testicular macrophages have different functional properties as compared with resident macrophages elsewhere.[47] The testicular macrophages have no known counterparts in the ovary, and thus constitute a dimorphic feature.

Ovarian stromal cells

By cellular morphology, stromal cells in the ovary resemble peritubular myoid cells in the testis, but their spatial organization is different. The ovarian stromal cells seem randomly distributed in the ovarian cortex and are not engaged in folliculogenesis, as can be judged from their appearance. Ovarian cells of the stromal connective tissue lineage may be mistaken for theca cells if not stained for steroidogenic enzymes which are selective expressed in the theca cells.

SEX DETERMINATION

Males

Around day 42 of human embryogenesis the undifferentiated gonadal anlagen in XY individuals show the first signs of testicular development. This critical event is referred to as sex determination. In males this happens when pre-Sertoli cells express the testis-determining gene SRY, followed by expression of Sox9. Gonocytes proliferate rapidly by mitoses and subsequently become quiescent without entering meiosis.

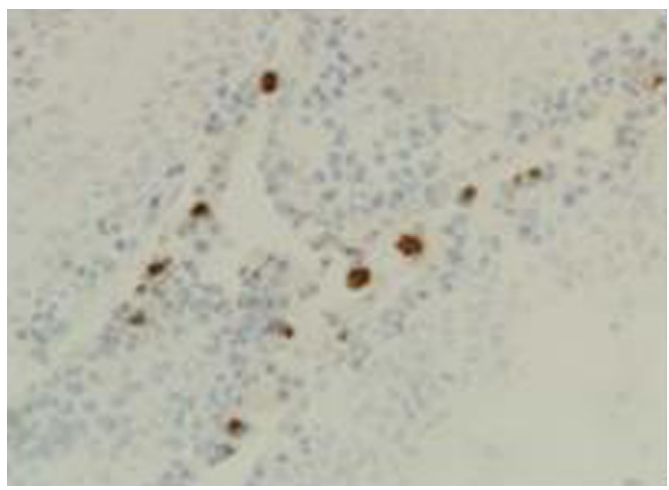

Figure 2. Resident macrophages in rat testis interstitium, stained for the macrophage marker ED2.

Testicular morphogenesis is apparent when Sertoli cells supported by peritubular cells form testis cords, also enclosing gonocytes. The morphology of the testes remains stable until puberty, when the cords are differentiated into seminiferous tubules at the start of spermatogenesis. Due to this process testicular volume increases, defining the onset of puberty in boys. At the tissue level this is reflected by quantitative onset of meiotic activity of male germ cells and formation of a lumen, changing the seminiferous cords into tubules.

Females

Female sex determination occurs 1 week later than in the male. In XX human embryos, the undifferentiated appearance of the gonad remains unchanged until the end of the 7th week pc. Ovarian gonocytes show intense mitotic proliferation and, in contrast to testicular gonocytes, enter into meiosis which later stops at the diplotene stage. Meiotic germ cells are first detectable at 9 weeks of gestation in the human developing ovary. Folliculogenesis starts at this point and is morphologically apparent in the end of the first trimester (week 12 pc) of pregnancy in humans. Gonocytes (now referred to as oogonia) are surrounded by granulosa-cell precursors forming a single layer of flattened cells resulting in joint cord-like structures referred to as ovigerous cords, later developing into primordial follicles.[22,48] Subsequently primordial follicles develop into primary and secondary follicles during mid-gestation. Before birth almost all female germ cells have entered meiosis. During prenatal development many oocytes undergo programmed cell death, and a definite number of around 2 million persist at term age in humans. Several genes with selective expression in ovaries have been characterized, particularly in mice, but no ovary-determining gene is yet apparent. AMH, which is crucial for male development, is also expressed by the ovary, albeit much later than in the testis, and is involved in regulation of folliculogenesis.[49] The major dimorphic features of the developing gonads are summarized in Table 3.

Table 3. Sexually dimorphic features of the gonads.

Function	Testis	Ovary
Determining gene	SRY	Unknown
Timing of sex determination	Day 42 pc	Day 47 pc
Germ-cell status and dependence	Mitotic arrest; gonocytes not required for differentiation	Meiotic arrest; gonocytes obligatory for differentiation
Steroidogenic cell types	Leydig cells (fetal type)	Theca and granulosa cells
Steroid hormones; regulation	Androgens; hCG/LH	Androgens, progesterone, oestrogens; hCG/LH, FSH
Connective tissue/ stromal cells	Organized peritubular myoid cell layer supporting cords	Stromal cells without obvious spatial organization
Temperature dependence of gametogenesis	Scrotal temperature	Body temperature
Immune/host defence	Interstitial macrophage population	Unknown

pc, post conception; hCG, human chorionic gonadotropin; LH, luteinizing hormone; FSH, follicle-stimulating hormone.

SUMMARY

In human males testicular development starts one week earlier than in the ovary due to a transient expression of the male sex-determining gene SRY in cells from the supporting cell lineage. These pre-Sertoli cells aggregate around gonocytes to form the seminiferous cords. This process is dependent on physical association of peritubular myoid cells coating the periphery of the cords. Further testis differentiation is regulated by the pre-Sertoli cells, whereas germ cells are dispensable. This is in contrast to ovarian development, where there is an absolute requirement for germ cells which supply the developing granulosa cells with survival signals. The pre-granulosa cells also originate from the supporting-cell lineage. The first morphological change in the developing ovary appears after 7 weeks of human gestation. Pre-granulosa cells and oocytes aggregate and differentiate progressively into primordial follicles first visible at 12 weeks. Meiotic germ cells are detectable at 9 weeks' gestation in the human female embryo. Fetal Leydig cells appear in the developing testis during the 7th week. They arise from steroidogenic precursors migrating to the gonadal anlagen from the mesonephros. Androgen production by Leydig cells is detectable from 7 weeks and peaks at 13–14 weeks. Male gonadal development can proceed in the absence of androgen action. In the ovary, CYP19 aromatase, required for oestrogen synthesis, is co-localized with and probably induced by Foxl2 in pre-granulosa cells, whereas androgen-producing theca cells originate from steroidogenic precursor cells. Ovarian interstitial stromal cells share a cellular origin with testicular peritubular cells in testes.

Research agenda

We eagerly look forward to the following discoveries:

- the ovary-determining gene
- the downstream targets and molecular function of Sry

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