Journal of Health Sciences Institute

cusbed.cumhuriyet.edu.tr Founded: 2016 Publisher: Sivas Cumhuriyet University

REVIEW

Embryonic Testis Development, Anatomy and the Role of Spermatogonial Stem Cells in Spermatogenesis

Vahit Ağır*, Mustafa Numan Bucak

Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Selçuk University, Konya, Türkiye

ARTICLE INFO

Received: *25 June 2025*
Accepted: *14 August 2025*

KEYWORDS
Spermatogenesis
Spermatogonial stem cells (SSCs)
Testosterone
Follicle-stimulating hormone (FSH)
Luteinizing hormone (LH)
Germ cell development

***Correspondence:**
vethekvahit@gmail.com

HOW TO CITE
Ağır V, Bucak MN (2025) Embryonic Testis Development, Anatomy and the Role of Spermatogonial Stem Cells in Spermatogenesis, Journal of Health Sciences Institute, 10(2): 169-187

ABSTRACT

Spermatogenesis is a highly coordinated process that ensures the continuous production of spermatozoa in adult males, originating from spermatogonial stem cells (SSCs) located in the basal compartment of the seminiferous tubules. Unique among adult stem cells, SSCs not only maintain the stem cell pool through self renewal but also generate differentiated germ cells that transmit genetic information to the next generation. This process proceeds through sequential stages of proliferation, meiosis, and morphological maturation, supported by Sertoli cells and regulated by endocrine signals from the hypothalamic pituitary gonadal axis. Within this regulatory framework, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) play critical roles by acting on Sertoli and Leydig cells, respectively, to sustain the microenvironment necessary for germ cell development, while Leydig cell-derived testosterone influences gene expression and cellular interactions via classical and non-classical pathways. Following testicular development, spermatozoa acquire motility and fertilizing capacity during epididymal transit, completing their functional maturation. A deeper understanding of SSC biology and the molecular and hormonal mechanisms underlying spermatogenesis holds significant promise for reproductive medicine, infertility therapies, and the conservation of endangered species, particularly in light of recent advances in SSC isolation, culture, and transplantation, which underscore their potential in regenerative and reproductive applications despite ongoing challenges in preserving their viability and purity during manipulation.

Introduction

Spermatogenesis is a tightly regulated process in which SSCs self-renew to maintain their population while also differentiating into mature spermatozoa. Originating from primordial germ cells during embryonic development, SSCs reside in the seminiferous tubules, where their proliferation, meiosis, and morphological maturation are supported by Sertoli cells and governed by endocrine and paracrine factors (Sharma et al., 2015). Testosterone, primarily produced by Leydig cells, is essential for this process, and its depletion leads to germ cell loss and impaired fertility. Despite the potential for high germ cell production, only 25–30% complete differentiation, with the rest undergoing apoptosis before meiosis, highlighting the importance of SSC survival and regulation (Plant & Zeleznik, 2014). Understanding the biology of SSCs is of great clinical and biotechnological interest, offering potential applications in infertility treatment, regenerative medicine, and the conservation of endangered species. This study aims to investigate the regulatory mechanisms influencing SSC function and spermatogenesis, providing insights that may support both therapeutic and biotechnological advancements.

Copyright © 2025 Journal of Health Sciences Institute. This work is licensed under Creative Commons Attribution 4.0 International License (CC BY-NC-ND 4.0)

Embryonic Development of the Male Reproductive System

Formation of the Primitive Gonads (Genital Ridges)

The genital ridges, which are the precursors of the testes and ovaries, form as a coelomic thickening on the ventral side of the mesonephros in the anterior posterior direction (Figure 1). These newly formed cell layers on the surface of the mesonephros are termed the crista genitalis (Hummitzsch et al., 2019). The crista genitalis appears at approximately day 33 of gestation in humans, between days 27 and 31 in cattle, on day 23 in sheep, on day 25 in goats, and on day 10 in mice. The primitive gonads consist of both somatic cells and germ cells (Nef et al., 2019a).

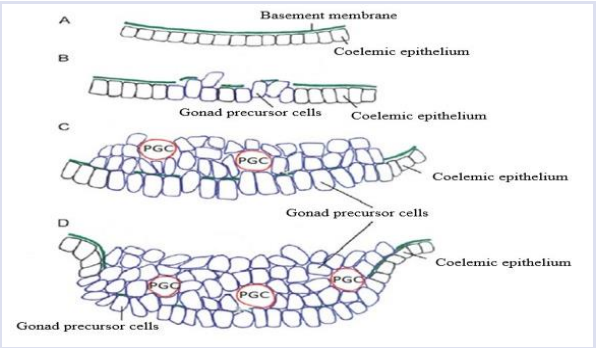


Figure 1. Formation of the primitive gonads (genital ridges). **A:** Basal membrane and coelomic epithelium. **B:** Some coelomic epithelial cells lose their epithelial characteristics, transform into gonadal precursor cells, and migrate inward through a fragmented basal membrane. **C:** The genital ridge forms as a cluster of proliferated coelomic epithelial cells. Primordial germ cells (PGCs) migrate and localize among the gonadal precursor cells. **D:** Gonadal precursor cells continue to proliferate, contributing to the development of the genital ridge, (Piprek et al., 2016) adapted from.

When the gonads are initially formed, they remain undifferentiated with respect to sex; they are unique organs possessing the potential to develop into either testes or ovaries, depending on the presence or absence of sex-specific factors. In the undifferentiated gonads, four types of cells are present: supporting cells, interstitial cells, PGCs, and connective tissue cells. After sex determination, these cell types differentiate in a sex-specific manner. Supporting cells differentiate into Sertoli cells in males and granulosa cells in females. Interstitial cells differentiate into Leydig cells in males and theca cells in females. Germ cells develop into spermatozoa or oocytes, while connective tissue cells differentiate into stromal cells in the ovary and peritubular myoid cells in the testes (Carré & Greenfield, 2016).

Key Transcription Factors in Gonad Development

The gonads initially develop as structures termed the genital ridges (crista genitalis), which are the precursors of the testes and ovaries, emerging on the ventromedial surface of the embryonic kidneys (mesonephros). Formation of the genital ridge begins with the proliferation of coelomic epithelial cells on the ventromedial surface of the mesonephros after implantation. The proliferating coelomic epithelium forms an epithelial cell layer constituting the genital ridge. The coelomic epithelium is a highly active cell layer capable of providing mesenchymal cells, which contribute to the mesodermal components of various organs and supply signals essential for their development (Ariza et al., 2016). As the coelomic epithelial cells proliferate, the basement membrane disintegrates, allowing many epithelial cells to migrate into the mesonephros (Kusaka et al., 2010). These cells undergo epithelial mesenchymal transition as they detach from the coelomic epithelium and begin to fill the space between the coelomic epithelium and the mesonephros (Schmahl et al., 2000). These mesenchymal cells of the genital ridge serve as progenitors capable of differentiating into somatic supporting cells and interstitial/stromal cells (Ito et al., 2006; Mork et al., 2012).

Several studies (Hu et al., 2013; Katoh-Fukui et al., 2012; Nef et al., 2019b) have identified a set of transcription factors required to initiate the formation, proliferation, and differentiation of the genital ridge (Figure 2). Mutations in the genes encoding these transcription factors can result in failure of primitive gonad development and the formation of non-functional fibrous tissue in the region where the genital ridge would normally form (Miura et al., 2018).

Key transcription factors involved in genital ridge formation:

SOX9: A critical regulator of sex differentiation, SOX9 promotes Sertoli cell differentiation during testis development and thus plays an essential role in sex determination. Together with SRY, it facilitates the differentiation of undifferentiated supporting cells into Sertoli cells (Nef et al., 2019b).

GATA4: A zinc finger transcription factor crucial for genital ridge development, GATA4 is synthesized in gonadal somatic cells. Among GATA family members (GATA1, GATA4, GATA6), only GATA4 is expressed during gonad formation (Hu et al., 2013).

WT1 (Wilms Tumor 1): Essential for the development of the urogenital system, particularly the gonads and kidneys. WT1 is expressed in primitive gonadal cells by day 9 of gestation in mice and continues throughout genital ridge formation. In the absence of WT1, coelomic epithelial thickening and gonad formation are impaired (Bandiera et al., 2013).

OSR1 (Odd-Skipped Related 1): Expressed during intermediate mesoderm development. Knockout studies of Osr1 and Wt1 in mice result in defective gonads, suggesting a role for Osr1 in supporting gonadal cell expansion (Wang et al., 2005).

NR5A1 (Nuclear Receptor Subfamily 5, Group A, Member 1): A nuclear receptor essential for the development of all steroidogenic tissues, including gonads and adrenal glands. NR5A1-deficient mice fail to develop these organs. It plays a key role in somatic gonadal cell proliferation and energy metabolism during early gonadogenesis (Morohashi et al., 2013).

CBX2 (Chromobox 2): Maintains transcriptional repression of many genes through chromatin remodeling

and histone modification. CBX2 is crucial for gonad development and sexual differentiation. Mutations in CBX2 lead to underdeveloped gonads and sex differentiation disorders (Katoh-Fukui et al., 2012).

LHX9 (LIM Homeobox Protein 9): A LIM homeodomain protein required for genital ridge development. In LHX9-deficient mice, somatic cells of the genital ridge fail to proliferate, and the ridge does not form (Mazaud et al., 2002).

EMX2 (Empty Spiracles Homeobox 2): Important for the development of the gonads, kidneys, ureters, and urogenital system. EMX2 expression is observed in the coelomic epithelium around embryonic day 10–11 in mice. EMX2-deficient mice exhibit defective gonad formation, with irregular cell clusters that are lost through apoptosis (Kusaka et al., 2010).

MFGE8 (Milk Fat Globule-EGF Factor 8 Protein): Also known as lactadherin, MFGE8 mediates cellular adhesion during genital ridge formation by encoding integrin-binding proteins (Ishii et al., 2005). These proteins are important for cell–cell adhesion during gonad development and also contribute to sperm–oocyte binding and phagocytosis of apoptotic cells in fetal and adult tissues (Hanayama et al., 2002).

PBX1 (Pre-B-cell Leukemia Homeobox 1): Expressed in the coelomic epithelium and later in the gonadal interstitium (Ota et al., 2008). PBX1-deficient mice lack adrenal glands and Müllerian ducts, and gonadal defects are associated with reduced cellular proliferation in the genital ridge (Schnabel et al., 2003).

INSR (Insulin Receptor) and IGF1R (Insulin-like Growth Factor 1 Receptor): These pathways are crucial for overall growth, proliferation, and differentiation. Genes encoding the receptor tyrosine kinases of the insulin pathway are required for proliferation of somatic progenitor cells in the gonads. Mice lacking INSR and IGF1R exhibit reduced expression of NR5A1, WT1, and LHX9, impairing gonad development (Pitetti et al., 2013).

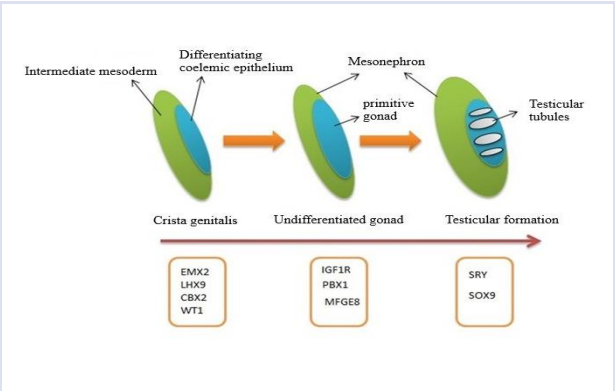


Figure 2. Formation of the genital ridge (crista genitalis) and key regulatory genes involved. Several genes play essential roles during the development of the genital ridge, including Empty Spiracles Homeobox 2 (EMX2), Insulin-Like Growth Factor 1 Receptor (IGF1R), Pre B-cell Leukemia Transcription Factor 1 (Pbx1), Milk Fat Globule-EGF Factor 8 Protein (MFGE8), LIM Homeobox Protein 9 (LHX9), Chromobox 2 (CBX2), Wilms Tumor 1 (WT1), and GATA-Binding Protein 4 (GATA4).

Formation and Migration of Primordial Germ Cells

In mammals, during the pre-implantation blastocyst stage, three distinct cell types emerge: the trophectoderm, the hypoblast, and the epiblast (Figure 3). The inner cell mass segregates into two layers: the epiblast and the hypoblast. The epiblast layer gives rise to both somatic cells and PGCs (Irie et al., 2014).

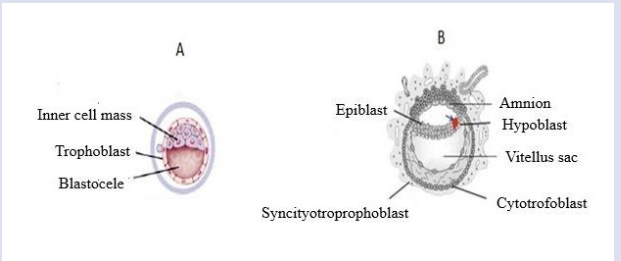


Figure 3. Formation of primordial germ cells. **A:** Pre-implantation blastocyst. **B:** Primordial germ cells derived from epiblast cells after implantation. The red-colored cells indicate primordial germ cells. (Piprek et al., 2016) adapted from.

The PGCs, which give rise to spermatogonia and oogonia, originate from epiblast cells after implantation. These large, round cells first migrate amoeboidly into the dorsal mesoderm, then reach the undifferentiated primordia of the testes and ovaries (crista genitalis), where they settle beneath the coelomic epithelium and proliferate to form an epithelial layer (germinal epithelium). Subsequently, cells of this layer invaginate together with the basement membrane into the mesenchymal tissue of the gonads in the form of tubules or cords, giving rise to follicular epithelium and oogonia in females, and convoluted seminiferous tubules, Sertoli cells, and prespermatogonia (also called gonocytes) in males (Smitz & Cortvrindt, 2002).

In cattle, between days 30 and 64 of gestation, PGCs migrate toward the gonadal region through amoeboid movements and pseudopodia. This migration induces epithelial proliferation within the mesenchyme of the genital ridges. PGCs migrate from their site of origin toward the dorsal mesoderm and settle in the genital ridge, where they localize near the Sertoli precursor cells and differentiate into gonocytes (Kenngott et al., 2013). The presence of the Y chromosome directs the differentiation of the undifferentiated gonads toward the male genital system. Male gonocytes proliferate for several days before entering a quiescent state in the G0/G1 phase, which persists until birth. Postnatally, these cells migrate to the basement membrane of the seminiferous tubules, where they differentiate into undifferentiated type A spermatogonia, also known as SSCs (Yamazaki et al., 2003). SSCs are responsible for the maintenance of spermatogenesis. The continuous production of type A spermatogonia and their differentiation into spermatozoa ensure the transmission of genetic material to subsequent generations (Young, 2006).

Factors Regulating PGC Formation

The differentiation and formation of PGCs occur under the influence of certain growth factors. One of these growth factors is bone morphogenetic protein 4 (BMP4), a member of the transforming growth factor β (TGF-β) superfamily. Other BMPs, such as BMP8b and BMP2, have also been identified as essential for the normal development of PGCs. A study reported that mice carrying mutations in the Bmp4, Bmp7, and Bmp8b genes exhibited severe defects in PGC development, with almost no PGCs being formed (Table 1) (McLaren, 1999). BMPs play critical roles in the development and function of PGCs, particularly BMP4, which regulates both the formation and proliferation of PGCs. BMP4 secreted from the extraembryonic ectoderm creates a permissive condition for epiblast cells to differentiate into PGCs (Beddington & Robertson, 1999; Starz-Gaiano & Lehmann, 2001). Another factor, B-lymphocyte-induced maturation protein-1 (Blimp1 or Prdm1), which also plays important roles in early cell fate decisions, cooperates with Prdm14 to suppress the somatic cell program, initiate PGC specification, and induce pluripotency-related genes. Blimp1 expression is detected in epiblast cells at around embryonic days 6–7 in mice (Bao et al., 2012).

Blimp1 (Prdm1) and Prdm14 act together during the transition of epiblast cells into PGCs after implantation, initiating the epigenetic reprogramming necessary for PGCs to achieve a pluripotent state (Nagamatsu et al., 2011). PGC formation begins after blastocyst implantation, when some cells located in the proximal region of the embryo are diverted from somatic differentiation and instead become specified as PGCs (Irie et al., 2014). The newly specified PGCs proliferate mitotically and reach the genital ridges at around day 11 in mice and day 13 in rats (Günesdogan et al., 2014). BMP and WNT (Wingless and Int-1) signaling pathways are required to direct epiblast cells toward the PGC fate, inducing the expression of three critical transcription factors necessary for PGC formation: BLIMP1 (PRDM1), PRDM14, and AP2γ (TFAP2C) (Welling & Geijsen, 2013).

BLIMP1: A Transcriptional Repressor

BLIMP1 (B-lymphocyte-induced maturation protein 1, also known as PRDM1) belongs to the PRDM family of proteins (Günesdogan et al., 2014). BLIMP1 expression is undetectable during the pre-implantation stage of embryogenesis (Chu et al., 2011), but it becomes evident in developing PGC progenitors and in the post-implantation endoderm (Ohinata et al., 2005).

During embryogenesis, BLIMP1 represses somatic gene expression programs and directs cells in the proximal epiblast toward the germ cell lineage. Since it is active during PGC formation in the post-implantation embryo, BLIMP1 is considered a key factor in PGC specification and proliferation (Ohinata et al., 2005). Disruptions or mutations in BMP and WNT signaling pathways may result in loss of BLIMP1, which reduces the proportion of epiblast cells adopting the PGC fate and inhibits the proliferation of specified PGCs (Ohinata et al., 2009).

PRDM14:PR domain containing 14

PRDM14 (PR domain-containing 14) is another member of the PRDM protein family (Ohinata et al., 2009). PRDM14 is expressed exclusively in PGCs and pluripotent cells (Chia et al., 2010). In mice, PRDM14 expression begins around embryonic day 6 in cells of the proximal epiblast after implantation (Ohinata et al., 2005). Both BLIMP1 and PRDM14 are required for the differentiation of embryonic stem cells into PGCs. Mice lacking PRDM14 are viable but sterile due to the absence of germ cells (Nakaki & Saitou, 2014). In the absence of functional BMP4 and WNT3, expression of PRDM14 and BLIMP1 is significantly reduced, resulting in few or no PGCs being formed (Bagci & Fisher, 2013).

Table 1. Key genes and transcription factors ınvolved in PGCs specification and genital ridge development.

Gene/Factor	Full Name	Function / Role	Reference
Blimp1 (PRDM1)	B-lymphocyte-induced maturation protein-1	Represses somatic program; directs cells toward PGCs lineage	(Ohinata et al., 2005)
PRDM14	PR domain containing 14	Epigenetic reprogramming; activation of pluripotency-associated genes	(Nakaki & Saitou, 2014)
AP2γ (TFAP2C)	Activating enhancer-binding protein 2 gamma	PGCs specification and proliferation; maintains *Blimp1* and *PRDM14* expression	(Kurimoto et al., 2008)
BMP4	Bone morphogenetic protein 4	Provides microenvironmental signals for epiblast-to-PGCs differentiation	(Irie et al., 2014)
BMP8b	Bone morphogenetic protein 8b	Regulates PGCs number; synergizes with BMP4	(Bagci & Fisher, 2013)
BMP2	Bone morphogenetic protein 2	Functions alongside BMP4/BMP8b in normal PGCs development	(Bagci & Fisher, 2013)
WNT3	Wingless-type MMTV integration site family 3	Activates *Blimp1* and *PRDM14* with BMP signaling; involved in PGCs specification	(Bagci & Fisher, 2013)
WT1	Wilms tumor 1	Involved in genital ridge (crista genitalis) and gonad development	(Ohinata et al., 2005)
LHX9	LIM homeobox 9	Regulates genital ridge formation and somatic cell proliferation	(Mork et al., 2012)
CBX2	Chromobox homolog 2	Essential for sex differentiation and gonadal development	(Katoh-Fukui et al., 2012)

AP2γ Transcription Factor

AP2γ (TFAP2C) is a member of the AP2 transcription factor family (Schäfer et al., 2011). During embryonic development, AP2γ is expressed in various locations, including PGCs and the trophectoderm. In mice, AP2γ expression begins around embryonic days 7–8 in PGCs, following the induction of Blimp1 and Prdm14 gene expression (Kurimoto et al., 2008). Complete loss of AP2γ expression results in embryonic lethality due to defects in placental development (Auman et al., 2002). AP2γ is considered a crucial mediator of PGC specification and supports the continued proliferation of PGCs (Taylor, 2020).

Sex Determination and Differentiation

Genetic Mechanisms

The sex determining region of the Y chromosome gene (SRY), responsible for testis development, is located very close to the pseudoautosomal region 1 (PAR1) of the Y chromosome, which is the region where crossing over most frequently occurs during meiotic prophase I. Occasionally, the SRY gene participates in this recombination event. Consequently, an embryo with XY chromosomes may develop as female if it loses the SRY gene, whereas an XX embryo may develop as male if it acquires the SRY gene (Seda O, 2006). The initial step in sex determination is the presence of the SRY gene in the nuclei of supporting cells of the undifferentiated gonad. In humans, the first six weeks constitute the "undifferentiated stage," in which primitive germ cells are identical in both sexes, and no differentiation toward male or female is yet apparent (Hassa O, 2003).

The factor initiating differentiation is the SRY gene expressed in supporting cells of the bipotential gonad, which becomes active around embryonic days 11–12 in mice (Park & Jameson, 2005). When the DNA surrounding this gene region loosens, it becomes accessible for transcription and supporting cells in the undifferentiated gonads of XY embryos differentiate into Sertoli cells. Sertoli cells stimulate the transformation of germ cells into spermatogonia, produce Müllerian inhibiting factor (MIF), which causes regression of the Müllerian duct, and promote the differentiation of mesenchymal cells into testosterone-secreting Leydig cells (Münsterberg & Lovell-Badge, 1991). Under the control of SRY, bipotential gonads develop into testes. After genetic determination of gonadal differentiation, the subsequent process of sexual development is regulated by hormones secreted from the gonads. Sry expression activates the Sox9 gene, which then activates fibroblast growth factor 9 (Fgf9), leading to the differentiation of pre-Sertoli cells and the formation of testis cords (Gonen & Lovell-Badge, 2019; Sekido & Lovell-Badge, 2008).

The chromatin remodeling factor SRY is characterized by its DNA-binding domain, which binds to A/T-rich DNA sequences and bends the DNA at an angle of 60–85°, a property essential for its biological function. If this DNA bending is impaired, the undifferentiated male gonad cannot develop into a testis (Parma & Radi, 2012). Supporting cells of the primordial gonad are the first to differentiate in the presence of sex-determining factors like Sry. These supporting cells are thought to direct other cells toward either the ovarian or testicular pathway and represent the first somatic cell population to differentiate in the embryonic gonad (Nicol & Yao, 2015).

In all vertebrates, the gonadal primordium consists mainly of supporting cell precursors, steroidogenic progenitor cells, and primordial germ cells. During gonadal sex differentiation, each gonadal cell population differentiates into either ovarian or testicular cell types (Liu et al., 2016). In XY embryos, supporting cells differentiate into Sertoli cells that surround germ cells and form testis cords, while steroidogenic cells differentiate into fetal Leydig cells that produce testosterone. In ovaries, supporting cell precursors become pre-granulosa cells, steroidogenic progenitors form theca cells, and germ cells differentiate into oogonia (Wear et al., 2017).

Hormonal Control of Sexual Differentiation

The target tissues of hormones secreted from the genetically determined gonads (testes and ovaries) are the Müllerian and Wolffian duct primordia, which form the precursors of female and male genital tracts, respectively. At this stage, hormones secreted by the fetal testes play the decisive role. The role of fetal testicular hormones in sex development was first demonstrated by Jost in 1947. He castrated sexually undifferentiated fetal rabbits and observed the development of female genitalia, showing that the Müllerian duct develops into female structures in the absence of testes and without requiring estrogen (Jost, 1947). Later studies established that two substances secreted from the fetal testes Müllerian inhibiting factor (MIF) and testosterone are essential for male development (Jost et al., 1973).

Subsequent studies (Mıchela Schultz & Wılson, 1974; Neumann et al., 1970) supported this concept. Up to about embryonic day 12–13 in mice and the 7th week in humans, both Müllerian and Wolffian ducts coexist. If the gonadal primordia develop into testes under genetic control (SRY), MIF secreted by Sertoli cells causes Müllerian duct regression, while testosterone secreted by Leydig cells stimulates Wolffian duct development into epididymis, vas deferens, and seminal vesicles. In contrast, if the gonads develop into ovaries, the absence of testosterone results in Wolffian duct regression, and the absence of MIF allows Müllerian duct development into uterus and oviducts.

Thus, the presence of testicular hormones determines male development, while their absence leads to female development. Male genital development is an active process under the influence of male hormones, while female genital development occurs passively in the absence of these hormones. Although estrogens and progesterone are secreted by the fetal ovaries and placenta in female embryos, they are not essential for female sexual development. Indeed, blocking estrogen synthesis or receptors in female embryos does not

prevent the development of the female genital tract, though it impairs its maturation and leads to infertility (Rey et al., 2020). For the development of external genitalia, the enzyme 5α-reductase is required to convert testosterone into dihydrotestosterone (DHT), which is responsible for male external genital development. In its absence, female external genitalia develop. Mutations in the gene encoding 5α-reductase result in a condition called "5α-reductase deficiency," characterized by defective development of male external genitalia. This condition has been observed in humans but not yet reported in animals (Stévant et al., 2018).

Adult Testis Structure

Gross Anatomy of the Testis and Scrotum

The testes are a pair of organs located within the scrotum. The scrotum, a skin-covered sac, has the crucial function of maintaining testicular temperature below intra-abdominal levels. Beneath its thin, sparsely haired skin lies a loose connective tissue layer. The organ itself is covered by a thick tunica albuginea and a thin tunica vaginalis, which also lines the inner surface of the scrotum (AL-Mamari, 2023). The tunica albuginea is a capsule of dense irregular connective tissue, rich in collagen fibers and containing few elastic fibers. The parietal layer of the tunica vaginalis adheres to the scrotum, while its visceral layer covers the testis and epididymis, contacting the tunica albuginea (Amann, 1989).

The space between the two layers of the tunica vaginalis communicates with the pelvic (abdominal) cavity. During development, the descent of the testes from the abdominal cavity into the scrotum (descensus testis) occurs through this pathway. Connective tissue septa (septula testis) extend inward from the tunica albuginea, dividing the testicular parenchyma into pyramid-shaped lobules, each containing 1–4 convoluted tubules (Sarma & Devi, 2012). In the adult rat testis, approximately 20 seminiferous tubules are present, each with a diameter of 200–400 µm. The ends of these coiled tubules connect to the rete testis, which transports spermatozoa from the tubules to the epididymis (Krinke, 2000).

Seminiferous Tubules

The seminiferous tubules are surrounded by a basal membrane and consist of multiple layers of cells. Spermatogonial cells form a thick epithelium 4–7 cell layers deep within the tubules. These cells differentiate progressively from the basal compartment toward the lumen, proliferating and moving upward. Broad-based Sertoli cells rest on the basal membrane, extending toward the lumen, interspersed with numerous spermatogonia to form the seminiferous tubules (Griswold, 2016). Spermatogonia detach from the basal membrane and move toward the lumen, undergoing mitotic divisions to form primary spermatocytes, which further divide into secondary spermatocytes. Round spermatids appear in the lumen as a result of meiotic division. These spermatids, embedded within the cytoplasm of Sertoli cells, gradually acquire the species-specific morphology of mature spermatozoa. Upon completing their differentiation, spermatozoa are released from the epithelial layer into the lumen (Nakata et al., 2015).

Sertoli Cells

Sertoli cells support spermatogonial cells and extend from the basal lamina of the seminiferous epithelium toward the lumen of the tubule. They are rich in smooth endoplasmic reticulum, have a well developed Golgi apparatus, numerous lysosomes, and mitochondria of the crista type, particularly abundant at the base. In addition, they contain various cytoplasmic inclusions, such as glycogen, lipid droplets, and albumin crystals (Griswold, 2016). Sertoli cells play crucial roles in spermatogenesis, including regulating and controlling the process, supporting, protecting, and nourishing developing germ cells, phagocytosing degenerated germ cells and residual cytoplasmic bodies from developing germ cells, and secreting substances to facilitate the transport of spermatozoa (Dalgaard et al., 2002).

The FSH and testosterone stimulate Sertoli cells to synthesize androgen-binding protein (ABP). The ABP testosterone complex in the seminiferous epithelium initiates the proliferation of spermatogonia. High intratubular testosterone concentration is essential for the maintenance of spermatogenesis. Sertoli cells also secrete the hormone inhibin, which inhibits FSH production by the anterior pituitary, either directly by affecting FSH secretion or indirectly by modulating GnRH secretion from the hypothalamus (Weinbauer & Nieschlag, 1998). Additionally, Sertoli cells secrete large amounts of potassium-rich intracellular fluid. This specialized fluid assists in releasing spermatozoa from the cytoplasm of Sertoli cells, in which they are deeply embedded, and facilitates their movement toward the lümen (Figure 4) (Mruk & Cheng, 2015).

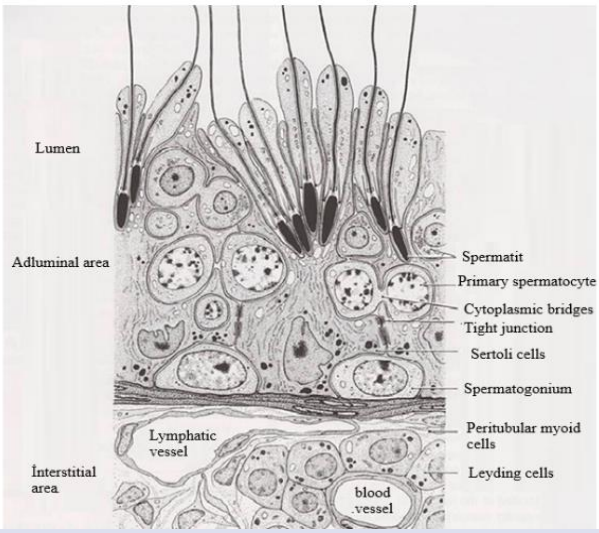


Figure 4. Structure of the Seminiferous Tubule (Nakata et al., 2015) adapted from.

Intertubular Areas

Loose connective tissue is present between the tubules (Russell et al., 1993). Within these interstitial areas, blood and lymphatic vessels, fibrocytes, collagen fibers, free mononuclear cells (lymphocytes, macrophages, and mast cells), and Leydig cells with endocrine function are located (Ariyaratne & Chamindrani Mendis-Handagama, 2000).

Leydig Cells

Leydig cells were first described in 1850 by Franz Leydig, who gave his name to them. These cells are found in small clusters within the interstitial tissue. They measure 15–20 µm in diameter and contain a centrally located, peripherally heterochromatic, round nucleus, often more than one. Leydig cells possess a well-developed Golgi apparatus near the nucleus, as well as lysosomes, peroxisomes, and Reinke crystals in their cytoplasm, although the function of these crystals remains unknown. The cells are also rich in mitochondria of the crista type. Leydig cells express LH receptors (Midzak et al., 2009).

LH is the primary factor required for Leydig cell differentiation and maintenance of their function (Ge et al., 2005). LH stimulates Leydig cell proliferation directly or indirectly by regulating the production of growth-promoting factors. In adult rat testes, Leydig cell proliferation is minimal. Leydig cells synthesize and secrete androgens, including testosterone, androstenedione, and dehydroepiandrosterone. Androgens enter the systemic circulation through nearby capillary fenestrations. Part of the testosterone produced is released into the interstitial fluid, where it exerts paracrine effects (on nearby cells) and autocrine effects (on Leydig cells themselves) during spermatogenesis (Ge et al., 2005; Krinke, 2000). The main functions of testosterone secreted by Leydig cells at puberty include the development of sexual desire (libido), maintenance of accessory gland function, manifestation of secondary sexual characteristics, regulation of spermatogenesis, negative feedback on the pituitary and hypothalamus, and general anabolic effects (Krinke, 2000). Circulating testosterone levels correlate with both the steroidogenic capacity of Leydig cells and their total number in the testes (Ge et al., 2005). Testosterone production by Leydig cells declines with age, though the mechanisms remain incompletely understood. Possible reasons include a decrease in Leydig cell number, reduced LH stimulation, and diminished testosterone-producing capacity of Leydig cells (Midzak et al., 2009).

Blood Testis Barrier

The blood testis barrier (BTB), formed by tight junctions between adjacent Sertoli cells, is located near the base of the seminiferous epithelium and divides the epithelium into two compartments: basal and adluminal. Spermatogonia and preleptotene spermatocytes reside in the basal compartment, whereas primary and secondary spermatocytes, round spermatids, and elongated spermatids are located in the adluminal compartment (Cheng & Mruk, 2002). The BTB provides a microenvironment necessary for completion of meiosis by isolating cells in the adluminal compartment from the systemic and lymphatic circulation, as well as through local immune suppression (Li et al., 2012).

The BTB differs from other tissue barriers by being composed of four types of cell junctions: tight junctions, ectoplasmic specializations, desmosomes, and gap junctions (Mruk & Cheng, 2010). Tight junctions, the most important component of the BTB, serve as both “gate” and “fence.” The gate function prevents the passage of water, solutes, and large molecules between intracellular spaces, creating a barrier, while the fence function restricts the movement of proteins and lipids, thereby establishing cell polarity. Tight junctions in the testis work in concert with ectoplasmic specializations. Desmosomes mediate strong adhesion between cells, whereas gap junctions allow the diffusion of metabolites, ions, and other molecules smaller than 1 kDa (Pointis & Segretain, 2005). Tight junctions encircle the cells in a belt-like continuous anastomosis to form a specialized microenvironment. The fibrils formed between adjacent cells close the intercellular spaces, creating an impermeable barrier. Each fibril consists of structural proteins (occludin, claudins, tricellulin), signaling and cytoskeletal proteins (zona occludens 1 (ZO1), ZO2, ZO3), and membrane proteins (Mruk & Cheng, 2015).

Spermatogonial Stem Cells (SSCs)

In the testes, the stem cells that serve as the cellular source of spermatogenesis are called spermatogonial stem cells (SSCs). The self-renewal and differentiation of SSCs ensure the continuity of spermatogenesis. In mammalian testes, SSC development begins shortly after birth, coordinated with testicular morphogenesis, to establish a stem cell pool and lay the groundwork for spermatogenesis. SSCs are located in the basal compartment of the seminiferous epithelium. These cells are capable of lifelong self-renewal and transmit genetic information to the next generation (De Rooıj & Russell, 2000). Spermatogenesis is the process by which millions of mature gametes, or spermatozoa, are produced daily in the testes. In addition to providing a continuous source of spermatozoa, the continuous replication of SSCs is responsible for maintaining the stem cell population. Within the testis, only SSCs possess the ability to self-renew, meaning they can undergo a series of mitotic divisions without differentiation. Through these divisions, SSCs sustain the continuity of spermatogenesis. The newly formed cells produced by mitosis retain the same potential for self-renewal and differentiation as their progenitors. SSCs, which are responsible for the maintenance of spermatogenesis, constitute only about 0.03% of the total testicular cell population in adult mice (de Barros et al., 2012).

Origin and Characteristics

Derived from PGCs, SSCs originate in the epiblast during embryogenesis and migrate to the genital ridges

through the allantois and hindgut. Upon reaching the genital ridges, PGCs contribute to the formation of the seminiferous tubules and become surrounded by differentiating Sertoli cells. At this stage, due to their morphological changes, these cells are referred to as gonocytes. During later stages of gestation, gonocytes increase in number through mitotic divisions. Prior to puberty, mitotic proliferation of these cells temporarily ceases. At the onset of puberty, elevated levels of gonadotropins trigger spermatogenesis and stimulate SSC division (de Barros et al., 2012).

SSCs are located on the basal lamina of the seminiferous tubules and remain in close contact with Sertoli cells in the seminiferous epithelium. SSCs renew themselves throughout the life of adult male animals to sustain spermatogenesis. Morphologically, SSCs in the seminiferous tubules are identified as Asingle (As) spermatogonia (Hamra et al., 2004). Through a series of mitotic divisions, these cells sequentially produce Apaired (Apr), Aaligned (Aal), A1, A2, A3, and A4 spermatogonia, and eventually type B spermatogonia (Figure 5). Finally, type B spermatogonia move from the basal compartment of the seminiferous epithelium toward the lumen and, through meiosis, give rise to haploid spermatocytes (Kostereva & Hofmann, 2008). For the continuous production of male gametes (spermatozoa), cells in the A single SSC pool must be converted into Apaired (Apr) spermatogonia (Ogawa et al., 2000).

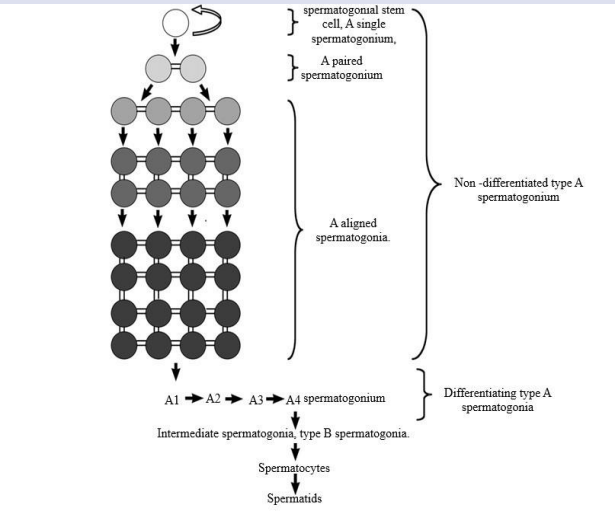


Figure 5. A spermatogonial stem cell, also known as an A single spermatogonium, can either self-renew (curved arrow) or differentiate into an A paired spermatogonium, which is connected by intercellular bridges (straight lines). The A paired spermatogonium subsequently undergoes mitotic divisions to form 4, 8, and eventually 16 A aligned spermatogonia. These cells ultimately give rise to differentiating type A spermatogonia. Through successive mitotic divisions, differentiating type A spermatogonia produce intermediate spermatogonia and then type B spermatogonia. Type B spermatogonia enter meiosis to form spermatocytes, which later differentiate into spermatids and eventually mature into spermatozoa.

Role of GDNF and Other Regulatory Factors

Our understanding of mammalian SSCs has advanced significantly with the discovery that the neurotrophic factor secreted by glial cells in the brain, glial cell line-derived neurotrophic factor (GDNF), is also expressed in the ovaries and testes. GDNF secreted by Sertoli cells is a key factor in the self-renewal of SSCs (Hofmann et al., 2005). In a study by Tadokoro and colleagues, it was found that the expression of GDNF by Sertoli cells depends on FSH (Tadokoro et al., 2002). Spermatogenesis is a complex and continuous process maintained by SSCs with self-renewal and differentiation potential. This process involves multiple factors acting along the hypothalamic pituitary testicular axis. Among these factors, GDNF secreted by Sertoli cells plays a critical role in maintaining the balance between SSC proliferation and differentiation (Ryu et al., 2005). The importance of GDNF in maintaining the SSC population was demonstrated in a study by Meng et al. The researchers reported that in GDNF-deficient mice, the SSC pool was depleted, whereas overexpression of GDNF led to an accumulation of undifferentiated spermatogonia. They concluded that the differentiation and division of SSCs are regulated by both GDNF and FSH (Meng et al., 2001).

As discussed in earlier sections, PGCs proliferate during migration to the genital ridges and contribute to gonad formation. Upon reaching the genital ridges, undifferentiated PGCs respond to signals from somatic cells of the primitive gonad and enter sex-specific differentiation pathways. In female embryos, PGCs enter meiosis and arrest in prophase I within primordial follicles as oogonia. In male embryos, PGCs proliferate for a short period before entering a quiescent phase after birth, and these cells are termed prespermatogonia (gonocytes). In male embryos, the retinoic acid that triggers meiosis in females is degraded by Cyp26b1, an enzyme secreted by Sertoli cell precursors, thereby prolonging mitotic proliferation of PGCs in males (Koubova et al., 2006).

Following sex determination, prespermatogonia migrate toward the center of the seminiferous tubules. At this stage, PGCs attached to the basal membrane exhibit structural changes compared to pre-migratory cells. Studies in mice have shown that PGCs acquire SSC properties from this point onward (Yoshida et al., 2006). After birth, gonocytes continue mitotic divisions, gradually migrate from the center to the basal membrane of the seminiferous tubules, and differentiate into spermatogonia (Culty, 2009). In pigs and goats, gonocytes differentiate into spermatogonia approximately two months after birth, whereas in sheep and cattle this transformation occurs at three and four months postnatally, respectively (Borjigin et al., 2010; Sarma & Devi, 2012).

Self-Renewal and Niche

In postnatal testes, SSCs reside in a specialized microenvironment within the basal compartment of the seminiferous epithelium, close to the basement membrane. This microenvironment, occupied by SSCs and

Sertoli cells, is called the niche. Other cell types or structures associated with the niche include Leydig cells, macrophages, lymphatic endothelial cells, blood vessels, and peritubular myoid cells. These components collectively regulate the balance between SSC self-renewal and differentiation, thus determining SSC fate (Potter & DeFalco, 2017). The self-renewal and differentiation of SSCs are controlled by several factors (Figure 6). Sertoli cells, the only cells in direct contact with SSCs within the niche, produce key molecular factors required for SSC maintenance, including GDNF and basic fibroblast growth factor (bFGF). Studies on GDNF-deficient animals have demonstrated that GDNF is crucial for SSC self-renewal. Consequently, GDNF is one of the most widely used molecular factors in in vitro culture systems to maintain SSCs (Huang et al., 2009). In addition, Leydig cells produce colony-stimulating factor 1 (CSF1) and insulin-like growth factor 1 (IGF1), both of which enhance SSC proliferation (Zheng et al., 2014).

The role of testicular macrophages within the SSC niche remains under investigation. Testicular macrophages produce 25-hydroxycholesterol, an intermediate in testosterone biosynthesis, and thus may enhance testosterone production. In a study it was suggested that testicular macrophages contribute to SSC proliferation and differentiation by secreting CSF1. They also reported that CSF1 is secreted by smooth muscle cells of testicular blood vessels (DeFalco et al., 2015). The testicular vascular system also plays a critical role in SSC proliferation and differentiation. In bulls, vascular endothelial growth factor (VEGF) has been shown to positively influence SSC differentiation (Caires et al., 2009).

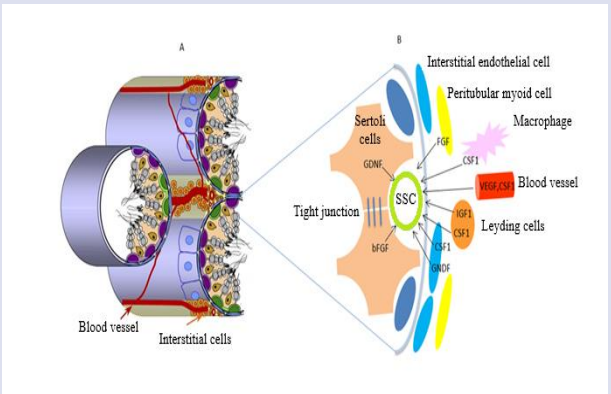


Figure 6. A: Seminiferous tubules, **B:** Cells and structures forming the niche: GDNF (glial cell line-derived neurotrophic factor), bFGF (basic fibroblast growth factor), CSF1 (colony stimulating factor 1), VEGF (vascular endothelial growth factor), IGF1 (insulin-like growth factor 1), and SSC (spermatogonial stem cell) (Shetty & Meistrich, 2007) adapted from.

Spermatogenesis

Spermatogenesis is the process by which mature spermatozoa are produced through the proliferation and differentiation of spermatogonial stem cells within the seminiferous tubules (Figure 7) (Griswold, 2016). In mammals, spermatogenesis takes place in the seminiferous tubules of the testes. Spermatogonia, which constitute the stem cell population in the testes, possess the ability to self-renew throughout adult life and to rapidly regenerate following tissue injury (Kanatsu-Shinohara & Shinohara, 2013). At all stages of their development, spermatogonial stem cells are supported and nourished by Sertoli cells, which divide the seminiferous tubule epithelium into basal and adluminal compartments. Spermatogonia are located on the basement membrane of the seminiferous tubules. The cytoplasm of the Sertoli cells extends fine projections to envelop and nourish the spermatogonial cells throughout the process of spermatogenesis (Kızılay & Altay, 2019).

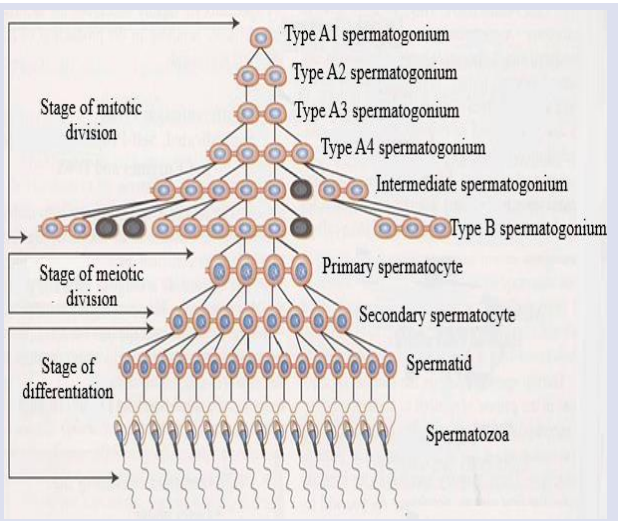


Figure 7. The stages of spermatogenesis in the seminiferous tubules are shown, with degenerated cells indicated in gray.

Spermatocytogenesis

Spermatogonia are diploid chromosome-containing cells located on the basement membrane. Spermatogonia are classified as type A, intermediate, and type B. Type A spermatogonia are further divided into undifferentiated and differentiated subtypes, which are located in the basal compartment of the seminiferous tubules (De Rooıj & Russell, 2000). Undifferentiated type A spermatogonia (spermatogonial stem cells) undergo mitotic divisions to form A single (As), A paired (Apr), and A aligned (Aal) spermatogonia. They then continue mitotic divisions as differentiated type A spermatogonia (A1, A2, A3, A4), subsequently giving rise to intermediate spermatogonia (In) and finally type B spermatogonia (Kanatsu-Shinohara & Shinohara, 2013).

Type B spermatogonia divide to form primary spermatocytes. Primary spermatocytes replicate their DNA and undergo meiotic divisions, passing through progressive nuclear changes known as leptotene, zygotene, pachytene, and diplotene stages to produce secondary spermatocytes. Secondary spermatocytes further divide to generate haploid cells called spermatids. In bulls, the process from spermatogonia to spermatids takes approximately 45 days (Clermont, 1972). During the

transition from spermatocyte to spermatid, the 60 chromosomes (30 pairs) of the spermatocyte segregate, with 30 chromosomes allocated to one spermatid and the other 30 to the second spermatid. This ensures that half of the fetal genetic material is derived from the spermatozoon and the other half from the oocyte provided by the mother (Hess & de Franca, 2008).

Spermiogenesis

During spermiogenesis, spermatids undergo a series of morphological changes to become mature spermatozoa. These changes include condensation of nuclear chromatin, formation of the sperm tail, and shaping of the acrosomal region. Spermatid development is divided into four phases: Golgi, cap, acrosomal, and maturation phases (Plant & Zeleznik, 2014; Russell et al., 1993).

Golgi Phase

In the Golgi phase of spermiogenesis, proacrosomal granules are formed within the Golgi apparatus. These proacrosomal granules subsequently fuse to form a single acrosomal granule, which adheres to the nuclear region. Simultaneously, tail development begins at the opposite pole of the cell. The proximal centriole migrates close to the nucleus, where it is thought to form the base for the attachment of the tail to the head (Kanatsu-Shinohara & Shinohara, 2013).

Cap Phase

The cap phase is characterized by the spreading of the adherent acrosomal granule over the surface of the spermatid nucleus. This process continues until the anterior two-thirds of each spermatid nucleus is tightly covered by a thin, double-membrane-bound vesicle. During the cap phase, the distal centriole contributes to the formation of the tail, and the axoneme begins to take shape (Fawcett, 1975).

Acrosomal Phase

The acrosomal phase of spermiogenesis is marked by major changes in the nuclei, acrosomes, and developing tails of the spermatids. These developmental changes are facilitated by the rotation of each spermatid, orienting the acrosome toward the basal compartment of the seminiferous tubule and the tail toward the lümen (Hafez & Hafez, 2013). Nuclear changes include the condensation of chromatin into dense granules and the reshaping of the spherical nucleus into an elongated, flattened structure. At this stage of development, nuclear histones are gradually replaced by transition proteins. The acrosome, situated very close to the nucleus, condenses and elongates to conform to the shape of the reshaped nucleus. These modifications in nuclear and acrosomal shape are thought to be “molded” by the Sertoli cells (Braun, 2001).

Maturation Phase

The maturation phase of spermiogenesis involves the final transformation of elongated spermatids into cells ready to be released into the lumen of the seminiferous tubule. Within the nucleus, chromatin granules undergo condensation as transition proteins are replaced by protamines, which uniformly fill the entire sperm nucleus. During the maturation phase, a fibrous sheath forms around the axoneme, with nine pairs of fibers beneath it. The fibrous sheath covers the axoneme from the neck to the beginning of the end piece (Russell et al., 1993). The annulus migrates from its position adjacent to the nucleus to a point where it demarcates the midpiece from the principal piece of the tail. Mitochondria become tightly coiled within the sheath extending from the neck to the annulus. In the later stages of spermiogenesis, Sertoli cells phagocytose the residual cytoplasmic material left after spermatid elongation, and the cell body becomes spherical (Braun, 2001).

Spermiation

The release of fully formed spermatids into the lumen of the seminiferous tubules is known as spermiation. The elongated spermatids, oriented perpendicular to the tubular wall, are released into the lumen. The residual cytoplasm, connected to spermatid groups via intercellular bridges, remains embedded in the seminiferous epithelium (Kanatsu-Shinohara & Shinohara, 2013). Sertoli cells phagocytose the residual bodies and degenerated germ cells following spermiation. In spermatogenesis, a large number of potential spermatocytes and spermatids degenerate before maturing into spermatozoa (Hafez & Hafez, 2013).

Spermatozoon Morphology

A fully developed spermatozoon is an elongated cell consisting of a flattened head, which contains the nucleus, and a tail essential for cell motility. The entire spermatozoon is covered by the plasma membrane (plasmalemma) (Abou-Haila & Tulsiani, 2000). The acrosome, or acrosomal cap, is a double-membraned structure located at the anterior portion of the sperm head. The neck region connects the head to the tail (flagellum), which is subdivided into the middle, principal, and end pieces (Figure 8) (Guraya, 2012).

The function of spermatozoa is not only to deliver the male genome to the oocyte but also to activate the oocyte arrested in the metaphase of the second meiotic division. A mature spermatozoon is a highly differentiated, haploid cell with a paddle-shaped head and a flagellum. The sperm head comprises the nucleus, acrosome, a small volume of cytoplasm, and cytoplasmic layers (Jin et al., 2011). The nucleus contains the male genome along with nuclear proteins and protamines. The acrosome of a mature spermatozoon is a cap-like vesicle covering the proximal region of the head, containing hydrolytic enzymes and matrix proteins, enclosed by the acrosomal membrane. This cap-like structure, containing enzymes such as acrosin, proacrosin, hyaluronidase, and others, is crucial in the fertilization process. The cytoplasm between the nucleus and overlying plasma membrane becomes reduced to thin layers (gaps) adjacent to the plasma membrane (Yamatoya et al., 2011).

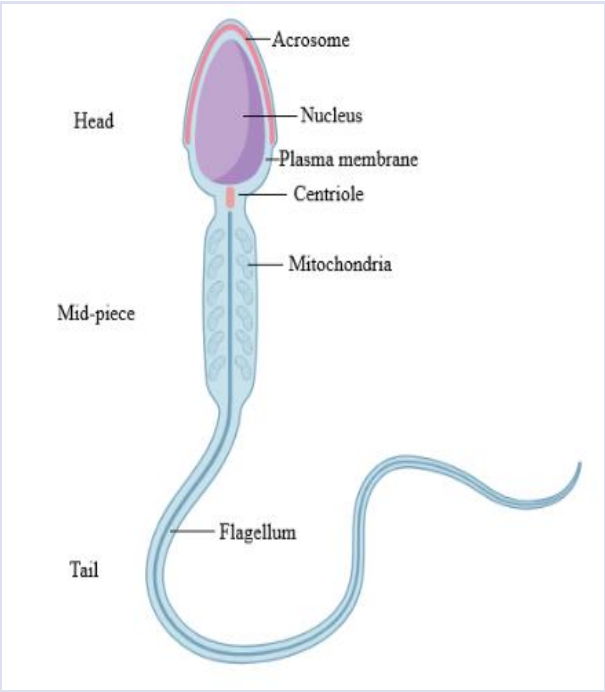


Figure 8. Parts of a bull spermatozoon. The sperm cell fulfills critical roles including movement, recognition of the oocyte, initiation of fertilization, and transmission of paternal DNA to the oocyte.

The sperm head contains an oval, flattened nucleus with highly compact chromatin. The condensed chromatin consists of deoxyribonucleic acid (DNA) and proteins called sperm protamines. The chromosome number and, hence, the DNA content of the sperm nucleus are haploid, representing half the DNA content of somatic cells of the same species (Balhorn, 2007). The tail, which confers motility to the spermatozoon, is divided into three regions: the middle piece, principal piece, and end piece. The neck, or connecting piece, forms a basal plate corresponding to an indentation at the posterior end of the nucleus. The region of the tail between the neck and the principal piece constitutes the middle piece (Eddy et al., 2003). The central core of the middle piece, and indeed of the entire tail, contains the axoneme, which is composed of nine doublet microtubules radially arranged around two central microtubules (the 9+2 structure) (Woolley, 2003). In the middle piece, this axonemal structure is surrounded externally by dense fibers. The axoneme and dense fibers of the middle piece are surrounded peripherally by numerous mitochondria, which are helically arranged around the longitudinal fibers, providing the energy required for sperm motility (Ounjai et al., 2012). The principal piece, extending from the annulus to near the tip of the tail, consists centrally of the axoneme and associated coarse fibers. The end piece, lacking the fibrous sheath, contains only the central axoneme covered by the plasma membrane. The axoneme is responsible for sperm motility, with microtubule doublets generating sliding movements between adjacent pairs, resulting in the bending waves of the tail (Garner & Hafez, 2000).

Epididymal Transport and Maturation

Following the completion of spermatogenesis in the testis, spermatozoa are not yet capable of fertilizing the oocyte. The ability to fertilize is acquired during passage through the epididymal duct, during transit through the female genital tract, through interactions with the oviductal epithelial cells, and through interactions with different female gamete structures. While the maturation of spermatozoa produced in the testis occurs in the epididymis, the changes that take place in the female genital tract are referred to as capacitation-related changes. Spermatozoa complete their epididymal maturation in approximately 7 days in bulls, 12 days in boars, and 16 days in rams (Caballero et al., 2011).

The epididymis is a long, convoluted ductal system that transports spermatozoa from the efferent ductules to the ductus deferens. Epididymal cell types exhibit specific structures and functions depending on their localization along the epididymis. The epithelial cells of the epididymis establish the blood–epididymis barrier, which maintains a unique luminal environment conducive to sperm maturation, protection, and storage (Gregory & Cyr, 2014). The epididymis consists of three main cell types: principal cells, clear cells, and basal cells. A fourth epithelial cell type, also known as narrow cells, is found in the proximal epididymal region known as the initial segments. Clear and narrow cells actively secrete protons into the lumen via a proton pump (V-ATPase) located in their apical microvilli and subapical vesicles (Breton et al., 2016). This process requires ATP hydrolysis, and thus, narrow and clear cells contain significantly more mitochondria compared to neighboring cells. The acidic environment established in the epididymis facilitates the transfer of proteins from epithelial cells to spermatozoa through extracellular vesicles called epididymosomes (Zhou et al., 2018). Basal cells do not contact the luminal compartment but regulate the function of other cells through cytoplasmic extensions between principal cells. Principal cells form tight junctions with each other to create the blood–epididymis barrier. This barrier provides an immune-privileged region within the epididymal lumen necessary for sperm maturation. Several androgen-dependent transmembrane proteins, including occludin and claudins, contribute to the formation of these tight junctions (Cyr et al., 2007).

Principal cells secrete proteins into the epididymal lumen via both merocrine and apocrine mechanisms (Hermo & Robaire, 2002). Merocrine secretion involves vesicles produced in the Golgi that fuse with the plasma membrane to release their contents into the extracellular space. Apocrine secretion delivers secretory material in the lumen through apical protrusions called epididymosomes (Farkaš, 2015). Epididymosomes are small membranous vesicles (25–300 nm in diameter) containing various proteins (approximately 250), lipids, and other molecules. Epididymosomes collected from different segments of

the epididymis are highly heterogeneous in size and content (Rejraji et al., 2006), which may explain the transfer of different proteins to spermatozoa. Proteins transferred from epididymosomes to spermatozoa can be incorporated into the sperm plasma membrane or intracellular structures. Proteins secreted by the epididymal epithelium bind to sperm and regulate calcium channels in the sperm membrane (Frenette et al., 2005).

Various proteins with different functions have been shown to be transferred to spermatozoa by epididymosomes during epididymal transit (Sullivan & Saez, 2013). Some of these proteins play roles in the development of sperm functions, such as motility (Murta et al., 2016), capacitation (Krapf et al., 2012), acrosome reaction (Joshi et al., 2013), sperm–zona pellucida interaction (Frenette & Sullivan, 2001), and fertilization (Caballero et al., 2012). The activation of sperm cells within the female reproductive system is primarily based on increased calcium ion influx. Uptake of Ca^{2+} from the female genital epithelium into the spermatozoon results in a marked increase in protein phosphorylation, the acquisition of hyperactivated motility, and sperm cell capacitation. Hyperactivation is defined as an increase in the amplitude of flagellar bending, which provides the strong tail strokes required for spermatozoa to reach the oocyte. Increased calcium concentration near the oocyte triggers the acrosome reaction in sperm. The release of acrosomal contents exposes oocyte-binding proteins on the inner acrosomal membrane, allowing gamete fusion (Brandenburger et al., 2011).

Hormonal Regulation of Spermatogenesis

Hypothalamic–Pituitary–Gonadal Axis Overview

Spermatogenesis is regulated by a coordinated hormonal balance between the hypothalamus, pituitary, and testes. Gonadotropin-releasing hormone (GnRH), a decapeptide neurohormone synthesized by the hypothalamus, is secreted into the hypophyseal portal circulation via the median eminence. GnRH binds to its receptors in the anterior lobe of the pituitary and stimulates the synthesis and secretion of LH and follicle-FSH. LH is secreted in a pulsatile manner in response to pulsatile GnRH release from the hypothalamus and reaches the testes through the vascular system. LH binds to its receptors on Leydig cells and stimulates testosterone synthesis and secretion. FSH also reaches the testes via the vascular system and acts on Sertoli cells through FSH receptors. Testosterone and its metabolite, 17β-estradiol (E2), exert negative feedback on the hypothalamus and pituitary to inhibit GnRH, LH and FSH secretion. This negative feedback mechanism is crucial for maintaining physiological hormone levels (Knobil, 2006).

Molecular Mechanism of FSH Action in Sertoli cells

FSH is a glycoprotein hormone that plays a central role in the reproductive system of mammals. In females, FSH regulates folliculogenesis and the synthesis of certain hormones, thus preparing the genital tract for fertilization, implantation, and pregnancy (Rastrelli et al., 2014). In males, this gonadotropin mediates testicular development and spermatogenesis. FSH, secreted pulsatilely in response to GnRH, reaches the testes through the vascular system (Santi et al., 2018). In the testis, FSH binds specifically to FSH receptors on the plasma membranes of Sertoli cells. The FSH receptor is a G-protein-coupled transmembrane receptor that interacts with FSH to detect extracellular signals and activate intracellular signaling pathways. Binding of FSH to its receptor induces a conformational change that triggers a cascade of biochemical reactions and a complex signaling network (Casarini & Crépieux, 2019).

The FSH binding activates adenylate cyclase (AC), increasing intracellular cAMP (cyclic adenosine monophosphate) levels (Sharpe, 2012). Elevated cAMP activates protein kinase A (PKA). In Sertoli cells, cAMP binding to PKA activates ERK1/2 through phosphorylation. ERK1/2 phosphorylates the nuclear transcription factor cAMP response element-binding protein (CREB), which induces transcription of target genes (Roth et al., 2010). Scobey et al showed that injecting non-replicating CREB into seminiferous tubules resulted in apoptosis of meiotic spermatocytes within 4 days, demonstrating that survival of germ cells depends on CREB-mediated factors produced by Sertoli cells (Scobey et al., 2001) CREB also induces transcription factors necessary for hormones regulating spermatogenesis (e.g., inhibin) and anti-apoptotic factors (e.g., bcl-2, SDF1). In addition to stimulating spermatogenesis, FSH also induces the production of androgen-binding protein (ABP) by Sertoli cells. This protein binds testosterone and helps maintain high intratesticular testosterone concentrations (Pugazhenthi et al., 2000).

Testosterone Production in Leyding Cells

Testosterone production in Leydig cells depends on pulsatile LH secretion from the anterior pituitary. The steroidogenic process involves mobilization of cholesterol to the Leydig cell membrane, its transport into mitochondria, conversion of cholesterol to pregnenolone by mitochondrial cytochrome P450, and subsequent testosterone synthesis in the smooth endoplasmic reticulum (Payne, 2007). Both testosterone and FSH stimulate Sertoli cells to secrete inhibin. Testosterone regulates Sertoli cell functions by binding to specific Sertoli cell receptors. Testosterone and inhibin exert negative feedback on FSH and LH synthesis by the anterior pituitary (Plant & Zeleznik, 2014). Cholesterol is converted to pregnenolone in the mitochondrial matrix by cytochrome P450. Pregnenolone is then metabolized to progesterone by 3β-hydroxysteroid dehydrogenase (3β-HSD), to 17-hydroxyprogesterone by 17α-hydroxylase, and finally to testosterone by 17β-hydroxysteroid dehydrogenase 3 (17β-HSD3) (Figure 9) (Miller, 2008).

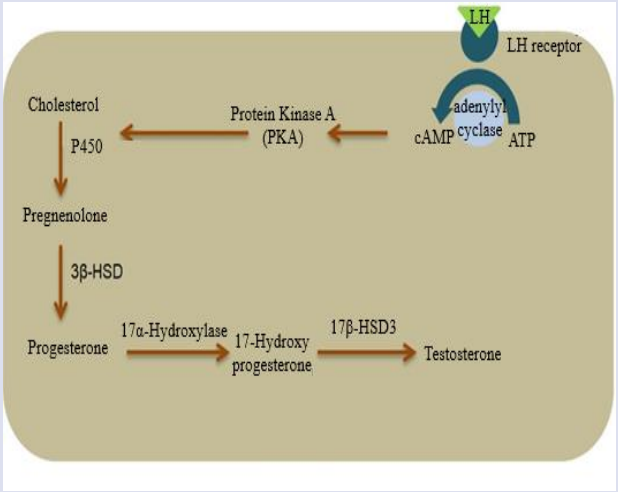


Figure 9. Leydig Cells and Testosterone Synthesis. The secretion of LH from the anterior pituitary stimulates Leydig cells via LH receptors, activating intracellular signaling cascades that enhance steroidogenesis, 3β-hydroxysteroid dehydrogenase (3β-HSD), 17β-hydroxysteroid dehydrogenase 3 (17β-HSD3), mitochondrial cytochrome P450.

Spermatogenesis is a finely regulated process involving cellular divisions and differentiations governed by FSH and testosterone. FSH acts directly on Sertoli cells through its membrane receptor and induces the secretion of inhibin, while in response to LH, testosterone is produced by the Leydig cells in the testicular interstitial compartment (Holdcraft & Braun, 2004). Leydig cell-derived testosterone works together with FSH on Sertoli cells to stimulate spermatogenesis, which depends on maintaining appropriate intratesticular testosterone levels. Testosterone is the androgen responsible for maintaining spermatogenesis. Androgen effects are mediated by the androgen receptor (AR), a 110 kDa protein localized in the cytoplasm and nucleus. In the absence of testosterone or AR, males are infertile (Chang et al., 2004). AR is essential for sexual differentiation and maintenance of normal spermatogenesis, and it is expressed in Leydig, peritubular myoid, and Sertoli cells (Wang et al., 2009).

Classical and Non-Classical Testosterone Signaling Pathways

Testosterone acts on spermatogenesis via two pathways: the classical and non-classical pathways (Roberts & Chauvin, 2019).

Classical Testosterone Pathway

In the classical pathway, testosterone diffuses through the Sertoli cell membrane and binds to AR in the cytoplasm. Upon binding, AR undergoes a conformational change, dissociates from heat shock proteins (HSP70, HSP90), and translocates into the nucleus. There, it binds to androgen response elements (AREs) on DNA and modulates gene expression (Shang et al., 2002). Although germ cells require androgens for survival and maturation, they do not express AR, and germ cell-specific AR is not required for maturation (Tsai et al., 2006). AR is expressed in Sertoli, Leydig, peritubular myoid, vascular endothelial, and vascular smooth muscle cells, and testosterone's role in spermatogenesis is mediated through these somatic cells (Zhou et al., 2002).

Non-Classical Testosterone Pathway

In the non-classical pathway, testosterone exerts its effects through two distinct mechanisms. In the first mechanism, the testosterone–androgen receptor complex binds to and activates the Src tyrosine kinase receptor. The proline-rich region of the androgen receptor (amino acids 352–359) interacts with the SH3 domain of Src tyrosine kinase (Migliaccio et al., 2000). Once Src kinase is activated, it induces the phosphorylation of the Epidermal Growth Factor Receptor (EGFR). Phosphorylation of EGFR leads to the activation of a cascade of kinases, including RAF, MEK, ERK, and MAP kinases, which transmit signals from the cell surface receptor to the DNA in the nucleus. This signaling ultimately results in the phosphorylation and activation of the transcription factor CREB (cAMP response element-binding protein) (Fix et al., 2004). Src activation is critical for spermatogenesis, regulating germ cell adhesion and sperm release from Sertoli cells (Walker, 2011). Src also phosphorylates β-catenin and N-cadherin, proteins involved in Sertoli–germ cell junctions (Xia & Cheng, 2005). The integrity of the blood–testis barrier also depends on the non-classical pathway (Figure 10) (McCabe et al., 2012).

The second mechanism involves testosterone-induced calcium influx into Sertoli cells via L-type Ca^{2+} channels, as well as GPCR-mediated activation of phospholipase C (PLC), which hydrolyzes PIP_2 to produce IP_3 and DAG. Decreased PIP_2 inhibits ATP-sensitive K^+ channels, causing membrane depolarization and further Ca^{2+} entry, which regulates multiple cellular processes (Von Ledebur et al., 2002).

Classical pathway testosterone diffuses through the plasma membrane, binds AR, which dissociates from HSPs and translocates into the nucleus, binding AREs and regulating gene expression (Fix et al., 2004). Non-classical kinase activation the testosterone-AR complex activates Src, EGFR, and MAPK signaling, leading to CREB activation and gene transcription. Non-classical calcium pathway testosterone activates GPCRs, leading to PLC activation, PIP_2 hydrolysis, K^+ channel inhibition, depolarization, and Ca^{2+} influx, influencing cellular processes (Hauser et al., 2008). The activation of kinases via non-classical pathways has been shown to regulate critical processes for maintaining spermatogenesis, including Sertoli–germ cell adhesion and spermatozoon release. Both classical and non-classical pathways in Sertoli cells are essential for sustaining spermatogenesis (Walker, 2011).

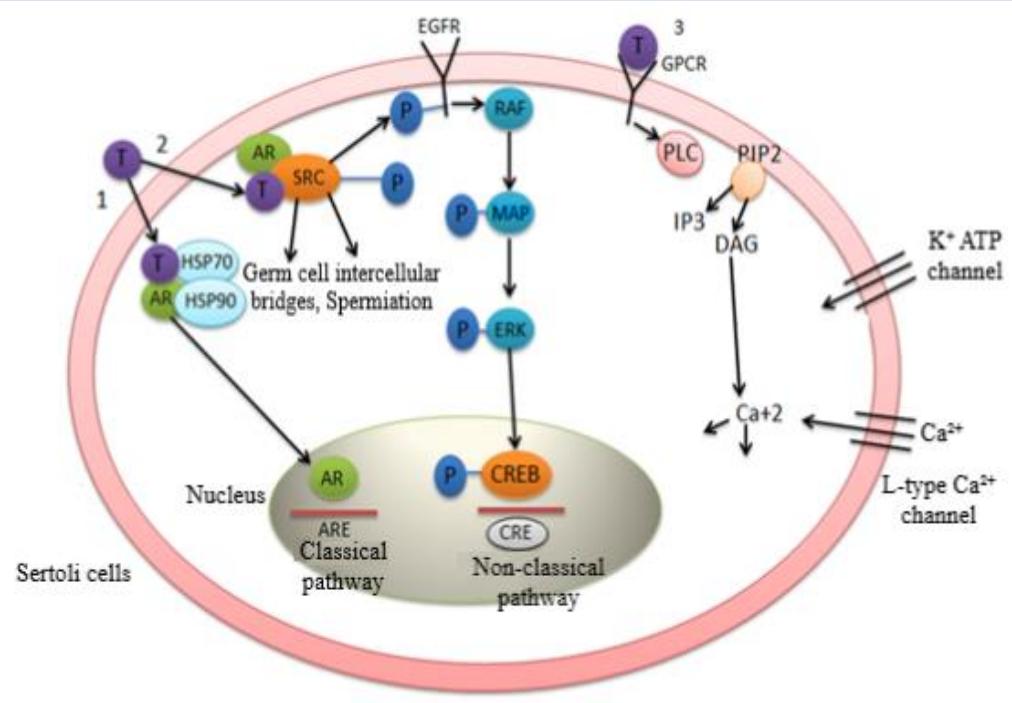


Figure 10. Testosterone signaling pathways in Sertoli cells.
1. Classical testosterone signaling pathway: Testosterone (T) diffuses across the plasma membrane and binds to the androgen receptor (AR), inducing a conformational change in AR and allowing it to dissociate from heat shock proteins (HSP70, HSP90) in the cytoplasm. The AR then translocates into the nucleus, where it binds to specific DNA sequences called androgen response elements (AREs). Binding of AR to AREs permits the activation of regulatory proteins (coactivators or corepressors), leading to altered gene expression and ultimately changes in cellular function. **2.** Non classical kinase activation pathway: Testosterone interacts with AR and can subsequently bind to and activate Src kinase, which is necessary for sperm release and germ cell intercellular bridges. Src also induces activation of the epidermal growth factor receptor (EGFR), which then leads to phosphorylation of MAP and RAF kinases, followed by sequential phosphorylation and activation of ERK, a regulator of germ cell adhesion. Consequently, genes containing cAMP response elements (CRE) can be induced by testosterone. **3.** Non-classical Ca^{2+} signaling pathway: Testosterone interacts with G protein coupled receptors (GPCR) on the plasma membrane. This activates phospholipase C (PLC), which hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP2) into inositol trisphosphate (IP3) and diacylglycerol (DAG). Decreased PIP_2 concentrations inhibit ATP sensitive potassium (K^+ ATP) channels, leading to membrane depolarization and Ca^{2+} influx through L-type Ca^{2+} channels.

Conclusion

A specialized population of adult stem cells resides within the testes, known as SSCs. These cells are unique among adult stem cells due to their ability to self-renew and differentiate, as well as being the only cell type capable of making a genetic contribution to the next generation. In recent years, numerous studies on the isolation, characterization, and transplantation of SSCs in experimental animals have advanced our understanding of their biology and highlighted their broad potential applications in reproductive and regenerative medicine. However, the very low number of SSCs present in the testes makes cell culture studies particularly important. The ability to safely isolate and transplant cells with high purity and viability remains one of the major challenges, but also constitutes the most critical step in this process.

Understanding the developmental mechanisms of germ cells is crucial for the development of treatments in veterinary and human medicine, as well as for addressing reproductive disorders. A comprehensive understanding of germ cell development can also contribute to the conservation of endangered species.

Declarations

Acknowledgments
Not applicable.

Conflict of Interest
Authors disclose no potential conflicts of interest.

Ethics Statement
Not applicable.

Informed Consent
Not applicable.

Author Contributions
Conceptualization: MNB, V.A; methodology: MNB, V.A; investigation: MNB, V.A; writing – review and editing: MNB, V.A; project administration: V.A.

Funding
Not applicable.

Data Availability
The data used to support the findings of this study can be made available upon request to the corresponding author.

References

Abou-Haila, A., & Tulsiani, D. R. P. (2000). Mammalian Sperm Acrosome: Formation, Contents, and Function. Archives of Biochemistry and Biophysics, 379(2), 173-182. DOI:10.1006/abbi.2000.1880

AL-Mamari, S. A. (2023). Anatomy of the Scrotum and Testicles. In Urogenital Trauma: A Practical Guide (pp. 355-370). Springer Nature Singapore. DOI:10.1007/978-981-99-6171-9_42

Amann, R. P. (1989). Structure and Function of the Normal Testis and Epididymis. Journal of the American College of Toxicology, 8(3), 457-471. DOI:10.3109/10915818909014532

Ariyaratne, H. B. S., & Chamindrani Mendis-Handagama, S. M. L. (2000). Changes in the Testis Interstitium of Sprague Dawley Rats from Birth to Sexual Maturity1. Biology of Reproduction, 62(3), 680-690. DOI:10.1095/biolreprod62.3.680

Ariza, L., Carmona, R., Cañete, A., Cano, E., & Muñoz-Chápuli, R. (2016). Coelomic epithelium-derived cells in visceral morphogenesis. Developmental Dynamics, 245(3), 307-322. DOI:10.1002/dvdy.24373

Auman, H. J., Nottoli, T., Lakiza, O., Winger, Q., Donaldson, S., & Williams, T. (2002). Transcription factor AP-2γ is essential in the extra-embryonic lineages for early postimplantation development. Development, 129(11), 2733-2747. DOI:10.1242/dev.129.11.2733

Bagci, H., & Fisher, A. G. (2013). DNA demethylation in pluripotency and reprogramming: the role of tet proteins and cell division. Cell Stem Cell, 13(3), 265-269.

Balhorn, R. (2007). The protamine family of sperm nuclear proteins. Genome Biology, 8(9), 227. DOI:10.1186/gb-2007-8-9-227

Bandiera, R., Vidal, V. P., Motamedi, F. J., Clarkson, M., Sahut-Barnola, I., von Gise, A., Pu, W. T., Hohenstein, P., Martinez, A., & Schedl, A. (2013). WT1 maintains adrenal-gonadal primordium identity and marks a population of AGP-like progenitors within the adrenal gland. Developmental cell, 27(1), 5-18.

Bao, S., Leitch, H. G., Gillich, A., Nichols, J., Tang, F., Kim, S., Lee, C., Zwaka, T., Li, X., & Surani, M. A. (2012). The germ cell determinant Blimp1 is not required for derivation of pluripotent stem cells. Cell Stem Cell, 11(1), 110-117. DOI:10.1016/j.stem.2012.02.023

Beddington, R. S., & Robertson, E. J. (1999). Axis development and early asymmetry in mammals. Cell, 96(2), 195-209.

Borjigin, U., Davey, R., Hutton, K., & Herrid, M. (2010). Expression of promyelocytic leukaemia zinc-finger in ovine testis and its application in evaluating the enrichment efficiency of differential plating. Reproduction, Fertility and Development, 22(5), 733-742.

Brandenburger, T., Strehler, E. E., Filoteo, A. G., Caride, A. J., Aumüller, G., Post, H., Schwarz, A., & Wilhelm, B. (2011). Switch of PMCA4 splice variants in bovine epididymis results in altered isoform expression during functional sperm maturation. Journal of Biological Chemistry, 286(10), 7938-7946. DOI:10.1074/jbc.M110.142836

Braun, R. E. (2001). Packaging paternal chromosomes with protamine. Nature Genetics, 28(1), 10-12. DOI:10.1038/ng0501-10

Breton, S., Ruan, Y. C., Park, Y.-J., & Kim, B. (2016). Regulation of epithelial function, differentiation, and remodeling in the epididymis. Asian journal of andrology, 18(1), 3. DOI:10.4103/1008-682X.165946

Caballero, J., Frenette, G., D'Amours, O., Belleannée, C., Lacroix-Pepin, N., Robert, C., & Sullivan, R. (2012). Bovine sperm raft membrane associated Glioma Pathogenesis-Related 1-like protein 1 (GliPr1L1) is modified during the epididymal transit and is potentially involved in sperm binding to the zona pellucida. Journal of cellular physiology, 227(12), 3876-3886. DOI:10.1002/jcp.24099.

Caballero, J., Frenette, G., & Sullivan, R. (2011). Post testicular sperm maturational changes in the bull: important role of the epididymosomes and prostasomes. Veterinary medicine international, 2011. DOI:10.4061/2011/757194.

Caires, K. C., de Avila, J., & McLean, D. J. (2009). Vascular endothelial growth factor regulates germ cell survival during establishment of spermatogenesis in the bovine testis. Reproduction, 138(4), 667.

Carré, G.-A., & Greenfield, A. (2016). The gonadal supporting cell lineage and mammalian sex determination: the differentiation of sertoli and granulosa cells. Molecular Mechanisms of Cell Differentiation in Gonad Development, 47-66. DOI:10.1007/978-3-319-31973-5_3

Casarini, L., & Crépieux, P. (2019). Molecular mechanisms of action of FSH. Frontiers in endocrinology, 10, 305. DOI:10.3389/fendo.2019.00305.

Chang, C., Chen, Y.-T., Yeh, S.-D., Xu, Q., Wang, R.-S., Guillou, F., Lardy, H., & Yeh, S. (2004). Infertility with defective spermatogenesis and hypotestosteronemia in male mice lacking the androgen receptor in Sertoli cells. Proceedings of the National Academy of Sciences, 101(18), 6876-6881. DOI:10.1073/pnas.0307306101.

Cheng, C. Y., & Mruk, D. D. (2002). Cell junction dynamics in the testis: Sertoli-germ cell interactions and male contraceptive development. Physiological reviews, 82(4), 825-874. DOI:10.1152/physrev.00009.2002

Chia, N.-Y., Chan, Y.-S., Feng, B., Lu, X., Orlov, Y. L., Moreau, D., Kumar, P., Yang, L., Jiang, J., & Lau, M.-S. (2010). A genome-wide RNAi screen reveals determinants of human embryonic stem cell identity. Nature, 468(7321), 316-320. DOI:10.1038/nature09531

Chu, L.-F., Surani, M. A., Jaenisch, R., & Zwaka, T. P. (2011). Blimp1 expression predicts embryonic stem cell development in vitro. Current Biology, 21(20), 1759-1765.

Clermont, Y. (1972). Kinetics of spermatogenesis in mammals: seminiferous epithelium cycle and spermatogonial renewal. Physiological Reviews, 52(1), 198-236. DOI:10.1152/physrev.1972.52.1.198

Connell, S. M. (2024). Aristotle on Memory and Emotion in Human and Non-human Animals. Memory and Emotions in Antiquity, acadcmia. edu.

Culty, M. (2009). Gonocytes, the forgotten cells of the germ cell lineage. Birth Defects Research Part C: Embryo Today: Reviews, 87(1), 1-26. DOI:10.1002/bdrc.20142

Cyr, D. G., Gregory, M., Dubé, É., Dufresne, J., Chan, P. T., & Hermo, L. (2007). Orchestration of occludins, claudins, catenins and cadherins as players involved in maintenance of the blood-epididymal barrier in animals and humans. Asian journal of andrology, 9(4), 463-475. DOI:10.1111/j.1745-7262.2007.00308.x

Dalgaard, M., Pilegaard, K., & Ladefoged, O. (2002). In Utero Exposure to Diethylstilboestrol or 4-n-Nonylphenol in Rats: Number of Sertoli Cells, Diameter and Length of Seminiferous Tubules Estimated by Stereological Methods. Pharmacology & toxicology, 90(2), 59-65. DOI:10.1034/j.1600-0773.2002.900202.x

de Barros, F. R. O., Giassetti, M. I., & Visintin, J. A. (2012). Spermatogonial stem cells and animal transgenesis. Innovations in biotechnology. 1st ed. Rijeka: InTech, 303-318.

De Rooıj, D. G., & Russell, L. D. (2000). All you wanted to know about spermatogonia but were afraid to ask. Journal of

andrology, 21(6), 776-798. DOI:10.1002/j.1939-4640.2000.tb03408.x

DeFalco, T., Potter, S. J., Williams, A. V., Waller, B., Kan, M. J., & Capel, B. (2015). Macrophages contribute to the spermatogonial niche in the adult testis. Cell reports, 12(7), 1107-1119. http://doi.org/10.1016/j.devcel.2013.09.003.

Eddy, E. M., Toshimori, K., & O'Brien, D. A. (2003). Fibrous sheath of mammalian spermatozoa. Microscopy Research and Technique, 61(1), 103-115. DOI:10.1002/jemt.10320

Farkaš, R. (2015). Apocrine secretion: new insights into an old phenomenon. Biochimica Et Biophysica Acta (BBA)-General Subjects, 1850(9), 1740-1750.

Fawcett, D. W. (1975). The mammalian spermatozoon. Developmental Biology, 44(2), 394-436. DOI:10.1016/0012-1606(75)90411-X

Fix, C., Jordan, C., Cano, P., & Walker, W. H. (2004). Testosterone activates mitogen-activated protein kinase and the cAMP response element binding protein transcription factor in Sertoli cells. Proceedings of the National Academy of Sciences, 101(30), 10919-10924. DOI:10.1073/pnas.0404278101

Frenette, G., Légaré, C., Saez, F., & Sullivan, R. (2005). Macrophage migration inhibitory factor in the human epididymis and semen. Molecular human reproduction, 11(8), 575-582. DOI:10.1093/molehr/gah197

Frenette, G., & Sullivan, R. (2001). Prostasome-like particles are involved in the transfer of P25b from the bovine epididymal fluid to the sperm surface. Molecular Reproduction and Development: Incorporating Gamete Research, 59(1), 115-121. DOI:10.1002/mrd.1013

Garner, D., & Hafez, E. (2000). Spermatozoa and seminal plasma. Reproduction in farm animals, 96-109. DOI:10.1002/9781119265306.ch7

Ge, R.-S., Dong, Q., Sottas, C. M., Chen, H., Zirkin, B. R., & Hardy, M. P. (2005). Gene expression in rat Leydig cells during development from the progenitor to adult stage: a cluster analysis. Biology of reproduction, 72(6), 1405-1415. DOI:10.1095/biolreprod.104.037499

Gonen, N., & Lovell-Badge, R. (2019). Chapter Eight - The regulation of Sox9 expression in the gonad. In B. Capel (Ed.), Current Topics in Developmental Biology (Vol. 134, pp. 223-252). Academic Press. DOI:10.1016/bs.ctdb.2019.01.004

Gregory, M., & Cyr, D. G. (2014). The blood-epididymis barrier and inflammation. Spermatogenesis, 4(2), e979619. DOI:10.4161/21565562.2014.979619

Griswold, M. D. (1998). The central role of Sertoli cells in spermatogenesis. Seminars in Cell & Developmental Biology, 9(4), 411-416. DOI:10.1006/scdb.1998.0203

Griswold, M. D. (2016). Spermatogenesis: The Commitment to Meiosis. Physiological Reviews, 96(1), 1-17. DOI:10.1152/physrev.00013.2015

Guraya, S. S. (2012). Biology of spermatogenesis and spermatozoa in mammals. Springer Science & Business Media.

Günesdogan, U., Magnúsdóttir, E., & Surani, M. A. (2014). Primoridal germ cell specification: a context-dependent cellular differentiation event. Philosophical Transactions of the Royal Society B: Biological Sciences, 369(1657), 20130543. DOI:10.1098/rstb.2013.0543

Hafez, E. S. E., & Hafez, B. (2013). Reproduction in farm animals. John Wiley & Sons.

Hamra, F. K., Schultz, N., Chapman, K. M., Grellhesl, D. M., Cronkhite, J. T., Hammer, R. E., & Garbers, D. L. (2004). Defining the spermatogonial stem cell. Developmental biology, 269(2), 393-410. DOI:10.1016/j.ydbio.2004.01.027

Hanayama, R., Tanaka, M., Miwa, K., Shinohara, A., Iwamatsu, A., & Nagata, S. (2002). Identification of a factor that links apoptotic cells to phagocytes. Nature, 417(6885), 182-187. DOI:10.1038/417182a

Hassa O, A. R. (2003). Embriyoloji. Yorum Basın Yayın Sanayi Ltd Şti, Ankara, 133–134.

Hauser, F., Cazzamali, G., Williamson, M., Park, Y., Li, B., Tanaka, Y., Predel, R., Neupert, S., Schachtner, J., Verleyen, P., & Grimmelikhuijzen, C. J. P. (2008). A genome-wide inventory of neurohormone GPCRs in the red flour beetle Tribolium castaneum. Frontiers in Neuroendocrinology, 29(1), 142-165. DOI:10.1016/j.yfrne.2007.10.003

Hermo, L., & Robaire, B. (2002). Epididymal cell types and their functions. In The epididymis: from molecules to clinical practice (pp. 81-102). Springer. DOI:10.1007/978-1-4615-0679-9_5

Hess, R. A., & de Franca, L. R. (2008). Spermatogenesis and Cycle of the Seminiferous Epithelium. In C. Y. Cheng (Ed.), Molecular Mechanisms in Spermatogenesis (pp. 1-15). Springer New York. DOI:10.1007/978-0-387-09597-4_1

Hofmann, M.-C., Braydich-Stolle, L., & Dym, M. (2005). Isolation of male germ-line stem cells; influence of GDNF. Developmental biology, 279(1), 114-124. DOI:10.1016/j.ydbio.2004.12.006

Holdcraft, R. W., & Braun, R. E. (2004). Androgen receptor function is required in Sertoli cells for the terminal differentiation of haploid spermatids. Development, 131(2), 459-467. DOI:10.1242/dev.00957

Hu, Y.-C., Okumura, L. M., & Page, D. C. (2013). Gata4 is required for formation of the genital ridge in mice. PLoS Genet, 9(7), e1003629. DOI:10.1371/journal.pgen.1003629

Huang, W., Eum, S. Y., András, I. E., Hennig, B., & Toborek, M. (2009). PPARα and PPARγ attenuate HIV-induced dysrégulation of tight junction proteins by modulations of matrix metalloproteinase and proteasome activities. The FASEB Journal, 23(5), 1596-1606.

Hummitzsch, K., Irving-Rodgers, H. F., Schwartz, J., & Rodgers, R. J. (2019). Chapter 4 - Development of the Mammalian Ovary and Follicles. In P. C. K. Leung & E. Y. Adashi (Eds.), The Ovary (Third Edition) (pp. 71-82). Academic Press. DOI:10.1016/B978-0-12-813209-8.00004-2

Irie, N., Tang, W. W., & Surani, M. A. (2014). Germ cell specification and pluripotency in mammals: a perspective from early embryogenesis. Reproductive medicine and biology, 13(4), 203-215. DOI:10.1007/s12522-014-0184-2

Ishii, M., Kanai, Y., Kanai-Azuma, M., Tajima, Y., Wei, T. T., Kidokoro, T., Sanai, Y., Kurohmaru, M., & Hayashi, Y. (2005). Adhesion activity of fetal gonadal cells to EGF and discoidin domains of milk fat globule-EGF factor 8 (MFG-E8), a secreted integrin-binding protein which is transiently expressed in mouse early gonadogenesis. Anatomy and embryology, 209(6), 485-494. DOI:10.1007/s00429-005-0463-0

Ito, M., Yokouchi, K., Yoshida, K., Kano, K., Naito, K., Miyazaki, J. I., & Tojo, H. (2006). Investigation of the fate of Sry-expressing cells using an in vivo Cre/loxP system. Development, growth & differentiation, 48(1), 41-47. DOI:10.1111/j.1440-169X.2006.00844.x

Jin, M., Fujiwara, E., Kakiuchi, Y., Okabe, M., Satouh, Y., Baba, S. A., Chiba, K., & Hirohashi, N. (2011). Most fertilizing mouse spermatozoa begin their acrosome reaction before contact with the zona pellucida during in vitro fertilization. Proceedings of the National Academy of Sciences, 108(12), 4892-4896. DOI:10.1073/pnas.1018202108

Joshi, C. S., Suryawanshi, A. R., Khan, S. A., Balasinor, N. H., & Khole, V. V. (2013). Liprin α3: a putative estrogen regulated acrosomal protein. Histochemistry and cell biology, 139(4), 535-548. DOI:10.1007/s00418-012-1044-y

Jost, A. (1947). Recherches sur la differentiation de embryon delapin. III. Arch. Anat. Microscop. Morphol. Exp, 36, 271–315.

Jost, A., Vigier, B., PrÉPin, J., & Perchellet, J. P. (1973). Studies on sex differentiation in mammals. Proceedings of the 1972 Laurentian Hormone Conference, DOI:10.1016/B978-0-12-571129-6.50004-X

Kanatsu-Shinohara, M., & Shinohara, T. (2013). Spermatogonial Stem Cell Self-Renewal and Development. Annual review of cell and developmental biology, 29(Volume 29, 2013), 163-187. DOI:10.1146/annurev-cellbio-101512-122353

Karkanaki, A., Praras, N., Katsikis, I., Kita, M., & Panidis, D. (2007). Is the Y chromosome all that is required for sex determination? Hippokratia, 11(3), 120-123.

Katoh-Fukui, Y., Miyabayashi, K., Komatsu, T., Owaki, A., Baba, T., Shima, Y., Kidokoro, T., Kanai, Y., Schedl, A., Wilhelm, D., Koopman, P., Okuno, Y., & Morohashi, K.-i. (2012). Cbx2, a Polycomb Group Gene, Is Required for Sry Gene Expression in Mice. Endocrinology, 153(2), 913-924. DOI:10.1210/en.2011-1055

Kenngott, R. A.-M., Vermehren, M., Ebach, K., & Sinowatz, F. (2013). The Role of Ovarian Surface Epithelium in Folliculogenesis during Fetal Development of the Bovine Ovary: A Histological and Immunohistochemical Study. Sexual Development, 7(4), 180-195. DOI:10.1159/000348881

Kızılay, F., & Altay, B. (2019a). Spermatogenez, spermiyogenezis ve klinik yansımaları. Androloji Bülteni, 21(4), 177-184. DOI:10.24898/tandro.2019.27443

Kızılay, F., & Altay, B. (2019b). Spermatogenez, spermiyogenezis ve klinik yansımaları.

Knobil, E. (2006). Knobil and Neill's physiology of reproduction (Vol. 2). Gulf Professional Publishing.

Kostereva, N., & Hofmann, M. C. (2008). Regulation of the spermatogonial stem cell niche. Reproduction in Domestic Animals, 43, 386-392. DOI:10.1111/j.1439-0531.2008.01189.x

Koubova, J., Menke, D. B., Zhou, Q., Capel, B., Griswold, M. D., & Page, D. C. (2006). Retinoic acid regulates sex-specific timing of meiotic initiation in mice. Proceedings of the National Academy of Sciences, 103(8), 2474-2479. DOI:10.1073/pnas.0510813103.

Krapf, D., Ruan, Y. C., Wertheimer, E. V., Battistone, M. A., Pawlak, J. B., Sanjay, A., Pilder, S. H., Cuasnicu, P., Breton, S., & Visconti, P. E. (2012). cSrc is necessary for epididymal development and is incorporated into sperm during epididymal transit. Developmental biology, 369(1), 43-53. DOI:10.1016/j.ydbio.2012.06.017 .

Krinke, G. (2000). The Laboratory Rat. Academic Press, 1st Ed, 150-152, 311-312.

Kurimoto, K., Yabuta, Y., Ohinata, Y., Shigeta, M., Yamanaka, K., & Saitou, M. (2008). Complex genome-wide transcription dynamics orchestrated by Blimp1 for the specification of the germ cell lineage in mice. Genes & development, 22(12), 1617-1635. http://doi.org/10.1101/gad.1649908.

Kusaka, M., Katoh-Fukui, Y., Ogawa, H., Miyabayashi, K., Baba, T., Shima, Y., Sugiyama, N., Sugimoto, Y., Okuno, Y., Kodama, R., Iizuka-Kogo, A., Senda, T., Sasaoka, T., Kitamura, K., Aizawa, S., & Morohashi, K.-i. (2010). Abnormal Epithelial Cell Polarity and Ectopic Epidermal Growth Factor Receptor (EGFR) Expression Induced in Emx2 KO Embryonic Gonads. Endocrinology, 151(12), 5893-5904. DOI:10.1210/en.2010-0915.

Li, N., Wang, T., & Han, D. (2012). Structural, cellular and molecular aspects of immune privilege in the testis. Frontiers in immunology, 3, 152. DOI:10.3389/fimmu.2012.00152.

Liu, C., Rodriguez, K., & Yao, H. H.-C. (2016). Mapping lineage progression of somatic progenitor cells in the mouse fetal testis. Development, 143(20), 3700-3710. Doi: 10.1242/dev.135756. DOI: 10.1242/dev.135756.

Mazaud, S., Oreal, E., Guigon, C., Carre-Eusebe, D., & Magre, S. (2002). Lhx9 expression during gonadal morphogenesis as related to the state of cell differentiation. Gene Expression Patterns, 2(3-4), 373-377. DOI: 10.1016/S1567-133X(02)00050-9.

McCabe, M. J., Allan, C. M., Foo, C. F., Nicholls, P. K., McTavish, K. J., & Stanton, P. G. (2012). Androgen initiates Sertoli cell tight junction formation in the hypogonadal (hpg) mouse. Biology of reproduction, 87(2), 38, 31-38. DOI: 10.1095/biolreprod.111.094318.

McLaren, A. (1999). Signaling for germ cells. Genes Development, 13, 373-376.

Meng, X., Pata, I., Pedrono, E., Popsueva, A., de Rooij, D. G., Jänne, M., Rauvala, H., & Sariola, H. (2001). Transient disruption of spermatogenesis by deregulated expression of neurturin in testis. Molecular and cellular endocrinology, 184(1-2), 33-39. DOI: 10.1016/S0303-7207(01)00649-9.

Mıchela Schultz, F., & Wılson, J. D. (1974). Virilization of the Wolffian duct in the rat fetus by various androgens. Endocrinology, 94(4), 979-986.

Midzak, A. S., Chen, H., Papadopoulos, V., & Zirkin, B. R. (2009). Leydig cell aging and the mechanisms of reduced testosterone synthesis. Molecular and cellular endocrinology, 299(1), 23-31. DOI: 10.1016/j.mce.2008.07.016.

Migliaccio, A., Castoria, G., Domenico, M. D., de Falco, A., Bilancio, A., Lombardi, M., Barone, M. V., Ametrano, D., Zannini, M. S., & Abbondanza, C. (2000). Steroid-induced androgen receptor–oestradiol receptor β–Src complex triggers prostate cancer cell proliferation. The EMBO journal, 19(20), 5406-5417. DOI:10.1093/emboj/19.20.5406.

Miller, W. L. (2008). Steroidogenic enzymes. In Disorders of the human Adrenal cortex (Vol. 13, pp. 1-18). Karger Publishers.

Miura, K., Tomita, A., & Kanai, Y. (2018). Sex Determination and Differentiation in Mammals. In Reproductive and Developmental Strategies (pp. 407-433). Springer. DOI:10.1007/978-4-431-56609-0_20

Mork, L., Maatouk, D. M., McMahon, J. A., Guo, J. J., Zhang, P., McMahon, A. P., & Capel, B. (2012). Temporal differences in granulosa cell specification in the ovary reflect distinct follicle fates in mice. Biology of reproduction, 86(2), 37, 31-39. DOI:10.1095/biolreprod.111.095208 .

Morohashi, K., Baba, T., & Tanaka, M. (2013). Steroid hormones and the development of reproductive organs. Sexual Development, 7(1-3), 61-79.

Mruk, D. D., & Cheng, C. (2010). Tight junctions in the testis: new perspectives. Philosophical Transactions of the Royal Society B: Biological Sciences, 365(1546), 1621-1635. DOI:10.1098/rstb.2010.0010 .

Mruk, D. D., & Cheng, C. Y. (2004). Sertoli-Sertoli and Sertoli-Germ Cell Interactions and Their Significance in Germ Cell Movement in the Seminiferous Epithelium during Spermatogenesis. Endocrine Reviews, 25(5), 747-806. DOI:10.1210/er.2003-0022 .

Mruk, D. D., & Cheng, C. Y. (2015). The mammalian blood-testis barrier: its biology and regulation. Endocrine reviews, 36(5), 564-591.

Murta, D., Batista, M., Silva, E., Trindade, A., Henrique, D., Duarte, A., & Lopes-da-Costa, L. (2016). Notch signaling in the epididymal epithelium regulates sperm motility and is transferred at a distance within epididymosomes. Andrology, 4(2), 314-327. DOI:10.1111/andr.12144.

Münsterberg, A., & Lovell-Badge, R. (1991). Expression of the mouse anti-Müllerian hormone gene suggests a role in both male and female sexual differentiation. Development, 113(2), 613-624. DOI:10.1242/dev.113.2.613.

Nagamatsu, G., Kosaka, T., Kawasumi, M., Kinoshita, T., Takubo, K., Akiyama, H., Sudo, T., Kobayashi, T., Oya, M., & Suda, T. (2011). A germ cell-specific gene, Prmt5, works in somatic cell reprogramming. Journal of Biological Chemistry, 286(12), 10641-10648. DOI 10.1074/jbc.M110.216390.

Nakaki, F., & Saitou, M. (2014). PRDM14: a unique regulator for pluripotency and epigenetic reprogramming. Trends in biochemical sciences, 39(6), 289-298.

Nakata, H., Wakayama, T., Sonomura, T., Honma, S., Hatta, T., & Iseki, S. (2015). Three-dimensional structure of seminiferous tubules in the adult mouse. Journal of anatomy, 227(5), 686-694. DOI:10.1111/joa.12375.

Nef, S., Stévant, I., & Greenfield, A. (2019a). Chapter Six - Characterizing the bipotential mammalian gonad. In B. Capel (Ed.), Current Topics in Developmental Biology (Vol. 134, pp. 167-194). Academic Press. DOI:10.1016/bs.ctdb.2019.01.002 .

Nef, S., Stévant, I., & Greenfield, A. (2019b). Characterizing the bipotential mammalian gonad. Current topics in developmental biology, 134, 167-194. DOI:10.1016/bs.ctdb.2019.01.002.

Neumann, F. v., von Berswordt-Wallrabe, R., Elger, W., Steinbeck, H., Hahn, J., & Kramer, M. (1970). Aspects of androgen-dependent events as studied by antiandrogens. Proceedings of the 1969 Laurentian Hormone Conference, DOI:10.1016/B978-0-12-571126-5.50013-3.

Nicol, B., & Yao, H. H.-C. (2015). Gonadal identity in the absence of pro-testis factor SOX9 and pro-ovary factor beta-catenin in mice. Biology of reproduction, 93(2), 35, 31-12. DOI:10.1095/biolreprod.115.131276.

Ogawa, T., Dobrinski, I., Avarbock, M. R., & Brinster, R. L. (2000). Transplantation of male germ line stem cells restores fertility in infertile mice. Nature medicine, 6(1), 29-34.

Ohinata, Y., Ohta, H., Shigeta, M., Yamanaka, K., Wakayama, T., & Saitou, M. (2009). A signaling principle for the specification of the germ cell lineage in mice. Cell, 137(3), 571-584. DOI 10.1016/j.cell.2009.03.014 .

Ohinata, Y., Payer, B., O'Carroll, D., Ancelin, K., Ono, Y., Sano, M., Barton, S. C., Obukhanych, T., Nussenzweig, M., & Tarakhovsky, A. (2005). Blimp1 is a critical determinant of the germ cell lineage in mice. Nature, 436(7048), 207-213. DOI:10.1038/nature03813.

Ota, T., Asahina, H., Park, S.-H., Huang, Q., Minegishi, T., Auersperg, N., & Leung, P. C. (2008). HOX cofactors expression and regulation in the human ovary. Reproductive Biology and Endocrinology, 6(1), 1-9. DOI:10.1186/1477-7827-6-49.

Ounjai, P., Kim, K. D., Lishko, P. V., & Downing, K. H. (2012). Three-dimensional structure of the bovine sperm connecting piece revealed by electron cryotomography. Biol Reprod, 87(3), 73. DOI:10.1095/biolreprod.112.101980 .

Park, S. Y., & Jameson, J. L. (2005). Minireview: Transcriptional Regulation of Gonadal Development and Differentiation. Endocrinology, 146(3), 1035-1042. DOI:10.1210/en.2004-1454 .

Parma, P., & Radi, O. (2012). Molecular mechanisms of sexual development. Sexual Development, 6(1-3), 7-17. DOI:10.1159/000332209.

Payne, A. H. (2007). Steroidogenic enzymes in Leydig cells. In The Leydig cell in health and disease (pp. 157-171). Springer. DOI:10.1007/978-1-59745-453-7_10.

Piprek, R. P., Kloc, M., & Kubiak, J. Z. (2016). Early development of the gonads: origin and differentiation of the somatic cells of the genital ridges. In Molecular Mechanisms of Cell Differentiation in Gonad Development (pp. 1-22). Springer. DOI:10.1007/978-3-319-31973-5_1.

Pitetti, J.-L., Calvel, P., Romero, Y., Conne, B., Truong, V., Papaioannou, M. D., Schaad, O., Docquier, M., Herrera, P. L., & Wilhelm, D. (2013). Insulin and IGF1 receptors are essential for XX and XY gonadal differentiation and adrenal development in mice. PLoS Genet, 9(1), e1003160. DOI:10.1371/journal.pgen.1003160.

Plant, T. M., & Zeleznik, A. J. (2014). Knobil and Neill's physiology of reproduction. Academic Press.

Pointis, G., & Segretain, D. (2005). Role of connexin-based gap junction channels in testis. Trends in Endocrinology & Metabolism, 16(7), 300-306.

Potter, S. J., & DeFalco, T. (2017). Role of the testis interstitial compartment in spermatogonial stem cell function. Reproduction (Cambridge, England), 153(4), R151. doi: 10.1530/REP-16-0588.

Pugazhenthi, S., Nesterova, A., Sable, C., Heidenreich, K. A., Boxer, L. M., Heasley, L. E., & Reusch, J. E.-B. (2000). Akt/protein kinase B up-regulates Bcl-2 expression through cAMP-response element-binding protein. Journal of Biological Chemistry, 275(15), 10761-10766.

Rastrelli, G., Corona, G., Mannucci, E., & Maggi, M. (2014). Factors affecting spermatogenesis upon gonadotropin-replacement therapy: a meta-analytic study. Andrology, 2(6), 794-808. DOI:10.1111/andr.262 .

Rejraji, H., Sion, B., Prensier, G., Carreras, M., Motta, C., Frenoux, J.-M., Vericel, E., Grizard, G., Vernet, P., & Drevet, J. R. (2006). Lipid remodeling of murine epididymosomes and spermatozoa during epididymal maturation. Biology of reproduction, 74(6), 1104-1113. DOI:10.1095/biolreprod.105.049304.

Rey, R., Josso, N., & Racine, C. (2020). Sexual differentiation. Endotext [Internet].

Roberts, K. P., & Chauvin, T. R. (2019). Molecular mechanisms of testosterone action on the testis. Current Opinion in Endocrine and Metabolic Research, 6, 29-33. DOI:10.1016/j.coemr.2019.03.003.

Roth, M. Y., Lin, K., Amory, J., Matsumoto, A., Anawalt, B., Snyder, C., Kalhorn, T., Bremner, W., & Page, S. (2010). Serum LH correlates highly with intratesticular steroid levels in normal men. Journal of andrology, 31(2), 138-145. DOI:10.2164/jandrol.109.008391.

Russell, L. D., Ettlin, R. A., Hikim, A. P. S., & Clegg, E. D. (1993). Histological and Histopathological Evaluation of the Testis. International Journal of Andrology, 16(1), 83-83. DOI:10.1111/j.1365-2605.1993.tb01156.x .

Ryu, B.-Y., Kubota, H., Avarbock, M. R., & Brinster, R. L. (2005). Conservation of spermatogonial stem cell self-renewal signaling between mouse and rat. Proceedings of the National Academy of Sciences, 102(40), 14302-14307.

Santi, D., Potì, F., Simoni, M., & Casarini, L. (2018). Pharmacogenetics of G-protein-coupled receptors variants: FSH receptor and infertility treatment. Best Practice & Research Clinical Endocrinology & Metabolism, 32(2), 189-200. DOI:10.1016/j.beem.2018.01.001.

Sarma, K., & Devi, J. (2012). Changes in the seminiferous epithelium of the testes during postnatal development in Assam goat. Anatomy research international, 2012. DOI:10.1155/2012/620924

Schäfer, S., Anschlag, J., Nettersheim, D., Haas, N., Pawig, L., & Schorle, H. (2011). The role of BLIMP1 and its putative downstream target TFAP2C in germ cell development and germ cell tumours. International journal of andrology, 34(4pt2), e152-e159. DOI:10.1111/j.1365-2605.2011.01167.x

Schmahl, J., Eicher, E. M., Washburn, L. L., & Capel, B. (2000). Sry induces cell proliferation in the mouse gonad. Development, 127(1), 65-73. DOI:10.1242/dev.127.1.65

Schnabel, C. A., Selleri, L., & Cleary, M. L. (2003). Pbx1 is essential for adrenal development and urogenital differentiation. Genesis, 37(3), 123-130. DOI:10.1002/gene.10235.

Scobey, M. J., Bertera, S., Somers, J. P., Watkins, S. C., Zeleznik, A. J., & Walker, W. H. (2001). Delivery of a cyclic adenosine 3', 5'-monophosphate response element-binding protein (creb) mutant to seminiferous tubules results in impaired spermatogenesis. Endocrinology, 142(2), 948-954. DOI:10.1210/endo.142.2.7948

Seda O, L. F., Sedova L (2006). Sex Determination. Genetics and Genomics, Multimedia E-textbook of Medical Biology.

Sekido, R., & Lovell-Badge, R. (2008). Sex determination involves synergistic action of SRY and SF1 on a specific Sox9 enhancer. Nature, 453(7197), 930-934. DOI:10.1038/nature06944

Shang, Y., Myers, M., & Brown, M. (2002). Formation of the androgen receptor transcription complex. Molecular cell, 9(3), 601-610. DOI:10.1016/S1097-2765(03)00237-5

Sharma, R., Bhat, R., Goyal, A., & Bhardwaj, J. (2015). Germ cells apoptosis during spermatogenesis in mammals. Journal of Entomology and Zoology Studies, 3(3), 506-515.

Sharpe, R. M. (2012). Sperm counts and fertility in men: a rocky road ahead: Science & Society Series on Sex and Science. EMBO reports, 13(5), 398-403. DOI:10.1038/embor.2012.50

Shetty, G., & Meistrich, M. L. (2007). The missing niche for spermatogonial stem cells: do blood vessels point the way? Cell Stem Cell, 1(4), 361-363. DOI 10.1016/j.stem.2007.09.013

Smitz, J., & Cortvrindt, R. G. (2002). The earliest stages of folliculogenesis in vitro. Reproduction, 123(2), 185-202. DOI:10.1530/rep.0.1230185

Starz-Gaiano, M., & Lehmann, R. (2001). Moving towards the next generation. Mechanisms of development, 105(1-2), 5-18. DOI:10.1016/S0925-4773(01)00392-6

Stévant, I., Kühne, F., Greenfield, A., Chaboissier, M.-C., Dermitzakis, E. T., & Nef, S. (2018). Single-cell transcriptomics of the mouse gonadal soma reveals the establishment of sexual dimorphism in distinct cell lineages. BioRxiv, 410407. DOI:10.1101/410407

Sullivan, R., & Saez, F. (2013). Epididymosomes, prostasomes, and liposomes: their roles in mammalian male reproductive physiology. Reproduction, 146(1), R21-R35. DOI:10.1530/REP-13-0058

Tadokoro, Y., Yomogida, K., Ohta, H., Tohda, A., & Nishimune, Y. (2002). Homeostatic regulation of germinal stem cell proliferation by the GDNF/FSH pathway. Mechanisms of development, 113(1), 29-39. DOI:10.1016/S0925-4773(02)00004-7

Taylor, R. M. (2020). Investigation into germ cell fate determination of rat embryonic stem cells. http://dx.doi.org/10.7488/era/281

Tsai, M.-Y., Yeh, S.-D., Wang, R.-S., Yeh, S., Zhang, C., Lin, H.-Y., Tzeng, C.-R., & Chang, C. (2006). Differential effects of spermatogenesis and fertility in mice lacking androgen receptor in individual testis cells. Proceedings of the National Academy of Sciences, 103(50), 18975-18980. DOI:10.1073/pnas.0608565103

Von Ledebur, E., Almeida, J., Loss, E., & Wassermann, G. (2002). Rapid effect of testosterone on rat Sertoli cell membrane potential. Relationship with K+ ATP channels. Hormone and metabolic research, 34(10), 550-555. DOI: 10.1055/s-2002-35426

Walker, W. H. (2011). Testosterone signaling and the regulation of spermatogenesis. Spermatogenesis, 1(2), 116-120. DOI:10.4161/spmg.1.2.16956

Wang, Q., Lan, Y., Cho, E.-S., Maltby, K. M., & Jiang, R. (2005). Odd-skipped related 1 (Odd1) is an essential regulator of heart and urogenital development. Developmental biology, 288(2), 582-594. DOI:10.1016/j.ydbio.2005.09.024

Wang, R.-S., Yeh, S., Tzeng, C.-R., & Chang, C. (2009). Androgen receptor roles in spermatogenesis and fertility: lessons from testicular cell-specific androgen receptor knockout mice. Endocrine reviews, 30(2), 119-132.

Wear, H. M., Eriksson, A., Yao, H. H.-C., & Watanabe, K. H. (2017). Cell-based computational model of early ovarian development in mice. Biology of reproduction, 97(3), 365-377. DOI:10.1093/biolre/iox089

Weinbauer, G. F., & Nieschlag, E. (1998). The role of testosterone in spermatogenesis. In Testosterone: action-deficiency-substitution (pp. 143-168). Springer. DOI:10.1007/978-3-642-72185-4_4

Welling, M., & Geijsen, N. (2013). Uncovering the true identity of naive pluripotent stem cells. Trends in cell biology, 23(9), 442-448.

Woolley, D. (2003). Motility of spermatozoa at surfaces. REPRODUCTION-CAMBRIDGE-, 126(2), 259-270. DOI:10.1530/rep.0.1260259

Xia, W., & Cheng, C. Y. (2005). TGF-β3 regulates anchoring junction dynamics in the seminiferous epithelium of the rat testis via the Ras/ERK signaling pathway: an in vivo study. Developmental biology, 280(2), 321-343. DOI:10.1016/j.ydbio.2004.12.036

Yamatoya, K., ITO, C., ARAKI, M., FURUSE, R., & TOSHIMORI, K. (2011). Dynamics of the mammalian sperm head: modifications and maturation events from spermatogenesis to egg activation Dynamics of the mammalian sperm head: modifications and maturation events from spermatogenesis to egg activation, 2009. Reproductive medicine and biology, 10(2), 97-103.

Yamazaki, Y., Mann, M. R. W., Lee, S. S., Marh, J., McCarrey, J. R., Yanagimachi, R., & Bartolomei, M. S. (2003). Reprogramming of primordial germ cells begins before migration into the genital ridge, making these cells inadequate donors for reproductive cloning. Proceedings of the National Academy of Sciences, 100(21), 12207-12212. DOI:10.1073/pnas.2035119100

Yoshida, S., Sukeno, M., Nakagawa, T., Ohbo, K., Nagamatsu, G., Suda, T., & Nabeshima, Y.-i. (2006). The first round of mouse spermatogenesis is a distinctive program that lacks the self-renewing spermatogonia stage. Development, 133(8), 1495-1505. DOI:10.1242/dev.02316

Young, L. E. (2006). Focus on stem cells in reproduction. Reproduction, 132(5), 671-672. DOI:10.1530/REP-06-0191

Zheng, Y., Zhang, Y., Qu, R., He, Y., Tian, X., & Zeng, W. (2014). Spermatogonial stem cells from domestic animals: progress and prospects. Reproduction, 147(3), R65-R74. DOI: 10.1530/REP-13-0466

Zhou, Q., Nie, R., Prins, G. S., Saunders, P. T., Katzenellenbogen, B. S., & Hess, R. A. (2002). Localization of androgen and estrogen receptors in adult male mouse reproductive tract. Journal of andrology, 23(6), 870-881. DOI:10.1002/j.1939-4640.2002.tb02345.x

Zhou, W., De Iuliis, G. N., Dun, M. D., & Nixon, B. (2018). Characteristics of the epididymal luminal environment responsible for sperm maturation and storage. Frontiers in endocrinology, 9, 59. DOI: 10.3389/fendo.2018.00059