Spring 2024 – Systems Biology of Reproduction
Lecture Outline – Testis Systems Biology
Michael K. Skinner – Biol 475/575
CUE 418, 10:35-11:50 am, Tuesday & Thursday
February 20, 2024
Week 7

Testis Systems Biology

- Cell Biology of the Testis
    - Cell Types and Organization
    - Cell Associations

- Spermatogenesis
    - Stages and Cycle
    - Germ Cell Differentiation
    - Genes Involved

- Endocrinology of the Testis
    - Gonadotropins
    - Testosterone and Leydig Cell

- Cell-Cell Interactions
    - Types of Interactions
    - Sertoli-Germ Cell Interactions
    - Other Cellular Interactions

Required Reading

de Kretser, et al. (2018) Structure/Cells Overview. In: Encyclopedia of Reproduction (Second Edition). Volume 1, Pages 10-16

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Structure/Cells Overview

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Development of Testes

The testes are located in the scrotum since sperm production requires a temperature that is cooler than that of the abdomen (de Kretser, 2016). They develop in the embryo just distal to the kidneys and descend into the scrotum shortly after birth through the inguinal canal. This canal, found on both sides in the region of the groin, is formed by the attachments of one of the muscles of the abdominal wall. The canal extends downwards and medially in the groin and links the abdominal cavity with the scrotum (de Kretser, 2016; de Kretser et al., 1982; Clermont and Huckins, 1961; Roosen-Runge and Holstein, 1978; Hutson et al., 1990).

Descent of the Testes

In some males, the inguinal canal does not close and the testes may retract from the scrotum into the abdominal cavity for brief periods. The descent of the testis is important because the temperature of the scrotum is lower than the intra-abdominal temperature and the germ cells require a lower temperature for their survival (Hutson et al., 1992). In some males, the inguinal canal which normally closes after the testes descend, remains patent and the testes may retract for varying periods causing damage to the germ cells because of the higher intra-abdominal temperature. Descent of the testis begins in the fetus at about 28 weeks of gestation and should be complete by birth and it is controlled by a Leydig cell secreted protein called insulin-like protein 3 which is a member of the insulin-like protein super family (de Kretser et al., 1982; Bowles and Koopman, 2007).

Failure of the testes to descend should be diagnosed as soon possible as spermatogenic damage and infertility may result. Surgery can be undertaken to close off the inguinal canal so that the testes are permanently located in the scrotum. The testes are ovoid in shape and in adults, their volume ranges from 15 to 35 mL. At birth they are about 1–3 mL in volume grow rapidly during pubertal development. The availability of an orchidometer, a range of spheres from 1 to 3 mL in progressively increasing volumes to 35 mL, is every helpful in determining testicular size (**Fig. 1**).

Fig. 1 *Orchidometer*. This set of models of differing testicular size helps the physician in assessing the size of the testes in patients with delayed puberty, infertility or potential testicular tumors.

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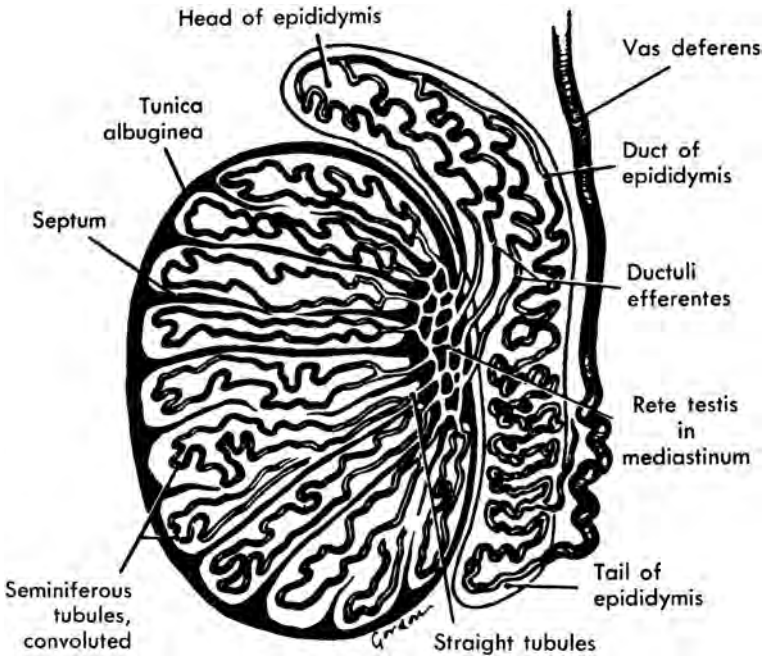

Fig. 2 The anatomical features of the testis, epididymis, vas deferens and the pampiniform plexus of veins, that surround the testicular artery, represent the venous drainage of the testis and epididymis.

The testes are covered by thick fibrous tissue that forms the tunica albuginea and should be smooth on their anterior and lateral surfaces. These surfaces are also covered by a serous membrane called the tunica vaginalis. Posteriorly, in the region under the epididymis, the tunica albuginea is thickened and projects into the parenchyma of the testes to form the mediastinum of the testis. A series of tubules traverse the mediastinum of the testis to link the seminiferous tubules to the efferent ducts that form the head of the epididymis (Roosen-Runge and Holstein, 1978) (**Fig. 2**).

In prepubertal boys, the germ cells in the testes are called gonocytes which are centrally placed in the seminiferous cords, that are the precursors of the seminiferous tubules. The other cellular components of the cords are the immature Sertoli cells that extend from the basement membrane of the tubule to the lumen of the seminiferous tubules. As development proceeds, the gonocytes move to the periphery of the cords to lie on the basement membrane of the cords and commence dividing by mitosis to give rise to the spermatogonial stem cells a process that requires retinoic acid and Oct 4 (de Rooji and Russell, 2000; de Rooij and Grootegoed, 1998; Dann et al., 2008; Bowles and Koopman, 2007). Continuation of spermatogonial mitosis requires the action of Foxo1 and the spermatogonial stem cells have the capacity for pluipotency, a characteristic marker of stem cells in many tissues (de Rooji and Russell, 2000; de Rooij, 2001; Goertz et al., 2011). When this occurs at the time of puberty, the basally placed Sertoli cells form specialized tight junctions just central to the gonocytes thereby preventing inter-cellular transport of substances and creating a blood–testis barrier (Dann et al., 2008). External to the basement membrane of the seminiferous tubules, there is a layer of myofibroblasts that can contract and increase the intra-tubular pressure. This facilitates the movement of sperm and the fluid produced by the Sertoli cells in to the rete testis (Simoni et al., 1999).

Functions

The testes have three functions, the production of sperm, the secretion of the steroid hormone, testosterone, after puberty and the production and secretion of protein hormones inhibin, activin, and follistatin. In addition, insulin and IGF1 are important in the control of Sertoli cell proliferation (Pitetti et al., 2013). Testosterone is synthesized and secreted by the Leydig cells of the testis that lie close to blood vessels found in the inter-tubular region of the testis. The Leydig cells have the characteristics of steroid secreting cells, namely a well developed smooth endoplasmic reticulum and mitochondria which have tubular cristae unlike "conventional" mitochondria in which the inner mitochondrial membrane forms "plate-like" cristae (de Kretser, 1967).

There are also lymphatic vessels in the inter-tubular compartment of the testes and these join abdominal lymphatics that also transport testosterone into the chest where they join the thoracic duct, the common duct of all lymphatic vessels in the body. The thoracic duct joins the venous system at the junction of the left subclavian vein and the left internal jugular vein (Stanton, 2016) (**Fig. 2**).

Spermatogenesis

The testes produce sperm in tubules termed seminiferous tubules by a process called spermatogenesis. These tubules are composed of germ cells and the supporting network of Sertoli cells. The tubules are surrounded by a layer of basement membrane, external to which lies a plate-like layer of peritubular cells which are contractile effectively "squeezing" the seminiferous tubules (Holstein et al., 1996; Simoni et al., 1999). Thereby they assist in moving sperm, released into the lumen of the seminiferous tubules, toward an irregular network of tubules located at the posterior and superior pole of the testis called the rete testis.

The rete testis is connected with the duct of the epididymis, a coiled tube that lies at the posterior aspect of the testis and is divided into the head, body and tail, the latter continuing as the vas deferens (Johnston and Whillis, 1954). In the epididymis, the sperm, which are still not motile, are moved by muscular contractions from the head to the tail of the epididymis. They acquire mobility as they pass through the epididymis to enter the vas deferens (Baker, 1989). The latter delivers the sperm during ejaculation to enter the prostatic urethra and pass through the penile urethra.

The cellular components in the seminiferous tubules are the germ cells that are the precursors of sperm and also the Sertoli cells. The latter are named after the person who first described them and they are a critical component of the seminiferous epithelium. They extend from the basement membrane of the tubule to the lumen and send projections between the surrounding germ cells not unlike the branches of a tree from the trunk. These projections contain microfilaments that provide a structural framework for the epithelium given that the germ cell components migrate from the basally placed spematogonia to the centrally placed spermatids and their final product, the spermatozoa (**Fig. 3**).

In prepubertal boys, the germ cells in the testes are called gonocytes and they are centrally placed in the seminiferous cords that comprise the testis (Clermont and Huckins, 1961; de Rooij and Grootegoed, 1998; de Rooji and Russell, 2000). The other cells comprising the cords are the immature Sertoli cells that extend from the basement membrane of the cords surrounding the gonocytes. At the commencement of puberty, the gonocytes move to the periphery of the cords and the Sertoli cells form specialized cell junctions central to the gonocytes which will progress to give rise to the population of spermatogonia, the precursors to the subsequent stages of spermatogenesis (Johnston and Whillis, 1954). These changes, under the influence of the pubertal increase in FSH, act through Foxo1 and Oct 4 (Dann et al., 2008).

Where adjacent Sertoli cell projections meet basally, they form specialized tight cell junctions that prevent inter-cellular transport creating a blood–testis barrier (Dym and Fawcett, 1970: Russell, 1977). These tight junctions are placed at such a position in the seminiferous epithelium that only the spematogonia are in contact with the basement membrane of the seminiferous tubules. All other germ cells lie central to the blood–testis barrier and are thus dependent on the Sertoli cells for transport of materials for optimal germ cell function and can be considered to "nurse" germ cells central to these inter-Sertoli cell junctions.

The inter-Sertoli cell junctions must open centrally to enable the progeny of spermatogonia, the primary spermatocytes, to leave the basal compartment and enter the luminal compartment. The inter-Sertoli cell junctions reform basally below the primary spermatocytes that now lie within adluminal compartment of the seminiferous tubule (Stanton, 2016).

Studies have shown that the number of Sertoli cells can affect the magnitude of sperm production. One of the important factors that controls Sertoli cell numbers is activin A which stimulates proliferation and inhibits differentiation of Sertoli cells (Baker, 1989; Kreuger et al., 1974). Increasing systemic levels of activin A using an adeno-associated virus expressing activin A (Russell, 1977) stimulated proliferation and prevented differentiation of Sertoli cells in mice. This was associated with disruption of the blood testis barrier formed by the inter-Sertoli cell junctions and resulted in a 23.5% decrease in testis weight due to diminished spermatogenesis linked to disordered Sertoli cell function. The latter was associated with increase in markers of juvenile Sertoli cells and a decrease in claudin-11, a marker of mature Sertoli cells. These data are consistent with studies of the levels of activin A in mice during normal post-natal development which established that activin A levels are elevated at birth but decline rapidly after day 4 postpartum (Meehan et al., 2000).

Other studies using treatment with FSH or thyroxine, a hormone secreted by the thyroid gland, can enhance Sertoli cell proliferation and thus increase sperm output.

In part, the action of FSH on spermatogenesis is exerted directly via spematogonia which are the only germ cells that have FSH receptors (Simoni et al., 1999). Germ cells also do not have androgen receptors and thus the requirement of testosterone for successful spermatogenesis is dependent on the presence of androgen receptors on the Sertoli cells.

Since in the human, testicular sperm production continues from puberty throughout life, there is clearly a need for a population of stem cells to produce the precursor cells that develop into sperm. The cells forming this stem cell population are the spermatogonia that undergo several mitotic divisions and have 46 chromosomes as do all other cells in organs throughout the body (Amory et al., 2011). They develop from the gonocyte population found in the testes of prepubertal boys. The gonocytes are initially placed in the centre of the seminiferous cords and migrate to lie between the precursors of the Sertoli cells and, as with the gonocytes, the spermatogonia are basally placed in contact with the basement membrane of the seminiferous tubules. These cells undergo several stages of development and are designated by their cytological features before commencing meiosis.

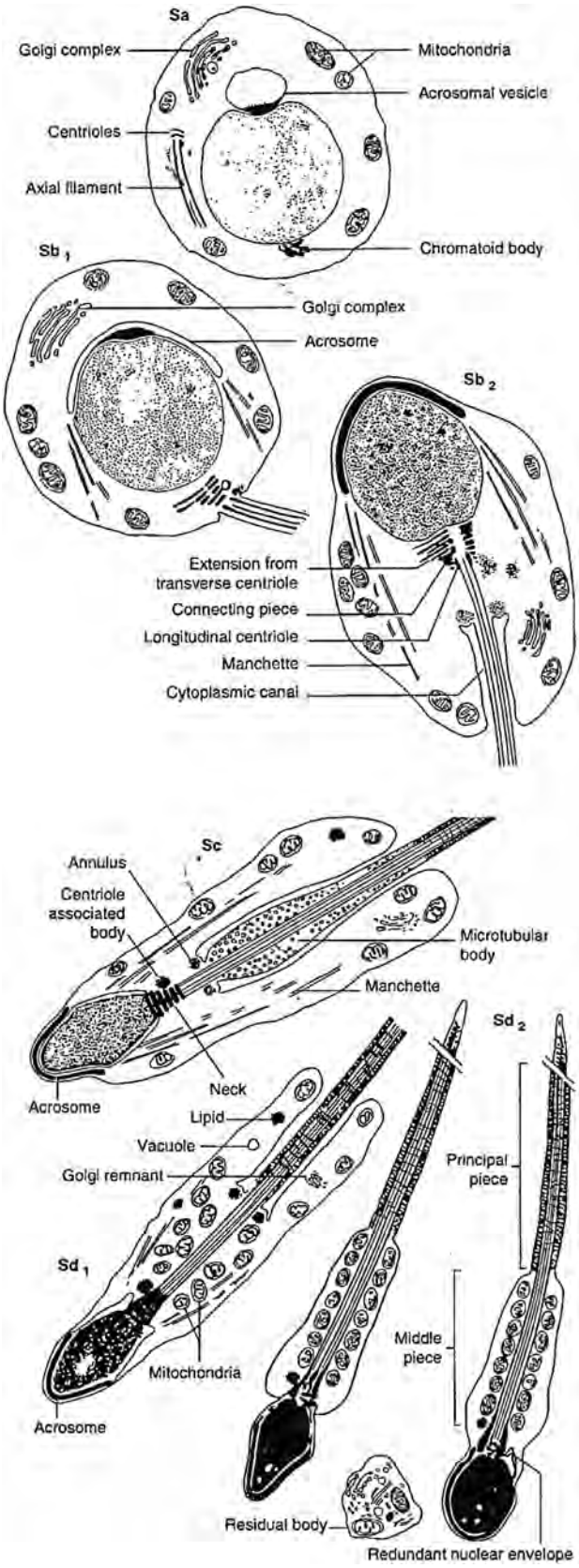

Fig. 3 The efferent ducts draining from the rete testis to form the head of the epididymis is illustrated together with the distal regions of the epididymis termed the body and tail.

The spermatogonia lose their contact with the basement membrane of the tubule when they begin another process of cell division called meiosis by which the chromosome numbers in these cells are reduced from 46 to 23. These cells are called primary spermatocytes.

During meiosis, the homologous chromosomes, derived originally from the fertilization of the egg, one provided by the mother, and the other member of the pair, provided by the father via his sperm, pair and replicate their DNA (deoxyribonucleic acid). The primary spermatocytes thus have nuclear features which enable the identification of the stages as the cells undergo the first meiotic division during which, one of each homologous chromosome pair, moves to the opposite pole of the cell. These cell types are named by the stage of meiosis that they have reached and can be identified by the chromatin pattern in their nucleus associated with the chromosome replication. Leptotene, zygotene, pachytene, diplotene, and diakinesis stages can be identified and, unlike cell division in somatic cells, these germ cells remain connected by intercellular bridges that link the cytoplasm of these cells. These bridges enable the development of the "chains" of germ cells and remain in place in the primary and secondary spermatocyte populations. This process requires the involvement of retinoic acid and androgens to proceed to completion. (Amory et al., 2011).

The completion the first meiotic division gives rise to cells called secondary spermatocytes that have half the number of chromosomes, 23, termed the haploid number, in contrast to their diploid precursor which had 46. The secondary spermatocytes then divide by mitosis to give rise to a further population of cells called round spermatids that are still connected by the cytoplasmic bridges.

Spermiogenesis

The round spermatids do not divide further but are transformed by a complex series of changes into a sperm, the process being called spermiogenesis (de Kretser, 1969). The basic changes in the developing spermatids during spermiogenesis are common to many mammalian species but the resulting sperm vary in their morphology especially in the shape of the head of the resulting sperm. The structure of the sperm tail however has many features in common across species. In the round spermatids the nucleus, which is centrally placed in the cell, is "capped" at one pole by a series of vesicles from the Golgi complex that coalesce to form a "cap" that is called the acrosome and is applied to that part of the nucleus closest to the acrosome. The acrosome covers approximately 30%–50% of the nuclear surface.

The nucleus, in the region of the acrosome, comes into close apposition with the cell membrane but remains separated from the nucleus by the acrosome.

Subsequently, as spermatid development continues, the nuclear chromatin undergoes a progressive condensation forming electron dense granules associated with stabilization of the DNA (Sassone-Corsi, 2002). That process involves the replacement of lysine-rich histones with transitional proteins, subsequently replaced by arginine-rich proteins called protamines. The nuclear chromatin granules condense as spermiogenesis progresses and it becomes more difficult to identify individual granules (**Fig. 4**).

At the pole of the nucleus opposite to the acrosome, a pair of centrioles, that participate in the development of the flagellum, lodge in a small fossa or indentation that still lies external to the nuclear membrane. This whole complex is called the connecting piece. The centriole closest to the nucleus, called the proximal centriole, lies at right angles to the plane of the distal centriole which gives rise to the core of the sperm tail called the axoneme. The axoneme is composed of a core of microtubules which forms the basis of the sperm tail sometimes called the axial filament (Fawcett, 1975).

The axial filament comprises nine pairs of doublet microtubules which surround two centrally placed single microtubules, a structure that is identical to the structure of cilia which also exhibit motility similar to the sperm tail.

A second set of nine outer dense fibers surround the axial filament distal to a dense ring termed the annulus. The annulus marks the distal end of the mid-piece and its mitochondrial sheath and defines the commencement of the fibrous sheath. The annulus marks the commencement of the region of the sperm tail called the principal-piece and the axonemal core, distal to the termination of the fibrous sheath, is termed the end-piece.

The final step, before sperm are released from the epithelium by a process called spermiation, is a movement of mitochondria, that up to this point have been distributed around the periphery of the spermatid cell membrane, to surround the mid-piece to form a "mitochondrial sheath" distal to the connecting-piece and ending at the annulus.

Spermiation, involves the release of sperm from the seminiferous epithelium. At this stage, the cytoplasm of the spermatid has migrated to a caudal position around the tail. Projections of Sertoli cells invaginate this caudal cytoplasmic collection to "literally" pull the residual cytoplasm off the spermatid, thereby releasing it into the lumen of the seminiferous tubule.

The residual bodies within the Sertoli cells, that contain the "unwanted" cellular components of the spermatids, are moved toward the base of the Sertoli cells and progressively "digested" by lysosomes. There is some data to suggest that these cellular components signal to the Sertoli cell that a "generation" of sperm have been released from that region of the epithelium.

The spermatozoa, that are released from the Sertoli cells are still immotile. They, together with fluid secreted by the Sertoli cells into the lumen of the seminiferous tubules, are moved toward the rete testis by the contractions of the peritubular myoid cells and enter the epididymis.

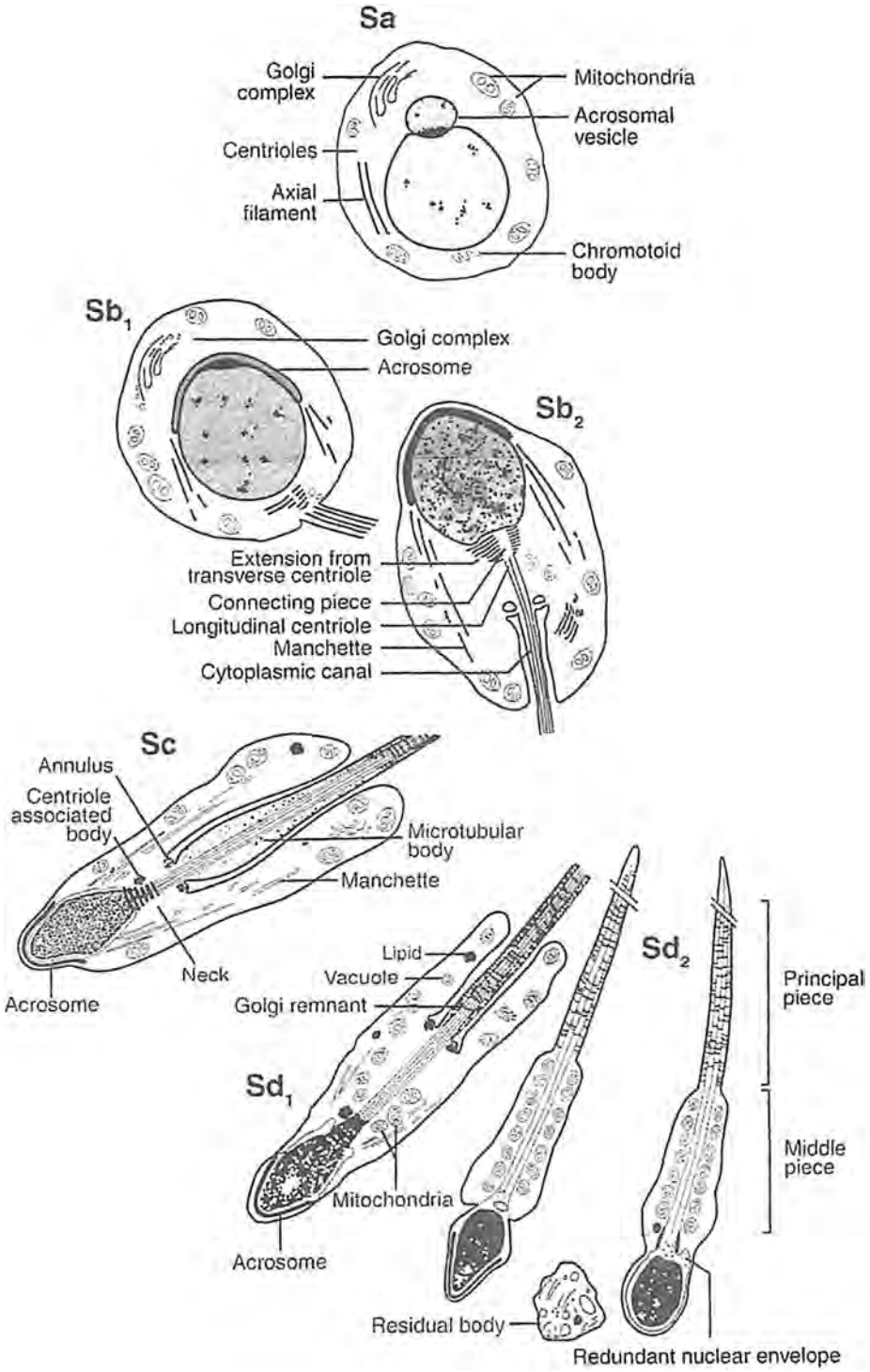

Fig. 4 The specific stages of spermiogenesis, the process by which a round spermatid (Sa) is transformed into a spermatozoon (Sd2) are illustrated. Reproduced with permission from de Kretser, D.M. (1969). Ultrastructural features of human spermiogenesis. *Zeitschrift für Zellforschung* **98**, 477–505.

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Russell, L. D. (1977). Movement of spermatocytes from the basal to the adluminal compartment of the rat testis. *The American Journal of Anatomy, 148*, 313–328.
Sassone-Corsi, P. (2002). Unique chromatin remodelling and transcriptional regulation in spermatogenesis. *Science, 296*, 2176–2178.
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Stanton, P. G. (2016). Regulation of the blood–testis barrier. *Seminars in Cell and Developmental Biology, 59*, 166–173.

Further Reading

Barakat, B., O'Connor, A. E., Gold, E., de Kretser, D. M., & Loveland, K. L. (2008). Inhibin, activin, follistatin and FSH serum levels and testicular production are highly modulated during the first spermatogenic wave in mice. *Reproduction, 136*, 345–359.
Cortes, D., Muller, J., & Skaakebaek, N. E. (1987). Proliferation of Sertoli cells during development of the human testis assessed by stereological methods. *International Journal of Andrology, 10*, 589–596.
Hogarth, C. A., Mitchell, D., Evanoff, R., Small, C., & Griswold, M. (2011). Identification and expression of potential regulators of the mitotic-to-meiotic transition. *Biology of Reproduction, 84*, 34–42.
de Kretser, D. M., Kerr, J. B., & Paulsen, C. A. (1981). Evaluation of the ultrastructural changes in the human Sertoli cell in testicular disorders and their relationship to the changes in the levels of serum FSH. *International Journal of Andrology, 4*, 124–144.
Nicholls, P. K., et al. (2012). Activin signalling regulates Sertoli cell differentiation and function. *Endocrinology, 153*, 6065–6077.

"Systems Biology of Reproduction"

Spring 2024 (Even Years) – Course Syllabus
Biol 475/575 Undergraduate/Graduate (3 Credit)
SLN: (475) – 06763, (575) – 06764
Time - Tuesday and Thursday 10:35 am-11:50 am
Course Lectures in person and recorded on Canvas/Panopto and Discussion Sessions live in person and on WSU Zoom for all campuses (Hybrid Course)
Room – CUE 418
Course Director – Michael Skinner, Abelson Hall 507, 335-1524, skinner@wsu.edu
Co-Instructor – Eric Nilsson, Abelson Hall 507, 225-1835, nilsson@wsu.edu
Learning Objective -
Current literature based course on the Systems Biology of Reproduction. Learning Systems approaches to the biology of reproduction from a molecular to physiological level of understanding.
Schedule/Lecture Outline –

January	9 & 11	Week 1	Systems Biology Introduction
	16 & 18	Week 2	Molecular/ Cellular/ Reproduction Systems
	23 & 25	Week 3	Sex Determination Systems
Jan /Feb	30 & 1	Week 4	Male Reproductive Tract Development & Function
February	6 & 8	Week 5	Female Reproductive Tract Development & Function
	13 & 15	Week 6	Gonadal Developmental Systems Biology
	20 & 22	Week 7	Testis Systems Biology
	27 & 29	Week 8	Ovary Systems Biology
March	5 & 7	Week 9	Epigenetics and Transgenerational Gonadal Disease
	11 – 15	**Week 10**	**Spring Break**
	19 & 21	Week 11	Gametogenesis/ Stem Cells/ Cloning
	26 & 28	Week 12	Hypothalamus-Pituitary Development & Function
April	2 & 4	Week 13	Reproductive Endocrinology Systems
	9 & 11	Week 14	Fertilization & Implantation Systems
	16 & 18	Week 15	Fetal Development & Birth Systems
	23 & 25	Week 16	Assisted Reproduction/Contraception
Apr/May	30 & 2	Week 17	Exam or Grant Review

Spring 2024 – Systems Biology of Reproduction
Lecture Outline – Testis Systems Biology
Michael K. Skinner – Biol 475/575
CUE 418, 10:35-11:50 am, Tuesday & Thursday
February 20, 2024
Week 7

Testis Systems Biology

- Cell Biology of the Testis
  - Cell Types and Organization
  - Cell Associations
- Spermatogenesis
  - Stages and Cycle
  - Germ Cell Differentiation
  - Genes Involved
- Endocrinology of the Testis
  - Gonadotropins
  - Testosterone and Leydig Cell
- Cell-Cell Interactions
  - Types of Interactions
  - Sertoli-Germ Cell Interactions
  - Other Cellular Interactions

Required Reading

de Kretser, et al. (2018) Structure/Cells Overview. In: Encyclopedia of Reproduction (Second Edition). Volume 1, Pages 10-16

Spring 2024 – Systems Biology of Reproduction
Discussion Outline – Testis Systems Biology
Michael K. Skinner – Biol 475/575
CUE 418, 10:35-11:50 am, Tuesday & Thursday
February 22, 2024
Week 7

Testis Systems Biology

Primary Papers:

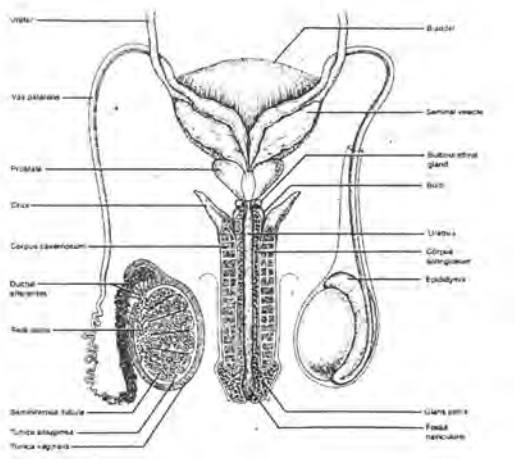
1. Guo, et al. (2021) Cell Stem Cell 28,764-778
2. Endo, et al. (2015) PNAS E2347-2356
3. Guo, et al. (2018) Cell Research 28:1141-1157

Discussion

Student 5: Reference 1 above
- What was the technology used?
- What experimental design was used?
- What insights were obtained on testis somatic cell and germ cell origins?

Student 6: Reference 2 above
- What was the experimental design and culture system used?
- What spermatogenic process occurred in vitro?
- How could this technology be applied?

Student 7: Reference 3 above
- What is the experimental and systems approach?
- What single cell expression and epigenetic relationships exist?
- What insights are provided on testis cell biology?



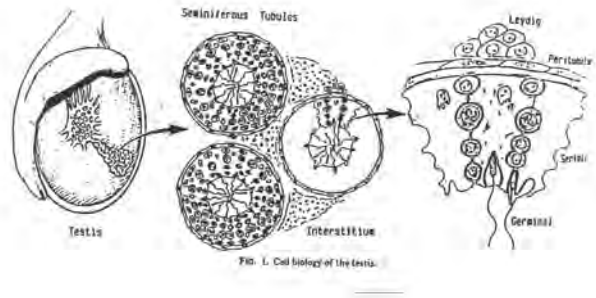
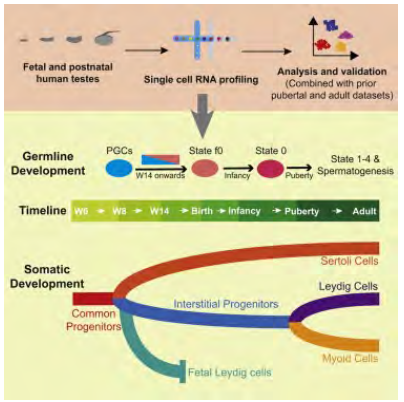
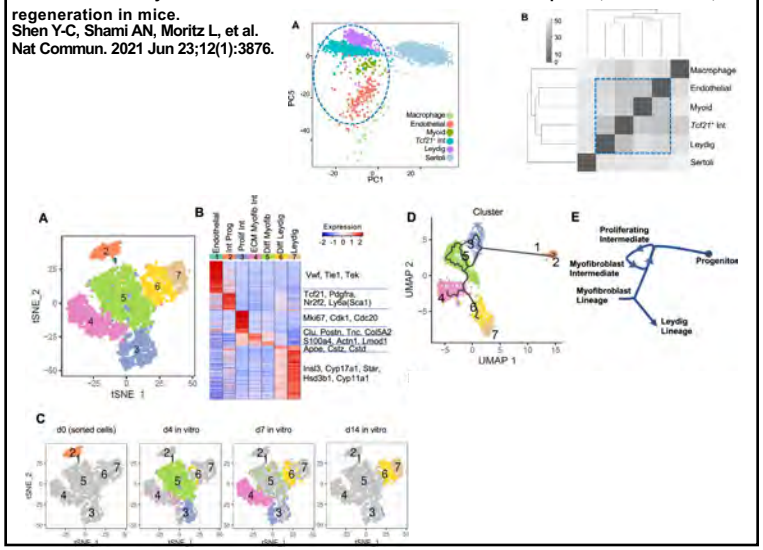


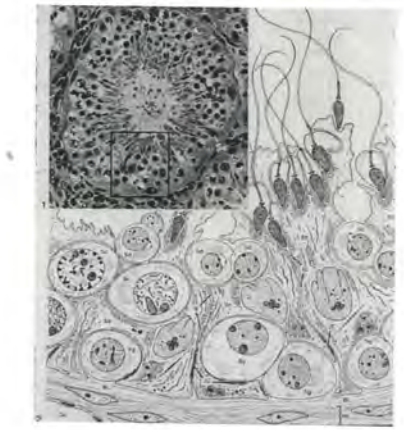
Fig. 1. Cell biology of the testis.

Single-cell analysis of the developing human testis reveals somatic niche cell specification and fetal germline stem cell establishment.
Guo J, Sosa E, Chitiashvili T, et al.
Cell Stem Cell. 2021 Apr 1;28(4):764-778.e4.



TCF21 + mesenchymal cells contribute to testis somatic cell development, homeostasis, and regeneration in mice.
Shen Y-C, Shami AN, Moritz L, et al.
Nat Commun. 2021 Jun 23;12(1):3876.





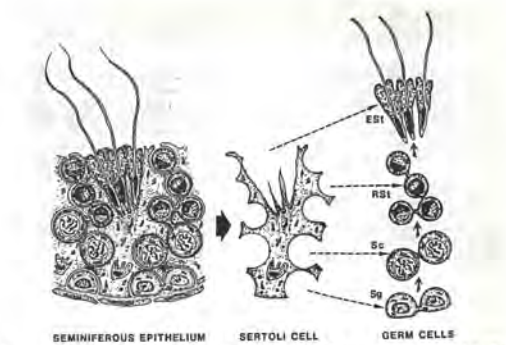
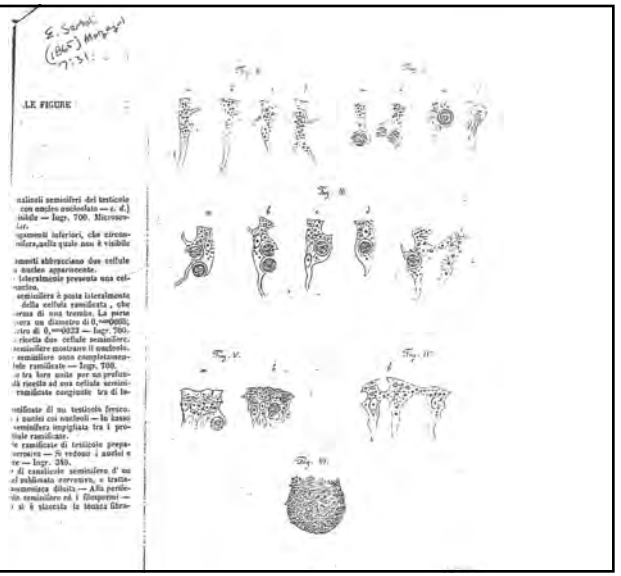
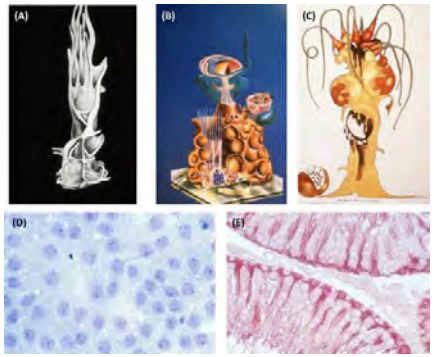
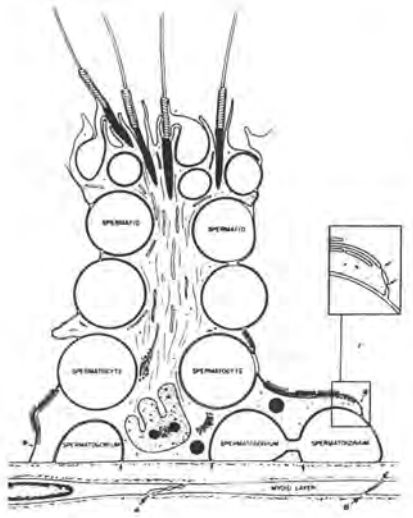


FIG. 9–4. The seminiferous epithelium showing the complex nature of the association between Sertoli cells and the developing germ cells along with an illustration depicting dissociation of this cellular complex. The developing germ cells occupy intracellular spaces between adjacent Sertoli cells and move from the basement membrane toward the lumen during spermatogenic process. The germ cells begin their developmental process as spermatogonia (Sg), become spermatocytes (Sc), then round spermatids (RSt) and finally elongated spermatids (ESt). Schematic dissociation of the seminiferous epithelium shows how the germ cells occupy the expanded intercellular spaces between adjacent Sertoli cells. (Adapted from Fawcett, D.W. (1974) In Male Fertility and Sterility. R.E. Mancini and L. Martini (eds.). New York, Academic Press.)



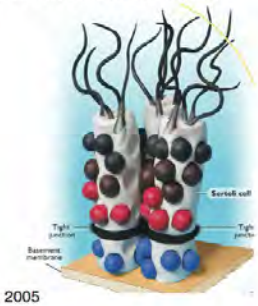


Representations of Sertoli cells from the archives of the author. (A) The artistic representation of a Sertoli cell from the model built by Lonnie Russell from serial sections in the electron microscope [13,14]. (B) Diagram illustrating the filaments and microtubules in mammalian Sertoli cells was on the cover of the first book on Sertoli cells [1]. (C) Artistic representation of Sertoli cells and associated germ cells by Holstein and Schafer and first printed in The Sertoli Cell [1]. (D) Field of Sertoli cells in culture from testis of 20-day old rat showing epithelial nature of cells. (E) Cross section of rat seminiferous tubules stained by immunocytochemistry with antibody to clusterin highlighting Sertoli cells in the tissue.



Sertoli cell – spans the entire seminiferous epithelium

Blood testis barrier – most exclusive component is specialized tight junctions of Sertoli cells.



Senger, 2005

Russell and Griswold, 1993

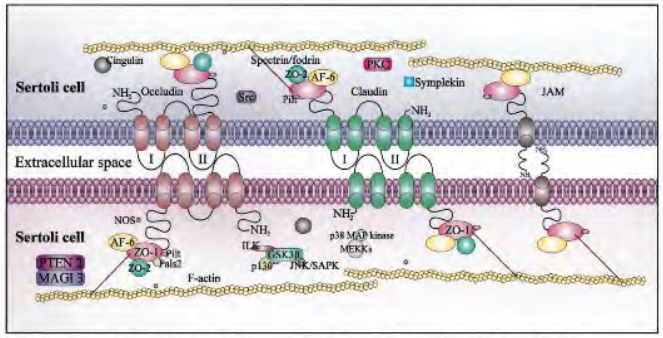
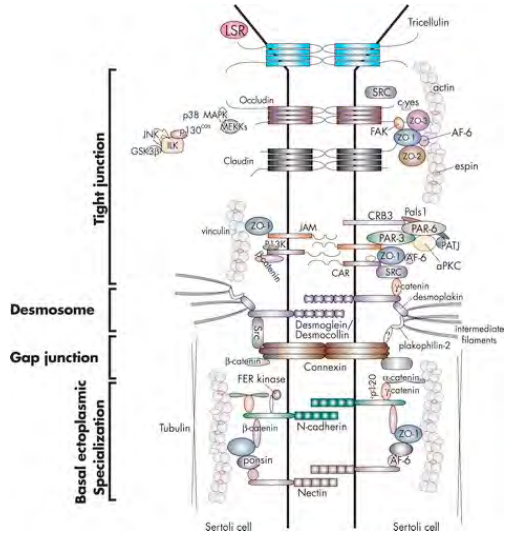
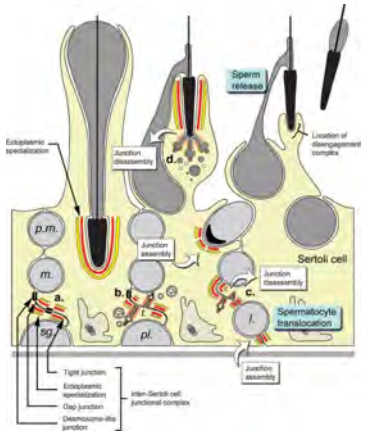


FIG. 6. Schematic drawing illustrating the molecular architecture of the three multiprotein complexes found at the Sertoli cell tight junction that constitute the blood-testis barrier. Shown are the three multiprotein complexes found at the Sertoli cell tight junction: 1) occludin –ZO-1/ZO-2; 2) claudin –ZO-1/ZO-2; and 3) JAM –ZO-1. Also shown are peripheral membrane proteins known to regulate Sertoli cell tight junction dynamics. This figure was prepared based on the following original research articles and reviews: (Refs. 4, 28, 114, 149, 150, 254 –256, and 341).





Blood testis barrier creates two compartments

Basal and adluminal side

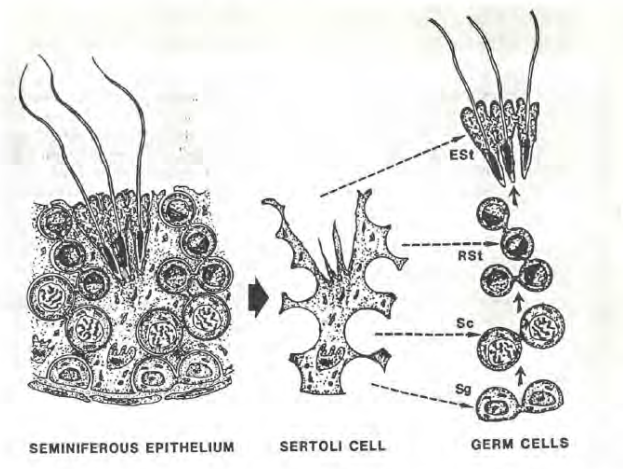
Basal – Spermatogonia, primary spermatocytes

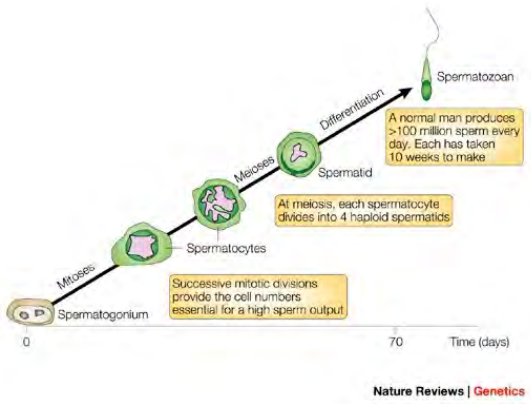
Primary spermatocytes

migrate through tight junctions so secondary spermatocytes and spermatids are located in adluminal side

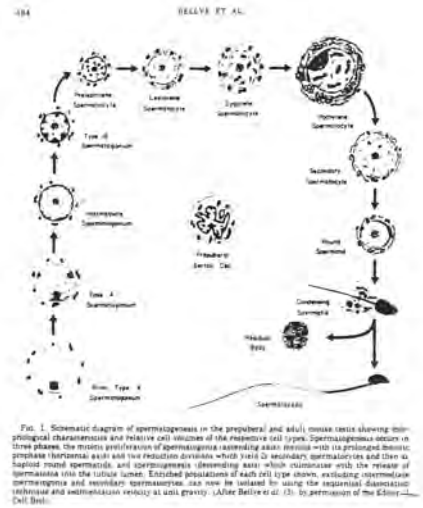
Seminiferous epithelium – Sertoli and differentiating germ cells of the seminiferous tubule

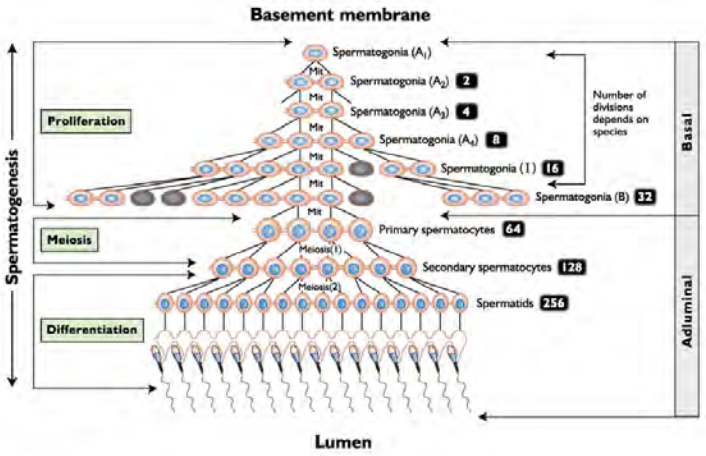
Cells of the seminiferous epithelium



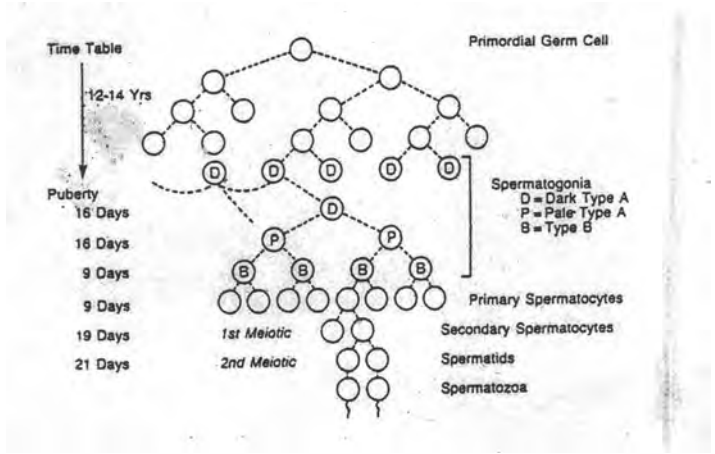


Cooke and Saunders, 2002





Senger, 2005



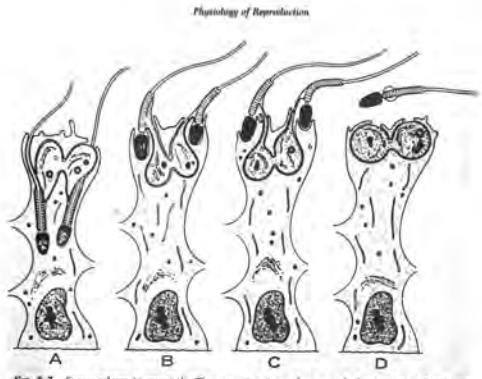


FIG. 9–7. Sperm release in mammals. The successive stages show a gradual extrusion of the elongate spermatid into the lumen with retention of the interconnected residual bodies. Release results from attenuation of the slender stalk of cytoplasm connecting the spermatid to the residual body. Once separated from the residual body, the cell becomes a spermatozoon. (From Fawcett, D.W. (1975) *In* Handbook of Physiology Vol. V, R.O. Greep and E.B. Astwood (eds.). Bethesda, American Physiological Society.)

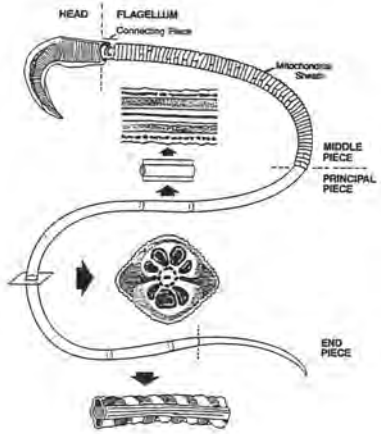
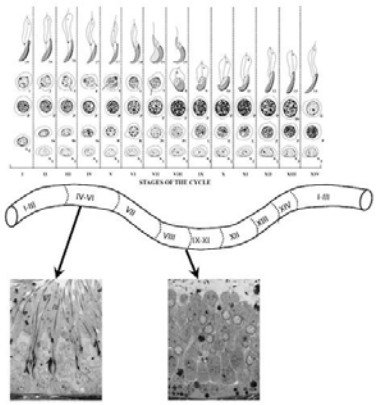


FIG. 1. General features of the mammalian sperm. The head of the spermatozoon is attached to the connecting piece of the flagellum. The other regions of the flagellum are the middle piece, the principal piece, and the end piece. The middle piece contains the mitochondrial sheath, while the principal piece contains the fibrous sheath. Longitudinal and cross-sectional views of the principal piece and a segment of fibrous sheath are indicated by arrows; the internal components of the flagellum are identified in Fig. 6.



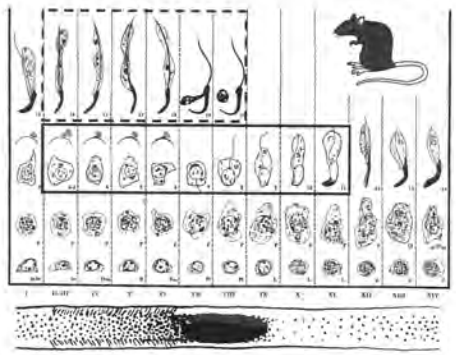
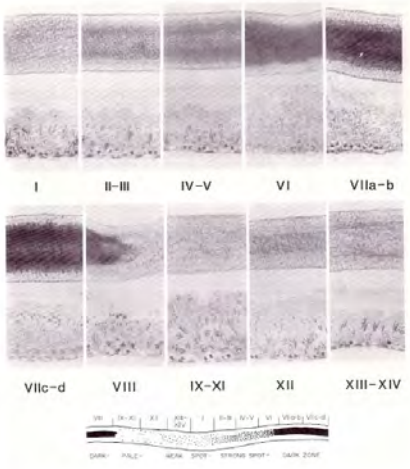


Fig. 1. The transillumination pattern of a segment of a rat seminiferous tubule correlated with 14 stages (indicated by Roman numerals) of the epithelial cycle. (From Dym and Clermont, 1970. Am. J. Anat. 128:265-282. Reproduced by

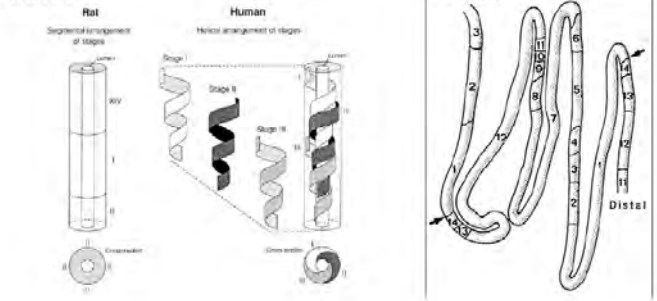


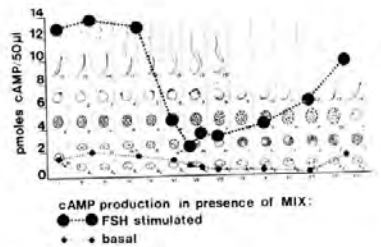
Parvinen M. Regulation of the seminiferous epithelium.
Endocr Rev. 1982, 3(4):404-17.

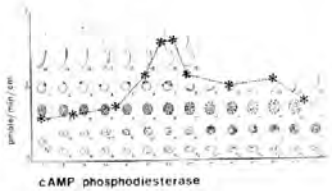
Spermatogenic wave - helical versus segmental waves

Most mammals have segmental spermatogenic waves – clearly defined space

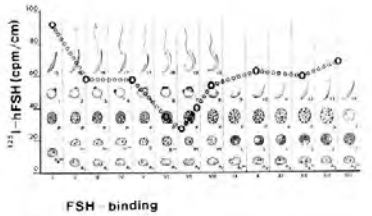
Humans, birds and stallions (limited extent) have helical waves.

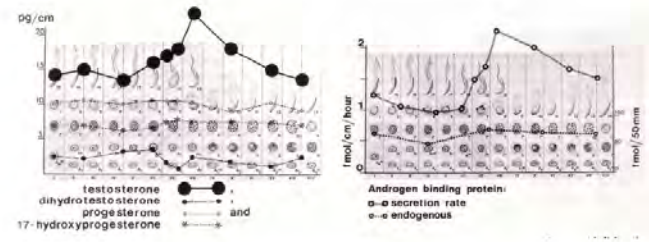






Parvinen M. Regulation of the seminiferous epithelium. Endocr Rev. 1982, 3(4):404-17.



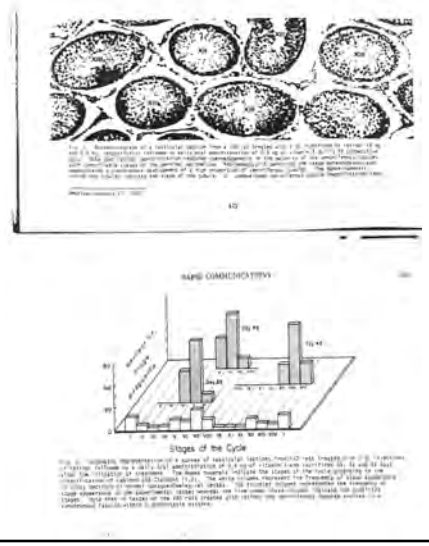


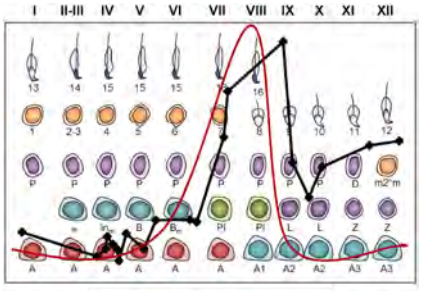
<u>Vitamin A deficient (VAD) model</u>
Vitamin A stored in the liver.

Rat – put on VAD diet at 20 days of age. In 9-10 weeks spermatogenesis has stopped. Only germ cells present are type A spermatogonia and a few preleptotene spermatocytes

Inject rat with retinol and return to a normal diet and spermatogenesis resumes.
Spermatogenesis is synchronous for 2-3 rounds.

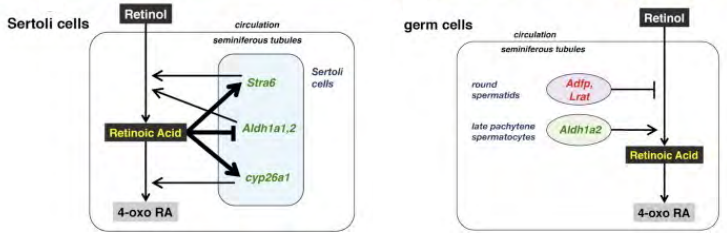
Sacrifice animals at specific times can get testis samples at 1-2 stages. Gene and protein expression associated with select stages.

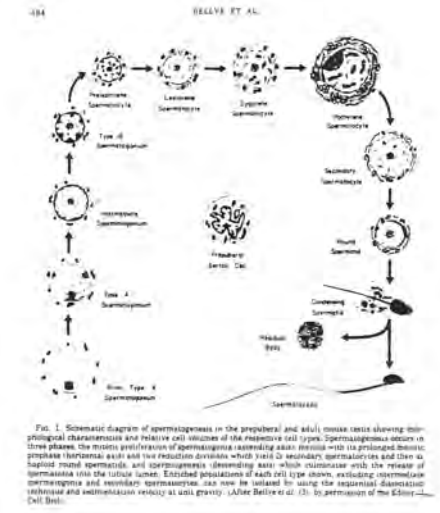


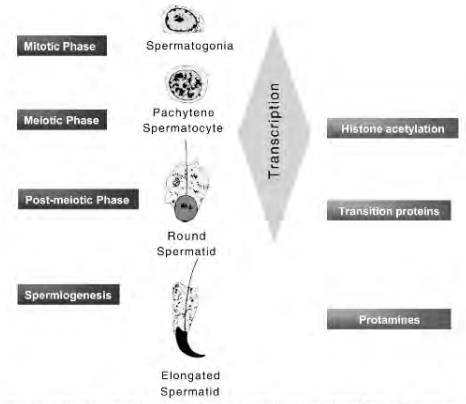


Retinoic acid pulse across the cycle of the seminiferous epithelium. Red line shows the predicted RA pulse based on previous data, black line shows actual, relative RA values across the cycle in stage-synchronized testes (data from.21 RA levels peak at late stage VIII/early stage IX and reach a maximum value of 35 picomoles per gram testis (pmoles/g testis). Minimum RA values around 3 pmoles/g testis were observed in stages II through V. In unsynchronized adult testes where all stages of the seminiferous epithelium are present, RA values averaged around 8-12 pmoles/g testis

Retinoic acid metabolism links the periodical differentiation of germ cells with the cycle of Sertoli cells in mouse seminiferous epithelium.
Sugimoto R, Nabeshima Y, Yoshida S.
Mech Dev. 2012 Jan-Feb;128(11-12):610-24.







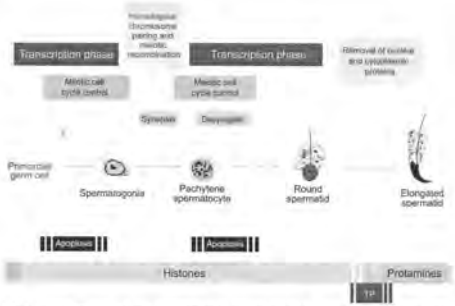


Fig. 1. Spermatogenesis is a cyclic developmental process by which spermatogonia cells generate the mature spermatozoon. These events are characterized by important modifications in chromatin organization, basically during two periods, meiosis—which includes the synapsis and desynapsis of the chromosomes—and the histone-protamine transition. Postmeiotically, a powerful wave of transcription occurs in haploid cells, which is governed by highly specialized molecular mechanisms. Specific genes operate at distinct steps of the spermatogenic process.

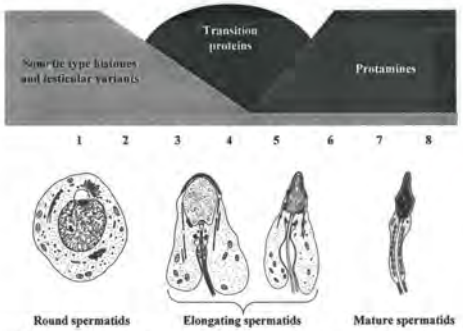


FIG. 2 Schematic diagram of histone-to-protamine replacement during human spermiogenesis that is subdivided into eight successive steps. Both somatic-type and testis-specific histones present in round spermatids are, for the most part, displaced by transition proteins at the beginning of the elongation phase. However, a low amount of somatic-type histones persists in late spermatids and mature spermatozoa.

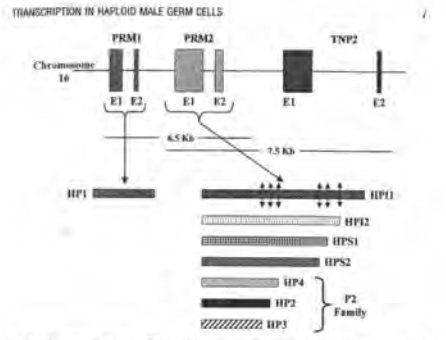


FIG. 3 The genes coding for PRM1, PRM2, and TNP2 are closely linked over a stretch of DNA 13–15 kb long. Whereas *Prm1* encodes directly for the 50-amino-acid-long HP1 protein, *Prm2* codes for a 101-amino-acid-long precursor, HP1. The latter, actually referred as to human preprotamine pHP2, is posttranslationally processed in several steps (HP2, HP51, HP52, HP4, HP2, and HP3, successively) via proteolytic cleavage at specific sites leading from HP1 to protamine HP3. E, exon.

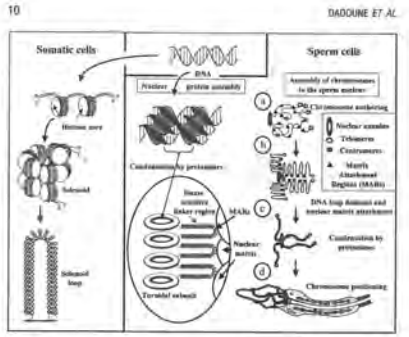
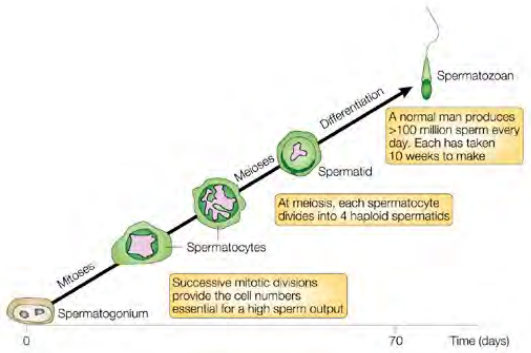


FIG. 5 Comparative chromatin organization in somatic and sperm cells. In somatic cells, DNA is bound to octamers of histone proteins forming the nucleosomal fiber. Chromatin is then coiled into a 30-nm solenoid filament which is further organized into loop domains. The latter play an important role in DNA replication and gene transcription. In sperm cells, chromosomal DNA is organized into loop domains attached at their bases to the nuclear annulus, a round-shaped structure located at the nuclear posterior pole (a) and to the nuclear matrix by specific sequences termed matrix attachment regions (MARs) (b). The protamine-bound DNA is coiled into toroid-shaped structures whose MARs are composed of DNase-sensitive linker regions disposed between them (c). Chromosome positioning is the final step in chromatin organization (d) (Drawn from data provided by Sotolongo *et al.*, 2003; Ward, 1993, 1997).



Cooke and Saunders, 2002

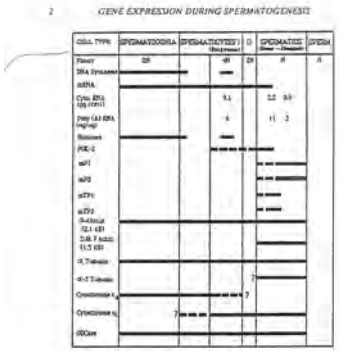


FIG. 1. Gene expression during spermatogenesis in the mouse. These data are compiled from the literature (reviewed in 14). A solid line indicates the approximate time period when a protein is synthesized. A dashed line represents the interval when mRNA for the protein has been detected but the protein has not yet been detected, i.e., the interval when the mRNA is stored as a ribonucleoprotein. A broken box indicates the time period when protamine 2 is synthesized as a precursor and subsequently processed to the form found in mature spermatozoa. Question marks represent uncertainty as to the exact time when synthesis starts or stops. PGK-2, phosphoglycerate kinase 2; MP, mouse protamine; TP, transition protein; S.M. γ actin, smooth muscle γ actin; ODCase, ornithine decarboxylase.

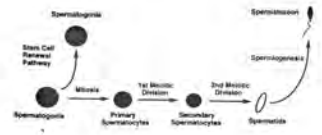


Figure 1 The Differentiation Program of Male Germ Cells

Mouse spermatogonia proliferate to generate a pool of undifferentiated stem cells. A small subset proceeds in the differentiation pathway, which includes two meiotic divisions and the spermiogenesis.

Table 1 · Partial List of the Genes Whose Deletion in the Mouse Generates Defects in the Male Germ Cell Lineage

Gene Disrupted	Phenotype	Female Phenotype	Increased Apoptosis	Spermatozoa	Reference
BAX	Complete arrest at pachytene spermatocyte stage. Irregular spermatogonia	Fertile	Yes	Absent	Knudson et al., 1995
CREM	Complete arrest at pachytene spermatocyte stage.	Fertile	Yes	Absent	Nantel et al., 1996
RAR_α	Complete arrest and degeneration of the epithelium. Normal interconnected tubuli	Fertile	No	Present	Lufkin et al., 1993
RXRβ	Partial arrest at primary spermatocytes. Oligo-asthero-teratozoospermia	Fertile	No	Present	Kastner et al., 1994
PMS2	Partial arrest at primary spermatocytes. Chromosome synaptic abnormalities.	Fertile	No	Present	Baker et al., 1995
MLH-1	Complete arrest at pachytene spermatocyte stage.	Sterile	Yes	Absent	Edelmann et al., 1996
HRB	Partial arrest at post-meiotic spermatids stage.	Fertile	Yes	Present	Ronni et al., 1995
ATM	Complete arrest at pachytene spermatocyte stage. Chromosome synaptic aberrations.	Sterile	Yes	Absent	Xu et al. 1996

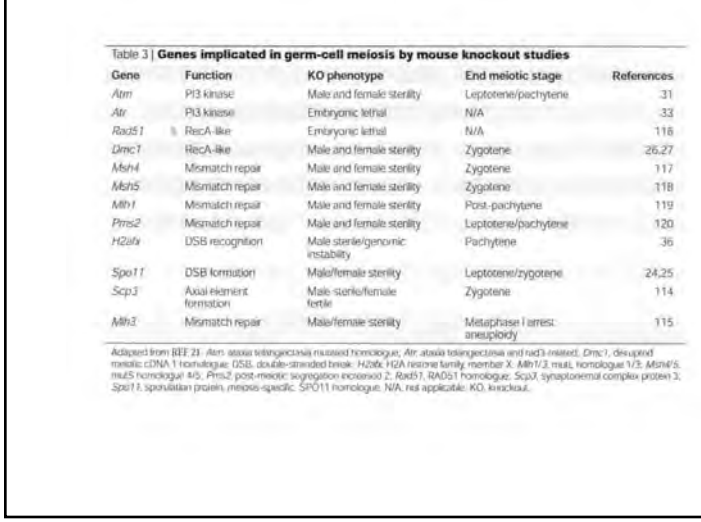


Table 3 | **Genes implicated in germ-cell meiosis by mouse knockout studies**

Gene	Function	KO phenotype	End meiotic stage	References
Atm	PI3 kinase	Male and female sterility	Leptotene/pachytene	31
Atr	PI3 kinase	Embryonic lethal	N/A	33
Rad51	RecA-like	Embryonic lethal	N/A	116
Dmc1	RecA-like	Male and female sterility	Zygotene	26,27
Msh4	Mismatch repair	Male and female sterility	Zygotene	117
Msh5	Mismatch repair	Male and female sterility	Zygotene	118
Mlh1	Mismatch repair	Male and female sterility	Post-pachytene	119
Pms2	Mismatch repair	Male and female sterility	Leptotene/pachytene	120
H2afx	DSB recognition	Male sterile/genomic instability	Pachytene	36
Spo11	DSB formation	Male/female sterility	Leptotene/zygotene	24,25
Scp3	Axial element formation	Male sterile/female fertile	Zygotene	114
Mlh3	Mismatch repair	Male/female sterility	Metaphase I arrest: aneuploidy	115

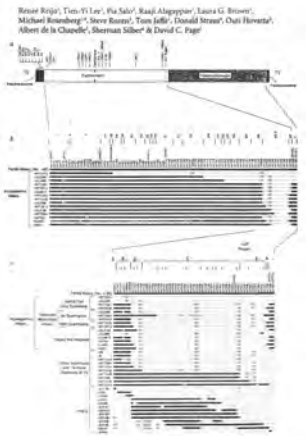
Adapted from REF 23. *Atm*, ataxia telangiectasia mutated homologue; *Atr*, ataxia telangiectasia and rad3-related; *Dmc1*, dosage suppressor of mitotic defects in *Saccharomyces cerevisiae* homologue; DSB, double-stranded break; *H2afx*, H2A histone family, member X; *Mlh1/3*, mutL homologue 1/3; *Msh4/5*, mutS homologue 4/5; *Pms2*, post-meiotic segregation increased 2; *Rad51*, RAD51 homologue; *Scp3*, synaptonemal complex protein 3; *Spo11*, sporulation protein, meiosis-specific; SPO11 homologue; N/A, not applicable; KO, knockout.

TABLE 2. Representative RNA-binding Proteins of the Testis*

TB-RBP (36, 37, 39, 62)
p48/52 (55, 61)
53 and 55 ×Da (40)
Prbp (41)
Sprr (42)
Tenr (43)
RBM (44, 72)
DAZ (44, 73)
SOD-RBP (50)
DBY (71)
EIFIAY (71)
ATP-dependent RNA-helicases (74, 75)
Poly (A) Binding Protein (76, 77)

*This table lists some of the currently identified RNA-binding proteins expressed, but often not solely expressed, in the testis. It does not include many ubiquitous RNA-binding proteins that also function during spermatogenesis.

Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene

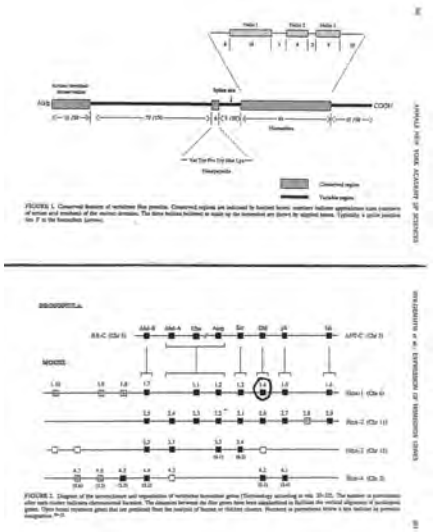


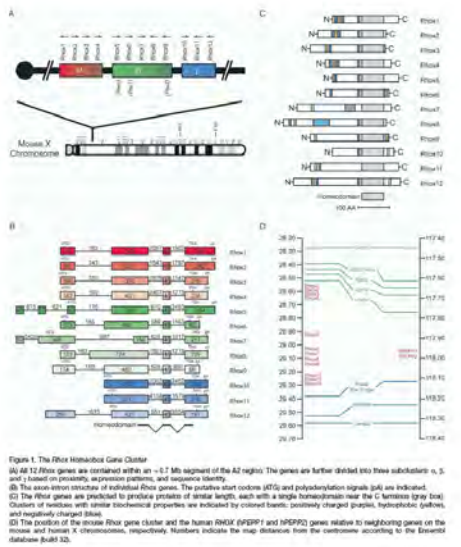
ONCOGENES AND SPERMATOGENESIS

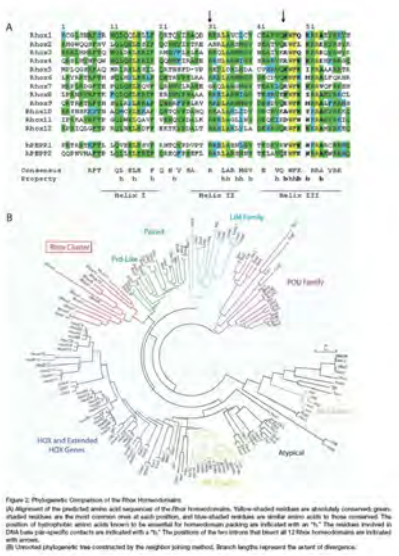
Oncogene	Function	Localization
c-fos	nuclear proto-oncogene	spermatogonia
c-jun	nuclear proto-oncogene	spermatogonia, early spermatocyte
c-myc	nuclear proto-oncogene	spermatogonia, early spermatocyte
c-raf	serine/threonine kinase	spermatogonia, spermatocytes, round spermatides
c-ras c-ras^{B} c-ras^{K} c-ras^{N}	membrane GTP binding proteins	all stages all stages spermatocytes all stages
c-mos	serine/threonine kinase	round spermatides
c-abl	membrane tyrosine kinase	early spermatides
pim-I	protein kinase	early spermatides

cfos cjun cmyc craf cras	craf cras	cabl cmos pim-I craf cras	craf cras

Spermatogonia → Spermatocyte → early spermatide → late spermatide
meiosis







Small RNAs just got bigger: Piwi-interacting RNAs (piRNAs) in mammalian testes

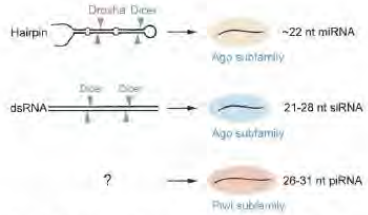


Figure 1. Classification of small RNAs. Definition and classification of small RNAs conventionally relies on their biogenesis mechanism. Two relatively well-defined classes of small RNAs include microRNAs (miRNAs) and small interfering RNAs (siRNAs). The biogenesis mechanism for piRNAs is currently unknown.

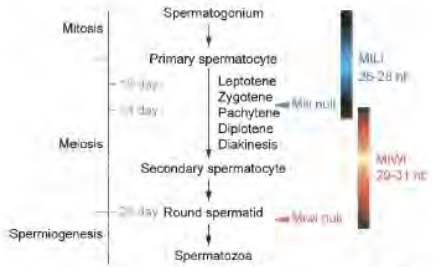
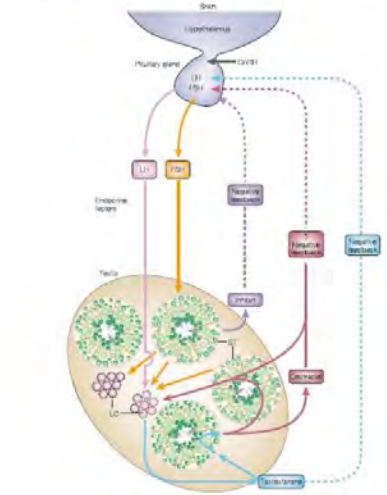


Figure 2. Expression of piRNAs and Piwi subfamily members during mouse spermatogenesis. Mammalian spermatogenesis occurs within the seminiferous epithelium inside the seminiferous tubule and can be divided into three phases.

Hypogonadal – pituitary – testis axis



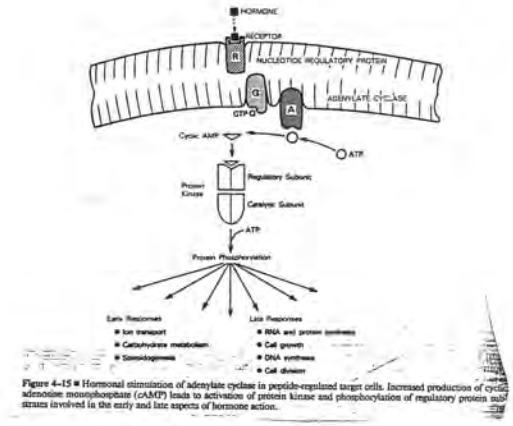


Figure 4–15 ■ Hormonal stimulation of adenylate cyclase in peptide-regulated target cells. Increased production of cyclic adenosine monophosphate (cAMP) leads to activation of protein kinase and phosphorylation of regulatory protein subunits involved in the early and late aspects of hormone action.

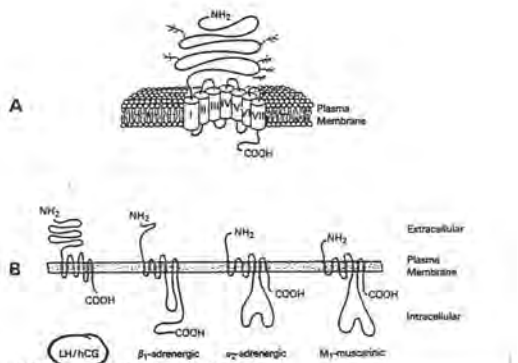


Figure 4–16 ■ Structure of the lutinizing hormone (LH) receptor.
A, General structure of extracellular region and transmembrane domains.
B, Comparison of LH receptor with other G protein–coupled receptors indicating the differences in extracellular regions and third intracellular loops.

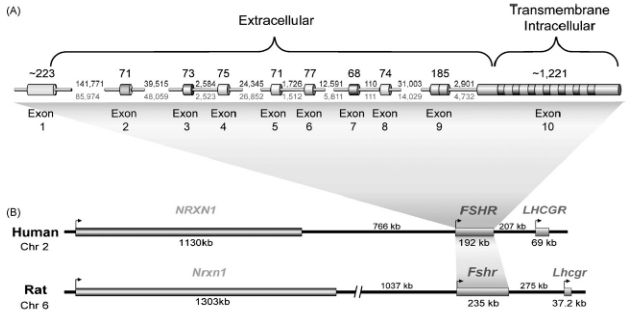


Fig. 1. *Fshr* gene structure and chromosomal location. (A) The exon–intron structure of *Fshr* corresponds to the domain structure of the protein. Exons 1 through 9 code for the extracellular ligand binding domain while exon 10 codes for the transmembrane and intracellular domains. Each of the small exons 2 through 8 encode individual leucine rich repeats and exon 9 codes for 2. The size of each exon in base pairs is shown above the diagram and the intron sizes are noted for rat (above) and human (below) between each exon. (B) In addition to the gene structure, synteny in the chromosomal environment surrounding *Fshr* is conserved between species. The size of *Fshr* and neighboring genes in humans and rats is shown above each gene and intergenic distances are noted. Adapted with permission from Heckert (2005) (Copyright 2005, Elsevier Academic Press).

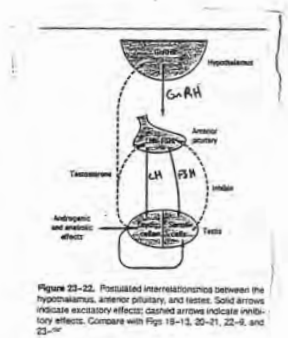


Figure 23–22. Postulated interrelationships between the hypothalamus, anterior pituitary, and testes. Solid arrows indicate excitatory effects; dashed arrows indicate inhibitory effects. Compare with Figs 18–13, 20–21, 22–9, and 23–7.

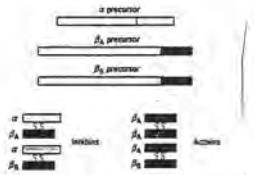


Figure 23–21. Inhibin precursor proteins and the various inhibins and activins that are formed from the C-terminal regions of these precursors. SS, disulfide bonds.

Testosterone (DHT)

50-100 ng/ml in testis
2-5 ng/ml in serum
Androgen receptors are saturated ~25 ng/ml

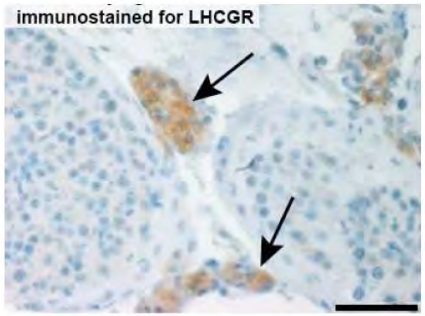
Produced by Leydig cells (LH regulation)

AR expressed: Sertoli, peritubular myoid, Leydig, endothelial cells of blood vessels, germ cells?

Interstitial cells – Leydig cells

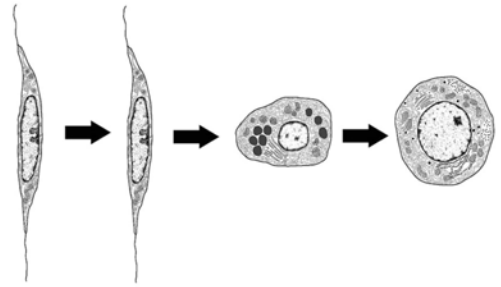
Receptors for LH
Produce testosterone

Kossack et al. PLoS Med 5(4)



De Novo Testosterone Production in Luteinizing Hormone Receptor Knockout Mice after Transplantation of Leydig Stem Cells.
Lo et al, 2004, Endo 145:4011

Leydig cell differentiation: stem to adult cells.



Stem Leydig Cell	Progenitor Leydig Cell	Immature Leydig Cell	Mature (Adult) Leydig Cell
Age: 7 d	*Age:* 21 d	*Age:* 35 d	*Age:* 90 d
Morphology: Spindle	*Morphology:* Spindle	*Morphology:* Round with Lipid Droplets	*Morphology:* Round without Lipid Droplets
T: No	*T:* Low	*T:* Higher	*T:* Highest
T Metabolites: No	*T Metabolites:* High	*T Metabolites:* Highest	*T Metabolites:* Low
LH Receptor: No	*LH Receptor:* Low	*LH Receptor:* Intermediate	*LH Receptor:* High
Mitotic Activity: Low	*Mitotic Activity:* High	*Mitotic Activity:* Low	*Mitotic Activity:* Probably none

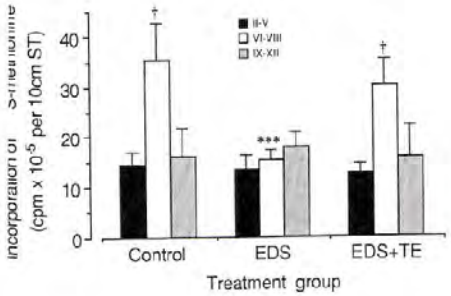
Investigation of testosterone regulation of spermatogenesis

Treat rats with ethane dimethane sulfonate (EDS) selectively kills Leydig cells in rats

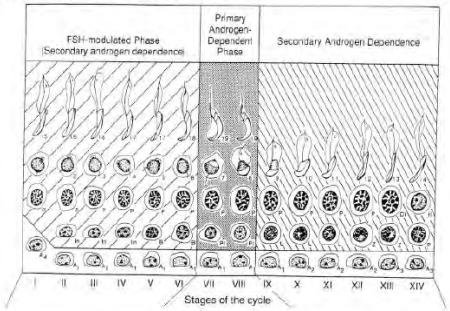
Protein production:
Stages II-V: no change

Stages VI-VIII: significantly suppressed

Stages IX-XII: no change



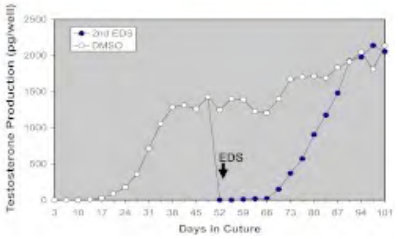
Degeneration of germ cells at stage VII (~4 days)
progressive increase
Day 8 post injection – 80% step 10-12 spermatids degenerating

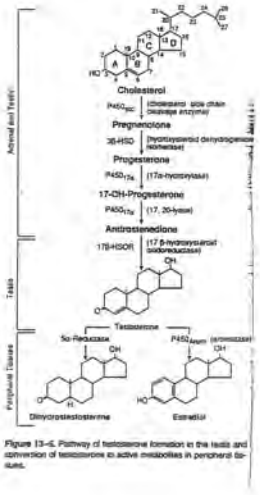


Testosterone supports germ cell differentiation during stages VII-VIII

Loss of germ cells – progressive loss
EDS – rescued by testosterone treatment

Identification, proliferation, and differentiation of adult Leydig stem cells.
Stanley E, Lin CY, Jin S, Liu J, Sottas CM, Ge R, Zirkin BR, Chen H.
Endocrinology. 2012 Oct;153(10):5002-10.





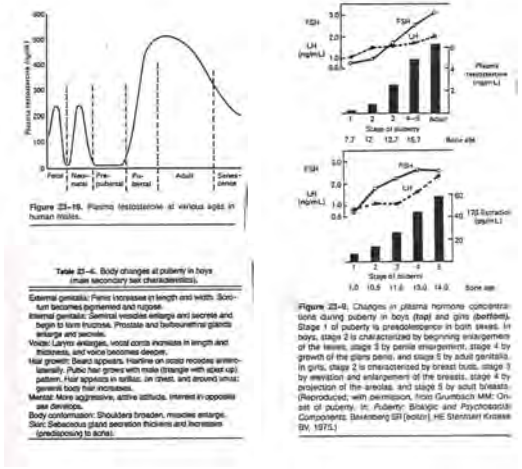
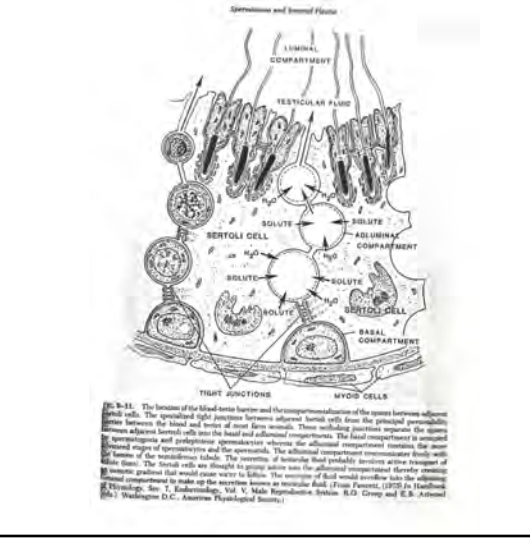


Figure 23–15. Plasma testosterone at various ages in human males.

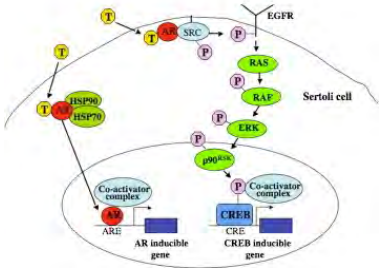
Table 23–6. Body changes at puberty in boys (male secondary sex characteristics).

External genitalia: Penis increases in length and width. Scrotum becomes pigmented and rugose.
Internal genitalia: Seminal vesicles enlarge and secrete and begin to form mucinous. Prostate and bulbourethral glands enlarge and secrete.
Voice: Larynx enlarges, vocal cords increase in length and thickness, and voice becomes deeper.
Hair growth: Beard appears. Hairline on scalp recedes anteriolaterally. Pubic hair grows with male (triangle with apex up) pattern. Hair appears in axillae, on chest, and around anus; general body hair increases.
Mental: More aggressive, active latitude, interest in opposite sex develops.
Body conformation: Shoulders broaden, muscles enlarge.
Skin: Sebaceous gland secretion thickens and increases (predisposing to acne).

Figure 23–8. Changes in plasma hormone concentrations during puberty in boys (top) and girls (bottom). Stage 1 of puberty is predominance in both sexes. In boys, stage 2 is characterized by beginning enlargement of the testes, stage 3 by penile enlargement, stage 4 by growth of the glans penis, and stage 5 by adult genitalia. In girls, stage 2 is characterized by breast buds, stage 3 by elevation and enlargement of the breasts, stage 4 by projection of the areolas, and stage 5 by adult breasts. (Reproduced, with permission, from Grumbach MM: Onset of puberty. In: *Puberty: Biologic and Psychosocial Components*. Bakwin-Berg SR [editor]. HE Stenmart Kristiansen BV, 1975.)



Classical and Non-classical Testosterone Pathways



Classical and non-classical pathways of testosterone action. The classical pathway of testosterone action is shown on the left. Testosterone diffuses through the plasma membrane and interacts with AR sequestered in the cytoplasm by heat shock proteins (HSP). As a result, the AR undergoes a conformational change, is released from the HSPs and then travels to the nucleus due to an intrinsic nuclear localization domain. In the nucleus AR binds to specific DNA motifs (AREs) and recruits co-activators or co-repressors (not shown) to regulate testosterone-mediated transcription. The non-classical pathway shown in the center and on the right is initiated with testosterone binding to the classical AR either localized near the plasma membrane or in the cytoplasm. AR then interacts with and causes the phosphorylation (P) of Src kinase, which may be tethered to the plasma membrane or present in membrane-associated protein complexes. The activated Src then phosphorylates the epidermal growth factor receptor (EGFR) directly or via intermediary factors. The EGFR then activates the MAP kinase cascade likely through the Ras small G protein that causes the phosphorylation of Raf kinases that activate MEK kinase that in turn activates ERK kinase. ERK then activates the p90RSK kinase to phosphorylate CREB on serine 133 allowing CREB bound to cAMP response elements (CREs) to recruit coactivators and induce gene transcription. It should also be noted that the kinases that are activated by the non-classical pathway are capable of phosphorylating other spermatogenesis-regulating proteins in Sertoli cells as well as activating other transcription factors to regulate additional webs of gene expression.

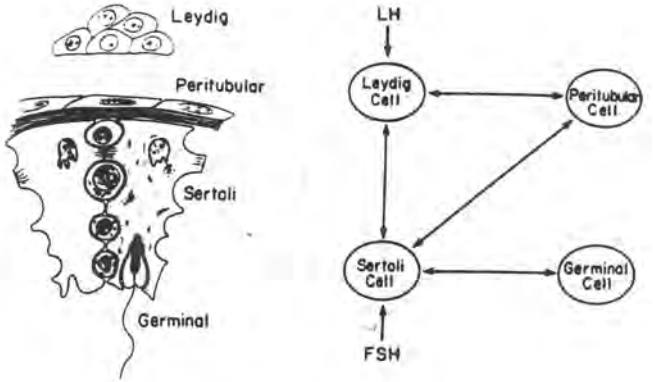
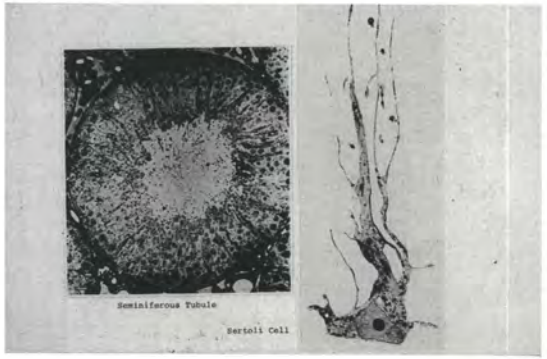
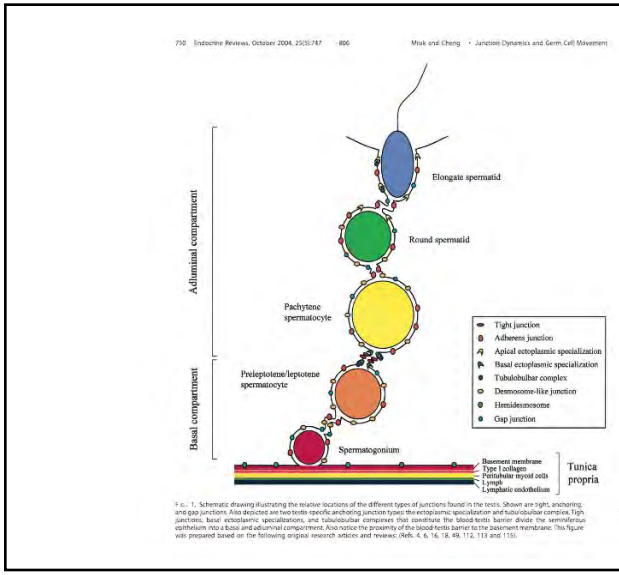


TABLE 1. Categorization of cell-cell interactions

Classification	Definition	Examples/mediators
Environmental	Interactions that influence the extracellular environment of the cell to affect cell contacts and cytoarchitecture	Extracellular matrix; cell adhesion molecules
Nutritional	Interactions involved in the delivery of essential nutrients between cells	Transfer of energy metabolites, metals, or vitamins
Regulatory	Agents provided by a cell that through a signal transduction event regulates another cell's function on a molecular level	Paracrine/autocrine factors; growth factors; differentiation factors; cytokines





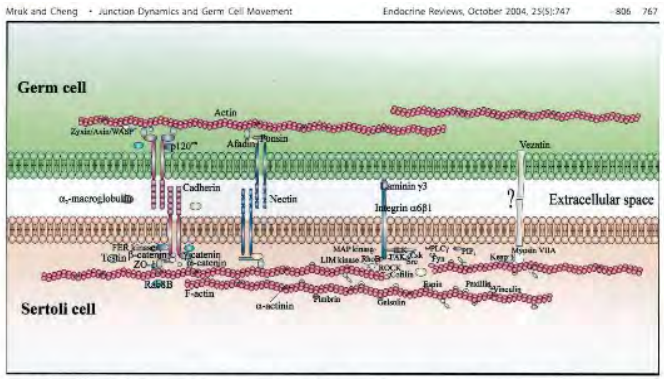
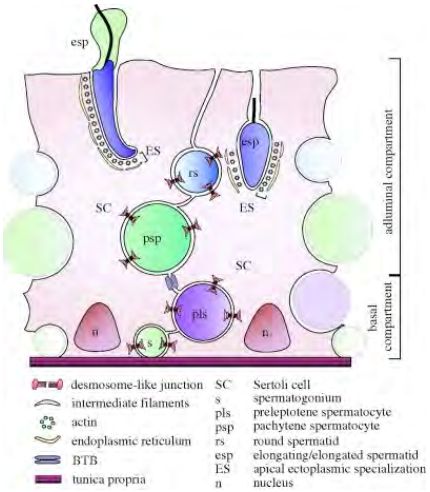
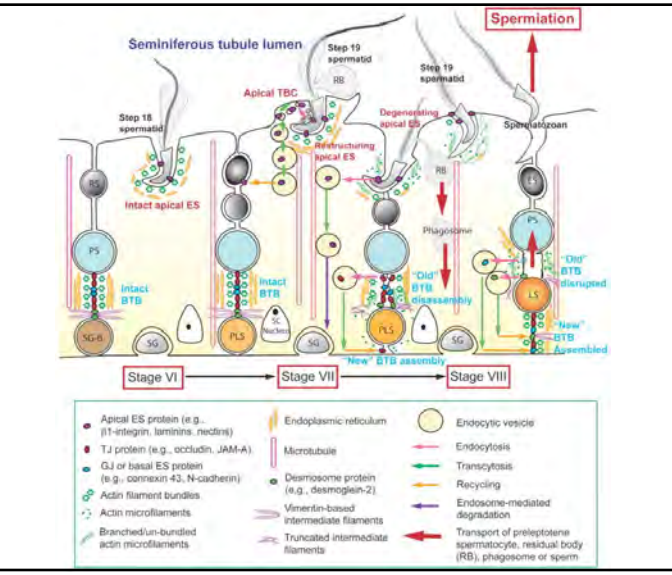


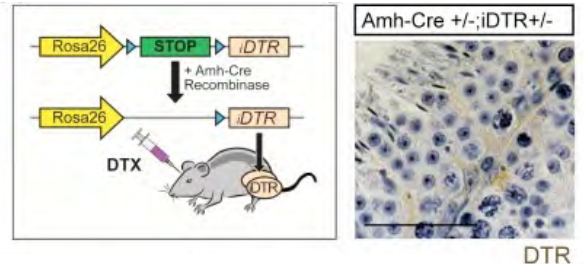
FIG. 9. Schematic drawing illustrating the molecular architecture of the four multiprotein complexes found at the Sertoli-germ cell adherens junction. Shown are the four multiprotein complexes found at the Sertoli-germ cell adherens junction: 1) cadherin–catenin, 2) nectin–afadin–ponsin, 3) integrin–laminin, and 4) vezatin-myosin (?). It is not yet known whether vezatin, the binding partner of myosin, is present in the testis. Also shown are signaling proteins known to regulate Sertoli-germ cell adherens junction dynamics. This figure was prepared based on the following original research articles and reviews: Refs. 4, 49, 53, 60, and 459).

Sertoli-germ cell junctions in the testis: a review of recent data.
Kopera IA, Bilinska B, Cheng CY, Mruk DD.
Philos Trans R Soc Lond B Biol Sci. 2010 May 27;365(1546):1593-605.





Sertoli cells maintain Leydig cell number and peritubular myoid cell activity in the adult mouse testis.
Rebourcet D, O'Shaughnessy PJ, Monteiro A, et al.
PLoS One. 2014 Aug 21;9(8):e105687.



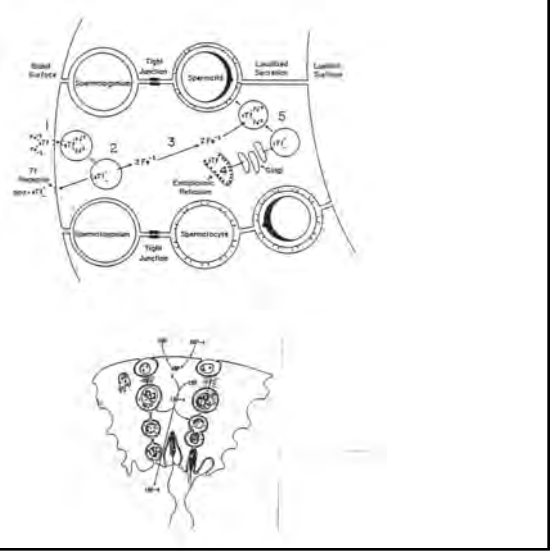


TABLE 1. Major Sertoli cell secretory products

Secretory product	Function and/or clearance/action	Potential coregulator
Transport/binding proteins		
ABP (65–67)	Androgen transport/facilitation	
Transferrin (62)	Iron transport	
Ceruloplasmin (64)	Copper transport	
Sulfated glycoprotein-1 (65–67)	Sphingolipid binding (68)	S22 (69) Band 4 (70) Testibumin (71)
Proteases/inhibitors		
Plasminogen activator (72, 73)	Activates plasminogen (74)	
Cystic protein-1 (75–78)	Cathepsin activity (77)	
α_2-Macroglobulin (78)	Protease inhibitor	
Extracellular matrix components		
Laminin (79)	Extracellular matrix component	
Collagen IV and I (79)	Extracellular matrix component	
Proteoglycans (80)	Extracellular matrix component	
Growth factors		
TGFα (81)	Growth stimulation	EC-EGF (67)
TGFβ (82)	Growth inhibition	
IGF-I (83–86)	Maintenance growth/differentiation	
IL-1 (88)	Growth regulation	
Regulatory proteins		
Inhibin (93)	Gonadotrophin-release agent	
Müllerian duct inhibitory agent (94)	Fetal Sertoli cells development	
Sulfated glycoprotein-2 (95, 96)	Sperm coating (96)	DAC-protein (83–88)
	Immunosuppressant (97, 98)	Clusterin (98–101) S40-S38 (88) CMH-II (101)
Metabolites		
Lactate/pyruvate (86–91)	Energy metabolites	
Estrogens (92)	Steroid hormone	

Trends in Endocrinology and Metabolism
Volume 15, Issue 7 , September 2004, Pages 345-350

Lactate and energy metabolism in male germ cells

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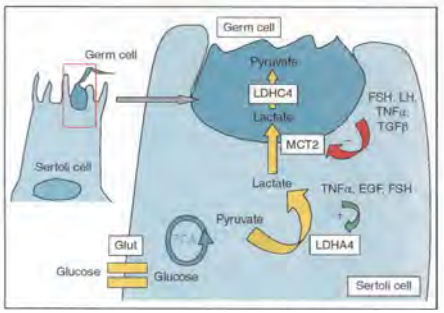
Available online 30 July 2004.

Various alterations in germ cell proliferation/differentiation, survival and energy metabolism are potentially involved in hypospermatogenesis leading to male infertility. Several reviews have been devoted to the different processes whose alteration might underlie hypospermatogenesis, except for energy metabolism in the testis. Energy metabolism in the testis exhibits some specificity in that lactate is the central energy metabolite used by germ cells. This metabolite is produced by somatic Sertoli cells, transported and used by germ cells in the context of an active cooperation under the control of the endocrine system and local cytokines. In this review, we present and discuss relevant published data on energy metabolism in male germ cells with a specific emphasis on lactate.



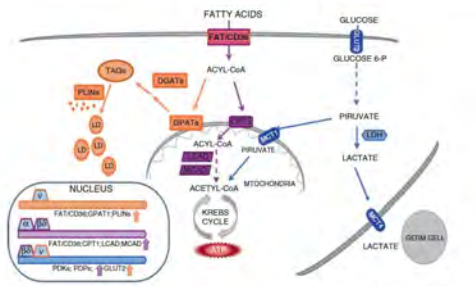
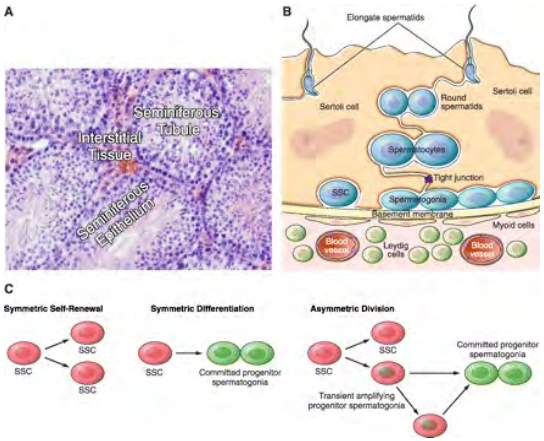
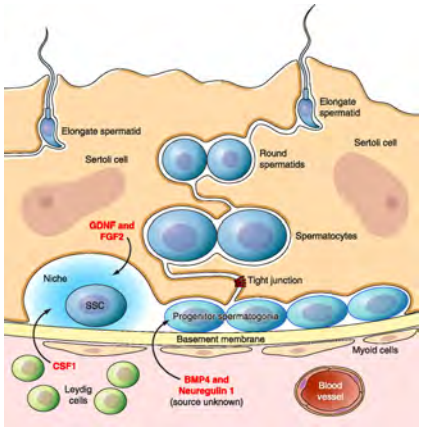


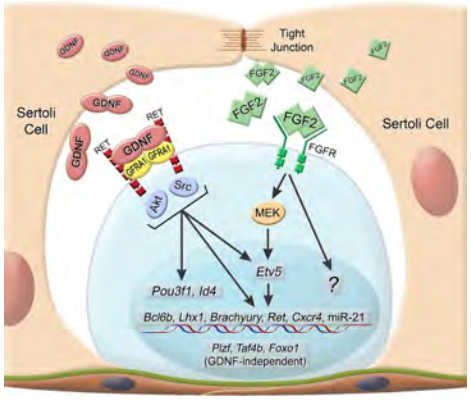
Fig. 3 Role of PPARs in the regulation of fatty acid metabolism and lactate production in Sertoli cells. Each isoform of peroxisome proliferator-activated receptor (PPAR) promotes different metabolic pathways in Sertoli cells. When PPARγ is activated, the expression of FAT/CD36, GPAT1 and PLINs is promoted, causing a shift toward lipogenesis. On the other hand, when PPARα and PPARβ/δ are activated, Sertoli cell metabolism moves toward full free fatty acid oxidation, due to an increase in FAT/CD36, CPT1, LCAD, and MCAD expression resulting in ATP augmentation. Simultaneously, activation of PPARγ and PPARβ/δ stimulates the glycolytic pathway, through the increase in GLUT2, PDKs and PDPs expression. In this scenario, lactate production and export to germ cells is also promoted, while a smaller portion of pyruvate is used to obtain ATP

The germline stem cell niche unit in mammalian testes.
Oatley JM, Brinster RL.
Physiol Rev. 2012 Apr;92(2):577-95.



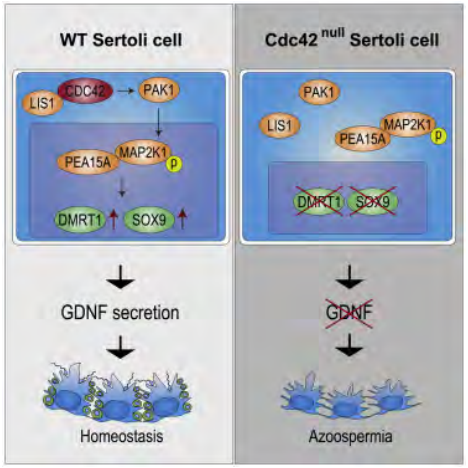


Schematic depicting the current understanding of determinants of the spermatogonial stem cell (SSC) niche in mammalian testes. Sertoli cells are known to dictate the formation of niche microenvironments and have been shown to produce the growth factors GDNF and FGF2 which regulate SSC proliferation and survival. Leydig cells are a source of CSF-1 which specifically regulates self-renewal of SSCs. The differentiation of SSCs is influenced by BMP4 and Neuregulin 1; however, the source of these factors is currently unknown. It is believed that upon differentiation from SSCs the resulting progenitor spermatogonia (i.e., Apr/Aal) migrate away from the niche and continue to develop as a cohort of maturing germ cells.

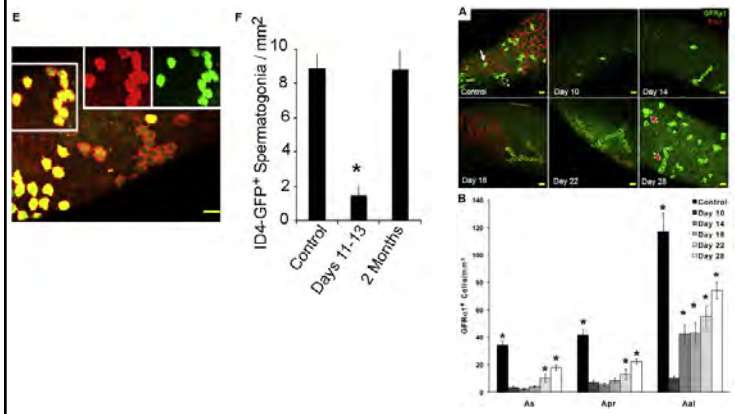


Molecular mechanisms of mouse SSC self-renewal. After GDNF binds to a ligand binding receptor GFRA1, the complex activates RET receptor tyrosine kinase following activation of Akt and Src-family kinases. The GDNF stimuli induces expression of many genes in SSCs, including transcription factor-encoding genes, *Etv5*, *Bcl6b*, *Lhx1*, *Brachyury*, *Ret*, *Cxcr4*, *Pou3f1*, and *Id4*. These transcriptional factors are involved in SSC self-renewal. *Plzf*, *Taf4b*, and *Foxo1* also play important roles on SSC self-renewal, but their expression is not regulated by GDNF. FGF2 is the second critical factor for SSC self-renewal, which induces *Etv5* expression through MEK activation. *Etv5* appears to be a key molecule, because this transcription factor upregulates other GDNF-inducing genes. MicroRNA-21 (miR-21) expression is regulated by *Etv5* and inhibits apoptosis in SSCs. GDNF and FGF2 are produced from Sertoli cells in the testis. Modified from [156].

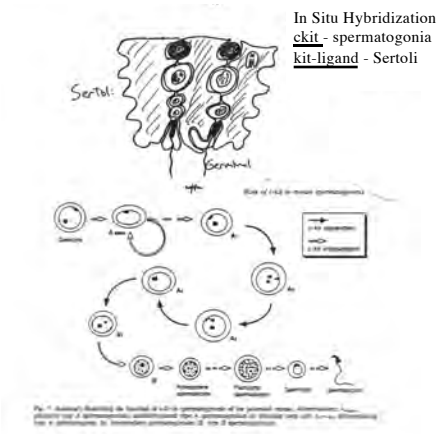
Cdc42 is required for male germline niche development in mice.
Mori Y, Takashima S, Kanatsu-Shinohara M, et al.
Cell Rep. 2021 Aug 17;36(7):109550.



Spermatogonial Stem Cell Numbers Are Reduced by Transient Inhibition of GDNF Signaling but Restored by Self-Renewing Replication when Signaling Resumes.
Parker N, Laychur A, Sukwani M, et al.
Stem Cell Reports. 2021 Mar 9;16(3):597-609.

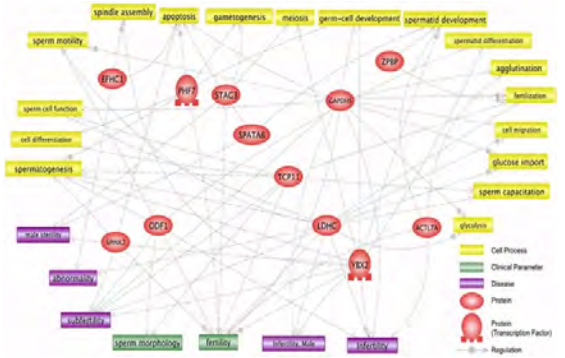


Kit and spermatogenesis -
ckit - oncogene, member PDGF receptor family
kit-ligand (i.e. Stem Cell factor, SMF) - growth factor ligand
- increase stem cell growth/early embryogenesis

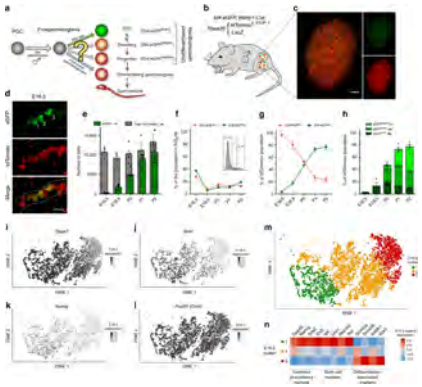


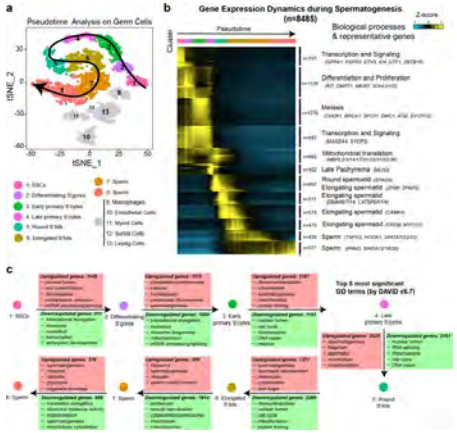
In Situ Hybridization
ckit - spermatogonia
kit-ligand - Sertoli

Comparative expression profiling of testis-enriched genes regulated during the development of spermatogonial cells
PLoS One. 2017 Apr 17;12(4):e0175787.
Ahn J, Park YJ, Chen P, Lee TJ, Jeon YJ, Croce CM, Suh Y, Hwang S, Kwon WS, Pang MG, Kim CH, Lee SS, Lee K.

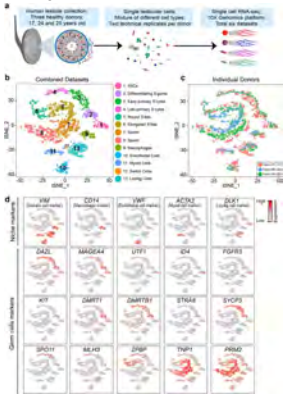


Developmental kinetics and transcriptome dynamics of stem cell specification in the spermatogenic lineage.
Law NC, Oatley MJ, Oatley JM.
Nat Commun. 2019 Jun 26;10(1):2787.

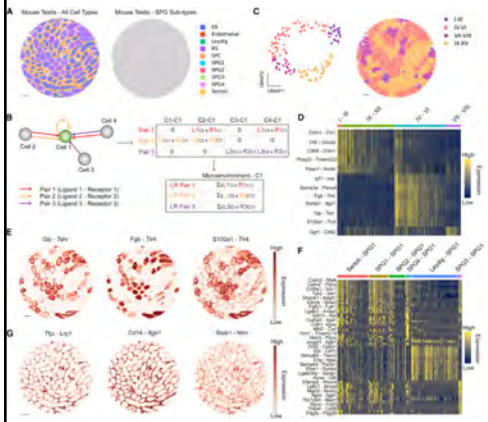




The adult human testis transcriptional cell atlas.
Guo J, Grow EJ, Mlcochova H, Maher GJ, Lindskog C, Nie X, Guo Y, Takei Y, Yun J, Cai L, Kim R, Carrell DT, Goriely A, Hotaling JM, Cairns BR.
Cell Res. 2018 Dec;28(12):1141-1157.

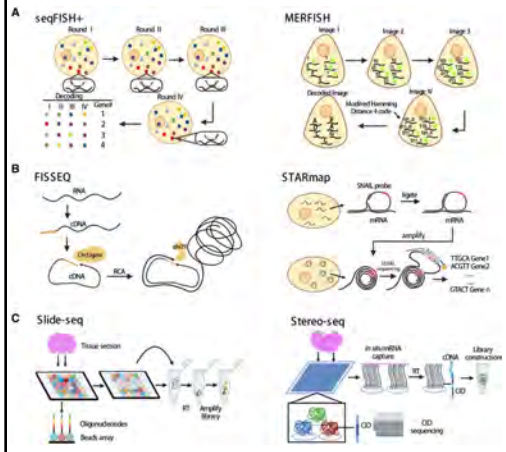


Dissecting the spermatogonial stem cell niche using spatial transcriptomics
Rajachandran S, Zhang X, Cao Q.
Cell Rep. 2023 Jul 25;42(7):112737.

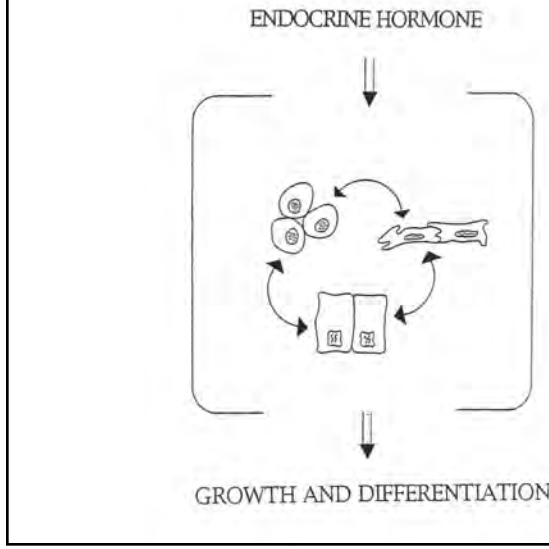


A spatially resolved ligand-receptor (LR) interaction map of the mouse testis
(A) Spatial mapping of major testicular cell types (left) and transcriptional states of spermatogonia (right) using a mouse testis Slide-seq dataset. ES elongating/elongated spermatid; RS, round spermatid; SPC, spermatocyte; SPG, spermatogonium. Scale bar, 150 μm.
(B) Schematic of the method to calculate spatially resolved LR interactions between a set of cells. Cell-cell interactions are calculated by multiplying ligand expression on the sending cell with receptor expression on the neighboring receiving cell for each LR pair.
(C) Left: uniform manifold approximation and projection (UMAP) of seminiferous tubules in the transcriptome space colored by the stages of the cycle of the seminiferous epithelium. Right: spatial mapping of the stages of the cycle of the seminiferous epithelium. Scale bar, 150 μm.
(D) Differentially expressed LR pairs across the stages of the seminiferous epithelium cycle.
(E) Spatial expression patterns of selective LR pairs enriched in stages IV–VI of the seminiferous epithelium cycle. Scale bar, 160 μm.
(F) Differentially expressed LR pairs among cell type-SPG1 pairs. The heatmap shows 60 representative LR pairs. Every other row of the heatmap is labeled because of space limitations. The full list of LR pairs is provided in Table S1.
(G) Spatial expression patterns of selective LR pairs enriched in the Leydig cell-SPG1 pair.

Dissecting mammalian reproduction with spatial transcriptomics.
Zhang X, Cao Q, Rajachandran S, Grow EJ, Evans M, Chen H.
Hum Reprod Update. 2023 Nov 2;29(6):794-810.



Representative spatial transcriptomics technologies. (**A**) Schematics of ISH-based ST technologies seqFISH+ and MERFISH. (**B**) Schematics of ISS-based ST technologies FISSEQ and STARmap. (**C**) Schematics of solid phase capture-based ST technologies Slide-seq and Stereo-seq. CID: co-ordinate identity; FISSEQ: fluorescent *in situ* sequencing; ISH: *in situ* hybridization; ISS: *in situ* sequencing; MERFISH: multiplexed error-robust fluorescence *in situ* hybridization; RCA: rolling circle amplification; RT: reverse transcription; seqFISH: sequential single-molecule fluorescence *in situ* hybridization; ST: spatial transcriptomics; STARmap: spatially resolved transcript amplicon readout mapping.



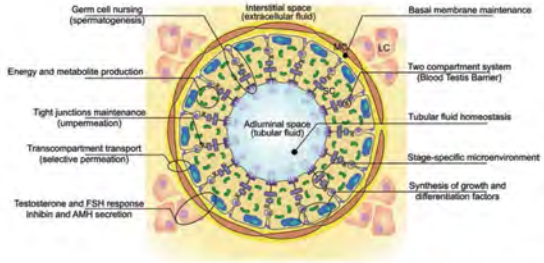
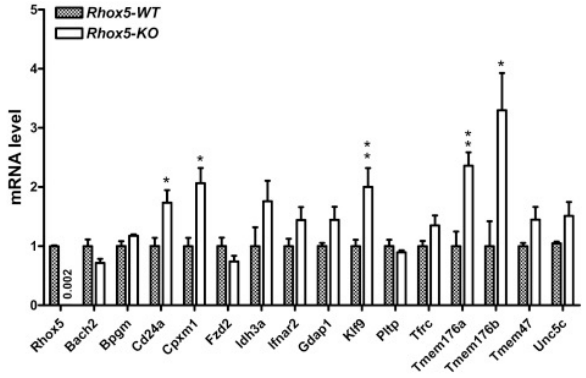
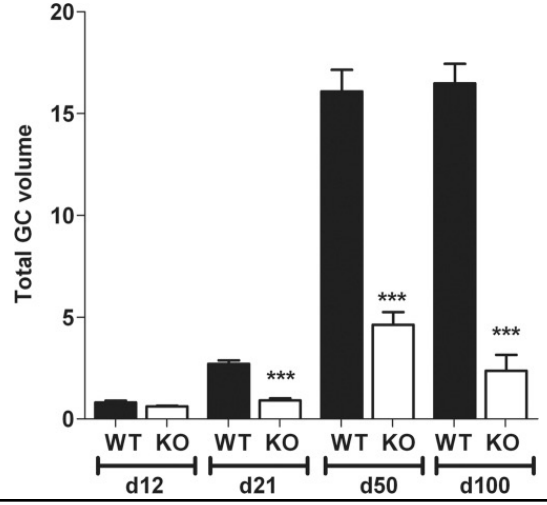


Fig. 1 The central role of Sertoli cells in testicular tissue environment and organization. Sertoli cells play several roles in testicular tissue's physiology. Besides their critical role on spermatogenesis, granting physical and nutritional support to developing germ cells, they also show paracrine and endocrine properties and are the main component of the blood-testis barrier, one of the tightest blood-tissue barriers of the human body

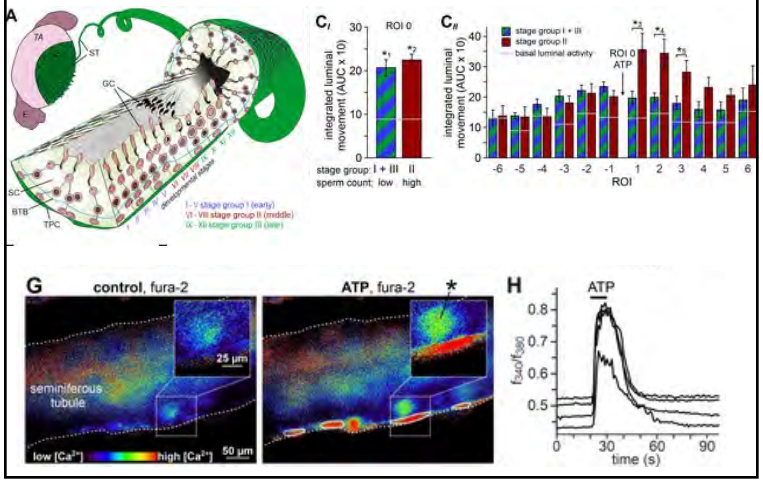
Androgen-induced Rhox homeobox genes modulate the expression of AR-regulated genes.
Hu Z, Dandekar D, O'Shaughnessy PJ, De Gendt K, Verhoeven G, Wilkinson MF.
Mol Endocrinol. 2010 Jan;24(1):60-75.



Androgen action via testicular peritubular myoid cells is essential for male fertility.
Welsh M, Saunders PT, Atanassova N, Sharpe RM, Smith LB.
FASEB J. 2009 Dec;23(12):4218-30.



ATP activation of peritubular cells drives testicular sperm transport.
Fleck D, Kenzler L, Mundt N, et al.
Elife. 2021 Jan 27;10:e62885.



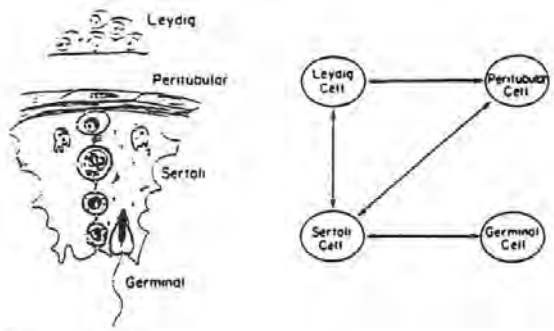


FIG. 2. Cell-cell interactions in the testis.

TABLE 6. Classification of testicular cell-cell interactions

Interaction	Environmental	Nutritional	Regulatory
Sertoli-germinal	Yes (e.g. cytoarchitecture)	Yes (e.g. transport proteins)	Yes (e.g. IGF-I)
Peritubular-Sertoli	Yes (e.g. basement membrane)	No	Yes (e.g. FMod5)
Sertoli-Leydig	No	No	Yes (e.g. POMC peptides)
Leydig-peritubular	No	No	Yes (e.g. androgen)

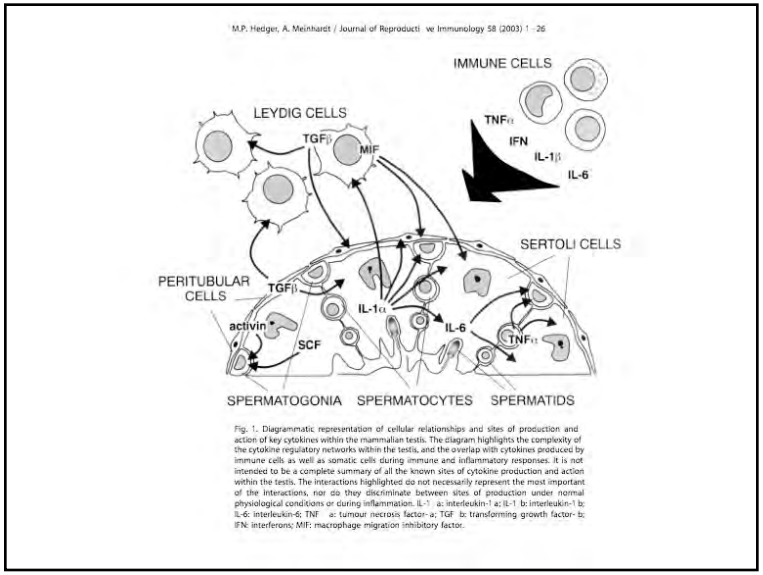
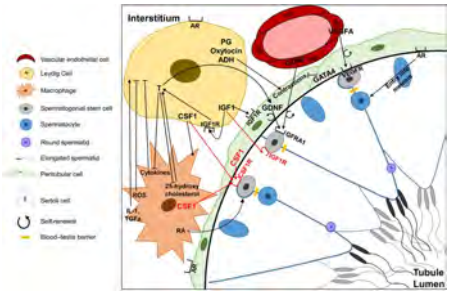


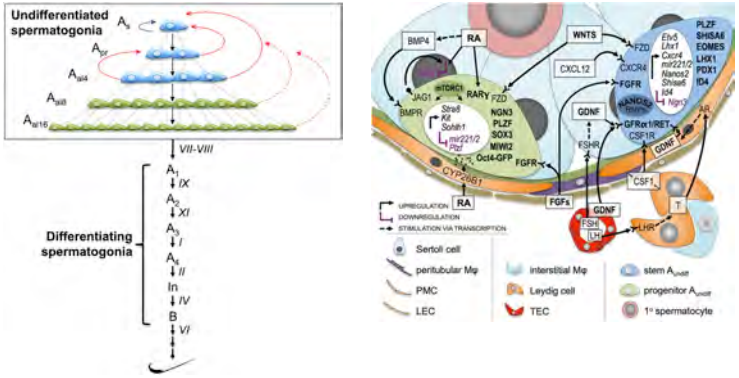
Fig. 1. Diagrammatic representation of cellular relationships and sites of production and action of key cytokines within the mammalian testis. The diagram highlights the complexity of the cytokine regulatory networks within the testis, and the overlap with cytokines produced by immune cells as well as somatic cells during immune and inflammatory responses. It is not intended to be a complete summary of all the known sites of cytokine production and action within the testis. The interactions highlighted do not necessarily represent the most important of the interactions, nor do they discriminate between sites of production under normal physiological conditions or during inflammation. IL-1: α: interleukin-1 α; IL-1: b: interleukin-1 b; IL-6: interleukin-6; TNF: α: tumour necrosis factor- α; TGF: b: transforming growth factor- b; IFN: interferons; MIF: macrophage migration inhibitory factor.

Essential roles of interstitial cells in testicular development and function.
Heinrich A, DeFalco T.
Andrology. 2019 Aug 24. doi: 10.1111/andr.12703.

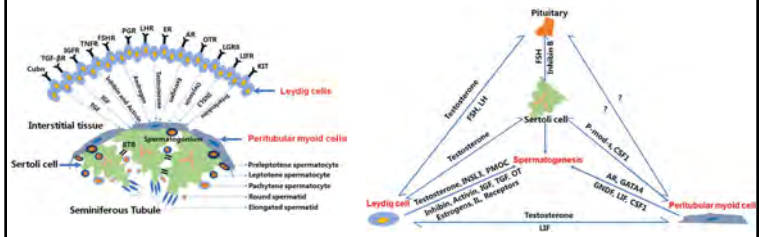


Role of interstitial cells in adult spermatogenesis. Illustration of a cross section of a seminiferous tubule and surrounding interstitium of a rodent testis, highlighting our current knowledge of the mechanisms through which testicular interstitial cells influence adult spermatogenesis. Text and receptors shown in red indicate interactions needing further study or are currently unclear. Arrows indicate a positive influence, and T-shaped lines indicate an inhibitory effect. ADH, vasopressin; AR, androgen receptor; CSF1/CSF1R, colony-stimulating factor 1/receptor; GDNF/GFRA1, glial cell-derived neurotrophic factor/receptor 1; IGF1/IGF1R, insulin-like growth factor 1/receptor; IL-1, interleukin-1; PG, prostaglandin; RA, retinoic acid; ROS, reactive oxygen species; T, testosterone; TGFa, transforming growth factor alpha; VEGFA/VEGFR, vascular endothelial growth factor/receptor.

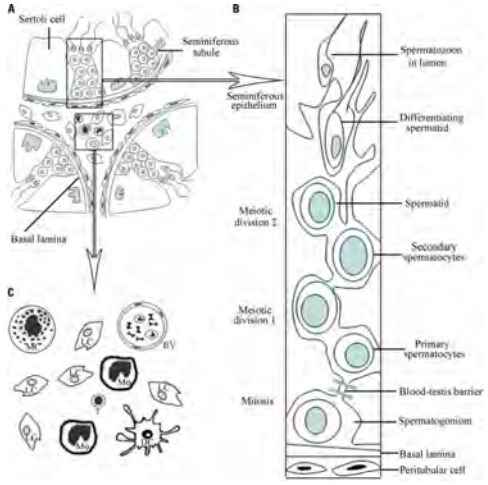
Molecular regulation of spermatogonial stem cell renewal and differentiation.
Mäkelä JA, Hobbs RM.
Reproduction. 2019 Jun 1. pii: REP-18-0476.R2.

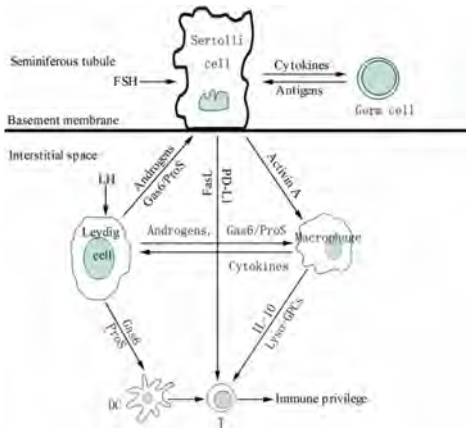


The roles and mechanisms of Leydig cells and myoid cells in regulating spermatogenesis.
Zhou R, Wu J, Liu B, Jiang Y, Chen W, Li J, He Q, He Z.
Cell Mol Life Sci. 2019 Jul;76(14):2681-2695.



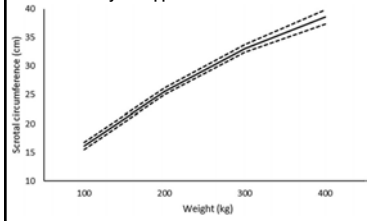
Structural, cellular and molecular aspects of immune privilege in the testis.
Li N, Wang T, Han D.
Front Immunol. 2012 Jun 11;3:152.



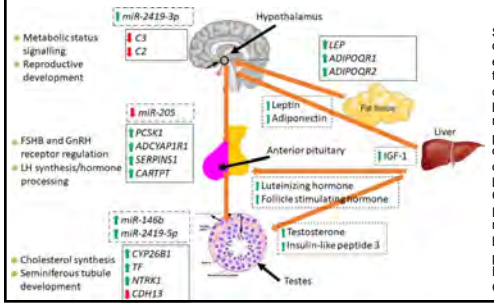


A schematic drawing illustrating intercellular communication via paracrine immunosuppressive molecules that favor immune privilege in the testis. Under the influence of follicle-stimulating hormone (FSH), androgens and germ cell antigens, Sertoli cells secrete immunosuppressive molecules that inhibit inflammatory responses of macrophages and T lymphocytes (T) in the interstitial compartments. Sertoli, Leydig cells and macrophages regulate activities of the other immune cells including dendritic cells (DC), T and mast cells (MC) via paracrine immunosuppressive molecules, favoring testicular immune privilege property.

Role of early life nutrition in regulating sexual development in bulls.
Byrne CJ, Keogh K, Kenny DA.
Animal. 2023 May:17 Suppl 1:100802.

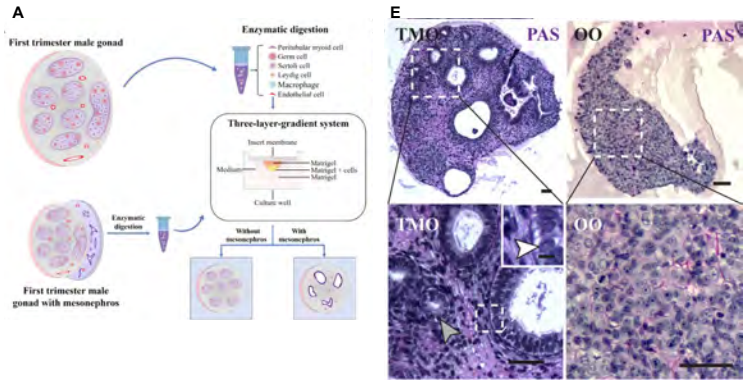


Relationship between scrotal circumference and live weight of Holstein-Friesian bulls on differing diets in early life (source: Byrne et al., 2017, Byrne et al., 2018a). The solid lines represent predicted values from models, with scrotal circumference as the dependent variable. Dashed lines represent ± SE. ($y = -0.001x^2 + 0.125x + 4.5912$; $R^2 = 0.85$).

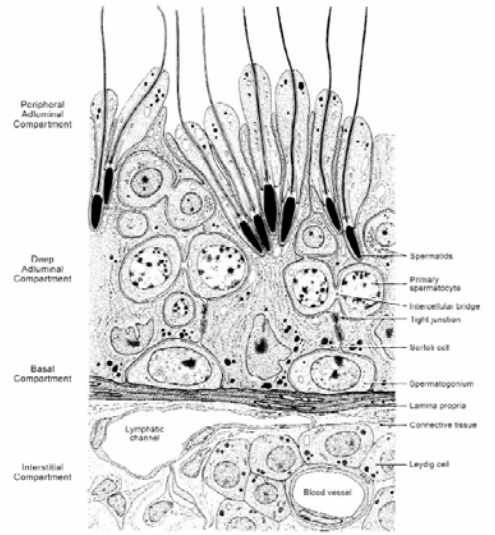


Schematic representation illustrating differential transcriptional and protein expression in key metabolic, neural and testicular tissues, as well as on systemic concentrations of metabolic and reproductive hormones in response to plane of nutrition/metabolic status of bull calves. The proposed biochemical responses are based on global transcriptomic and protein data and on bioinformatic network analyses reported by Byrne et al., 2018a, Byrne et al., 2018b, Coen et al., unpubl, English et al., 2018a, English et al., 2018b, Keogh et al., unpubl. mRNA – closed boxes, miRNA – dash dot boxes, hormones – dashed boxes, biological processes altered –. Abbreviations: FSH = follicle stimulating hormone, GnRH = gonadotrophin-releasing hormone.

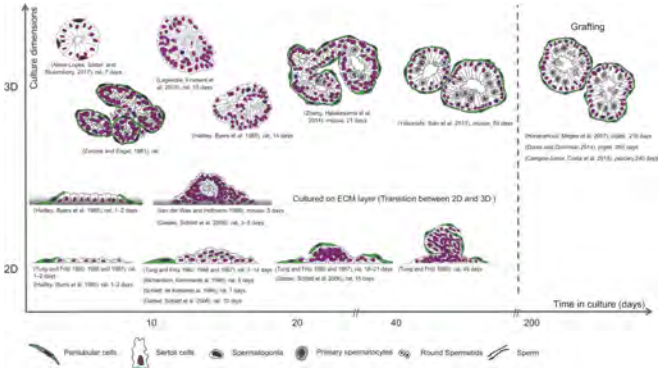
Two populations of self-maintaining monocyte-independent macrophages exist in adult epididymis and testis.
Wang M, Yang Y, Cansever D, et al.
Proc Natl Acad Sci U S A. 2021 Jan 5;118(1):e2013686117.

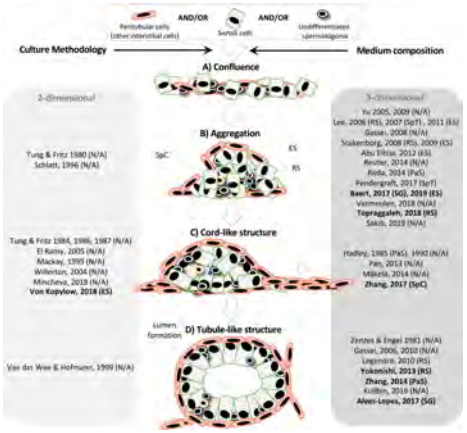


The 3-LGS generates compartmentalised human gonadal organoids. **A** Schematic illustrating culture preparation and the 3-LGS setup. **E** Tissue reorganisation fails to occur when mesonephros is included in the testicular tissue digest (testicular mesonephric organoid (TMO); representative organoid image from a 7.5 wpc embryonic tissue sample). The inclusion of mesonephric tissue resulted in the formation of mesonephric-like tubules throughout the interstitial space, observed as small tubular lumens lined by a simple cuboidal epithelium (grey arrow) or larger ducts with a pseudostratified columnar epithelium (white arrow; inset). The 3-LGS can also be used to generate ovarian organoids (OO) from dissociated ovarian tissue (representative organoid image from a 10 wpc embryonic tissue sample).

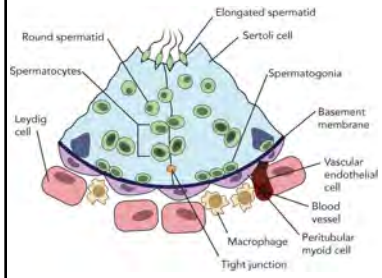


Testicular organoids: a new model to study the testicular microenvironment in vitro?
Hum Reprod Update. 2017 Dec 21. (Epub ahead of print)
Alves-Lopes JP, Stukenborg JB.

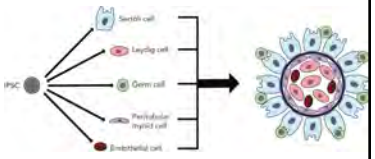




Testicular organoids to study cell-cell interactions in the mammalian testis.
Sakib S, Goldsmith T, Voigt A, Dobrinski I.
Andrology. 2019 Jul 21. doi: 10.1111/andr.12680.

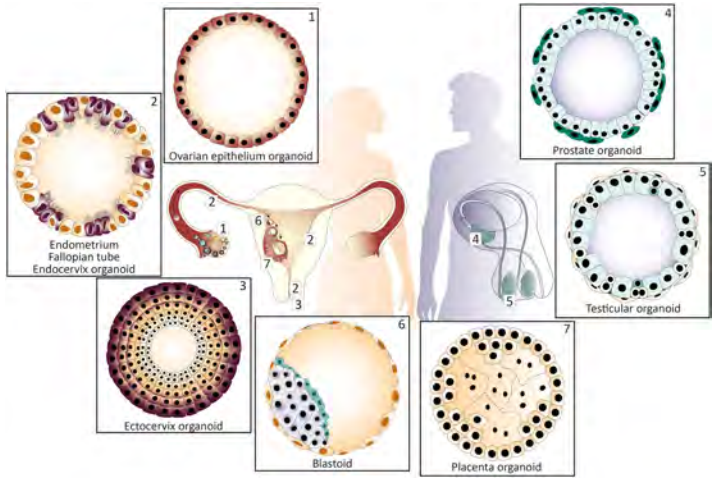


Schematic representation of the germ cell niche. Undifferentiated germ cells including spermatogonial stem cells are localized at the basement membrane and remain in close contact with the Sertoli cells inside the seminiferous epithelium. Peritubular myoid cells line the outer perimeter of the basement membrane, and Leydig cells, vascular cells, and testicular macrophages are located in the interstitium. As spermatogonia differentiate, they traverse the tight junctions and move from the basal to the adluminal compartment of the seminiferous epithelium.



Schematic representation of testicular organoids to study development. Different testicular cells can be derived from iPS cells to generate testicular organoids.

Human organoid systems in modeling reproductive tissue development, function, and disease.
Haider S, Beristain AG.
Hum Reprod. 2023 Aug 1;38(8):1449-1463.



3D culture systems for reproductive tissue organogenesis

Organoids as tools to investigate the molecular mechanisms of male infertility and its treatments
Kanbar M, Vermeulen M, Wyns C.
Reproduction. 2021 May;161(5):R103-R112.

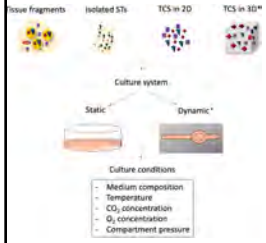


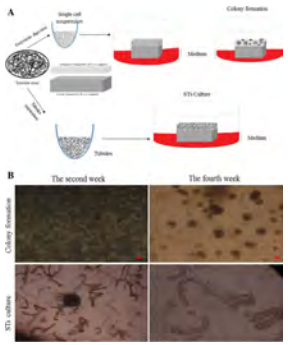
Table 1 Most commonly used scaffolds in 3D culture of testicular cells reporting germ cell differentiation up to the haploid stage.

Scaffolds	Species	Reference
Natural		
ECM-derived scaffolds		
Collagen	Rat	Lee et al. (2006b)
Matrigel™*	Rat	Lee et al. (2006b), Legeréte et al. (2010)
	Humans^	Sun et al. (2018)
Decellularized ECM*	Humans^	Ashouri Movassagh et al. (2020)
Seaweed-derived scaffolds		
Alginate	Mouse	Baert et al. (2019)
Soft-Agarose	Mouse	Stukenborg et al. (2008), Stukenborg et al. (2009), Abu Elhija et al. (2012)
Synthetic		
PLGA	Rat	Lee et al. (2011)
Methylcellulose	Mouse	Stukenborg et al. (2009)
	Rhesus monkey	Hultkhel et al. (2015)
	Humans^	Abofoul-Azab et al. (2019)

*Extracted from mice Engelbreth–Holm–Swarm tumour cells and mainly composed of laminin, collagen IV, and entactin (Hughes et al. 2010); ^solid or solubilized form; All reports used prepubertal testicular tissue except studies marked with a ^ that used adult tissue.
ECM, extracellular matrix; PLGA, Poly (lactic-co-glycolic acid).

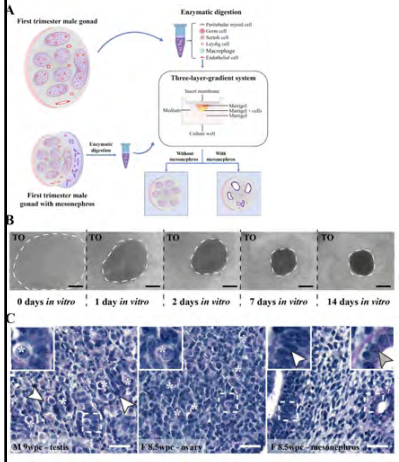
Different available options for germ cell in vitro maturation from intact or disaggregated testicular tissue. *: with or without scaffold – including testicular organoids; ▲: Microfluidic, shaking/rotating culture or shaken bioreactors; ST: seminiferous tubule; TCS: testicular cell suspension.

Organ culture of seminiferous tubules using a modified soft agar culture system.
Gholami K, Pourmand G, Koruji M, Ashouri S, Abbasi M.
Stem Cell Res Ther. 2018 Sep 26;9(1):249.



In-vitro spermatogenesis using culture of seminiferous tubules (STs) or testicular cells from 3- or 6-day-old mice. a Schematic presentation of experimental procedures. b Stereomicroscopic appearance of colony formation and seminiferous tubules. Arrow indicates complete canalization of seminiferous tubules in the fourth week. Scale bars in STs = 1 mm and in colonies = 50 µm

Self-organising human gonads generated by a Matrigel-based gradient system.
Oliver E, Alves-Lopes JP, Femke Harteveld F., et al.
BMC Biol. 2021 Sep 23;19(1):212.



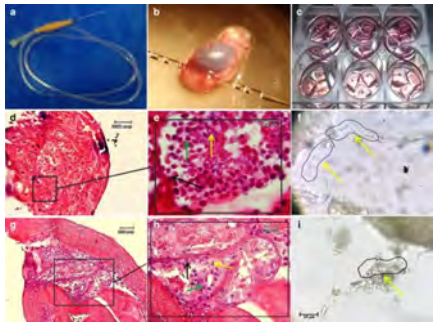
Abstract

Background: Advances in three-dimensional culture technologies have led to progression in systems used to model the gonadal microenvironment in vitro. Despite demonstrating basic functionality, tissue organisation is often limited. We have previously detailed a three-dimensional culture model termed the three-layer gradient system to generate rat testicular organoids in vitro. Here we extend the model to human first-trimester embryonic gonadal tissue.

Results: Testicular cell suspensions reorganised into testis-like organoids with distinct seminiferous-like cords situated within an interstitial environment after 7 days. In contrast, tissue reorganisation failed to occur when mesonephros, which promotes testicular development in vivo, was included in the tissue digest. Organoids generated from dissociated female gonad cell suspensions formed loosely organised cords after 7 days. In addition to displaying testis-specific architecture, testis-like organoids demonstrated evidence of somatic cell differentiation. Within the 3-LGS, we observed the onset of AMH expression in the cytoplasm of SOX9-positive Sertoli cells within reorganised testicular cords. Leydig cell differentiation and onset of steroidogenic capacity was also revealed in the 3-LGS through the expression of key steroidogenic enzymes StAR and CYP17A1 within the interstitial compartment. While the 3-LGS generates a somatic cell environment capable of supporting germ cell survival in ovarian organoids germ cell loss was observed in testicular organoids.

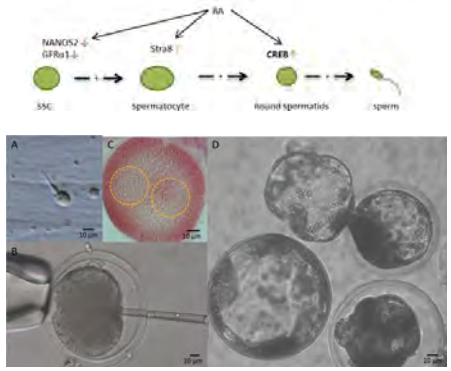
Conclusion: The 3-LGS can be used to generate organised whole gonadal organoids within 7 days. The 3-LGS brings a new opportunity to explore gonadal organogenesis and contributes to the development of more complex in vitro models in the field of developmental and regenerative medicine.

In vitro transplantation of spermatogonial stem cells isolated from human frozen-thawed testis tissue can induce spermatogenesis under 3-dimensional tissue culture conditions.
Mohaqiq M, Movahedin M, Mazaheri Z, Amirjannati N.
Biol Res. 2019 Mar 27;52(1):16.

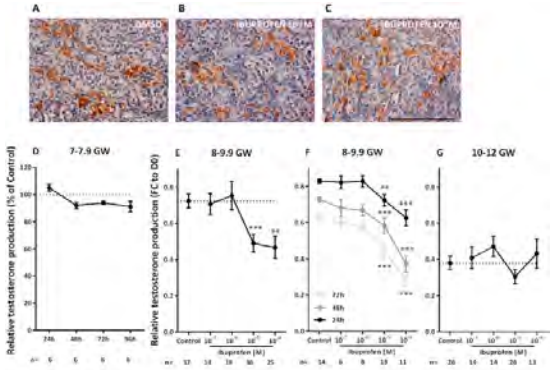


Transplantation of SSCs to host testes and following in organ culture results. IVT of SSs to host testis and organ culture **(a–c)**. H&E staining of tissue sections IVT group **(d, e)** and control group **(g, h)**. Dynamic dissection of testis fragments after 8 weeks in IVT group **(f)** and control group **(i)**. Black arrow: SCs, green arrow: spermatocyte and yellow arrow: long spermatid or sperm like cells

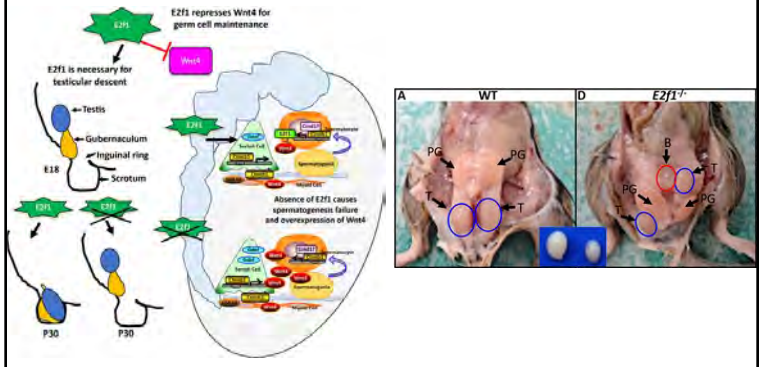
In-vitro differentiation of early pig spermatogenic cells to haploid germ cells.
Yu K, Zhang Y, Zhang BL, Wu HY, Jiang WQ, Wang ST, Han DP, Liu YX, Lian ZX, Deng SL.
Mol Hum Reprod. 2019 Sep 1;25(9):507-518.



Ibuprofen results in alterations of human fetal testis development
Sci Rep. 2017 Mar 10;7:44184.
Ben Maamar M, Lesné L, Hennig K, et al.



E2F1 regulates testicular descent and controls spermatogenesis by influencing WNT4 signaling
Jorgez CJ, Seth A, Wilken N, et al.
Development. 2021 Jan 13;148(1):dev191189.



Genetic dissection of spermatogenic arrest through exome analysis: clinical implications for the management of azoospermic men.
Krausz C, Riera-Escamilla A, Moreno-Mendoza D, et al.
Genet Med. 2020 Dec;22(12):1956-1966.

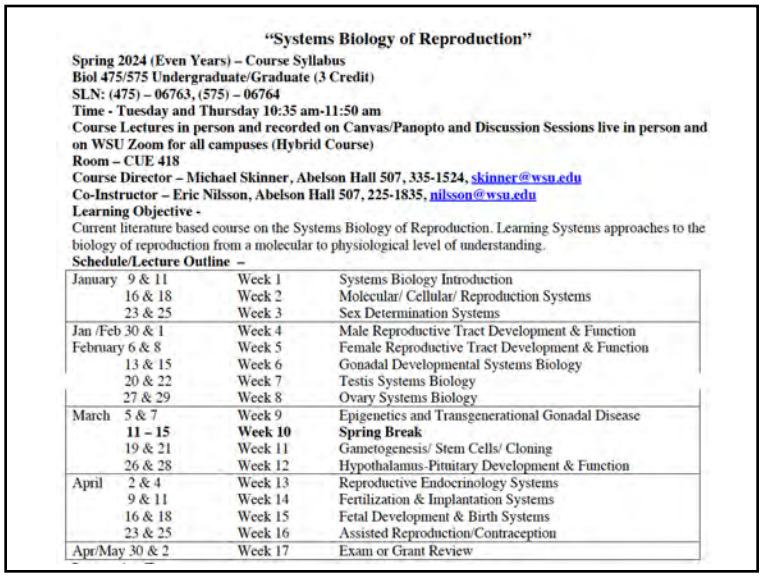
Abstract

Purpose: Azoospermia affects 1% of men and it can be the consequence of spermatogenic maturation arrest (MA). Although the etiology of MA is likely to be of genetic origin, only 13 genes have been reported as recurrent potential causes of MA.

Methods: Exome sequencing in 147 selected MA patients (discovery cohort and two validation cohorts).

Results: We found strong evidence for five novel genes likely responsible for MA (ADAD2, TERB1, SHOC1, MSH4, and RAD21L1), for which mouse knockout (KO) models are concordant with the human phenotype. Four of them were validated in the two independent MA cohorts. In addition, nine patients carried pathogenic variants in seven previously reported genes-TEX14, DMRT1, TEX11, SYCE1, MEIOB, MEI1, and STAG3-allowing to upgrade the clinical significance of these genes for diagnostic purposes. Our meiotic studies provide novel insight into the functional consequences of the variants, supporting their pathogenic role.

Conclusion: Our findings contribute substantially to the development of a pre-testicular sperm extraction (TESE) prognostic gene panel. If properly validated, the genetic diagnosis of complete MA prior to surgical interventions is clinically relevant. Wider implications include the understanding of potential genetic links between nonobstructive azoospermia (NOA) and cancer predisposition, and between NOA and premature ovarian failure.



"Systems Biology of Reproduction"

Spring 2024 (Even Years) – Course Syllabus
Biol 475/575 Undergraduate/Graduate (3 Credit)
SLN: (475) – 06763, (575) – 06764
Time - Tuesday and Thursday 10:35 am-11:50 am
Course Lectures in person and recorded on Canvas/Panopto and Discussion Sessions live in person and on WSU Zoom for all campuses (Hybrid Course)
Room – CUE 418
Course Director – Michael Skinner, Abelson Hall 507, 335-1524, skinner@wsu.edu
Co-Instructor – Eric Nilsson, Abelson Hall 507, 225-1835, nilsson@wsu.edu
Learning Objective -
Current literature based course on the Systems Biology of Reproduction. Learning Systems approaches to the biology of reproduction from a molecular to physiological level of understanding.
Schedule/Lecture Outline –

January	9 & 11	Week 1	Systems Biology Introduction
	16 & 18	Week 2	Molecular/ Cellular/ Reproduction Systems
	23 & 25	Week 3	Sex Determination Systems
Jan /Feb	30 & 1	Week 4	Male Reproductive Tract Development & Function
February	6 & 8	Week 5	Female Reproductive Tract Development & Function
	13 & 15	Week 6	Gonadal Developmental Systems Biology
	20 & 22	Week 7	Testis Systems Biology
	27 & 29	Week 8	Ovary Systems Biology
March	5 & 7	Week 9	Epigenetics and Transgenerational Gonadal Disease
	11 – 15	**Week 10**	**Spring Break**
	19 & 21	Week 11	Gametogenesis/ Stem Cells/ Cloning
	26 & 28	Week 12	Hypothalamus-Pituitary Development & Function
April	2 & 4	Week 13	Reproductive Endocrinology Systems
	9 & 11	Week 14	Fertilization & Implantation Systems
	16 & 18	Week 15	Fetal Development & Birth Systems
	23 & 25	Week 16	Assisted Reproduction/Contraception
Apr/May	30 & 2	Week 17	Exam or Grant Review