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Review

# Regulation of spermatogenesis: An evolutionary biologist's perspective

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ABSTRACT

This review describes the regulation of spermatogenesis taking into consideration the hypothalamic–pituitary gonadal axis, the male reproductive organs and the endocrine and paracrine factors involved in the control of sperm production and the release of androgens. Instead of detailed descriptions of many hormones and growth factors, we attempt to provide an integrative and evolutionary view by comparing different species and considering their specific needs for successful male reproduction. The review focuses on species specific differences in the structural organization of spermatogenesis and indicates that the crucial regulatory mechanisms controlling sperm output are targeted toward differentiating spermatogonia when they initiate clonal expansion. We argue that the further differentiation of germ cells is following a highly coordinated and strictly predetermined morphogenetic cascade widely independent of hormonal control. We propose a hypothetical "ancient" model. Spermatogenesis and steroidogenesis are controlled by a master switch (GnRH pulse generator) under whose control two separate feedback systems provide independent control of androgen (LH–testosterone) and sperm production (FSH–inhibin). This scenario offers high flexibility and has seen uncountable adaptions to optimize the specific needs of different species. Models for the hormonal regulation in hamsters, laboratory rodents and primates are presented to illustrate the species specific diversity.



**Contents**



## 1. Introduction

Male fertility in mammals depends on the continuous daily production of millions of spermatozoa. The process of spermatogenesis is one of exquisite complexity, requiring 6–9 weeks for completion, and involves a coordinated series of mitotic and meiotic divisions, elaborate cyto-differentiative steps, and constantly changing intercellular interactions, all overseen by an extraordinary interplay of autocrine, paracrine, and endocrine factors. The histology of spermatogenesis has been described in detail [1–4] and the endocrine regulation of spermatogenesis has been a long standing scientific topic which has been debated in many research areas e.g. developmental biology, veterinary and clinical medicine, and cell biology. Due to the importance of hormones for testicular function and the knowledge of the hypothalamus-pituitary gonadal axis described in the 1960s the discussion was dominated by views from endocrinologists. A PubMed search (dated November 2013) with the term "Regulation of spermatogenesis" returned 622 review

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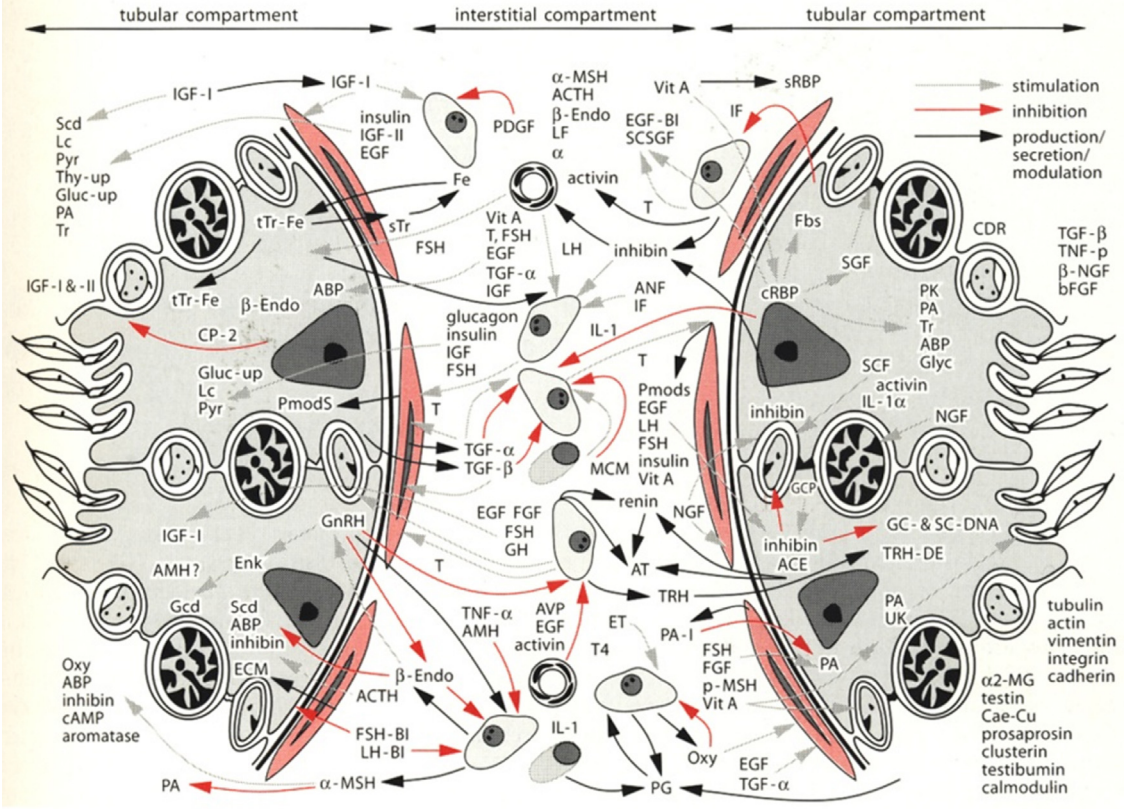


**Fig. 1.** Complexity of factors involved in endocrine and paracrine regulation of the testis.
Reprint with permission from Weinbauer et al. [17].

articles published since 1971. This indicates that this topic has enriched many textbooks and journals. The field was originally dominated by analysis of the actions of gonadotropins (follicle stimulating hormone (FSH), and luteinizing hormone (LH)) and steroids. However, the range of themes covered in the hundreds of reviews on the regulation of spermatogenesis illustrates a highly versatile and translational research field. Many different animal models were applied to study physiological aspects of spermatogenesis from invertebrates to primates. In vitro studies using testicular tissue and specific somatic cells generated knowledge on cellular mechanisms primarily in the signal transduction mechanisms of hormones and growth factors. A variety of control mechanisms of spermatogenesis and steroidogenesis were described which are required to adjust testicular function to naturally occurring changes in order to optimize reproductive outcome (e.g. seasonality, stress, nutrition). Manipulations of the endocrine control axes were performed to explore associated changes of sperm production. Our knowledge of the endocrine regulation of spermatogenesis arises from numerous studies suppressing and replacing endocrine factors and then examining the ensuing effects on testicular function and spermatogenesis in rodents and primates [5–14]. These studies have been particularly focussed on elucidating the role of hormones in adult spermatogenesis. Also, recent gene knockout studies and the creation of mice congenitally deficient in gonadotropin subunits, steroid hormone biosynthetic enzymes or hormone receptors have been invaluable in revealing which factors are crucial for the initiation of testis function and have provided important clues as to their cellular sites of action (see [15,16] for reviews).

Translational aspects range from potential treatment options for infertile patients up to the development of hormone based male contraceptive strategies. Naturally occurring or induced disturbances of spermatogenesis were elucidated to specific genes or endocrine/paracrine factors. As a consequence, it is reasonable to say that we have a good understanding of the endocrine factors involved in the regulation of spermatogenesis; at least we understand which hormones are required for spermatogenesis and how we can impair or restore spermatogenesis via hormonal stimulation or withdrawal.

On the other hand after many years of research the field generated a long list of hormones and growth factors controlling many specific aspects of spermatogenesis. A variety of signaling cascades and intracellular signal transduction pathways were postulated to be involved in the control of testicular functions. The large number of endocrine, paracrine, intracrine and juxtacrine factors and mechanisms of action for control of testis function generated a sometimes confusing scenario (Fig. 1).

The introduction of molecular analytical procedures generating expression patterns of thousands of gene products has only helped to a certain degree to enlighten the supposedly complex regulatory networks. The evaluation of these complex networks is greatly facilitated by the availability of in vitro culture systems providing access to pure somatic cells and specific germ cell stages. However, these systems are not yet fully established and do not yet generate strong strategies for stringent experimental research [18]. Potentially these systems will facilitate the in vitro manipulation under well-defined experimental conditions providing an opportunity to correlate distinct physiological changes with specific changes of mRNA expression covering thousands of genes. Unfortunately strategies for enrichment of specific germ cell stages and the creation of valid co-culture system for somatic and germ cells presents still a significant hurdle. Breakthroughs in expanding spermatogonia in culture are promising and may help to overcome this problem [19]. Innovative approaches to induce spermatogenesis

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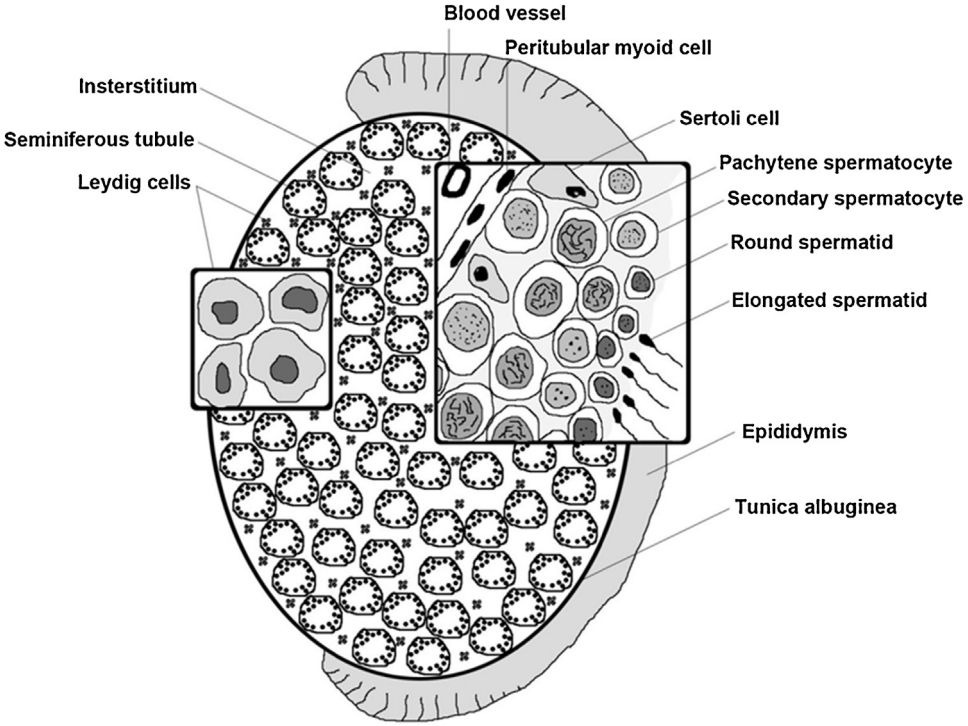


**Fig. 2.** Generalized schematic drawing depicting the mammalian testicular anatomy and cell biology.

in the culture dish create novel research strategies for future male germ cell research [18]. Currently the known regulatory networks can serve as a foundation to build physiologically relevant models that include both the endocrine and paracrine control of spermatogenesis under different physiological and pathological contexts.

This introduction delineates that the testis-specific multicompartmental organization of the testis, the synchronous differentiation of large germ cell cohorts and the many testis specific cellular processes require a unique regulation. On the other hand many previous studies have revealed a highly robust species-specific organization and kinetics of spermatogenesis contrasting with the postulated abundance of factors assumed to control spermatogenesis. Throughout evolution the process of spermatogenesis was crucial for maintaining any species and any fatal deviation would have led to immediate extinction. The importance of a robust regulation of male reproduction removes quickly any failure prone control mechanism.

In this review we will present a zoological perspective on the endocrine regulation of testis function. At first we will focus on the clonal expansion as a principle of male germ cell differentiation. We will introduce germ line stem cells and their niches as crucial cellular differentiation processes at the starting point of spermatogenesis. Depending on the species germ cell expansion occurs in different anatomical arrangements as cysts or multi-layered seminiferous epithelia. We will introduce the relevant hormones as constituents of the hypothalamic–pituitary gonadal axis and pay attention to hormonal feedback systems and major paracrine mechanisms. Finally we will discuss species-specific modifications of regulatory mechanisms which are implemented to adapt male germ cell development to species-specific needs. In doing so, we focus on evolutionary and comparative aspects. We follow the concept that the highly conserved processes of meiosis and spermiohistogenesis are rather autonomous morphogenetic series of differentiation steps whereas the initial entry of male germ cells into differentiation may or may not be regulated depending on the needs of the species.

## 2. Structural and functional compartmentalization of the testis

When comparing different families of vertebrates, it becomes apparent that the anatomy, relative size, and location of the testes differ greatly, sometimes even between closely related species. In fish and amphibians, the testes present as dual elongated lobes stretched along both sides of the abdominal cavity. These are attached to the dorsal mesenterium. A species dependent number of efferent ductules connect each testicular lobe to a distinct cranial part of the adjacent kidney (Pars sexualis) to facilitate sperm transport via specialized ducts of the original renal systems. In contrast, the mammalian testis is generally of elliptical shape and can be located either permanently in the abdominal cavity (e.g. elephants and marine mammals) or in the scrotum (e.g. man). Latter location provides a cooler environment compared to core body temperature. In homeothermic species specialized blood supply involving testicular arteries and veins and/or retraction by the cremaster muscle toward or away from the warmer body cavity creates an unusual and efficient temperature control system stabilizing the testis core temperature at 1–2 °C below body core temperature. The mammalian testis is enclosed by a fibrous capsule, the Tunica albuginea which is connected with the retracting muscles and is highly vascularized.

The mammalian testis is a bicompartmental organ consisting of seminiferous tubules (approx. 90% of adult mass) and an interstitium (approx. 10% of adult mass) as depicted in Fig. 2. The tubular wall consists of a basement membrane with underlying contractile peritubular cells [20]. The tubules are home to the sperm generating compartment, the seminiferous epithelium. The seminiferous epithelium is a unique and exquisitely complex epithelium, with both germ cells and Sertoli cells undergoing major structural and morphological changes during the spermatogenic process. Irrespective of testicular size or anatomical arrangement the testicular interstitium contains the steroidogenically active Leydig cells as a second testis specific somatic cell type [21]. The interstitium is also

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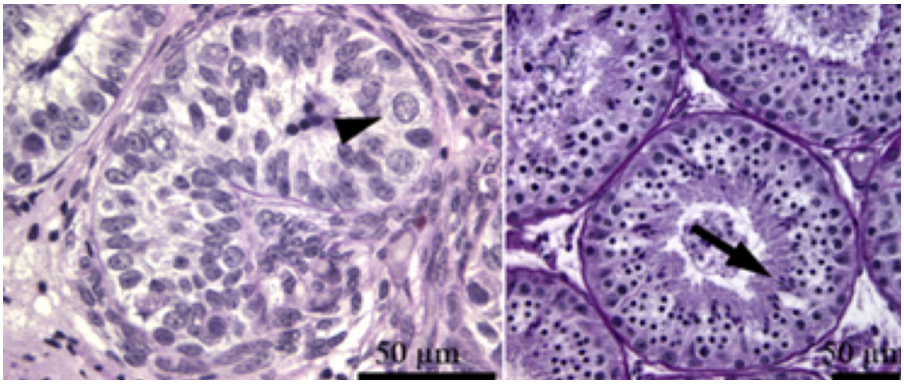


**Fig. 3.** Left panel: micrograph showing immature rhesus monkey testis tissue containing only Sertoli cells and spermatogonia (arrowhead). Right panel: micrograph of adult rhesus monkey testicular tissue containing a full complement of differentiating germ cells including elongated spermatids (arrow).

populated by many more cell types including fibroblasts, immune cells and blood vessels.

Sertoli cells present the first testis-specific cell type during ontogenesis. Male sexual differentiation of the indifferent gonad relies on appearance and subsequent aggregation of pre-Sertoli cells. This process is the initial event for tubulogenesis as a first indication of male gonadal differentiation. Sertoli cells undergo intense morphological changes to assume a terminally differentiated state capable of supporting germ cell development [22]. Sertoli cells divide in the fetal and neonatal period in rodents. The end of the proliferative period is around day 15 in rats [23]. Species undergoing puberty show accelerated periods of Sertoli cell proliferation during the postnatal period and during early puberty. In the prepubertal period Sertoli cells are relatively quiescent and seminiferous tubules grow slowly. Exposure to factors that potentiate Sertoli cell proliferation [24,25] or interfere with their maturation [26,27] generate changes in testicular size and spermatogenic potential of the adult. Since Sertoli cell are capable of supporting a finite number of germ cells the final number of Sertoli cells established during puberty determines the postpubertal spermatogenic potential of the testis.

When testicular organogenesis terminates during puberty, Sertoli cells form a syncytium-like epithelial monolayer in which germ cells are embedded. Tight and gap junctions between Sertoli cells generate an effective division of the epithelium into subcompartments. This epithelial barrier is known as blood–testis barrier and creates a basal and an adluminal compartment hindering regulatory molecules like protein hormones and growth factors to freely diffuse across the seminiferous epithelium. Transition of germ cells through the blood–testis barrier depends on testis-specific cellular processes. The timed establishment of new junctions between Sertoli cells and their appropriate removal guarantees an always intact epithelial barrier which renders only germ cells in the basal compartments accessible for protein hormones or growth factors. The blood testis barrier and epithelial sub-compartmentalization evokes a unique mode of intraepithelial secretion. Basolateral factors can control mitotic phases of germ cell development whereas adluminal secretions reach and influence meiotic and postmeiotic germ cell differentiation [28,29].

This special feature of the seminiferous epithelium renders it demanding to analyze regulatory mechanisms by standard in vitro culture of Sertoli cells. Since the barrier function is usually not established in monolayers of cultured Sertoli cells the detection of protein or mRNA in cultured cells or conditioned medium cannot reveal a directed secretion into the one or the other epithelial subcompartment which may be of relevance for the action of factors in the intact seminiferous epithelium.

At the completion of spermatogenesis, mature spermatids are released from the seminiferous epithelium into the seminiferous tubule lumen and proceed through the excurrent duct system, known as the rete testis, until they enter the epididymis via the efferent ducts. In smaller mammalian testes (e.g. rodents) the seminiferous tubules are attached to the superficial *rete testis* and extend throughout the entire testis. A fine network of arterial and venous blood vessels extends within the interstitial space between the seminiferous tubules. Larger testes (e.g. carnivores, primates) are divided into several lobes of which each is supported by blood vessels via fibrous septae. Here the seminiferous tubules are attached to a central intratesticular *rete testis* which extends into the initial segment as an external part of the *rete testis*.

The seminiferous epithelium consists of Sertoli cells and germ cells. The number of germ cells varies with the status of spermatogenesis. In the immature period or during testicular recrudescence the only germ cell types are spermatogonia populating the basement membrane in close contact with Sertoli cells (Fig. 3). At this developmental stage, germ cells represent only 5–10% of the seminiferous epithelium. During periods of active spermatogenesis this ratio changes and germ cells become very abundant making up 90% of the tubular mass. Within the seminiferous tubules differentiating germ cells form intimate associations with Sertoli cells, which have numerous cup-shaped processes that encompass the various germ cell types [30].

Gametogenesis and generation of sex steroids were originally different tasks occurring in different organs. Throughout evolution of vertebrates the endocrine cells specializing on sex steroid production and the gamete producing tissues were combined into one bifunctional organ [31,32]. In the female the gonad developed into ovaries and in the male into testes. A close association of androgen releasing endocrine cells and gamete producing tissue components enables intricate endocrine and paracrine crosstalk between both compartments providing options for an often useful synchronization of the endocrine and gametogenic output. Although combining both functions into one organ the seminiferous epithelium and the steroidogenic cells of the testis remained compartmentally separated: the seminiferous tubules with Sertoli cells as somatic constituents became the site of spermatogenesis. Leydig cells in the interstitial compartment became the site of androgen production.

The bicompartmental testicular organization is a striking difference to the ovary. Steroid generating granulosa cells are structurally and functionally associated with the maturation of eggs in the ovarian follicles. In the testis, but not in the ovary, sex steroid production is spatially separated and is therefore in principle independent of gamete production. However, due to the close proximity of Sertoli and Leydig cells only negligible diffusion barriers exist and

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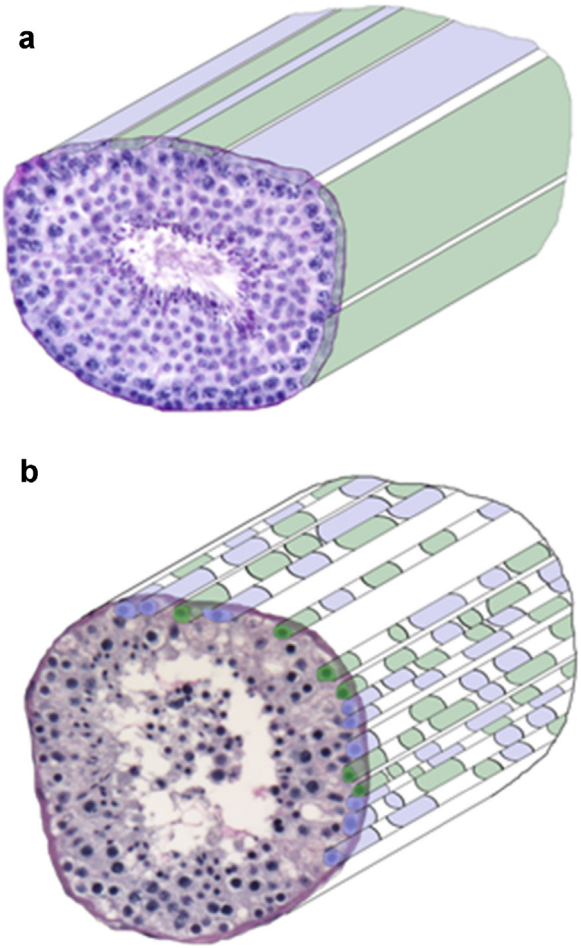


**Fig. 4.** Arrangement of germ cells along the basement membrane of the seminiferous tubules. In (a) the situation in the mouse is shown as a representative example of a species showing a single seminiferous epithelial stage arrangement. All germ cells in a cross-section of a seminiferous tubules are in developmental synchrony and reveal identical morphological features. In (b) the situation of man is shown representing species with mixed seminiferous epithelial stages. Germ cells at different stages of development can be recognized in each tubular cross-section. For both species the longitudinal extension of the premeiotic germ cell cohorts attached to the basement membrane area are schematically indicated. In mice the cohorts of germ cells are much larger compared to primates.

soluble factors released into the interstitium facilitate rapid and intense paracrine communication between the two testicular compartments.

In mammals, during periods of active spermatogenesis large numbers of germ cells are embedded into the epithelium in concentric layers. The premeiotic diploid germ cells are the spermatogonia which are arranged either as single cells, small interconnected cohorts or small chains in the basal compartment in contact with the basement membrane. Incomplete cytokinesis during mitotic and meiotic divisions generates expanding germ cell cohorts. Cells in interconnected cohorts show synchrony of all subsequent steps during the differentiation process. All meiotic (spermatocytes) and postmeiotic (spermatids) germ cells are located in the adluminal compartment. They present large interconnected cohorts with up to several thousand cells in species with numerous premeiotic divisions (Fig. 4). Only after removal of the spermatid cytoplasm during elongation germ cells are considered individual cells.

The various steps of germ cell development from spermatogonia to elongated spermatids can be separated and staged using morphological criteria. These were for the first time described in rats [33,34]. The most immature germ cells, the spermatogonia, include type A (denoted $A_{1-4}$), intermediate (found only in rodents), and type B forms, the latter being considered to be committed to differentiation into meiosis [1,35]. The actual stem cell pool for the germ cell line is considered to be a subset of the type A spermatogonia population, yet the identity of the "true" stem cell cannot unequivocally be discerned on the basis of morphology [1,35] nor as yet by biochemical markers. However, techniques such as the transplantation of germ cells can discern stem cells from differentiating germ cells and provided new information on the biology of spermatogonial stem cells [36,37].

Three models are currently proposed to describe the spermatogonial renewal kinetics in rodents [38]. In the $A_s$ model, one $A_{single}$ cell divides to become either two new separate $A_s$ (stem) cells, or to become two joined $A_{pair}$ (differentiating) cells. In the $A_0/A_1$ model, the $A_s$ and $A_{pr}$ cells are considered $A_0$ cells with putative stem cell characteristics, and the $A_{1-4}$ spermatogonia are considered $A_1$ differentiating stermatogonia, with the $A_{aligned}$ not being assigned to one of the groups specifically. The clone fragmentation model is a variation of the $A_s$ model, in which the formation of $A_{pr}$ out of $A_s$ is considered the commitment toward differentiation. But in this last model, all cells within a group of $A_{pr}$ or $A_{al}$ are considered a clone, because it is comprised of daughter cells. This model takes into account the existence of groups of cells with numbers different than $2^n$, because clone fragmentation can take place at any existing intracellular bridge between daughter cells.

In the monkey and human, there are two morphologically distinct type A spermatogonia; $A_{dark}$ ($A_d$) and $A_{pale}$ ($A_p$), as well as type B spermatogonia (1). $A_p$ spermatogonia divide to give rise to type B spermatogonia as well as to renew their own population, whereas $A_d$ spermatogonia are considered to be the nonproliferative reserve spermatogonial population [39–42]. Indeed, $A_d$ spermatogonia have been suggested to be able to undergo transition to $A_p$ following testicular insult, allowing repopulation of the testis in times of injury or insult [41,42]. $A_p$ spermatogonia (or a subset of) have been suggested to be the "true" stem cell of the testis, because $A_p$ (not $A_d$) spermatogonia are seen in humans after radiation therapy [43] and in the postpubertal cryptorchid testes [44]. Various studies have suggested that $A_p$ spermatogonia can undergo transition without division into $A_d$ [41,42,45,46].

In all mammals, both type A and B spermatogonia undergo a series of mitotic divisions to produce germ cell cohorts that enter into meiosis. Thus the size of the spermatogonial population and the extent of premeiotic expansion is a key determinant of the number of mature sperm eventually produced. The size of this population is likely controlled by a balance of proliferation and anti-apoptotic events [35].

Meiosis is initiated by the production of preleptotene spermatocytes from type B spermatogonia. During the prophase of the first meiotic division, germ cells undergo morphological transitions that can be classified on the basis of nuclear size and morphology [1–3]. DNA is replicated in the preleptotene phase and pairing of homologous chromosomes occurs in the zygotene phase. Cells with completely paired chromosomes are termed pachytene spermatocytes. The pachytene spermatocyte phase takes some weeks and as such these cells are seen frequently within the seminiferous epithelium [1,3]. A brief diplotene phase follows in which the chromosome pairs partially separate. The cells then undergo the first meiotic division to yield secondary spermatocytes, which quickly progress through a second meiotic division to yield haploid round spermatids.

Spermiogenesis is the process by which the round spermatid transforms, without further division, into the specialized elongated spermatid via a series of complex cytodifferentiative steps. Nineteen defined steps of spermiogenesis have been categorized in the rat [33]. These steps involve the formation and development of the

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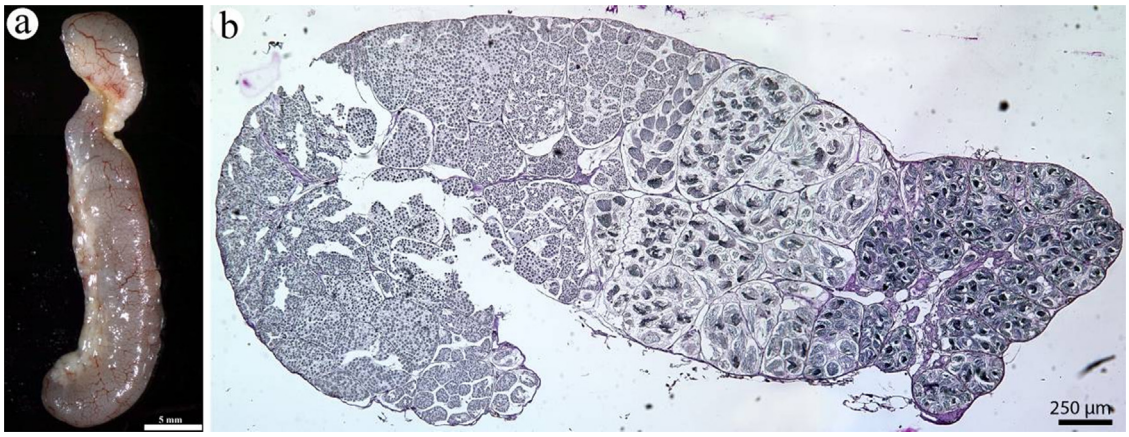


**Fig. 5.** Testicular histology of an adult testis from Ambystoma mexicanum with cystic arrangement of spermatogenesis. (a) Image of one entire testis. Note that the testis of this two-year old individual is composed of two distinct lobes, one cranial (upper part of the image) and a larger caudal (lower part of the image) lobe. (b) Micrograph of a cross-section (hematoxylin staining) of one lobe of an adult Axolotl. Note that each cyst contains only one germ cell type, with spermatogonia and spermatocytes present in cysts on the left side, elongated spermatids in the center, and spermatozoa on the right side of the image.

acrosome and flagellum, condensation of the chromatin, reshaping and elongation of the nucleus and removal of the cytoplasm prior to release of the spermatid during spermiation [3,34]. An important physiological consideration during spermiogenesis is that the spermatid nucleus becomes incapable of transcription as it condenses, and thus round spermatids transcribe high levels of messenger ribonucleic acids (mRNAs) that are subject to translational delay until translation of protein is required during elongation [47]. Spermiation is the final step of spermatogenesis, and involves removal of spermatid cytoplasm to yield the streamlined spermatozoon capable of motility, retraction of the Sertoli cell away from the spermatid and, finally, the release of the mature spermatid into the tubule lumen [4,30].

In other vertebrate species, such as fish or amphibians, differentiating germ cells are also developing as large cohorts. However, in these species differentiating germ cells are organized as spermatogenic cysts instead of layers of germ cells as in mammals (Fig. 5). Each cyst is surrounded by somatic cells forming a lining. A spermatogenic cyst contains one interconnected clone of germ cells going through the differentiation process in complete synchrony. The gonad consists of many cysts with the most primitive ones containing A-spermatogonia and premeiotic germ cells at the apical zone. Expanding cysts contain differentiating spermatogonia, spermatocytes and finally spermatids. The cysts are sequentially arranged throughout the lobes up to the distal tip of the gonad where cysts containing mature sperm rupture and are released. In cystic arrangements it is obvious that the number of premeiotic germ cell divisions determines the size of germ cell cysts. Assuming no germ cells die during differentiation, each additional premeiotic division leads to a doubling of final cell numbers in the synchronized germ cell cohorts and therefore also a doubling in the size of the cyst. Spermatogenic cyst sizes differ greatly between species indicating different numbers of mitotic divisions during premeiotic stages of spermatogenesis.

In a seminiferous epithelium of a mammal the cohorts of germ cells are principally also arranged as cysts. However, instead of round cysts located vertically in the testicular lobe the germ cell cohorts in mammals are arranged in horizontal layers of an epithelium. The cysts are flattened and spread out as expanded interconnected single or dual layer cohorts in the epithelium (Fig. 6). Several such monolayers are packed on top of each other. The basement membrane is populated by individual stem cells or small clones of undifferentiated spermatogonia among larger chains of differentiating spermatogonia with highly synchronous cell cycles (Fig. 6). The cells pass the blood testis barrier as clones and populate the adluminal compartment as expanded cohorts of spermatocytes and spermatids. Depending on the species a variable number of premeiotic divisions lead to the presence of small to very large synchronized germ cell clones stretching along the seminiferous tubules for several hundred micrometers in mice and rats.

Albeit anatomical and histological differences are obvious between cystic and epithelial arrangements of spermatogenesis, the basic organizational features of germ cells is similar. In all species male germ cell differentiation is initiated with a differentiating mitotic division of duplets or small chains (2–16 cells) of undifferentiated A-spermatogonia which are created by incomplete cytokinesis. The subsequent differentiation steps are highly synchronized. Ongoing incomplete mitotic and meiotic divisions maintain intercellular cytoplasmic bridges creating expanding cohorts of interconnected germ cells.

The continuous progression of postmeiotic germ cell development can be subjectively subdivided into developmental stages according to morphological criteria. Fig. 7 reveals an anatomist's description of 14 discernible stages of spermiogenesis of macaques from the appearance of round spermatids until spermiation [40]. Germ cell development is highly synchronized within the seminiferous epithelium. Since the fully active epithelium contains several generations of germ cells the developmental synchrony leads to the appearance of defined associations of germ cells. Since the developmental progression occurs in synchrony large germ cell cohorts with specific morphological features can be recognized in cross-sections of the seminiferous epithelium. The changes in germ cell associations occur as a consecutive wave. In many species the wave is longitudinally arranged along the seminiferous tubule. The stages of the seminiferous epithelial cycle are primarily defined by morphological features of round spermatids. Other morphological features can also be used for staging like the presence of residual bodies or specific germ cell types (Fig. 7). The initial appearance of round spermatids following the second meiotic division is always defined as stage I of the seminiferous epithelial cycle. Roman numerals are used to denote the different spermatogenic stages. The completion of one series of stages is known as one cycle of the seminiferous epithelium [3]. In the macaque Clermont [40] subdivided the seminiferous epithelial cycle into 12 stages. In subsequent years unique staging systems were proposed for a large number of species [1,48,33,34]. For example 12 stages of the seminiferous epithelial cycle are described for the mouse, 14 for the rat, 12 in monkeys [40,49]. Only 6 stages are differentiated in man [3].

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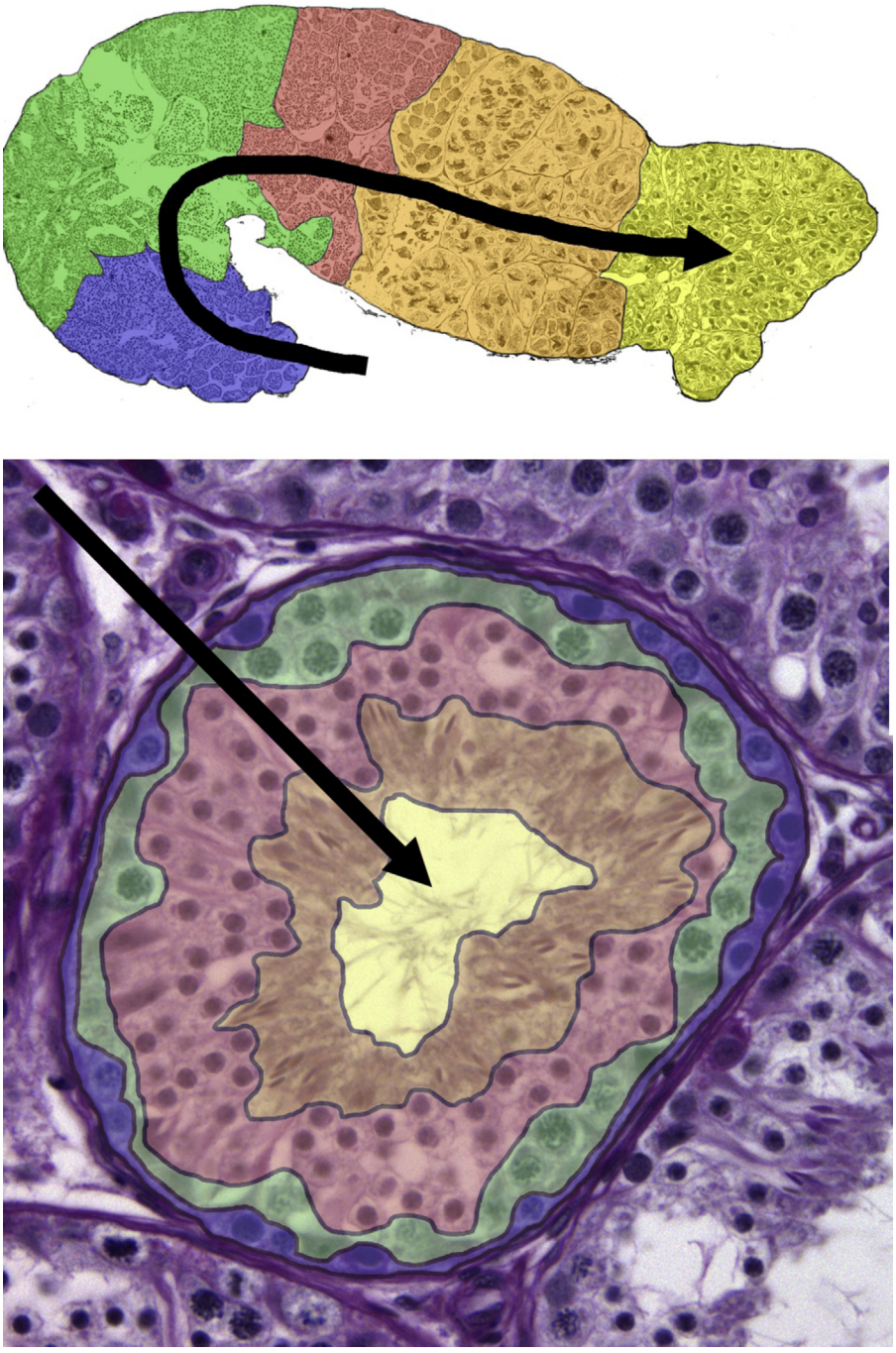

**Fig. 6.** Schematic representation of the arrangement of germ cells in testes with cystic (upper image; Mexican Axolotl, Ambystoma mexicanum) or epithelial spermatogenesis (lower image, human, Homo sapiens) testes. Black arrows show the direction of germ cell developmental progression. Blue: spermatogonia; green: spermatocytes; red: round spermatids; orange: elongated spermatids; yellow: sperm.

In some species (e.g. marmoset, man) spermatogenesis is arranged in stages that are intertwined and do not present a strict longitudinal pattern [2]. The synchronized germ cell cohorts in these species are smaller and therefore cover less space (Fig. 4). Then a single tubular cross section contains several germ cell associations at different stages of the seminiferous epithelial cycle rendering it more difficult to determine stages of the seminiferous epithelial cycle and discern individual germ cells to specific associations [50–52].

It always needs to be considered that the characterization of morphological features in developing germ cells is influenced by fixation, embedding and staining techniques. Since the stages of germ cell development (and consequently the seminiferous epithelial cycle) are postulated using subjective criteria, the staging system is a man made artificial, highly useful experimental tool to dissect the continuous process of germ cell development (and the long time period of the seminiferous epithelial cycle) into well-defined subunits. The definition of spermatogenic stages has greatly helped to describe specific processes at defined developmental stages during spermatogenesis. However, spermatogenic stages should not be considered as functional definitions. In the coming years molecular markers of germ cell development need to be defined to functionally enrich the morphology based staging system.

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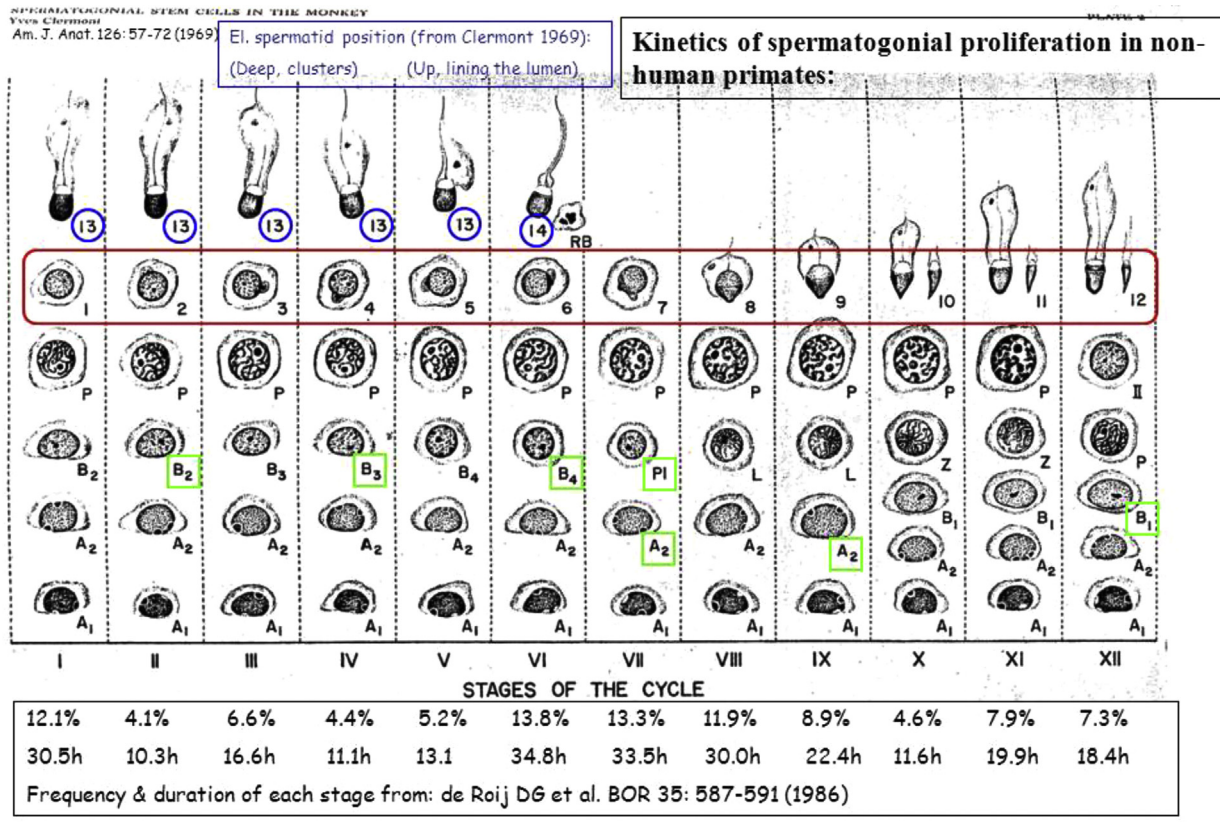


**Fig. 7.** The 12 stages of the seminiferous epithelial cycle in rhesus monkeys (modified from [40]). Morphological features of round spermatids, in particular the shape of the acrosome (red box) are used to define spermatogenic stages. In addition mitotic activities in spermatogonia and the presence of specific germ cell types (B-spermatogonia, preleptotene spermatocytes, green boxes) are used as additional criteria. Note that the early stages are characterized by the presence of round and elongating spermatids (blue circles). Stage frequencies can be evaluated as relatives values enabling calculation of the time each stage is present (see results and calculation from [49]; black box). *Note*: Clermont refers to the $A_{dark}$ as $A_1$, and to the $A_{pale}$ as $A_2$ cells.

The kinetics of spermatogenesis differs between species but is highly stable for a given species: after initiation of spermatogonial differentiation in the mouse spermiation occurs after approximately 35 days whereas in man it takes approximately 64 days [3,53]. The differentiation of germ cells appears to be an autonomous species-specific morphogenetic series of events. Xenogeneic germ cell transplantation experiments revealed that the kinetics of germ cell development is inherited in germ cells [54,55]. For example rat germ cells transplanted into the mouse testis developed with the rat specific kinetics inside the mouse seminiferous epithelium. Since the morphogenetic program in male germ cells appears highly stable the exact timing of the initial event is of utmost importance to initiate and maintain the high synchrony inside the seminiferous epithelium. The somatic environment does therefore not dictate the progression of germ cell development but may be involved in the timing of the initial event. It is known from the embryonic and fetal testis that testicular somatic cells show cyclic patterns of gene expression [56].

## 3. Spermatogonial differentiation and expansion/proliferation

Spermatogonia are located at the basal membrane of the seminiferous compartment [57,58]. A small subpopulation of spermatogonia functions as unipotent stem cells to generate exclusively sperm. In contrast to other adult stem cells in the organism, spermatogonial stem cells show additional unique features. As progeny from primordial germ cells and constituents of the germ line they are theoretically immortal. Mouse spermatogonia have indeed a very long lifespan as was shown by studies using serial germ cell transplantation [59,60]. Spermatogonia inherit the germline of the species at a critical point when germ cells need to mitotically expand prior to meiosis. A very high number of sperm is needed to maintain fertility of males. Therefore stem cell spermatogonia must maintain DNA-integrity against the accumulation of replication errors and exposure to environmental mutagens. Spermatogonial stem cells have therefore a key position in controlling balanced evolutionary changes of the genome.

Based on histological studies it has been postulated that spermatogonia do not undergo unequal divisions. Thus, each spermatogonial division generates two identical daughter cells [61,62,40]. On a biomolecular level, however, the two daughter cells do not share the same complement of proteins [63,64]. Therefore, two histologically "identical" daughter cells might still show different biomolecular fingerprints. Biomolecular markers of spermatogonia have been studied in order to establish a molecular "fingerprint" for each different subpopulation of spermatogonia both in primates and in rodents [65–73]. One of the main goals has been the identification of the "real" spermatogonial stem cells [74–79]. Several studies in the recent past had some success in pinpointing spermatogonial markers and relate a specific marker to a particular spermatogonial subpopulation, slowly advancing our knowledge on spermatogonial markers [79–81]. However, it is still unresolved if a fixed subpopulation of "true" spermatogonial stem cells exist or whether rapidly changing and stochastically selected subfractions of spermatogonia act as stem cells at any given time as was proposed using three-dimensional modeling in the mouse [82].

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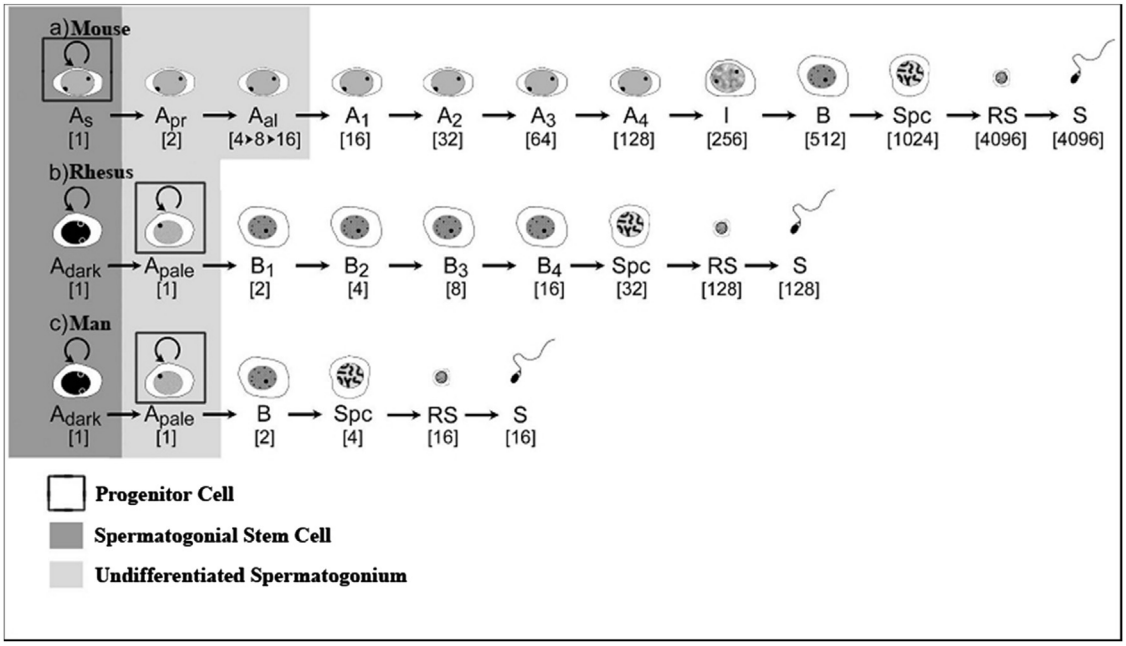


**Fig. 8.** Modified from [85]: scheme of male germ cell differentiation in mouse, rhesus monkey and man. Spermatogonial subtypes: $A_s$, $A_{single}$; $A_{pr}$, $A_{pair}$; $A_{al}$, $A_{aligned}$; I, intermediate; B, B-spermatogonium; Spc, spermatocyte; RS, round spermatid; S, sperm.

In rodents spermatogonial stem cells are morphologically recognized as a small subpopulation of single spermatogonia which rarely divide [83,84,48]. During initial expansion spermatogonia form pairs or chains of up to 16 cells known as $A_{paired}$ or $A_{aligned}$ spermatogonia. The divisions of these small cohorts are synchronized with the spermatogenic wave and the cells show already the first markers of differentiation. In rodents, the clonal expansion of germ cells is intense and in theory leads to 4096 spermatids arising from one stem cell division (Fig. 8).

In primates, at least two distinct types of undifferentiated spermatogonia exist. These two spermatogonial subtypes are – throughout life – commonly named $A_{dark}$ and $A_{pale}$ spermatogonia [61,62,85–89,40]. $A_{dark}$ spermatogonia are considered reserve stem cells which are only proliferatively active during pubertal expansion [90] and following depletion of spermatogonia due to irradiation or toxic exposure [41,42]. In contrast, $A_{pale}$ spermatogonia are considered self renewing progenitors which proliferate regularly during each spermatogenic cycle [77,78]. The limited number of premeiotic spermatogonial divisions has strong implications, as in contrast to rodents where one stem cell division gives rise to several thousand germ cells, in man a maximum number of 32 spermatids can be created from one stem cell division [91]. These species-specific differences in spermatogonial physiology of rodents and in primates are summarized in Fig. 8 (modified from [85]).

Primates are in need of either a higher number or a higher expansion of spermatogonial stem cells. In the rhesus monkey cohorts of pairs or quadruplets of spermatogonia proliferate twice during each seminiferous epithelial cycle resulting in replenishment of the $A_{pale}$-population and creation of the differentiating population of B-spermatogonia [77,78]. A similar model can be proposed for man [91]. Our model reveals several significant differences between primates and rodents as no unequal division of any of the premeiotic germ cells is involved and a large population of self-renewing progenitors ($A_{pale}$) exist generating appropriate numbers of differentiating progeny in the absence of efficient mitotic expansion in primates [91].

As yet the existence of a testicular stem cell niche is well documented but its cellular and molecular function is poorly understood [92]. In the mouse, primordial germ cells isolated from the embryonic epiblast or teratocarcinoma cells have the potential to function as germ line stem cells when transplanted into the testicular niche [93,94]. These findings indicate that the testicular microenvironment offers a unique microenvironment to germ cells at any developmental stage. Testicular stem cell niches may not be randomly distributed along the seminiferous tubules as regions with preference for stem cells or differentiating spermatogonia have been described [95,96]. The exact cellular and molecular details of the niche microenvironment have not yet been elucidated and may be species dependent. However, settlement and colonization of testicular stem cells from a large number of species after infusion into the mouse testis reveals that stem cell recognition and settlement are highly conserved between species [55].

The number of sperm produced depends primarily on testicular size. Great variability exists in respect to relative testis weight with some species like hamsters showing very large testes while other species like whales showing comparatively small testes in relation to their body mass. In active adult mammalian testes seminiferous tubules make up 75–90% of the testicular weight. When the testes are fully active all species show a tubule diameter of 300–400 μm accommodating the fully active seminiferous epithelium and the tubular lumen. As the tubule diameter is fixed testicular size depends on the extent of longitudinal outgrowth of seminiferous tubules. The exact mechanisms controlling testicular size are unknown. However, FSH and thyroxin are known to affect Sertoli cell expansion and testicular size.

In many species spermatogenic output correlates with Sertoli cell numbers. This is true when the number of differentiating germ cells shows no modulations as is observed in many rodents. In these species the efficiency of sperm output is usually running at a maximum capacity. Other species show a modulation of sperm output and the germ cell load of the epithelium can be controlled by hormonal stimulation. The regulatory mechanisms do, however, not affect the efficiency of germ cell development as all analyzed species show a comparable transit efficiency from differentiating spermatogonia to spermatids. This finding indicates that modulations in the efficiency of germ cell production are not dependent on survival of differentiating germ cells. As a consequence the number

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of A-spermatogonia transiting into differentiating spermatogonia is the limiting step for the extent of sperm production.

In light of this the number of stem cell niches and the ratio of stem cell niches occupied by actively proliferating stem cells are the two critical parameters to determine sperm output. In many species including rodents only small undifferentiated A-spermatogonia are required due to high expansion of germ cells during premeiotic stages. Therefore a reliable low level production of germ cell precursors is managed by a well functioning autonomous stem cell system relying on rare stem cells ($A_s$) and numerous subsequent divisions ($A_{pr}$, $A_{al}$). Clonal splitting maintains a pool of undifferentiated spermatogonia but most undifferentiated spermatogonia transit into spermatogenic differentiation as $A_1$ spermatogonia as 8 or 16 cell clones.

## 4. Hormonal control of the testis by feedback mechanisms

The two gonadotropic hormones FSH and LH are the central actors in the HPG-axis. Their release from pituitary gonadotropes is controlled by GnRH. The GnRH pulse generator in the hypothalamus is considered the master ON/OFF switch of the reproductive axis. A wide range of information e.g. age, nutrition, social status and stress are used to create a decision that reproductive functions of an individual should be (re)initiated or halted. The GnRH pulse generator is a master switch as it controls the entire reproductive system by affecting both gonadotropins. Fig. 6 depicts a hypothetical model of hormonal regulation in which the GnRH master switch controls two fully independent regulatory feedback loops which are either responsible for spermatogenesis (FSH–inhibin) or steroidogenesis (LH–testosterone). FSH acts specifically on Sertoli cells regulating the sperm output of the seminiferous epithelium by controlling the expansion of premeiotic germ cells. Inhibin B functions as endocrine signal for the integrity and activity of the stem cell system providing a signal to the pituitary on the capacity of sperm production. LH acts on Leydig cells stimulating the release of androgens which induce or maintain a male phenotype, stimulate sexual organs and androgen controlled actions in the periphery. At the same time androgens function as feedback hormone at the hypothalamic level. In this scenario both gonadotropins have separate functions and independent regulatory feedback loops: The FSH and inhibin axis controlling the production of sperm and the LH–testosterone axis the androgenization of the organism. We will later discuss species specific modifications of this basic model.

Changes in the photoperiod are perceived as stable indicators of seasonal changes. The duration of daylight is transduced via the retina and the pineal organ into melatonin patterns which are perceived by the hypothalamus to switch the GnRH pulse generator into on or off mode [97]. Length of the photoperiod can therefore be registered as one of the most important environmental factors. The GnRH master switch can use this information to control activity of the reproductive organs in synchrony with seasonal changes. Depending on the species shortening (short day breeders) in fall or lengthening (long day breeders) of photoperiods in spring induces the activity of the GnRH pulse generator. This promotes the birth of offspring at favorable seasonal conditions and provides a clear advantage to species living in habitats with seasonal changes.

In most circumstances spermatogenesis and steroidogenesis are synchronously controlled via the GnRH pulse generator by seasonal signals as only in combination do gamete production and sexual activity guarantee reproductive success. As the master switch acts on both gonadotropins, GnRH cannot separately modulate spermatogenesis or steroidogenesis. Under specific circumstances FSH and LH stimulation need to be dissociated to some extent. Variations in pulse frequency and amplitude of GnRH can modulate the response of gonadotropic cells enabling the preferential release of either FSH or LH. The LH–testosterone axis functions as a negative feedback loop (Fig. 9). LH acts on Leydig cells promoting the release of androgens after activation of various intracellular signaling cascades. Serum levels of androgens are controlled by balanced stimulatory action of LH on Leydig cells versus inhibitory actions of androgens in parts of the brain reducing the release of LH from gonadotropes. In the male testosterone is the dominant steroid hormone communicating to all peripheral organs that sex differentiation has been directed toward male gonadal differentiation. In some fish showing gender transformation during life the information on sexual differentiation may need to be modified several times during life. However, in mammals sexual differentiation is genetically determined and gender transformation is naturally not observed. The most significant androgen actions occur only once and incur irreversible anatomical changes usually during embryogenesis (e.g. induction of sexual accessory and copulatory organs, male differentiation of brain areas) or at puberty (e.g. increase in body mass, male muscle and hair growth patterns, growth of larynx or teeth, modified organogenesis of skin inducing marking or sex glands, modified teeth, horn or antler development). Due to these anatomical and usually irreversible actions of testosterone many species-specific features occur during sex differentiation and render the appearance of male and female vertebrates different.

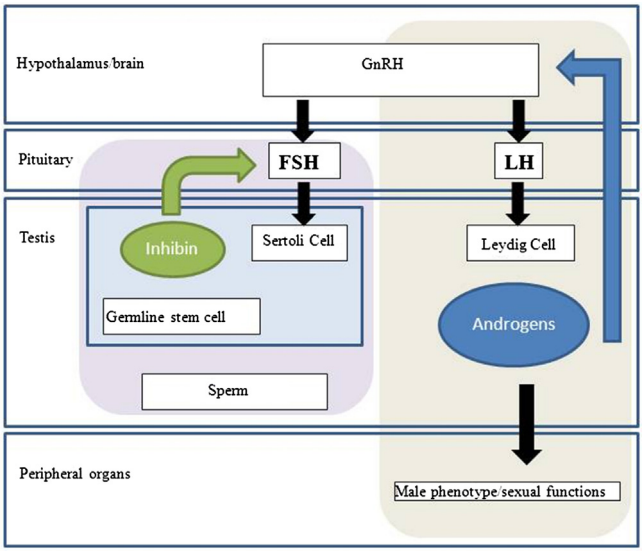


**Fig. 9.** Schematic diagram illustrating a "hypothetical ancient" model for hormonal control of testicular function. The two gonadotropic axes are fully separated providing independent feedback control for spermatogenesis (FSH–inhibin axis) and steroidogenesis (LH–testosterone axis).

In addition to irreversible anatomical sex-specific features during development, androgens control reversible short and long term changes which may be needed to adapt males to recurring changes in the environment. It is intriguing that diurnal and seasonal fluctuations of androgens control many circadian physiological features. Peripheral testosterone levels in men for example are usually increased at nighttime and affect many functions like brain activity and secretory actions of glands well as sexual activities (morning erections). Seasonal fluctuations of androgens limit sexual activities to mating seasons. In mammals the seasonal testosterone-dependent parameters usually affect sexual accessory organs but also secondary characteristics (e.g. body mass, antler growth, activity of marking glands). Non-mammalian species developed an even wider and versatile range of androgen-controlled parameters. In fish and birds androgen-controlled seasonal features affect body color or contours by specific overgrowth of pigmented cells, fins or feathers. In general

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high androgen serum levels in vertebrates promotes aggressiveness.

Albeit stringent control mechanisms for establishment of adequate serum levels of testosterone are mandatory, the androgen serum patterns differ greatly between species. The release of androgens is always pulsatile. Every LH pulse evokes a testosterone release. In species like mice which do not have binding globulins the resulting androgen peaks are brief and of high amplitude. In contrast species with serum binding globulins show extended peaks with lower amplitudes and more elevated basal serum levels. Studies on androgen effects in mice are therefore very tedious as determination of testosterone in normal adult mice is difficult. However, the determination of androgen dependent parameters like seminal vesicle or prostate weight presents a more reliable bioassays to determine the overall androgen status in mice.

The pulsatile release of LH is centrally controlled by the GnRH pulse generator and androgens show circadian rhythms as well as seasonal and age related variations. Albeit the abundance of Leydig cells in the testis shows great variability between species under normal conditions their number seems not to be critical as reduction by 50% through hemicastration has no major impact on serum testosterone levels [98]. Mice carrying testicular xenografts [99,100] in the absence or presence of one or both of their own testes revealed no change in androgen levels confirming a central control of testosterone release which is unrelated to the number of Leydig cells present in the animal. It is, however, remarkable that xenografts from different species in mice provoke a different level of androgens indicating that the target tissue plays a role to define the setpoint of androgen levels in the feedback control loop [101].

In all thus far analyzed vertebrate species androgens play an important paracrine role during testicular differentiation as well as in the initiation and maintenance of spermatogenesis. The advantage of this paracrine action is that spermatogenesis and steroidogenesis can be synchronized. However, when the paracrine action of testosterone becomes dominant spermatogenesis and steroidogenesis can no longer be separately modulated. We will explore diverse species-specific needs and resulting variations of endocrine control of testis functions later in this chapter.

In contrast to the LH–testosterone axis the role of the second gonadotropin is less obvious at least in rodents and appears much more versatile in different species. Albeit showing some degree of subfertility male mice lacking the gene encoding the FSHβ subunit (FSHβKO) have normal sexual development and are fertile. There is normal progression of germ cells through spermatogenesis although fewer germ cells are present [102–104]. Leydig cell steroidogenesis pathways were altered in adult FSHβKO mice as the levels of mRNAs encoding 17βHSD and StAR were increased [105]. Transgenic mice that express FSH but not LH permitted the support of spermatogenesis through the completion of meiosis but lack completion of germ cell maturation [106]. Knock out of the FSH receptor in mice (FSHRKO) results in delayed spermatogenesis and sexual maturity plus the sperm produced are aberrant with reduced fertilizing capability. Although neither spermatogenesis nor steroidogenesis are fully normal and these mice have small testes they are fertile [104,107–111]. Experiments on hpg mice showed that FSH-mediated expansion of the germ cell population occurs independently of androgen action through the Sertoli cell or any other androgen responsive cell. FSH stimulated the development of germ cells to round spermatids but only to about 5% of normal levels. However, FSH was unable to generate spermatids in the absence of androgen action [112–114]. Taking these data into consideration it was postulated that FSH is not essential to maintain fertility in mice (reviewed in [115]).

This situation is totally different in other species. The Djungarian hamster is a rodent contrasting the mouse as a highly FSH-dependent species. This hamster needs to adapt to extreme environmental conditions with very long and intense Siberian winters providing only few months per year for reproduction. This has evoked a very stringent seasonal control of reproduction [116]. Testis weights in these hamsters fluctuate between 30 mg under inhibitory photoperiods and 400 mg under stimulatory photoperiods. When photoperiods expand the GnRH pulse generator is activated. The activation also occurs spontaneously under extended exposure to short photoperiods for more than 12 weeks [117]. Administering FSH to photoinhibited adult animals restores spermatogenesis in the absence of testosterone [118]. The FSH-only induced sperm have full fertilizing capabilities [119]. During spontaneous recrudescence an increase of FSH several weeks prior to reactivation of the LH–testosterone axis re-induces testis growth and spermatogenesis [117]. FSH stimulates Sertoli cell proliferation and junctional organization in photoregressed adult hamsters. While these processes are irreversible and occur once during postnatal development in mice, they are reversible in hamsters and remain under control of FSH even in adulthood [120]. In conclusion the Djungarian hamster provides a model whose spermatogenic function is primarily controlled by FSH.

In most other animals FSH synergizes with testosterone to increase the efficiency of spermatogenesis. In primates FSH receptors are specifically expressed in Sertoli cells [121] and FSH appears to be crucial for quantitatively normal spermatogenesis. However, whether or not FSH is essential to maintain fertility in primates has been debated for more than a decade (reviewed in [122]). In men, there is evidence for FSH being essential for fertility as homozygous inactivating mutations in the gene encoding the FSH-β subunit result in infertility [123–127]. Three examples of FSH-β subunit mutations have been described in men. Small testes and azoospermia result from each of the C100R, Y94X and V79X FSH-β mutations [123–125]. In contrast to men having FSH-β mutations, 4 of 5 men having the only reported homozygous inactivating mutation of human FSH receptor (A181V) remained fertile, although all had testes that were smaller than normal and 4 of the men had oligospermia. Only one example has been characterized for a male having an activating mutation in the FSH receptor in the absence of systemic FSH. Testis volume and spermatogenesis of the affected individual were within normal parameters [128]. In primates FSH targets premeiotic germ cells via Sertoli cells and controls their expansion. FSH deprivation in monkeys inhibits spermatogonial proliferation and their transition to spermatocytes [129]. The number of spermatogonia increased after FSH stimulation of in intact as well as FSH depleted monkeys [130,7,131]. FSH treatment of prepubertal monkeys increased the number of Sertoli cells and initiated spermatogenesis resulting in the production of spermatogonia and spermatocytes [132–134]. Taking together the role of FSH for regulation of testicular function is highly diverse and appears to be highly adaptable to the needs of any species.

In contrast to the LH–testosterone feedback loop the feedback mechanism for FSH is less obvious albeit inhibin B is generally considered the feedback hormone for FSH in the male (for review [122]). In principle only species modulating sperm output are in need to establish a fine-tuned regulatory feedback system for control of spermatogenic output. The activity of the seminiferous epithelium in rodent testes is usually maintained at a 100% level which creates no need for regulatory mechanisms. Autonomous cellular processes guarantee an always efficient germ cell load of the seminiferous epithelium. In the absence of any modulations of sperm output FSH lost its function as modulator of spermatogenic efficiency. Therefore rodents are inappropriate models to explore FSH feedback mechanisms. However, those species with variable sperm output need to know the maximal level of sperm production to then adapt the hormonal stimulation to the desired level of sperm production. For estimation of the maximal capacity of sperm

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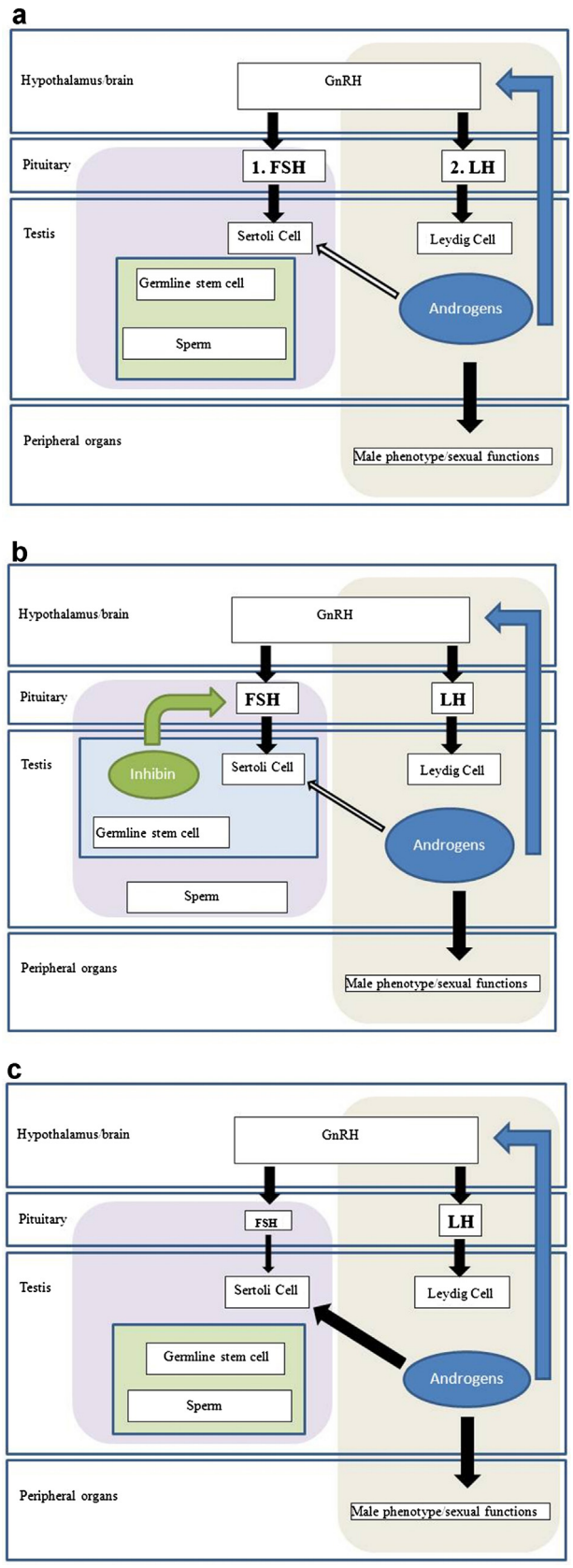


**Fig. 10.** Schematic drawings illustrating species-specific models for hormonal control of testicular function which present modifications from the "hypothetical ancient" model depicted in Fig. 9. In all three species the two feedback loops are not fully independent. Testosterone exerts positive paracrine effects by stimulating sperm production via Sertoli cells. (a) shows a model for the Djungarian hamster which shows a subsequent activation of the FSH and LH axis during photorecrudescence. Inhibin plays no major role since spermatogenic output is not hormonally modulated. (b) reveals the primate situation. While both gonadotropic axes are active the FSH–inhibin axis functions as modulator of the spermatogenic output. The mouse or rat situation is depicted in (c). The LH axis is dominant. Steroidogensis and spermatogenesis are activated synchronously via paracrine action of testosterone. As in hamsters inhibin plays no major role as spermatogenic efficiency is not hormonally modulated. In adulthood the role of FSH is widely diminished.

production the most relevant information is the number of stem cell niches occupied by spermatogonial stem cells and their activity status. Inhibin B functions as a readout of functional stem cell niches in the primate testis. It is well known that primate spermatogenesis is not running at maximal capacity [135]. Strong evidence and a physiological model for an FSH–inhibin feedback had been presented by Plant and Marshall already in 2001 [122]. In primates the level of inhibin B depends on the amount of testicular tissue since hemicastration leads to a rapid and persistent 50% reduction of serum inhibin B levels [99]. In addition inhibin B levels in monkeys are influenced by both gonadotropins [136]. However, inhibin B is not just constitutively released by each Sertoli cell. Experimental germ cell depletion by testicular irradiation in primates is followed by a rapid reduction of inhibin B [137,138]. Already few days after irradiation when all A-spermatogonia are depleted but the more differentiating germ cells are still present, inhibin B levels are low. During subsequent recovery of spermatogenesis when stem cells slowly colonize the germ cell deficient tubules inhibin B levels rise. The levels of inhibin B correlate well with A-spermatogonia declining rapidly during their depletion and recovering slowly during recolonization. It is therefore obvious that inhibin B levels indicate the integrity and activity of the testicular stem cell system and provide an endocrine signal for the number of functional testicular stem cells. The inhibin level therefore can be perceived by the brain as a marker for the maximal potential of sperm production. Depending on the number of functional units the release of more or less FSH can control the total sperm output. At least in male primates inhibin B functions therefore as a feedback hormone for FSH as it is can be perceived centrally as a marker for the capacity of sperm production (Fig. 10b).

## 5. Species-specific variability of endocrine control mechanisms of testis functions

Every species has adapted its reproductive needs to the environmental conditions to guarantee maximal reproductive success. Endocrine mechanisms provide flexible options to control spermatogenesis and steroidogenesis and to adapt reproductive strategies to the species-specific needs. Fig. 10 depicts three different species-specific scenarios for the regulation of spermatogenesis and steroidogenesis. As outlined in the hypothetical model depicted in Fig. 9 in principle both processes could be controlled independently since they are located in separate compartments and the hormones controlling each process are associated with specific target cells. In reality this extreme scenario with two completely separated feedback loops controlling spermatogenesis and steroidogenesis independently has not been described for any species. In the following three plots several adaptations and modifications of the regulatory control system is shown. Fig. 10a reveals the situation in the Djungarian hamster, a photoperiod-sensitive species in which temporal asynchrony of spermatogenesis and steroidogenesis is observed. Both gonadotropic axes are maintained and can independently activate spermatogenesis or steroidogenesis. During recrudescence FSH is first released to initiate the spermatogenic process while the animal is still sexually inactive. LH is secreted several weeks later to stimulate sexual functions and mating behavior. Testosterone has

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stimulatory paracrine function in order to support an efficient spermatogenic output during mating season. As hamsters like other rodents do not modulate the sperm output inhibin B may be of less relevance for this species. The following plot (Fig. 10b) depicts the scenario in primates. Both gonadotropic axes are synchronously active. The FSH–inhibin B axis controls an adequate sperm output at the stem cell and premeiotic germ cell stage. Germ cell expansion may not be maximally stimulated and the capacity of the seminiferous epithelium is not always reached. The degree of FSH stimulation is modulated centrally via input from the brain. The LH–testosterone axis controls the peripheral androgenization status. Testosterone has relevant paracrine activity in the primate testis stimulating many aspects the efficiency of germ cell survival. Fig. 10c reveals the situation in mice and rats. Both species are prepared for maximal reproductive success throughout life and the seminiferous epithelium is loaded permanently with a maximal number of germ cells creating a constant non-modulated optimal sperm output. As no modulation of sperm production is needed, the inhibin B feedback role is diminished. Since sperm output is constant and therefore FSH modulation is not required synchronous activation of steroidogenesis and spermatogenesis is sufficient and the LH–testosterone axis controls both, the peripheral androgenization status via endocrine action and the activity of the seminiferous epithelium via paracrine actions. The role of FSH is diminished.

These three models reveal a high diversity of gonadotropic regulation of testis function. However, despite of a high degree of modulations a complete loss of the one or the other gonadotropic axis has not been observed in any species. The loss of a complete axis may lead to a decline of adaptive options which may eliminate necessary modifications of reproductive strategies when changes in environmental conditions occur.

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## References

[1] Clermont Y. Kinetics of spermatogenesis in mammals: seminiferous epithelium cycle and spermatogonial renewal. Physiol Rev 1972;52:198–236.
[2] de Kretser DM, Kerr JB. The cytology of the testis. In: Knobil E, Neill JD, editors. The physiology of reproduction, vol. 1. New York: Raven Press; 1988. p. 837–932.
[3] Russell LD, Ettlin RA, Sinha Hikim AP, Clegg ED. Histological and histopathological evaluation of the testis. Clearwater, FL: Cache River Press; 1990.
[4] Russell L. Role in spermiation. In: Russell LD, Griswold MD, editors. The Sertoli cell. Clearwater, FL: Cache River Press; 1993. p. 269–302.
[5] Awoniyi CA, Santulli R, Sprando RL, Ewing LL, Zirkin BR. Restoration of advanced spermatogenic cells in the experimentally regressed rat testis: quantitative relationship to testosterone concentration within the testis. Endocrinology 1989;124:1217–23.
[6] El Shennawy A, Gates RJ, Russell LD. Hormonal regulation of spermatogenesis in the hypophysectomized rat: cell viability after hormonal replacement in adults after intermediate periods of hypophysectomy. J Androl 1998;19:320–34.
[7] Marshall GR, Zorub DS, Plant TM. Follicle-stimulating hormone amplifies the population of differentiated spermatogonia in the hypophysectomized testosterone-replaced adult rhesus monkey (*Macaca mulatta*). Endocrinology 1995;136:3504–11.
[8] McLachlan RI, Wreford NG, de Kretser DM, Robertson DM. The effects of recombinant follicle stimulating hormone on the restoration of spermatogenesis in the gonadotropin-releasing hormone-immunized adult rat. Endocrinology 1995;136:4035–43.
[9] Meachem SJ, Wreford NG, Stanton PG, Robertson DM, McLachlan RI. Follicle-stimulating hormone is required for the initial phase of spermatogenic restoration in adult rats following gonadotropin suppression. J Androl 1998;19:725–35.
[10] Meachem SJ, McLachlan RI, Stanton PG, Robertson DM, Wreford NG. FSH immunoneutralization acutely impairs spermatogonial development in normal adult rats. J Androl 1999;20:756–62.
[11] Russell LD, Clermont Y. Degeneration of germ cells in normal, hypophysectomized and hormone treated hypophysectomized rats. Anat Rec 1977;187:347–66.
[12] Sinha Hikim AP, Swerdloff RS. Temporal and stage-specific effects of recombinant human follicle-stimulating hormone on the maintenance of spermatogenesis in gonadotropin-releasing hormone antagonist-treated rat. Endocrinology 1995;136:253–61.
[13] Weinbauer GF, Behre HM, Fingscheidt U, Nieschlag E. Human follicle-stimulating hormone exerts a stimulatory effect on spermatogenesis, testicular size, and serum inhibin levels in the gonadotropin-releasing hormone antagonist-treated nonhuman primate (*Macaca fascicularis*). Endocrinology 1991;129:1831–9.
[14] Weinbauer GF, Gockeler E, Nieschlag E. Testosterone prevents complete suppression of spermatogenesis in the gonadotropin-releasing hormone antagonist-treated nonhuman primate (*Macaca fascicularis*). J Clin Endocrinol Metab 1988;67:284–90.
[15] Cooke HJ, Saunders PT. Mouse models of male infertility. Nat Rev Genet 2002;3:790–801.
[16] Couse JF, Korach KS. Estrogen receptor null mice: what have we learned and where will they lead us? Endocr Rev 1999;20:358–417.
[17] Weinbauer GF, Gromoll J, Simoni M, Nieschlag E. Physiology of testicular function. In: Nieschlag E, Behre HM, editors. Andrology, male reproductive health and dysfunction. 1st ed. 1997. p. 25–57 [Chapter 3].
[18] Reuter K, Schlatt S, Ehmcke J, Wistuba J. Fact or fiction: in vitro spermatogenesis. Spermatogenesis 2012;2:245–52.
[19] Kanatsu-Shinohara M, Shinohara T. Culture and genetic modification of mouse germline stem cells. Ann N Y Acad Sci 2007;1120:59–71.
[20] Dym M, Fawcett DW. The blood–testis barrier in the rat and the physiological compartmentation of the seminiferous epithelium. Biol Reprod 1970;3:308–26.
[21] Christensen AK. Leydig cells. In: Greep RO, Astwood EB, editors. Handbook of physiology. Section 7, vol. 5. Washington, DC: American Physiological Society; 1975. p. 57–94.
[22] Gondos B, Berndston WE. Postnatal and pubertal development. In: Russell LD, Griswold MD, editors. The Sertoli cell. Clearwater, FL: Cache River Press; 1994. p. 116–53.
[23] Orth JM. Proliferation of Sertoli cells in fetal and postnatal rats: a quantitative autoradiographic study. Anat Rec 1982;203:485–92.
[24] Meachem SJ, McLachlan RI, de Kretser DM, Robertson DM, Wreford NG. Neonatal exposure of rats to recombinant follicle stimulating hormone increases adult Sertoli and spermatogenic cell numbers. Biol Reprod 1996;54:36–44.
[25] Cooke PS, Zhao YD, Bunick D. Triiodothyronine inhibits proliferation and stimulates differentiation of cultured neonatal Sertoli cells: possible mechanism for increased adult testis weight and sperm production induced by neonatal goitrogen treatment. Biol Reprod 1994;51:1000–5.
[26] Huhtaniemi IT, Nevo N, Amsterdam A, Naor Z. Effect of postnatal treatment with a gonadotropin-releasing hormone antagonist on sexual maturation of malerats. Biol Reprod 1986;35:501–7.
[27] Sharpe RM, Atanassova N, McKinnell C, Parte P, Turner KJ, Fisher JS, et al. Abnormalities in functional development of the Sertoli cells in rats treated neonatally with diethylstilbestrol: a possible role for estrogens in Sertoli cell development. Biol Reprod 1998;59:1084–94.
[28] Janecki A, Steinberger A. Polarized Sertoli cell functions in a new two-compartment culture system. J Androl 1986;7(1):69–71.
[29] Handelsman DJ, Spaliviero JA, Kidston E, Robertson DM. Highly polarized secretion of inhibin by Sertoli cells in vitro. Endocrinology 1989;125(2):721–9.
[30] Russell LD. Form, dimensions and cytology of mammalian Sertoli cells. In: Russell LD, Griswold MD, editors. The Sertoli cell. Clearwater, FL: Cache River Press; 1993. p. 1–38.
[31] Blüm V. Vergleichende Reproduktionsbiologie der Wirbeltiere. Berlin: Springer Verlag; 1985. p. 1–109.
[32] Penzlin H. Lehrbuch der Tierphysiologie. Stuttgart: Gustav Fischer Verlag; 1970. p. 1–513.
[33] Leblond CP, Clermont Y. Definition of the stages of the cycle of the seminiferous epithelium in the rat. Ann N Y Acad Sci 1952:548–73.
[34] Leblond CP, Clermont Y. Spermiogenesis of rat, mouse and guinea pig as revealed by the 'periodic acid-fuchsin sulfurus acid' technique. Am J Anat 1952;90:167–206.
[35] de Rooij DG, Russell LD. All you wanted to know about spermatogonia but were afraid to ask. J Androl 2000;21:776–98.
[36] Meachem S, von Schönfeldt V, Schlatt S. Spermatogonia: stem cells with a great perspective. Reproduction 2001;121:825–34.
[37] McLean DJ, Johnston DS, Russell LD, Griswold MD. Germ cell transplantation and the study of testicular function. Trends Endocrinol Metab 2001;12:16–21.
[38] Meistrich ML, van Beek ME. Spermatogonial stem cells. In: Desjardins C, Ewing LL, editors. Cell and molecular biology of the testis. New York: Oxford University Press; 1993. p. 266–95.
[39] Schlatt S, Weinbauer GF. Immunohistochemical localization of proliferating cell nuclear antigen as a tool to study cell proliferation in rodent and primate testes. Int J Androl 1994;17:214–22.

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[40] Clermont Y. Two classes of spermatogonial stem cells in the monkey (*Cercopithecus aethiops*). Am J Anat 1969;126:57–71.
[41] van Alphen MM, van de Kant HJ, de Rooij DG. Depletion of the spermatogonia from the seminiferous epithelium of the rhesus monkey after X irradiation. Radiat Res 1988;113:473–86.
[42] van Alphen MM, van de Kant HJ, de Rooij DG. Repopulation of the seminiferous epithelium of the rhesus monkey after X irradiation. Radiat Res 1988;113:487–500.
[43] Schulze C. Morphological characteristics of the spermatogonial stem cells in man. Cell Tissue Res 1979;198:191–9.
[44] Schulze C. Survival of human spermatogonial stem cells in various clinical conditions. Fortschr Androl 1981;7:58–68.
[45] Fouquet JP, Dadoune JP. Renewal of spermatogonia in the monkey (*Macaca fascicularis*). Biol Reprod 1986;35:199–207.
[46] O'Donnell L, Narula A, Balourdos G, Gu YQ, Wreford NG, Robertson DM, et al. Impairment of spermatogonial development and spermiation after testosterone-induced gonadotropin suppression in adult monkeys (*Macaca fascicularis*). J Clin Endocrinol Metab 2001;86:1814–22.
[47] Braun RE. Post-transcriptional control of gene expression during spermatogenesis. Semin Cell Dev Biol 1998;9:483–9.
[48] De Rooij DG, Lok D, Weenk D. Feedback regulation of the proliferation of the undifferentiated spermatogonia in the Chinese hamster by the differentiating spermatogonia. Cell Tissue Kinet 1985;18:71–81.
[49] de Rooij DG, van Alphen MM, van de Kant HJ. Duration of the cycle of the seminiferous epithelium and its stages in the rhesus monkey (*Macaca mulatta*). Biol Reprod 1986;35(3):587–91.
[50] Schulze W, Rehder U. Organization and morphogenesis of the human seminiferous epithelium. Cell Tissue Res 1984;237(3):395–407.
[51] Schulze W, Riemer M, Rehder U, Hohne KH. Computer-aided three-dimensional reconstructions of the arrangement of primary spermatocytes in human seminiferous tubules. Cell Tissue Res 1986;244:1–7.
[52] Millar MR, Sharpe RM, Weinbauer GF, Fraser HM, Saunders PT. Marmoset spermatogenesis: organizational similarities to the human. Int J Androl 2000;23:266–77.
[53] Heller CG, Clermont Y. Spermatogenesis in man: an estimation of its duration. Science 1963;140:184–6.
[54] Franca LR, Ogawa T, Avarbock MR, Brinster RL, Russell LD. Germ cell genotype controls cell cycle during spermatogenesis in the rat. Biol Reprod 1998;59:1371–7.
[55] Dobrinski I. Transplantation of germ cells and testis tissue for the study and preservation of fertility. Soc Reprod Fertil Suppl 2007;65:447–58.
[56] Timmons PM, Rigby PW, Poirier F. The murine seminiferous epithelial cycle is pre-figured in the Sertoli cells of the embryonic testis. Development 2002;129:635–47.
[57] de Rooij DG. The spermatogonial stem cell niche. Microsc Res Tech 2009;72:580–5.
[58] Stukenborg JB, Wistuba J, Luetjens CM, Elhija MA, Huleihel M, Lunenfeld E, et al. Coculture of spermatogonia with somatic cells in a novel three-dimensional soft-agar-culture-system. J Androl 2008;29:312–29.
[59] Kanatsu-Shinohara M, Toyokuni S, Morimoto T, Matsui S, Honjo T, Shinohara T. Functional assessment of self-renewal activity of male germline stem cells following cytotoxic damage and serial transplantation. Biol Reprod 2003;68:1801–7.
[60] Ogawa T, Ohmura M, Yumura Y, Sawada H, Kubota Y. Expansion of murine spermatogonial stem cells through serial transplantation. Biol Reprod 2003;68:316–22.
[61] Clermont Y, Leblond CP. Differentiation and renewal of spermatogonia in the monkey, *Macaca rhesus*. Am J Anat 1959;104:237–73.
[62] Clermont Y. Renewal of spermatogonia in man. Am J Anat 1966;118:509–24.
[63] Luo J, Megee S, Dobrinski I. Asymmetric distribution of UCH-L1 in spermatogonia is associated with maintenance and differentiation of spermatogonial stem cells. J Cell Physiol 2009;220:460–8.
[64] Suzuki H, Sada A, Yoshida S, Saga Y. The heterogeneity of spermatogonia is revealed by their topology and expression of marker proteins including the germ cell-specific proteins Nanos2 and Nanos3. Dev Biol 2009;336:222–31.
[65] Schrans-Stassen BH, van de Kant HJ, de Rooij DG, van Pelt AM. Differential expression of c-kit in mouse undifferentiated and differentiating type A spermatogonia. Endocrinology 1999;140:5894–900.
[66] Buaas FW, Kirsh AL, Sharma M, McLean DJ, Morris JL, Griswold MD, et al. Plzf is required in adult male germ cells for stem cell self-renewal. Nat Genet 2004;36:647–52.
[67] Wu X, Schmidt JA, Avarbock MR, Tobias JW, Carlson CA, Kolon TF, et al. Prepubertal human spermatogonia and mouse gonocytes share conserved gene expression of germline stem cell regulatory molecules. Proc Natl Acad Sci U S A 2009;106:21672–7.
[68] Gassei K, Ehmcke J, Schlatt S. Efficient enrichment of undifferentiated GFR alpha 1+ spermatogonia from immature rat testis by magnetic activated cell sorting. Cell Tissue Res 2009;337:177–83.
[69] Gassei K, Ehmcke J, Dhir R, Schlatt S. Magnetic activated cell sorting allows isolation of spermatogonia from adult primate testes and reveals distinct GFRa1-positive subpopulations in men. J Med Primatol 2010;39:83–91.
[70] Hermann BP, Sukhwani M, Simorangkir DR, Chu T, Plant TM, Orwig KE. Molecular dissection of the male germ cell lineage identifies putative spermatogonial stem cells in rhesus macaques. Hum Reprod 2009;24:1704–16.
[71] Hermo L, Pelletier RM, Cyr DG, Smith CE. Surfing the wave, cycle, life history, and genes/proteins expressed by testicular germ cells. Part 1: Background to spermatogenesis, spermatogonia, and spermatocytes. Microsc Res Tech 2010;73:241–78.
[72] von Kopylow K, Kirchhoff C, Jezek D, Schulze W, Feig C, Primig M, et al. Screening for biomarkers of spermatogonia within the human testis: a whole genome approach. Hum Reprod 2010;25:1104–12.
[73] Phillips BT, Gassei K, Orwig KE. Spermatogonial stem cell regulation and spermatogenesis. Philos Trans R Soc Lond B Biol Sci 2010;365:1663–78.
[74] De Rooij DG, Grootegoed JA. Spermatogonial stem cells. Curr Opin Cell Biol 1998;10:694–701.
[75] de Rooij DG. Stem cells in the testis. Int J Exp Pathol 1998;79:67–80.
[76] Buageaw A, Sukhwani M, Ben-Yehudah A, Ehmcke J, Rawe VY, Pholpramool C, et al. GDNF family receptor alpha1 phenotype of spermatogonial stem cells in immature mouse testes. Biol Reprod 2005;73:1011–6.
[77] Ehmcke J, Luetjens CM, Schlatt S. Clonal organization of proliferating spermatogonial stem cells in adult males of two species of non-human primates, *Macaca mulatta* and *Callithrix jacchus*. Biol Reprod 2005;72:293–300.
[78] Ehmcke J, Simorangkir DR, Schlatt S. Identification of the starting point for spermatogenesis and characterization of the testicular stem cell in adult male rhesus monkeys. Hum Reprod 2005;20:1185–93.
[79] Hermann BP, Sukhwani M, Hansel MC, Orwig KE. Spermatogonial stem cells in higher primates: are there differences from those in rodents? Reproduction 2010;139:479–93.
[80] Simorangkir DR, Marshall GR, Plant TM. A re-examination of proliferation and differentiation of type A spermatogonia in the adult rhesus monkey (*Macaca mulatta*). Hum Reprod 2009;24:1596–604.
[81] Day IN, Thompson RJ. UCHL1 (PGP 9.5): neuronal biomarker and ubiquitin system protein. Prog Neurobiol 2010;90:327–62.
[82] Klein AM, Nakagawa T, Ichikawa R, Yoshida S, Simons BD. Mouse germ line stem cells undergo rapid and stochastic turnover. Cell Stem Cell 2010;6:214–24.
[83] Huckins C, Oakberg EF. Morphological and quantitative analysis of spermatogonia in mouse testes using whole mounted seminiferous tubules. I: The normal testes. Anat Rec 1978;192:519–28.
[84] Lok D, Weenk D, de Rooij DG. Morphology, proliferation and differentiation of undifferentiated spermatogonia in the Chinese hamster and the ram. Anat Rec 1982;203:83–99.
[85] Ehmcke J, Wistuba J, Schlatt S. Spermatogonial stem cells: questions, models and perspectives. Hum Reprod Update 2006;12:275–82.
[86] Clermont Y. The cycle of the seminiferous epithelium in man. Am J Anat 1963;112:35–51.
[87] Clermont Y. Stermatogenesis in man. A study of the spermatogonial population. Fertil Steril 1966;17:705–21.
[88] Clermont Y, Antar M. Duration of the cycle of the seminiferous epithelium and the spermatogonial renewal in the monkey, *Macaca arctoides*. Am J Anat 1973;136:153–65.
[89] Ehmcke J, Hübner K, Schöler HR, Schlatt S. Spermatogonia: origin, physiology and prospects for conservation and manipulation of the male germ line. Reprod Fertil Dev 2006;18:7–12.
[90] Simorangkir DR, Marshall GR, Ehmcke J, Schlatt S, Plant TM. Prepubertal expansion of dark and pale type A spermatogonia in the rhesus monkey (*Macaca mulatta*) results from proliferation during infantile and juvenile development in a relatively gonadotropin independent manner. Biol Reprod 2005;73:1109–15.
[91] Ehmcke J, Schlatt S. A revised model for spermatogonial expansion in man: lessons from non-human primates. Reproduction 2006;132:673–80.
[92] Ogawa T, Ohmura M, Ohbo K. The niche for spermatogonial stem cells in the mammalian testis. Int J Hematol 2005;82:381–8.
[93] Chuma S, Kanatsu-Shinohara M, Inoue K, Ogonuki N, Miki H, Toyokuni S, et al. Spermatogenesis from epiblast and primordial germ cells following transplantation into postnatal mouse testis. Development 2005;132:117–22.
[94] Nayernia K, Drabent B, Meinhardt A, Adham IM, Schwandt I, Müller C, et al. Triple knockouts reveal gene interactions affecting fertility of male mice. Mol Reprod Dev 2005;70:406–16.
[95] Chiarini-Garcia H, Raymer AM, Russell LD. Non-random distribution of spermatogonia in rats: evidence of niches in the seminiferous tubules. Reproduction 2003;126:669–80.
[96] Yoshida S, Sukeno M, Nabeshima Y. A vasculature-associated niche for undifferentiated spermatogonia in the mouse testis. Science 2007;317:1722–6.
[97] Niklowitz P, Lerchl A, Nieschlag E. Photoperiodic responses in Djungarian hamsters (*Phodopus sungorus*): importance of light history for pineal and serum melatonin profiles. Biol Reprod 1994;51:714–24.
[98] Ramaswamy S, Marshall GR, McNeilly AS, Plant TM. Evidence that in a physiological setting Sertoli cell number is the major determinant of circulating concentrations of inhibin B in the adult male rhesus monkey (*Macaca mulatta*). J Androl 1999;20:430–4.
[99] Schlatt S, Gassei K, Westernströer B, Ehmcke J. Modulating testicular mass in xenografting: a model to explore testis development and endocrine function. Endocrinology 2010;151:4018–23.
[100] Schlatt S, Westernströer B, Gassei K, Ehmcke J. Donor-host involvement in immature rat testis xenografting into nude mouse hosts. Biol Reprod 2010;82(5):888–95.
[101] Schlatt S, Kim SS, Gosden R. Spermatogenesis and steroidogenesis in mouse, hamster and monkey testicular tissue after cryopreservation and heterotopic grafting to castrated hosts. Reproduction 2002;124:339–46.

Please cite this article in press as: Schlatt S, Ehmcke J. Regulation of spermatogenesis: An evolutionary biologist's perspective. Semin Cell Dev Biol (2014), http://dx.doi.org/10.1016/j.semcdb.2014.03.007

[102] Wreford NG, Rajendra Kumar T, Matzuk MM, de Kretser DM. Analysis of the testicular phenotype of the follicle-stimulating hormone beta-subunit knockout and the activin type II receptor knockout mice by stereological analysis. Endocrinology 2001;142:2916–20.
[103] Kumar TR, Wang Y, Lu N, Matzuk MM. Follicle stimulating hormone is required for ovarian follicle maturation but not male fertility. Nat Genet 1997;15:201–4.
[104] Johnston H, Baker PJ, Abel M, Charlton HM, Jackson G, Fleming L, et al. Regulation of Sertoli cell number and activity by follicle-stimulating hormone and androgen during postnatal development in the mouse. Endocrinology 2004;145:318–29.
[105] Baker PJ, Pakarinen P, Huhtaniemi IT, Abel MH, Charlton HM, Kumar TR, et al. Failure of normal Leydig cell development in follicle-stimulating hormone (FSH) receptor-deficient mice, but not FSHbeta-deficient mice: role for constitutive FSH receptor activity. Endocrinology 2003;144:138–45.
[106] Allan CM, Haywood M, Swaraj S, Spaliviero J, Koch A, Jimenz M, et al. A novel transgenic model to characterize the specific effects of follicle-stimulating hormone on gonadal physiology in the absence of lutenizing hormone actions. Endocrinology 2001;142:2200–13.
[107] Dierich A, Sairam MR, Monoco L, Fimia GM, Gansmuller A, Lemeur M, et al. Impaired follicle-stimulating hormone (FSH) signaling in vivo: targeted disruption of the FSH receptor leads to aberrant gametogenesis and hormonal imbalance. Proc Natl Acad Sci U S A 1998;95:13612–7.
[108] Abel MH, Wootton AN, Wilkins V, Huhtaniemi I, Knight PG, Charlton HM. The effect of a null mutation in the follicle-stimulating hormone receptor gene on mouse reproduction. Endocrinology 2000;141:1795–803.
[109] Krishnamurthy H, Kats R, Danilovich N, Javeshghani D, Sairam MR. Intercellular communication between Sertoli cells and Leydig cells in the absence of follicle-stimulating hormone-receptor signaling. Biol Reprod 2001;65:1201–7.
[110] Grover A, Sairam MR, Smith CE, Hermo L. Structural and functional modifications of sertoli cells in the testis of adult follicle-stimulating hormone receptor knockout mice. Biol Reprod 2004;71:117–29.
[111] Abel MH, Baker PJ, Charlton HM, Monteiro A, Verhoeven G, De Gendt K, et al. Spermatogenesis and sertoli cell activity in mice lacking sertoli cell receptors for follicle-stimulating hormone and androgen. Endocrinology 2008;149:3279–85.
[112] Haywood M, Spaliviero J, Jimemez M, King NJ, Handelsman DJ, Allan CM. Sertoli and germ cell development in hypogonadal (hpg) mice expressing transgenic follicle-stimulating hormone alone or in combination with testosterone. Endocrinology 2003;144:509–17.
[113] O'Shaughnessy PJ, Monteiro A, Verhoeven G, De Gendt K, Abel MH. Effect of FSH on testicular morphology and spermatogenesis in gonadotrophin-deficient hypogonadal mice lacking androgen receptors. Reproduction 2010;139:177–84.
[114] Singh J, Handelsman DJ. The effects of recombinant FSH on testosterone-induced spermatogenesis in gonadotropin deficient (hpg) mice. J Androl 1996;17:382–93.
[115] Siegel ET, Kim HG, Nishimoto HK, Layman LC. The molecular basis of impaired follicle-stimulating hormone action: evidence from human mutations and mouse models. Reprod Sci 2013;20(3):211–33.
[116] Niklowitz P, Khan S, Bergmann M, Hoffmann K, Nieschlag E. Differential effects of follicle-stimulating hormone and luteinizing hormone on Leydig cell function and restoration of spermatogenesis in hypophysectomized and photoinhibited Djungarian hamsters (*Phodopus sungorus*). Biol Reprod 1989;41:871–80.
[117] Schlatt S, de Geyter M, Kliesch S, Nieschlag E, Bergmann M. Spontaneous recrudescence of spermatogenesis in the photoinhibited male Djungarian hamster, *Phodopus sungorus*. Biol Reprod 1995;53:169–177.
[118] Lerchl A, Sotiriadou S, Behre HM, Pierce J, Weinbauer GF, Kliesch S, et al. Restoration of spermatogenesis by follicle-stimulating hormone despite low intratesticular testosterone in photoinhibited hypogonadotropic Djungarian hamsters (*Phodopus sungorus*). Biol Reprod 1993;49:1108–16.
[119] Niklowitz P, Lerchl A, Nieschlag E. In vitro fertilizing capacity of sperm from FSH-treated photoinhibited Djungarian hamsters (*Phodopus sungorus*). J Endocrinol 1997;154:475–81.
[120] Tarulli GA, Stanton PG, Lerchl A, Meachem SJ. Adult Sertoli cells are not terminally differentiated in the Djungarian hamster: effect of FSH on proliferation and junction protein organization. Biol Reprod 2006;74:798–806.
[121] Dankbar B, Brinkworth MH, Schlatt S, Weinbauer GF, Nieschlag E, Gromoll J. Ubiquitous expression of the androgen receptor and testis-specific expression of the FSH receptor in the cynomolgus monkey (*Macaca fascicularis*) revealed by a ribonuclease protection assay. J Steroid Biochem Mol Biol 1995;55:35–41.
[122] Plant TM, Marshall GR. The functional significance of FSH in spermatogenesis and the control of its secretion in male primates. Endocr Rev 2001;22:764–86.
[123] Phillip M, Arbelle JE, Segev Y, Parvari R. Male hypogonadism due to a mutation in the gene for the β-subunit of follicle-stimulating hormone. N Engl J Med 1998;338:1729–32.
[124] Lindstedt G, Nystrom E, Mathews C, Ernest I, Janson PO, Chatterjee K. Follitropin (FSH) deficiency in an infertile male due to FSH-beta gene mutation: a syndrome of normal puberty and virulization but underdeveloped testicles with azoospermia, low FSH but high lutropin and normal serum testosterone concentrations. Clin Chem Lab Med 1998;36:663–5.
[125] Layman LC, Porto AL, Xie J, da Motta LA, da Motta LD, Weiser W, et al. FSH beta gene mutations in a female with partial breast development and a male sibling with normal puberty and azoospermia. J Clin Endocrinol Metab 2002;87:3702–7.
[126] Mantovani G, Borgato S, Beck-Peccoz P, Romoli R, Borretta G, Persani L. Isolated follicle-stimulating hormone (FSH) deficiency in a young man with normal virilization who did not have mutations in the FSHbeta gene. Fertil Steril 2003;79:434–6.
[127] Giltay JC, Deege M, Blankenstein RA, Kastrop PM, Wijmenga C, Lock TT. Apparent primary follicle-stimulating hormone deficiency is a rare cause of treatable male infertility. Fertil Steril 2004;81:693–6.
[128] Gromoll J, Simoni M, Nieschlag E. An activating mutation of the follicle-stimulating hormone receptor autonomously sustains spermatogenesis in a hypophysectomized man. J Clin Endocrinol Metab 1996;81:1367–70.
[129] Aravindan GR, Gopalakrishnan K, Ravindranath N, Moudgal NR. Effect of altering endogenous gonadotrophin concentrations on the kinetics of testicular germ cell turnover in the bonnet monkey (*Macaca radiata*). J Endocrinol 1993;137:485–95.
[130] van Alphen MMA, van de Kant HJG, de Rooij DG. Follicle-stimulating hormone stimulates spermatogenesis in the adult monkey. Endocrinology 1988;129:1831–9.
[131] Simorangkir DR, Ramaswamy S, Marshall GR, Pohl CR, Plant TM. A selective monotropic elevation of FSH, but not that of LH, amplifies the proliferation and differentiation of spermatogonia in the adult rhesus monkey (*Macaca mulatta*). Hum Reprod 2009;24:1584–95.
[132] Arslan M, Weinbauer GF, Schlatt S, Shahab M, Nieschlag E. FSH and testosterone, alone or in combination, initiate testicular growth and increase the number of spermatogonia and Sertoli cells in a juvenile non-human primate (*Macaca mulatta*). J Endocrinol 1993;136:235–43.
[133] Schlatt S, Arslan M, Weinbauer GF, Behre HM, Nieschlag E. Endocrine control of somatic cell and premeiotic germ cell development in the male rhesus monkey (*Macaca mulatta*). Eur J Endocrinol 1995;133:235–47.
[134] Ramaswamy S, Plant TM, Marshall GR. Pulsatile stimulation with recombinant single chain human lutenizing hormone elicits precocious Sertoli cell proliferation in the juvenile male rhesus monkey. Biol Reprod 2000;63:82–8.
[135] Ramaswamy S, Marshall GR, McNeilly AS, Plant TM. Dynamics of the follicle-stimulating hormone (FSH)–inhibin B feedback loop and its role in regulating spermatogenesis in the adult male rhesus monkey (*Macaca mulatta*) as revealed by unilateral orchidectomy. Endocrinology 2000;141:18–27.
[136] Ramaswamy S, Marshall GR, Pohl CR, Friedman RL, Plant TM. Inhibitory and stimulatory regulation of testicular inhibin B secretion by luteinizing hormone and follicle-stimulating hormone, respectively, in the rhesus monkey (*Macaca mulatta*). Endocrinology 2003;144:1175–85.
[137] Foppiani L, Schlatt S, Simoni M, Weinbauer GF, Hacker-Klom U, Nieschlag. Inhibin B is a more sensitive marker of spermatogenetic damage than FSH in the irradiated non-human primate model. J Endocrinol 1999;162:393–400.
[138] Schlatt S, Foppiani L, Rolf C, Weinbauer GF, Nieschlag E. Germ cell transplantation into X-irradiated monkey testes. Hum Reprod 2002;17:55–62.

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