Endocrinology of the Male Reproductive System

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GENERAL STRUCTURE OF THE MALE REPRODUCTIVE SYSTEM

TESTIS

The testis lies within the scrotum and is covered on all surfaces except its posterior border by a serous membrane called the tunica vaginalis. This structure forms a closed cavity representing the remnants of the processus vaginalis into which the testis descended during fetal development (Figure 1). Along its posterior border, the testis is loosely linked to the epididymis which at its lower pole gives rise to the vas deferens (1) .

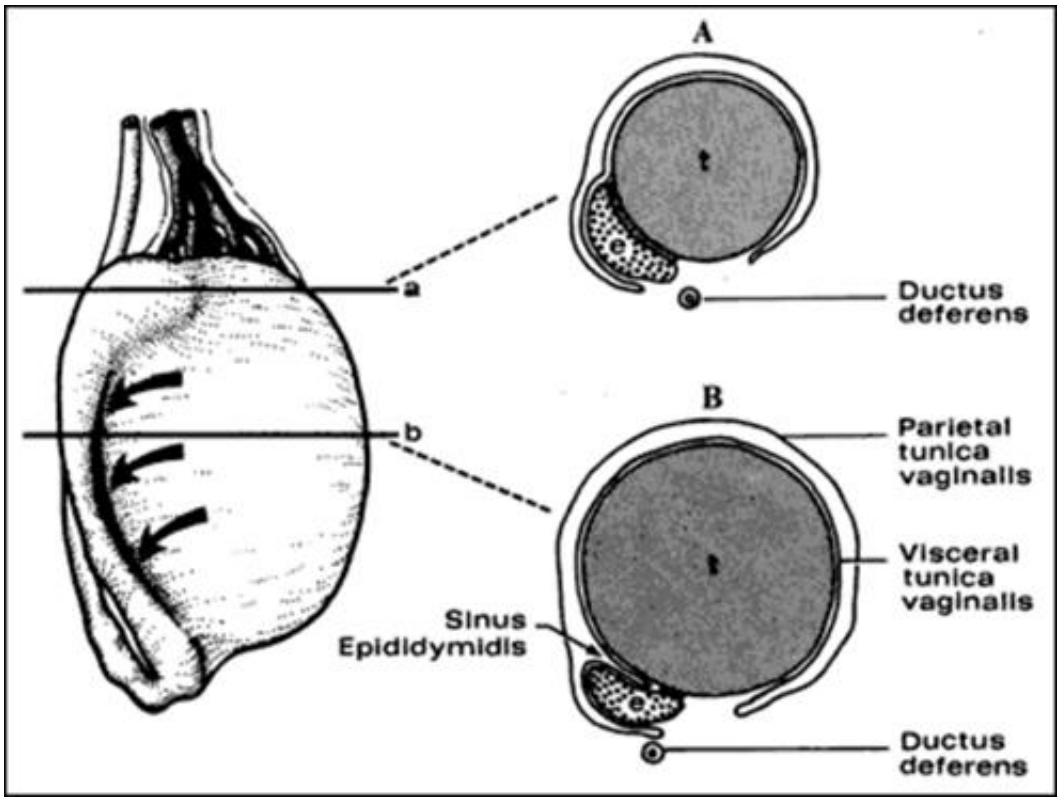

Figure 1. The relationships of the tunica vaginalis to the testis and epididymis is illustrated from the lateral view and two cross sections at the level of the head and mid-body of the epididymis. The large arrows indicate the sinus of the epididymis posteriorly. Reproduced with permission from de Kretser et.al.1982 in 'Disturbances in Male Fertility' Eds K Bandhauer and J Frick, Springer – Verlag Berlin.

The testis is covered by a thick fibrous connective tissue capsule called the tunica albuginea. From this structure, thin imperfect septa run in a posterior direction to join a fibrous thickening of the posterior part of the tunica albuginea called the mediastinum of the testis. The testis is thus incompletely divided into a series of lobules.

Within these lobules, the seminiferous tubules form loops, the terminal ends of which extend as straight tubular extensions, called tubuli recti, which pass into the mediastinum of the testis and join an anastomosing network of tubules called the rete testis. From the rete testis, in the human a series of six to twelve fine efferent ducts join to form the duct of the epididymis. This duct is extensively coiled and forms the structure of the epididymis that can be divided into the head, body and tail of the epididymis (1) . At its distal pole, the tail of the epididymis gives rise to the vas deferens (Figure 2).

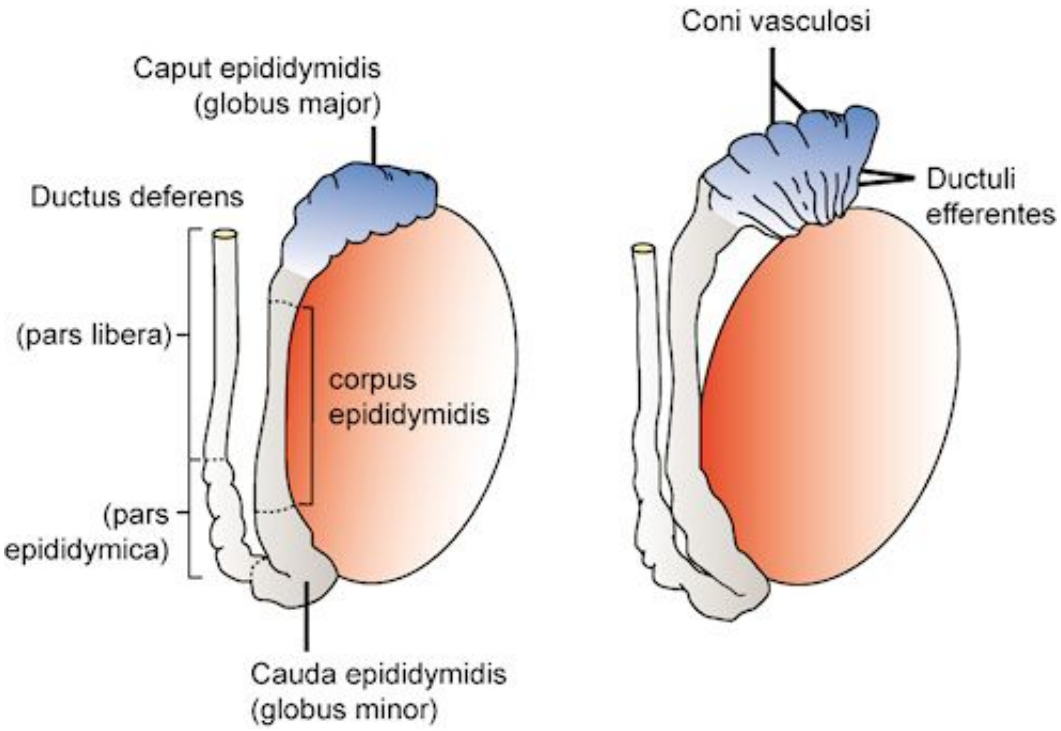

Figure 2. The arrangement of the efferent ducts and the subdivisions of the epididymis and vas are shown. Reproduced with permission from de Kretser et.al.1982 in ‘Disturbances in Male Fertility’ Eds K Bandhauer and J Frick, Springer – Verlag Berlin.

The arterial supply to the testis arises at the level of the second lumbar vertebra from the aorta on the right and the renal artery on the left and these vessels descend retroperitoneally to descend through the inguinal canal forming part of the spermatic cord. The testicular artery enters the testis on its posterior surface sending a network of branches that run deep to the tunica albuginea before entering the substance of the testis (2) . The venous drainage passes posteriorly and emerges at the upper pole of the testis as a plexus of veins termed the pampiniform plexus (Figure 3). As these veins ascend they surround the testicular artery, forming the basis of a countercurrent heat exchange system which assists in the maintenance of a temperature differential between the scrotally placed testis and the intra-abdominal temperature (3) .

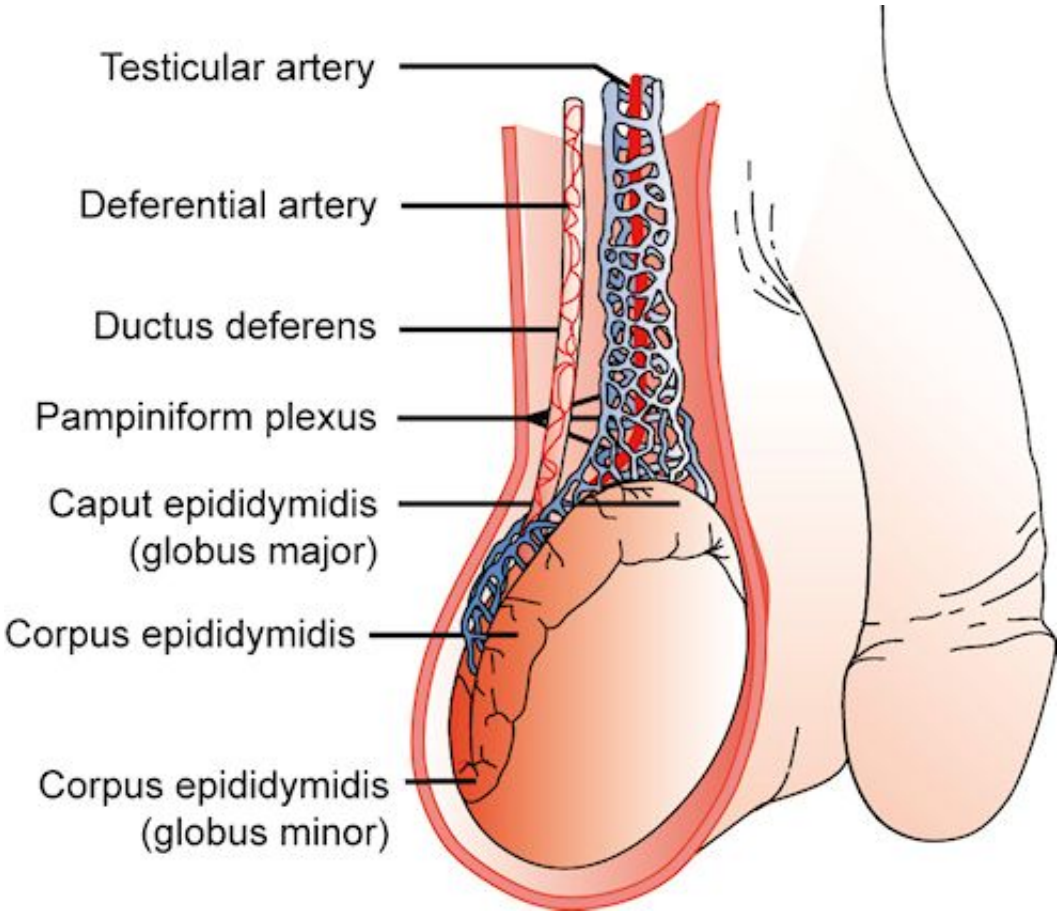

Figure 3. The arrangement of the vasculature of the testis in the region of the distal spermatic cord and testis is shown. Reproduced with permission from de Kretser et.al.1982 in 'Disturbances in Male Fertility' Eds K Bandhauer and J Frick, Springer – Verlag Berlin.

The Distal Reproductive Tract

The vas deferens ascends from the testis on its posterior surface as a component of the spermatic cord passing through the inguinal canal and descends on the posterolateral wall of the pelvis to reach the posterior aspect of the bladder where its distal end is dilated forming the ampulla of the vas (Figure 4). At this site it is joined by the duct of the seminal vesicle, on each side, to form an ejaculatory duct that passes through the substance of the prostate to enter the prostatic urethra. Together with seminal vesicles, the prostate which opens by a series of small ducts into the prostatic urethra, contribute approximately 90-95% of the volume of the ejaculate. During the process of ejaculation, these contents, together with sperm transported through the vas, are discharged through the prostatic and penile urethra. Retrograde ejaculation is prevented by contraction of the internal sphincter of the bladder during ejaculation. Failure of this sphincter to contract results in retrograde ejaculation and a low semen volume.

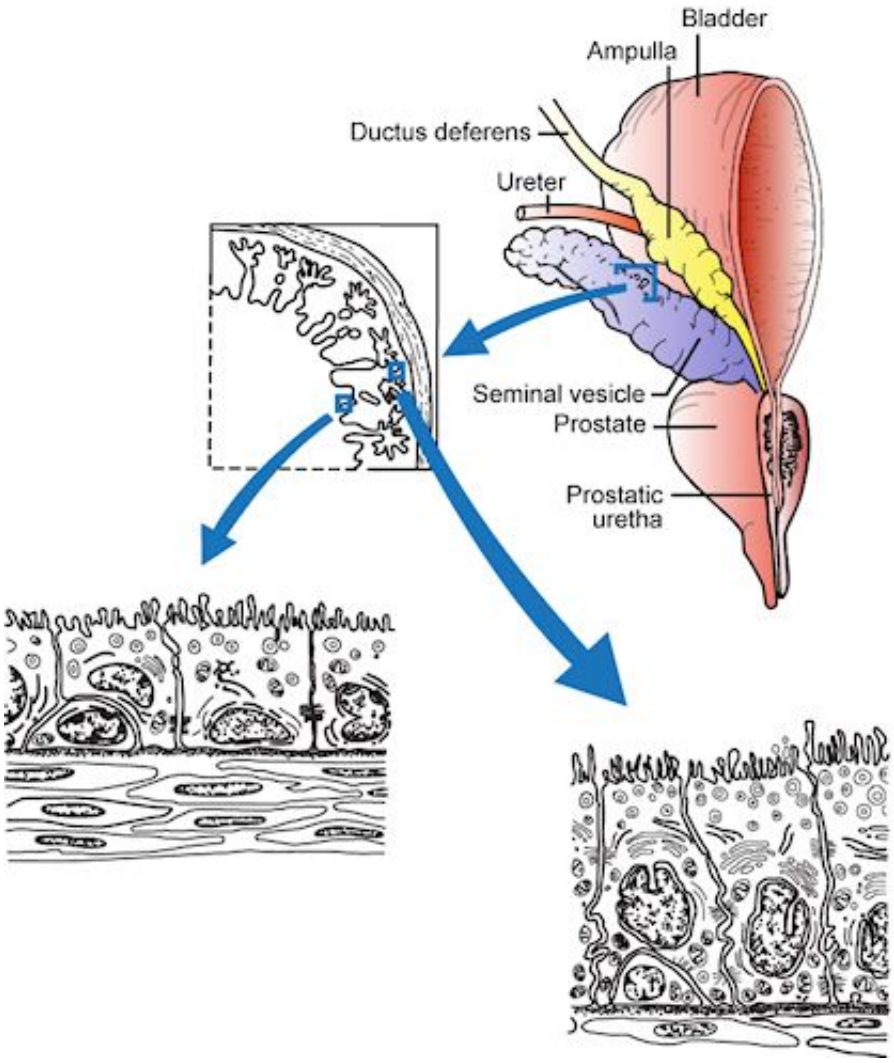

Figure 4. The diagram depicts the relationship between the vas deferens, the seminal vesicles, the posterior aspect of the bladder and the prostate gland. The cytological features of the epithelium of the seminal vesicles is shown: this tissue is androgen dependent. Reproduced with permission from de Kretser et.al.1982 in ‘Disturbances in Male Fertility’ Eds K Bandhauer and J Frick, Springer – Verlag Berlin.

SPERMATOGENESIS

Spermatogenesis represents the process by which precursor germ cells termed spermatogonia undergo a complex series of divisions to give rise to spermatozoa (4 , 5) . This process takes place within the seminiferous epithelium which is a complex structure composed of germ cells and radially oriented supporting cells called Sertoli cells (Figure 5). The latter cells extend from the basement membrane of the seminiferous tubules to reach the lumen. The cytoplasmic profiles of the Sertoli cells are extremely complex as this cell extends a series of processes that surround the adjacent germ cells in an arboreal pattern (5-7) .

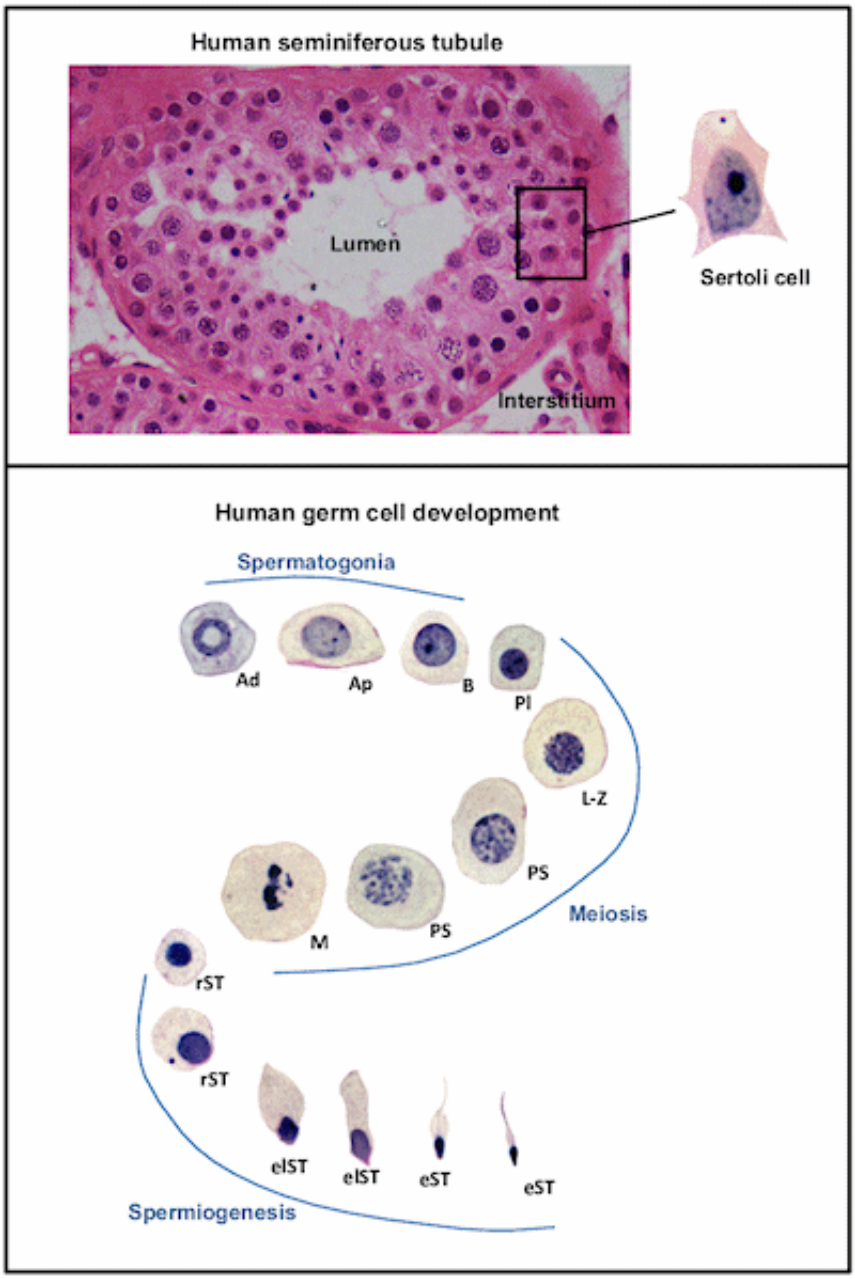

Figure 5. The top panel illustrates the typical structure of the human seminiferous epithelium containing the germ cells and Sertoli cells. The position of Sertoli cell nuclei within the epithelium is indicated, as is the tubule lumen. The tubules are surrounded by thin plate-like contractile cells called peritubular myoid cells. The Leydig cells and blood vessels lie within the interstitium. The bottom panel illustrates the nuclear morphology of the major cell types found within the human seminiferous epithelium, showing the progress of spermatogenesis from immature spermatogonia through meiosis and spermiogenesis to produce mature elongated spermatids. Abbreviations: Ad: A dark spermatogonia, Ap: A pale spermatogonia, B: type B spermatogonia, Pl: preleptotene spermatocyte, L-Z: leptotene to zygotene spermatocyte, PS: pachytene spermatocyte, M: meiotic division, rST: round spermatid, elST: elongating spermatid, eST: elongated spermatid. All germ cell micrographs were taken at the same magnification to indicate relative size. Micrograph of seminiferous epithelium was provided by Dr S. Meachem.

Spermatogenesis can be divided into three major phases (i) proliferation and differentiation of spermatogonia, (ii) meiosis, and (iii) spermiogenesis which represents a complex metamorphosis involved in the transformation of round spermatids arising from the final division of meiosis into the complex structure of the spermatozoon (Figure 5).

Spermatogonial Renewal and Differentiation

Spermatogonia are precursor male germ cells that reside near the basement membrane of the seminiferous epithelium.. Spermatogonial stem cells (SSC) divide to renew the stem cell population and to provide spermatogonia that are committed to the spermatogenic differentiation pathway. Adult mouse and human SSC are pluripotent, and have the ability to differentiate into derivatives of all three germ layers (8 , 9) . During normal spermatogenesis the SSC divide only rarely, however they divide more frequently in response to onslaughts from radiation or chemical exposure, reviewed in (10) .

In general, two main types of spermatogonia, known as Type A and B, can identified in mammalian testes on the basis of nuclear morphology (5) . Type A spermatogonia exhibit fine pale staining nuclear chromatin and are considered to be the undifferentiated spermatogonial population, which also includes the SSC pool. Type B spermatogonia show coarse chromatin collections close to the nuclear membrane (11) and represent the more differentiated spermatogonia that are committed to entry into meiosis (12) . Spermatogonia do not separate completely after mitosis due to incomplete cytokinesis and remain joined by intercellular bridges (13) . These intercellular bridges persist throughout all stages of spermatogenesis and are thought to facilitate biochemical interactions allowing synchrony of germ cell maturation.

In many mammals, the Type A spermatogonia can be divided into several subtypes that represent different phases of proliferation and differentiation. In rodents, the least differentiated spermatogonia are known as $A_{s(single)}$, which divide to produce $A_{pr(paired)}$ which then subsequently divide to produce $A_{al\ (aligned)}$ cells. The A_{al} cells divide and expand to produce chains (or cysts) of spermatogonia, termed $A_{al4\text{-}16}$. The A_{s} cells are considered to include the SSC population however A_{pr} and A_{al} cells can also fragment and recover their stem cell potential (14) . The pioneering technique of spermatogonial transplantation (15-18) together with immunohistochemical approaches in whole mount tissues and transgenic mouse models, see (19) , has allowed the molecular properties of spermatogonial subtypes to be investigated, primarily in rodents. Undifferentiated spermatogonia express a variety of marker proteins, see (10 , 19) , and recent work suggests that SSC specifically express the transcriptional repressor ID4 (20) . The transition from undifferentiated to differentiated spermatogonia is marked by the gain of C-KIT expression and the loss of neurogenenin 3 expression, see (10) .

In humans and other primates, the Type A spermatogonia can only be classified into two subtypes; A dark (Ad) and A pale (Ap) spermatogonia (12) . Some investigators have proposed that the Ad spermatogonia are similar to A_{s} in the rodent, and thus represent the SSC or reserve spermatogonial population (21-23) whereas others have suggested that the Ap spermatogonia are the true stem cell of the testis (24) . More recent studies suggest that Ap

spermatogonia also show characteristics of A_s spermatogonia in rodents, reviewed in (25) , however it remains unclear how primate Type A spermatogonial subtypes relate to those in rodents. Further studies on markers of rodent spermatogonial subtypes, including SSC, and their analysis in primate testes will inform our understanding of primate spermatogonial biology (25) .

Meiosis

Meiosis is the process by which gametes undergo reductional division to provide a haploid spermatid, and in which genetic diversity of the gamete is assured via the exchange of genetic material. During meiosis I, DNA synthesis is initiated, resulting in a tetraploid gamete. The exchange of genetic information is achieved during meiotic recombination, which involves the induction of DNA double-strand breaks (DSBs) during pairing of homologous chromosomes and the subsequent repair of DSBs using homologous chromosomes as templates. Once exchange of genetic material is complete, the cells proceed through two successive reductive divisions to yield haploid spermatids. This process is governed by genetically programmed checkpoint systems.

Meiosis commences when Type B spermatogonia lose their contact with the basement membrane to form preleptotene primary spermatocytes. The preleptotene primary spermatocytes commence DNA synthesis and the condensation of individual chromosomes begins, resulting in the appearance of thin filaments in the nucleus which identify the leptotene stage (26) . At this stage, each chromosome consists of a pair of chromatids (Figure 6). As the cells move into the zygotene stage, there is further thickening of these chromatids and the pairing of homologous chromosomes. The further enlargement of the nucleus and condensation of the pairs of homologous chromosomes, termed bivalents, provides the nuclear characteristics of the pachytene stage primary spermatocyte. During this stage, there is exchange of genetic material between homologous chromosomes derived from maternal and paternal sources, thus ensuring genetic diversity of the gametes. The sites of exchange of genetic material are marked by the appearance of chiasmata and these become visible when the homologous chromosomes separate slightly during diplotene. The exchange of genetic material involves DNA strand breakage and subsequent repair (27) .

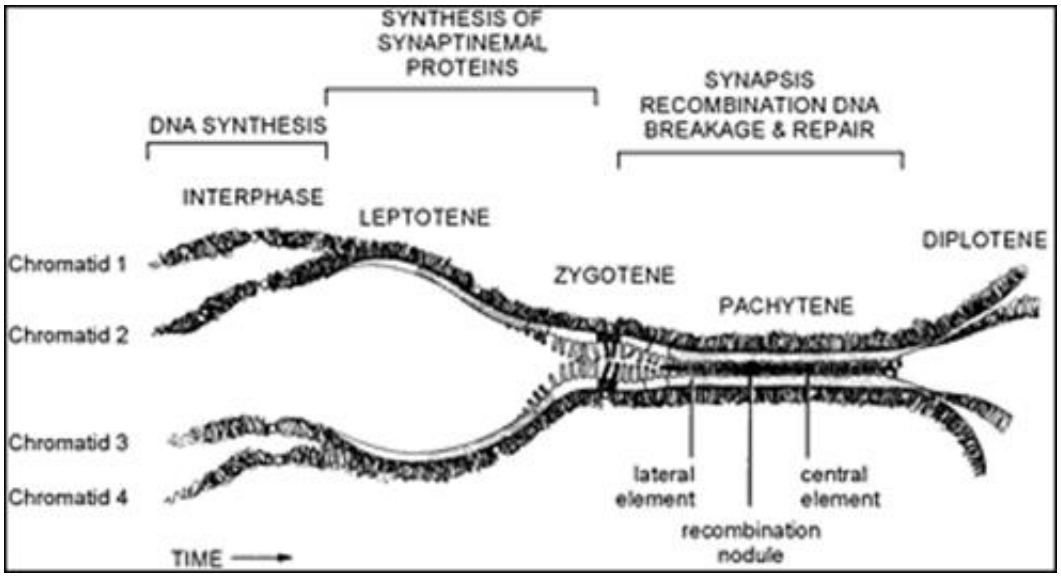

Figure 6. The diagrammatic representation of the events occurring between homologous chromosomes during the prophase of the first meiotic division shows the period of DNA synthesis, the formation of the synaptonemal complex and the processes involved in recombination. Reproduced with permission from de Kretser and Kerr (1994) in “The Physiology of Reproduction” Ed E Knobil & J D Neill, Lippincott, Williams & Wilkins.

The diplotene stage is recognized by partial separation of the homologous pairs of chromosomes that still remain joined at their chiasmata and each is still composed of a pair of chromatids. With dissolution of the nuclear membrane, the chromosomes align on a spindle and each member of the homologous pair moves to opposite poles of the spindle during anaphase. The resultant daughter cells are called secondary spermatocytes and contain the haploid number of chromosomes but, since each chromosome is composed of a pair of chromatids, the DNA content is still diploid. After a short interphase, which in the human represents approximately six hours, the secondary spermatocytes commence a second meiotic division during which the chromatids of each chromosome move to opposite poles of the spindle forming daughter cells that are known as round spermatids (12 , 28) . Meiotic maturation in the human takes about 24 days to proceed from the preleptotene stage to the formation of round spermatids.

Spermiogenesis

The transformation of a round spermatid into a spermatozoon represents a complex sequence of events that constitute the process of spermiogenesis. No cell division occurs, but a conventional round cell becomes converted into a spermatozoon with the capacity for motility. The basic steps in this process (Figure 7) are consistent between all species and consist of (a) the formation of the acrosome (b) nuclear changes (c) the development of the flagellum or sperm tail (d) the reorganisation of the cytoplasm and cell organelles and (e) the process of

release from the Sertoli cell termed spermiation (5 , 29 , 30) .

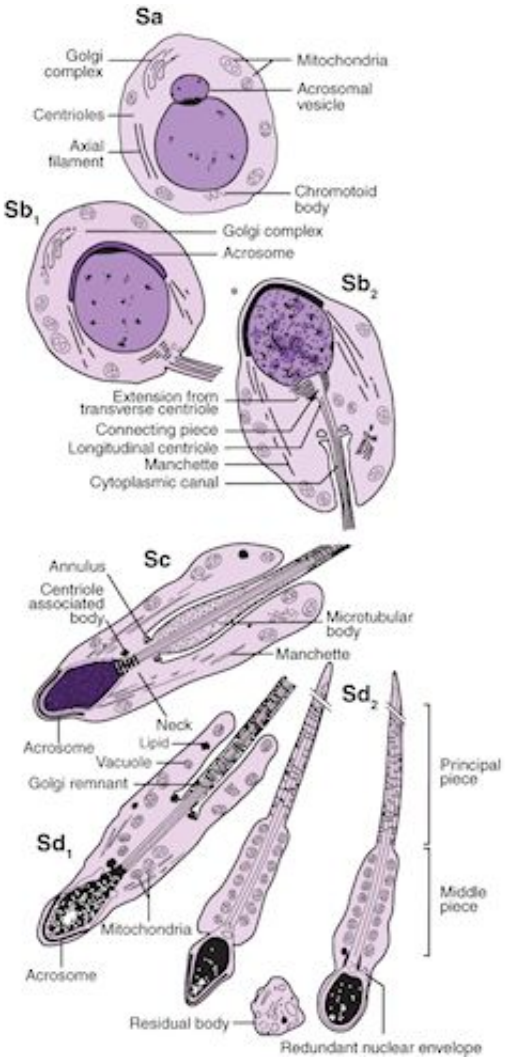

Figure 7. The changes during spermiogenesis involving the transformation of a round spermatid to a mature spermatozoon are shown. Redrawn with permission from de Kretser and Kerr (1994) in “The Physiology of Reproduction” Ed E Knobil & J D Neill, Lippincott, Williams & Wilkins.

The formation of the acrosome commences by the coalescence of a series of granules from the Golgi complex. These migrate to come into contact with the nuclear membrane where they form a cap-like structure which becomes applied over approximately 30-50% of the nuclear surface (29) . Acrosome biogenesis begins early in round spermatid development, and progressively extends as a “cap” over the nucleus as round spermatids differentiate further.

Once the acrosome is fully extended, round spermatids begin what is known as the elongation phase of spermiogenesis. As spermatid elongation commences, the nucleus polarizes to one

side of the cell (Figure 7) and comes into close apposition with the cell membrane in a region where it is covered by the acrosomal cap. Soon after this polarization, the spermatid's chromatin starts to visibly condense, forming progressively larger and more electron dense granules together with a change in the shape of the condensed nucleus. This change in nuclear shape varies significantly between species. The condensation of chromatin is achieved by the replacement of lysine-rich histones with transitional proteins which in turn are subsequently replaced by arginine-rich protamines (31 , 32) . The spermatid chromatin subsequently becomes highly stabilized and resistant to digestion by the enzyme DNAse. Associated with these changes is a marked decrease in nuclear volume and, importantly, the cessation of gene transcription (33) . Therefore, the subsequent spermatid elongation phase proceeds in the absence of active gene transcription, and different mechanisms controlling post-transcriptional gene expression are tightly regulated (see (34)).

At the commencement of spermatid elongation, a complex, microtubule-based structure known as the manchette is formed. The microtubule network originates from a perinuclear ring at the base of the acrosome and extends outwards into the cytoplasm. The manchette is closely opposed to the nuclear membrane, and is thought to participate in nuclear head shaping, perhaps by exerting a force on the nucleus as it progressively moves distally towards the posterior portion of the nucleus (35 , 36) .

The formation of the tail commences early in spermiogenesis in the round spermatid phase, when a filamentous structure emerges from one of the pair of centrioles which lie close to the Golgi complex. Associated with the changing nuclear cytoplasmic relationships, the developing flagellum and the pair of centrioles become lodged in a fossa in the nucleus at the opposite pole to the acrosome. The central core of the flagella's axial filament, called the axoneme, consists of nine doublet microtubules surrounding two single central microtubules, which represents a common pattern found in cilia. This basic structure is modified at the region of its articulation with the nucleus through the formation of a complex structure known as the connecting piece (37) .

Metamorphosis of the flagella proceeds during the elongation phase, as it acquires its characteristic neck region, mid-, principal- and end-pieces (38) . The development of the flagella is thought to involve a mechanism known as Intra-Manchette Transport (IMT), which is proposed to be similar to the Intra-Flagellar Transport (IFT) systems used in other ciliated cells. IMT involves proteins being "shuttled" from the spermatid nucleus down to the developing flagellum via molecular motors travelling along "tracks" of microtubules and filamentous actin (36 , 39) .

The middle and principal pieces contain the mitochondrial and fibrous sheath components, respectively, and include the outer dense fibers. The biochemical characteristics of these components of the sperm tail are emerging (40-45) , reviewed in (38) . While these components provide some structural stability to the tail, recent evidence suggests that they may serve as a molecular scaffold to position key enzymes critical to successful sperm motility. For instance, CatSper 1 is an ion channel plasma membrane-located protein that has been shown to regulate calcium ion fluxes critical for the process of hyperactivation of sperm motility associated with capacitation (46) which is located on the principal-piece. Recent studies

demonstrate that CatSper, or a directly associated protein, is a non-genomic progesterone receptor that mediates the effects of progesterone on sperm hyperactivation and acrosome reaction (47 , 48) . Further studies have shown that plasma membrane calcium-ATPase 1 is also located to the principal-piece and has been shown to be critical for the process of hyperactivation of sperm motility (49) . While these are plasma membrane-located complexes, tpx1 (also called CRISP2), a protein localized to the outer dense fibers of the tail and acrosome (50) has been shown to regulate ryanodine receptor calcium signalling (51) .

The formation of the mitochondrial sheath occurs at the time of the final reorganization of the cytoplasm and organelles of the spermatid (5 , 29 , 52) . The mitochondria that had remained around the periphery of the spermatid aggregate around the proximal part of the flagellum to form a complex helical structure (Figure 8).

The mature elongated spermatids undergo a further complex remodeling during the process of spermiation, which is the process by which the mature spermatids are released from the Sertoli cells prior to their passage to the epididymis, see (53) for review. This remodeling includes the removal of specialized adhesion junctions that have ensured tight adhesion of the spermatid to the Sertoli cell during its elongation process, further remodeling of the spermatid head and acrosome and removal of the extensive cytoplasm to produce the streamlined spermatozoan. The cytoplasm of the spermatid migrates to a caudal position around the tail and is markedly reduced in volume. Some observations suggest that prolongations of Sertoli cell cytoplasm send finger-like projections which invaginate the cell membrane of the spermatid cytoplasm and literally ‘pull’ the residual cytoplasm off the spermatid (29) . The remnants of the spermatid cytoplasm form what is termed the residual body. The residual bodies contain mitochondria, lipid and ribosomal particles and are phagocytosed and moved to the base of the Sertoli cell where they are broken down by lysosomal mechanisms. The morphological features of spermiation are relatively conserved between species, particularly among mammals (54) . Spermiation is highly susceptible to perturbation by pharmacological modulators and by agents that suppress gonadotropins, reviewed in (53) .

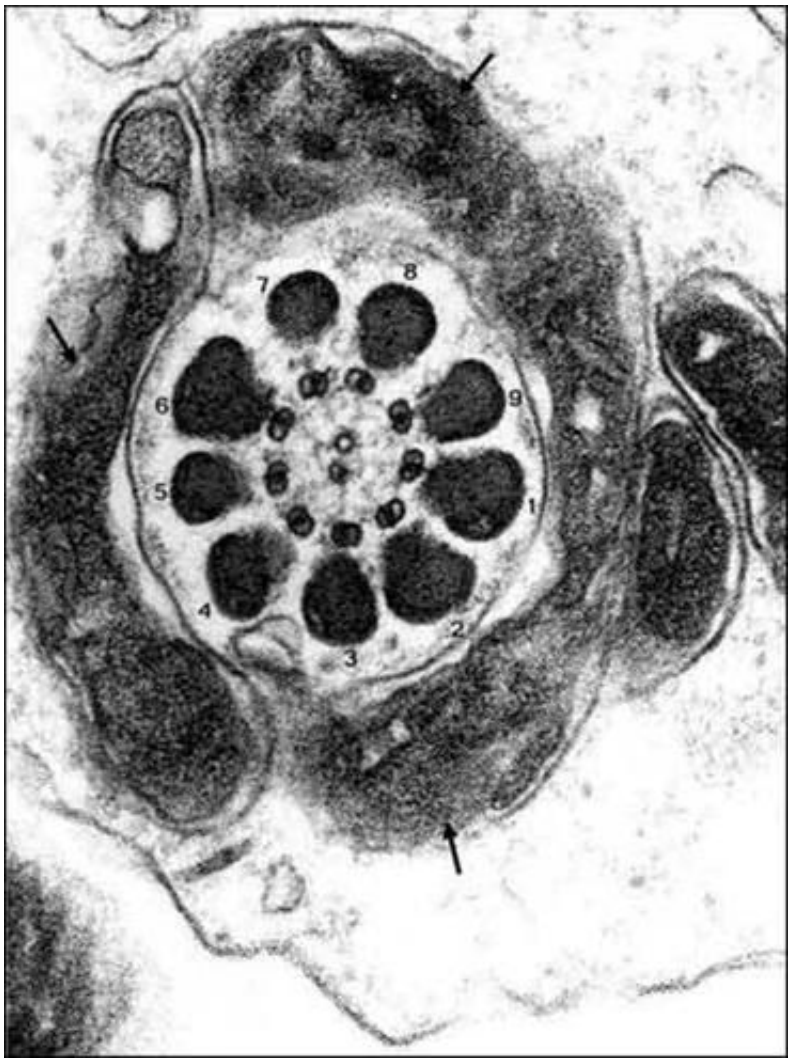

Figure 8. A cross-section through the developing mid-piece of the sperm tail shows the aggregation of mitochondria (arrows) surrounding the outer dense fibres (labelled 1-9) which in turn surround the axoneme composed of 9 doublet microtubules surrounding two central microtubules. Reproduced with permission from “Visual atlas of human sperm structure and function for assisted reproductive technology” Ed A.H. Sathanathan 1996.

The Cycle of the Seminiferous Epithelium

Within the seminiferous epithelium, the cell types that constitute the process of spermatogenesis are highly organized to form a series of cell associations or stages. These cell associations, or stages of spermatogenesis, result from the fact that a particular spermatogonial cell type when it appears in the epithelium is always associated with a specific stage of meiosis and spermatid development. The cycle of the seminiferous epithelium was defined by LeBlond and Clermont (55) , as the series of changes in a given area of the seminiferous tubule between two appearances of the same developmental stage or cell association. They defined

14 stages in the rat cycle based on the 19 phases of spermiogenesis as identified by the periodic acid Schiff (PAS) stain (Figure 9). In effect, if it was possible to observe the same region of the seminiferous epithelium by phase contrast microscopy over time, the appearance would progress through the 14 stages before stage I reappeared. They also demonstrated that the duration of any one stage was proportional to the frequency with which it was observed in the testis. As type A spermatogonia in any one area of the epithelium progress through meiosis and spermiogenesis to become spermatozoa, the specific area of the tubule would pass through the 14 stages four times. In each progression, the progeny of the spermatogonia progressively move toward the lumen of the tubule.

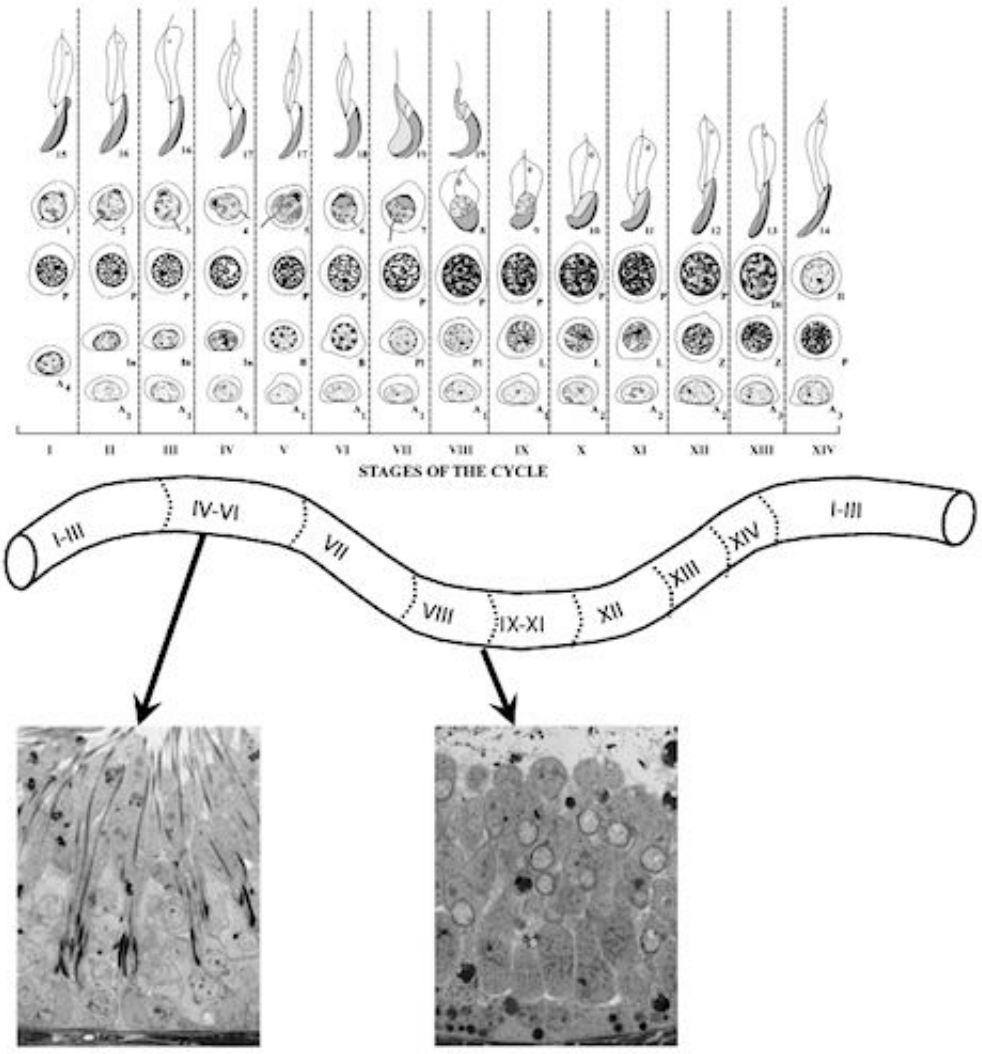

Figure 9. This is a diagrammatic representation of the stages of the seminiferous cycle in the rat and shows the types of germ cell associations which form the stages. The most mature spermatids are shed at stage VIII and this is reflected by the alteration in the transillumination patterns seen in the tubules (central representation of the tube). The stages at which proliferative events occur are shown as well as some key physiological events.

Studies in many mammalian species demonstrated that the cycle of spermatogenesis could be identified for each species but showed that the duration of the cycle varied for each species (12) . In many species, especially the rat, the same stage of spermatogenesis extends over several millimetres of the adjacent tubule and it is possible, by observation under transillumination, to dissect lengths of seminiferous tubules at the same phase of spermatogenesis (56) . Such observations amply confirmed the earlier studies of Perey and colleagues (57) , that the stages of spermatogenesis were sequentially arranged along the length of the tubule (Figure 10). As the cycle progress, this arrangement resulted in a “wave of spermatogenesis” along the tubule. Regaud (58) interpreted his observations correctly by the statement “the wave is in space what the cycle is in time”.

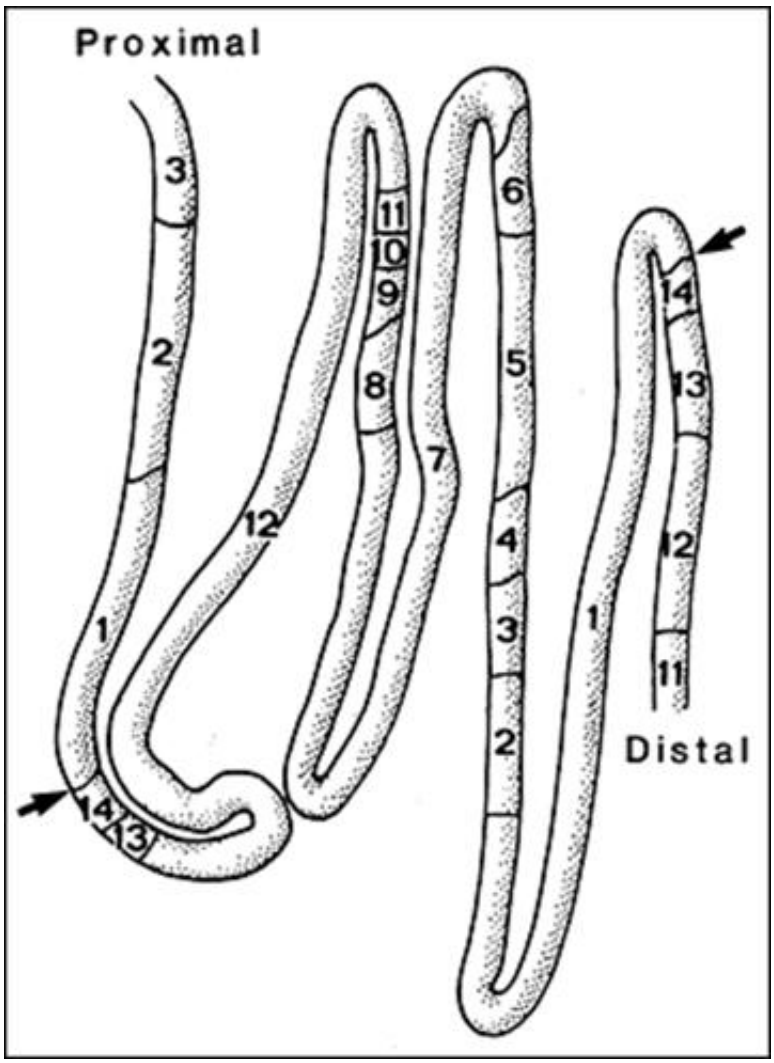

Figure 10. The pattern of the stages of spermatogenesis as they occur along the tubule are shown. Data based on Perey et. al. (30). Reproduced with permission from de Kretser and Kerr (1994) in “The Physiology of Reproduction” Ed E Knobil & J D Neill, Lippincott, Williams & Wilkins.

For many years, investigators believed that such a cycle did not occur in the human testis but the careful studies of Clermont (59) showed that human spermatogenesis could be subdivided into 6 stages. However unlike the rat, each stage often only occupied one quadrant of a tubule giving the disorganized appearance. By careful studies using tritiated thymidine injections into the testis, Clermont and Heller (28) demonstrated that the duration of the cycle in the human took 16 days and the progression from spermatogonia to sperm took 70 days or four and a half cycles of the seminiferous cycle. Other studies showed that the cycle length was specific for each species (eg rat 49 days) and the progression of each cell type in spermatogenesis involved a defined duration (12). It is likely that the relatively poor definition of stages in human seminiferous tubules compared to the rat, is due to a greater number of spermatogonia entering each phase of the cycle in the rat, their cell progeny therefore occupying a greater length of the tubule.

Recent transcriptional profiling studies described the changing patterns of gene expression across the rat spermatogenic cycle, and demonstrated that Sertoli cells and germ cells showed highly co-ordinated stage-dependent changes in gene expression (60) . The mechanisms underlying these temporal constraints on spermatogenesis have been the subject of speculation as to whether these were intrinsic or were imposed by the Sertoli cells. The latter observation is supported by the demonstration that when rat germ cells were transplanted into the mouse testis, spermatogenesis proceeded at the normal rate for the rat, indicating that the kinetics of the spermatogenic cycle are determined by intrinsic mechanisms within germ cells (61) . In contrast however, Sertoli cells demonstrate cyclic expression of certain proteins in the embryonic and pre-pubertal period, even in the absence of germ cells (62) . Taken together, these observations suggest that the Sertoli cell contains a “clock” that modulates cyclic gene and protein expression, but that the precise timing of this clock can be modulated by germ cells.

THE ROLE OF THE SERTOLI CELL IN SPERMATOGENESIS

As indicated earlier, the Sertoli cells have an intimate physical relationship with the germ cells (Figure 11) during the process of spermatogenesis (5 , 7 , 63) . The cytoplasmic extensions that pass between the germ cell populations surrounding the Sertoli cell provides structural support through a microfilament and microtubular network present in the cytoplasm of the Sertoli cell (64) . This architecture is not static but changes in the tubule depending on the stage of the spermatogenic process. Additionally, the Sertoli cells have a role in regulating the internal environment of the seminiferous tubule. This arises from specialized inter-Sertoli cell junctions which are formed at the sites where processes of Sertoli cell cytoplasm from adjacent cells meet (65) . The junctions are predominantly located at the baso-lateral regions of the cell and form occluding-type junctions. As a result of these junctional specializations, intercellular transport between the Sertoli cell and spermatogonia is possible but does not extend to the more centrally placed germ cells that are effectively sequestered from the extra-tubular environment by the tight occluding junctions. The physiological counterpart of these anatomical structures is known as the blood testis barrier that regulates the entry of a variety of substances into the central compartment of the seminiferous tubule (66) . The siting of the cell junctions

effectively divides the seminiferous epithelium into a basal compartment which contains the bases of the Sertoli cells and spermatogonia and an adluminal compartment containing the central regions of the Sertoli cell and the other germ cell types. It is clear that as Type B spermatogonia migrate from the basement membrane of the tubule into the adluminal compartment, these tight junctions must open up to allow this cellular migration to take place (Figure 12) and reform beneath the Type B spermatogonia which have now left the basement membrane to form preleptotene spermatocytes. Recent studies have indicated that the formation and dissolution of these junctional specializations are under the control of numerous physiological regulators including endocrine (67 , 68) and paracrine (69) factors.

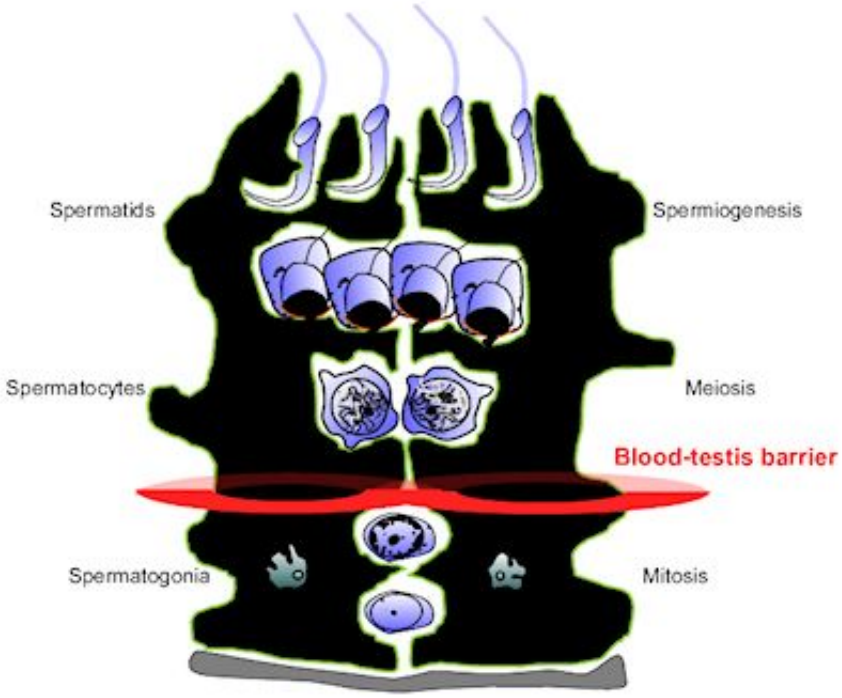

Figure 11. The general architecture of the Sertoli cell is shown. Note the thin cytoplasmic processes that extend between the germ cells. The Sertoli cell is in contact with a variety of germ cells and adjacent Sertoli cells when three dimensional perspectives are considered.

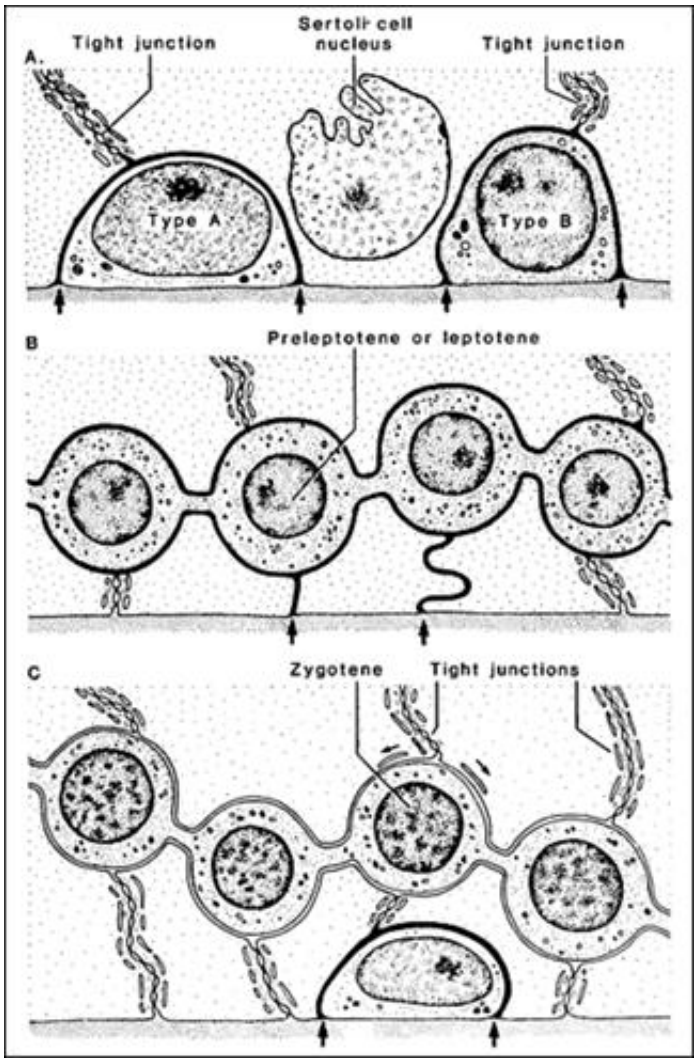

Figure 12. The relationship between the Sertoli cells and spermatogonia as they proceed through the leptotene and zygotene stages of meiosis is shown. Note that the tight inter-Sertoli cell junctions “open up” to allow germ cells to move from the basal to the adluminal compartments.

These physical and biological relationships between Sertoli cells and germ cells is reflected in observations that have led to the conclusion that the number of Sertoli cells in a testis determines the ultimate spermatogenic output (70-73) . There is considerable data to indicate that experimental manipulation of Sertoli cell number by such agents as FSH or the induction of neonatal hypothyroidism can increase Sertoli cell number and increase the spermatogenic output of the testis. These observations suggest that there are a maximum number of germ cells that can be supported by an individual Sertoli cell. Whether this is a functional or physical relationship is unclear. As discussed later in this chapter, the endocrine regulation of spermatogenesis by androgens and FSH is dependent on the expression of receptors within Sertoli cell, rather than in the germ cells.

ROLE OF THE PERITUBULAR MYOID CELLS

External to the basement membrane of the seminiferous tubule, there are several layers of modified myofibroblastic cells termed myoid cells (74 , 75) . These cellular layers are responsible for the irregular contractions of the seminiferous tubules which propel fluid secreted by the Sertoli cells, together with testicular spermatids into the lumen and through the tubular network to the region of the rete testis. These cells also participate in regulating processes within the seminiferous tubules by the production of growth factors such as activin A and platelet derived growth factors (76 , 77) . Recent studies on transgenic mice in which androgen receptor (AR) was specifically ablated from most peritubular myoid cells revealed that these cells influence Sertoli cell number, function and ability to support germ cell development (78) , as well as Leydig cell development and steroidogenesis (79) .

Cellular Processes in Steroidogenesis

The Leydig cells lie within the intertubular regions of the testis and are found adjacent to blood vessels and the seminiferous tubules (5 , 80) . They are the cell type responsible for testosterone production which is essential for the maintenance of spermatogenesis. There are very significant organizational differences in the intertubular tissue betweens species reflecting the number of Leydig cells and differing architecture involving blood vessels and lymphatic sinusoids (81) . Additionally, fibroblasts and macrophages and small numbers of mast cells are found in the intertubular regions of the testis (82 , 83) .

In most species there are two generations of Leydig cells (84 , 85) . A fetal generation under the stimulation of hCG results in the production of testosterone during gestation (86) . These cells in the human, decrease in number towards term and degenerate and are lost from the intertubular region at about twelve months of age (87) . The adult generation of Leydig cells in the human results from LH stimulation commencing at the time of puberty. This generation arises by division and differentiation of mesenchymal precursors under the influence of LH (88) .

The Leydig cells have the capacity to synthesize cholesterol from acetate or to take up this substrate for steroidogenesis from lipoproteins (80 , 89) . Typical of any steroid secreting cell, the Leydig cell contains abundant smooth endoplasmic reticulum and mitochondria which have tubular cristae which are unique to steroidogenic cells. The enzymes required for steroidogenesis are located in the mitochondria and in endoplasmic reticulum requiring intracellular transport of substrates between these organelles to achieve successful androgen production.

INTRINSIC CONTROL SYSTEMS

The spermatogenic process is controlled by both classic endocrine mechanisms as well as by intrinsic mechanisms that are mediated by growth factors, cytokines and other molecules (5 , 6 , 90) . It is not possible within this chapter to provide a full description of all of these processes, some of which are still unclear. This section provides the reader with an understanding of some

examples of these intrinsic processes over which external hormonal mechanisms exert important control. In some cases the manner in which hormones influence these intrinsic mechanisms remain unclear.

Pivotal in our understanding of intrinsic mechanisms is the central role played by the Sertoli cell. As discussed earlier, the Sertoli cell, through the formation of the blood-testis barrier divides the seminiferous epithelium into a basal and adluminal compartment and limits intercellular transport to the centrally placed germ cells (65) . These cells therefore control the environment in which all germ cells other than spermatogonia develop. By modulating entry of substances into the adluminal compartment, the Sertoli cells are required to provide such factors as substrates for glycolysis (91-93) . Several studies have established that lactate rather than glucose is the preferred substrate for glycolysis in primary spermatocytes and lactate is generated from glucose by Sertoli cells under the influence of FSH. The production of lactate leads to changes in the pH resulting in the alterations of the processing of stem cell factor from a soluble to a cell bound form, thereby influencing its action on spermatogonial stimulation (94) .

Further examples of the involvement of Sertoli cells arise from their ability to produce locally, a range of proteins which are essential for spermatogenesis but cannot gain access to the seminiferous epithelium because of the blood-testis barrier. For instance, testicular transferrin is an iron binding protein which is a secretory product of the Sertoli cell that is regulated by FSH and delivers iron to primary spermatocytes through a receptor-mediated endocytotic process (95 , 96) . Another example is the copper binding protein, ceruloplasmin that is involved in the delivery of copper to the germ cells (97) . All cells require iron to maintain respiration and cytochrome function, and copper is required as a coenzyme for a number of proteins.

There is ample evidence to document the importance of vitamin A in spermatogenesis, reviewed in (98 , 99) While the retinoid binding proteins are localised in Sertoli cells themselves, some evidence suggests that there may be a direct effect on the germ cells (100) further supported by the disrupted sperm production in mice with targeted inactivation of the retinoic acid receptor (101) , reviewed in (98 , 99) .

There are many more examples of the cooperative linkages between Sertoli cells and germ cells and numerous reviews can provide detailed information (6 , 7 , 90 , 102) . The close association between germ cells and Sertoli cells is reflected by the capacity for germ cells to influence the metabolic activity of the Sertoli cells. This view can be illustrated by the cyclic changes in Sertoli cell function that occur during the stages of the cycle of spermatogenesis as shown by studies, on rats, involving transillumination of the seminiferous tubules to enable stage specific segments of tubules (Figure 9, see above) to be dissected for metabolic experiments (56) . This approach has revealed the remarkable stage-specificity in the expression patterns of many Sertoli cell genes (60) . Furthermore, the metabolic activity of the Sertoli cell is influenced by the populations of germ cells that are found at a particular stage of the seminiferous cycle (103-105) . For example maximal transferrin production occurs at stages X to XIV (106) , while the greatest concentrations of androgen binding protein is found at stages VII-VIII (107) . Some of these data can be linked to pathophysiological studies; for instance the greatest concentrations of androgen receptors are found at stages VII-VIII (108) ,

which are the stages that show maximal apoptosis when the testis is exposed to androgen withdrawal induced by treating rats with the Leydig cell cytotoxin, ethane dimethane sulphonate (109) .

It is now clear that the number of Sertoli cells in the testis determines the total sperm output of the testis, reviewed in (110) , as emphasized by studies showing that the perinatal induction of hypothyroidism extends the duration of Sertoli cell proliferation, which in turn leads to increased Sertoli cell numbers and increased sperm output of the adult testis (70 , 73) . Other Sertoli cell mitogens such as FSH and activin (111 , 112) , together with thyroxine can also exert significant changes in the number to Sertoli cells in the testis depending on the temporal pattern of their secretion. The latter must occur before the cessation of Sertoli cell proliferation. In the rat, this occurs at about 20 days whereas in the human, Sertoli cells cease to divide during the pubertal process (113) . It is possible that the failure of many men with hypogonadotropic hypogonadism to achieve normal testicular size and normal sperm counts, when treated by gonadotropic stimulation, may result from abnormal Sertoli cell proliferation during fetal and prepubertal life resulting in a decreased Sertoli cell complement (114) .

The delay in germ cell maturation that occurs when perinatal hypothyroidism is induced to extend the duration of Sertoli cell proliferation suggests that there is a link between the degree of “maturity” of the Sertoli cell and its capacity to support spermatogenesis (73 , 115 , 116) . Alternatively, the continuing proliferation of Sertoli cells in this state prevents the formation of the occluding tight junctions between processes of adjacent Sertoli cells and the consequent development of the blood testis barrier. Despite the profound delay in germ cell development during the hypothyroid state, when the hypothyroidism is reversed, spermatogenesis resumes and produces an augmented total sperm output.

Given that there is increasing evidence that a range of cytokines and growth factors are important regulators of spermatogenesis through a variety of mechanisms (see reviews (90 , 117)), the delay in spermatogenic development in the hypothyroid model may be a reflection of the inability of the Sertoli cells in that state to supply the necessary cytokines and growth factors at specified times. The production of these proteins by the Sertoli cells is closely linked to specific stages of the spermatogenic cycle, implying but not proving that they are required to modulate yet to be defined mechanisms during spermatogenesis.

These mechanisms are likely to be involved in the expansion and survival of germ cell populations since the successful production of a normal sperm output, several germ cell populations must proliferate during mitosis and meiosis, and differentiate during spermiogenesis. There is no doubt that these events are influenced by the hormones discussed earlier but emerging data also implicate locally produced growth factors and cell survival molecules that influence the process of apoptosis.

Spermatogonia

In the neonatal period, the migration and proliferation of the primordial germ cells and the subsequent pre-spermatogonia (gonocytes) represents a crucial step in the establishment of spermatogenesis (118 , 119) . This process is dependent on the interaction of stem cell factor

(SCF) produced by the Sertoli cells and the receptor, c-KIT, which is located on germ cells, particularly spermatogonia. Recent studies suggest that the production of a membrane bound form of SCF by alternative splicing may be essential for spermatogonial multiplication (119) . The production of the membrane-bound form is stimulated by a decrease in pH facilitated by the generation of lactate by Sertoli cells, an essential element in facilitating the survival of primary spermatocytes whose preferred substrate for glycolysis is lactate (92) . This is an example of the close interaction between Sertoli cells and germ cells both in the production of growth factors and the interplay of their metabolic pathways.

Spermatogonia and SSC reside within a specialized microenvironment within the testis known as the “niche”, where the balance between SSC renewal and differentiation is regulated. This niche is comprised of cells, extracellular matrix and soluble factors that regulate the functions of cells within the niche. The number and function of stem cell niches within the seminiferous epithelium is directly dictated by the Sertoli cell (120) . Sertoli cells secrete Glial-cell line Derived Neurotrophic Factor (GDNF) which acts on receptors on undifferentiated spermatogonia to control differentiation and self-renewal of SSC (121-125) . Sertoli cells can also regulate the stem cell niche via the expression of the *Etv5* gene and by mediating FGF9 responses, reviewed in (19) . Colony stimulating factor (Csf1), expressed by the surrounding peritubular myoid cells and Leydig cells, has been demonstrated to regulate SSC self-renewal (126) , highlighting the likely role of other surrounding somatic cells to the regulation of the spermatogonia and the stem cell niche. In summary, the regulation of SSC and spermatogonial commitment to the spermatogenic differentiation pathway is reliant on interactions with the surrounding somatic cells.

Meiosis

Many studies, including those in transgenic mouse models, have identified key proteins necessary for the completion of male meiosis, see (127) . Targeted gene disruption approaches have also identified sexually dimorphic meiosis-associated proteins, suggesting different levels of checkpoint control between males and females, particularly in terms of chromosome recombination and homologous pairing, see (128) .

Failure of normal meiotic recombination events is related to an increased incidence of gamete aneuploidy, which has a higher incidence in infertile men compared to case controls, reviewed in (129) . Many proteins have been shown to be essential for male meiotic recombination events, including those involved in synaptonemal complexes and DNA repair mechanisms, reviewed in (127 , 129) . For example, genetic ablation of the DNA repair protein PMS2 results in very few synaptonemal complexes forming and improper homologous chromosome pairing (130) . Meiosis is not arrested however, and some abnormal sperm are produced (130) . The induction of spermatocyte apoptosis and arrest at the spermatocyte phase is commonly observed in other transgenic models in which the expression of other meiotic recombination proteins is perturbed , reviewed in (127) .

Many proteins are required for male meiotic division, see (127) . For example, the testis-specific heat shock protein, HSP 70-2, is essential for male meiosis. It is required for desynapsis of the synaptonemal complexes and for the activation of CDC2 to form the active CDC2/cyclin

B1 complex to enable progression into the first meiotic division (30 , 131) . The ability of HSP 70-2 to activate CDC2 is regulated by the interaction with a testis-specific linker histone chaperone, tNASP (132) .

Recent studies have shown that Mybl1 is likely a key regulator of male meiosis (133) . Mybl1 is a DNA binding protein that acts as transcriptional regulator. A point mutation in Mybl1 in mice causes spermatocyte arrest, aberrant chromosome synapsis, defects in DSB repair and abnormal cell cycle progression. Chromatin immunoprecipitation experiments revealed that Mybl1 likely directly targets various genes involved in different aspects of meiosis, suggesting that Mybl1 is a “master” transcriptional regulator of male meiosis (133) .

Spermiogenesis

As discussed earlier in this chapter, the steps in the formation of a sperm from its precursor, the haploid round spermatid, represent a fascinating process in cell biology. The development of the sperm tail, the remarkable nuclear changes involving the condensation and complexing of DNA and the changes in the relative positions of the nucleus, cell organelles and the cytoplasm, all pose innumerable questions as to how these events are controlled. Many proteins have been implicated in the control of these specific cellular processes, as demonstrated by the various spermiogenic abnormalities in transgenic mouse models, reviewed in (34 , 38) .

The intrinsic and tightly-regulated control of gene transcription and translation is especially important for the complex cellular differentiation occurring during spermiogenesis. Haploid spermatids, as well as meiotic spermatocytes, express many unique genes that are not expressed in somatic cells (134) . In addition, alternative splicing is highly prevalent in the testis, and generates many germ cell-specific transcripts likely important for carrying out the ordered procession of germ cell development (135) . One example of the importance for alternative splicing in spermiogenesis is the CREM gene, whereby the use of alternative polyadenylation mechanisms regulates the expression of either repressor or activator forms of the CREM transcription factor (136) .

Further studies on the CREM transcription factor have detailed unique mechanisms of transcriptional regulation during spermiogenesis, reviewed in (137) . CREM is a master regulator of the transcription of many genes involved in haploid spermatid development. The activation of CREM target genes is influenced by CREM binding to a spermatid-specific co-activator protein known as ACT. The localization of ACT in the nucleus of spermatids is controlled by a kinesin, whereby the kinesin effectively exports ACT out of the nucleus at certain stages thus inhibiting CREM-dependent gene transcription (138) . These studies reveal sophisticated and unique mechanisms governing the control of gene transcription during spermiogenesis.

As mentioned earlier, developing spermatids lose their ability to perform active gene transcription as their DNA is remodeled into the compact sperm nucleus. Therefore the post-transcriptional and translational control of gene expression is particularly important in spermatids. All mRNA transcripts expressed in meiotic and post-meiotic germ cells are subject to translational repression and there are many examples whereby genes transcribed earlier on

in germ cell development are translationally repressed until the proteins are required during spermatid elongation, reviewed in (139) .

Recent studies have indicated that small RNA species play an essential role in controlling post-transcriptional gene expression during spermiogenesis. These small RNAs include small interfering RNA (siRNA), micro RNA (miRNA) and piwi-interacting RNA (piRNA), each of which have specific modes of action and roles in spermatogenesis, reviewed in (140) . miRNAs result in either the degradation or translational repression of their target mRNA transcript, reviewed in (141) . miRNAs are produced and processed via the actions of the enzymes DROSHA and Dicer, both of which are required in the testis for male fertility, see (141) . Spermiogenesis is impaired when DROSHA and Dicer are ablated from postnatal germ cells (142) and a number of miRNAs are preferentially expressed in germ cells, including in spermatids and spermatozoa (143) . These studies highlight an essential requirement for miRNA species in spermiogenesis.

Some aspects of small RNA processing and mRNA translational regulation may occur within the chromatoid body. This is a spermatid-specific cellular organelle that moves around the perinuclear region of the round spermatid, periodically “delivering” factors to and from the pores on the nuclear surface to the spermatid cytoplasm (144) . The chromatoid body contains polyA-containing RNA molecules and is suggested to be involved in the regulation of unique mRNAs required for spermiogenesis, reviewed in (144 , 145) . The chromatoid body also contains piRNAs and is enriched with proteins involved in piRNA-mediated RNA regulation, suggesting that this organelle may contribute to the control of haploid gene expression by small RNAs (145) .

The proper development of the sperm flagella is essential for sperm motility and hence fertility. Many proteins are now known to be required for flagella development and motility, reviewed in (38) . Even structurally normal sperm can fail to move as shown by the genetic inactivation of the gene encoding a sperm calcium ion channel (146) . Mutations in a number of genes required for assembly of the axoneme, such as dyenin, are associated with a syndrome known as Primary Ciliary Dyskinesia (PCD). PCD is associated with a range of pathologies, including male infertility, and is caused by a failure of proper development and function of cilia in various organs, including the sperm flagellum (147) . The identification of the molecular mechanisms governing flagellar development and motility is important for the development of new therapies for male infertility.

Cell survival mechanisms

It has been recognized for some time that germ cells degenerate at various stages during spermatogenesis however this occurs by apoptosis rather than necrosis. These apoptotic processes result in the removal of germ cells from the testis during the onset of spermatogenesis as well as under a variety of hormonal manipulations, see (148) for review. The requirement for apoptosis during the onset of spermatogenesis was highlighted by studies of the inactivation of the gene encoding bcl-w, a cell survival molecule (149-151) . These studies showed that the pubertal wave of spermatogenesis almost reached completion but the entire process collapsed rendering homozygous male mice infertile with an ultimate phenotype of Sertoli cell only tubules. Further studies of the reasons for the successful completion of the

pubertal wave of spermatogenesis showed that while bcl-w was one of several pro-survival molecules expressed in the seminiferous epithelium during the onset of spermatogenesis, it was the only pro-survival protein expressed in the adult (152) .

The apoptosis of particular germ cell types during testosterone and/or FSH suppression is an important response of the testis to gonadotropin suppression, reviewed in (153) . The suppression of either testosterone or FSH, or both, induces germ cell apoptosis, in particular subtypes of spermatogonia, spermatocytes and round spermatids, and apoptosis can be mediated via the extrinsic or intrinsic apoptotic pathways. The prevention of germ cell apoptosis is therefore an important mechanism by which the hormones testosterone and FSH support spermatogenesis (see below).

In a recent review, Young and Nelson (154) emphasized the role of apoptosis in the mediation of seasonal testicular regression. This data is consistent with the increase in apoptosis demonstrated in the testis following FSH withdrawal by passive immunization or in hypophysectomized rats (155 , 156) . In their summary of potential mechanisms, Young and Nelson (154) proposed two options (a) Withdrawal of support by Sertoli cells for germ cells leading to alteration in the balance of Bcl-2 family members which in turn activates the caspase cascade (b) Activation of death pathways through the binding of Fas ligand to its receptor Fas (157 , 158) .

CONTROL OF TESTICULAR FUNCTION

In the earlier part of this Chapter, the process of spermatogenesis and the organization of the seminiferous epithelium have been discussed together with intrinsic mechanisms that are crucial to establish successful spermatogenesis. These intrinsic mechanisms are subjected to external influences exerted through the pituitary gonadotrophins follicle stimulating hormone (FSH) and luteinizing hormone (LH). These two hormones together with testosterone, produced by the Leydig cells in the intertubular regions of the testis are important for successful spermatogenesis. The secretion of the gonadotrophins FSH and LH are regulated by the episodic secretion of gonadotrophin releasing hormone (GnRH) produced in the hypothalamus. There is now a substantial body of evidence that indicates that the kisspeptins, a family of neuropeptides are key upstream regulators of GnRH secretion (159) . For instance, arcuate kisspeptin-neurokinin B-dynorphin expressing hypothalamic neurons are critically involved in the increase in gonadotropin secretion that occurs after gonadectomy (160) . The regulation is further complicated by the isolation and characterization of gonadotropin- inhibitory hormone (GnIH), which acts both upstream of GnRH and also may operate at the levels of the gonads as an autocrine/paracrine regulator of steroidogenesis (161 , 162) .

The pituitary secretion of FSH and LH by the gonadotropes is also controlled by the feedback inhibition that occurs via the steroids, testosterone and estradiol, as well as the protein inhibitors, inhibin, secreted by the gonads and follistatin, produced locally within the pituitary by the follicular-stellate cells (163) . Follistatin exerts its inhibition of FSH secretion by its capacity to bind and block the actions of the activins A and B, the latter locally produced by the pituitary gland (164) . Further details of this system are considered elsewhere in this text. Through the

stimulation of the Leydig cells by LH, testosterone is produced locally within the inter tubular regions of the testis in high concentrations and exerts a very important influence on spermatogenesis. This section of the Chapter is divided into two parts (1) the production of testosterone and its control, (2) the control of spermatogenesis.

Control of Testosterone Production

Testosterone is the major androgen secreted by the Leydig cells found in the inter-tubular spaces of the testis. These cells arise from mesenchymal precursors and studies in the rat have identified that these precursors express the platelet-derived growth factor-α but not 3β hydroxysteroid dehydrogenase (165) . Further, they suggest that many of these precursors are situated in proximity to the external surfaces of the seminiferous tubules. A normal male produces approximately 7 mg testosterone daily but also produces lesser amounts of weaker androgens such as androstenedione and dihydroepiandrosterone. In addition to testosterone, through the actions of the enzyme 5α reductase, dihydrotestosterone is produced by the testis in smaller amounts. The testis also contributes approximately 25% of the total daily production of 17βestradiol through the local action of the enzyme aromatase which converts androgenic substrates to this estrogen (166) . The remainder of the circulating estradiol is produced by the adrenal and peripheral tissues through the actions of aromatase.

Cholesterol represents the major substrate for androgen production by the Leydig cells and is derived by an uptake mechanism involving the binding of circulating low density lipoprotein to specific receptors on Leydig cells which, following internalisation provides a significant source of cholesterol (167 , 168) . In addition, the Leydig cells are able to undertake de novo synthesis of cholesterol from acetate and relative contributions of these two sources is partly dependent on species and the state of stimulation of the Leydig cells. The conversion of cholesterol to testosterone involves a number of steps that are catalyzed by enzymes, predominantly belonging to cytochrome P450 family (Figure 13).

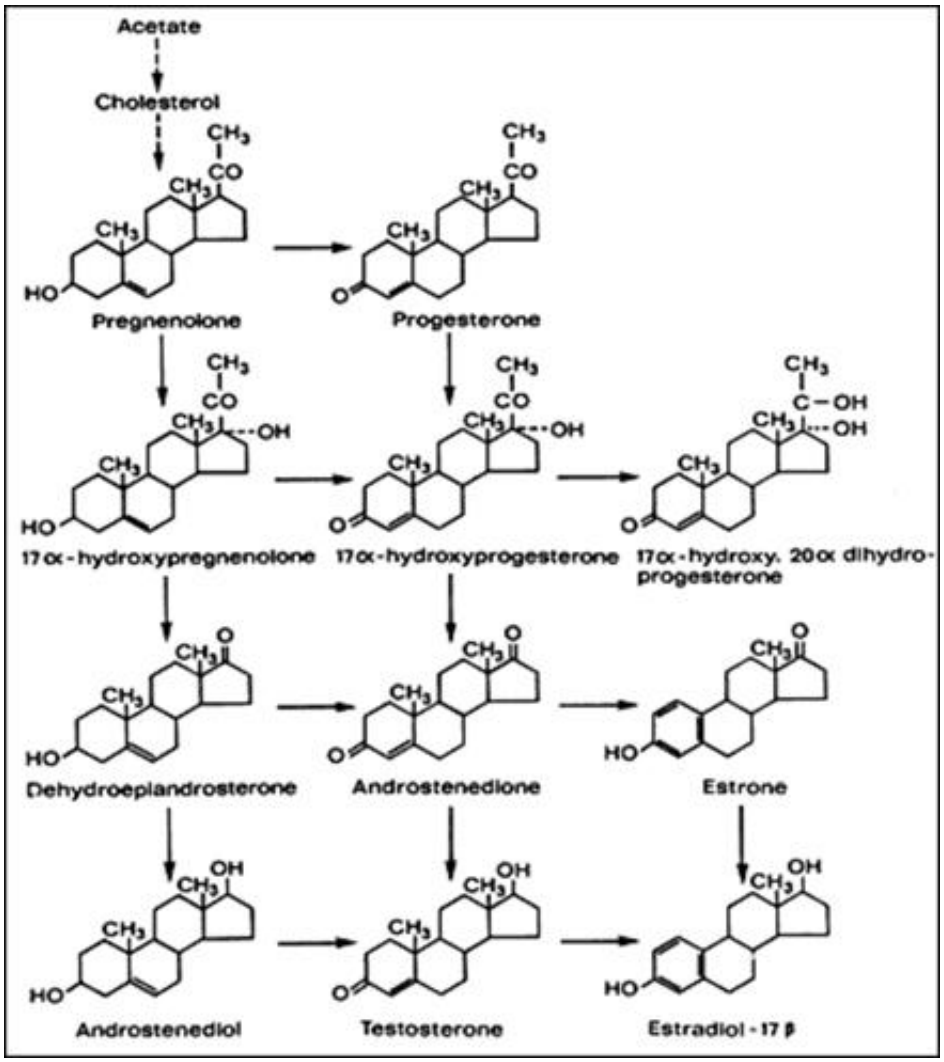

Figure 13. This diagram shows the steps in steroidogenesis leading to testosterone production.

The mobilization of cellular sources of cholesterol is achieved through the action of cholesterol ester hydrolase and subsequently, this is converted to pregnenolone by the enzyme cholesterol side-chain cleavage termed cytochrome P450SCC (169) . The conversion of cholesterol to pregnenolone is a key step at which regulation of androgen production within the Leydig cells occurs. Availability of cholesterol substrate can be rate-limiting and the intracellular trafficking of cholesterol across mitochondrial membranes is dependent on the steroidogenic acute regulatory protein (STAR) (170-172) . The role of this protein has been well demonstrated in patients with mutations in the gene encoding STAR in the disorder termed congenital lipoid adrenal hyperplasia wherein the mitochondria from the adrenals and gonads of these patients are unable to convert cholesterol to pregnenolone (173) . Further, the results of studies involving targeted disruption of the mouse gene encoding STAR support the data derived from human studies (174) .

Pregnenolone may progress to testosterone production through two pathways. It can be converted to progesterone through the enzyme 3β hydroxysteroid dehydrogenase (the D4 pathway) or can be hydroxylated at the 17α position by the enzyme 17ahydroxylase to form 17α hydroxypregnenolone (the D5 pathway). The relative importance of these two pathways vary with the species and the physiological status of the male (175) . The further conversion of 17α hydroxypregnenolone through the D5 pathway involves the formation of the C19 steroid dehydroepiandrosterone catalyzed by the enzyme 17,20 lyase and both steps appear to be catalyzed by a single microsomal enzyme cytochrome P450 c17 encoded by a single copy gene on chromosome 10 (176 , 177) . The conversion of dehydroepiandrosterone to androstenediol is mediated by a microsomal enzyme 17β hydroxysteroid dehydrogenase encoded by a single gene (178 , 179) .

The conversion of substrates from the D5 to the D4 pathway are catalyzed by the enzyme 3β hydroxysteroid dehydrogenase (180) . In the D4 pathway 17α hydroxyprogesterone proceeds through the action of cytochrome P450 c17 to androstenedione and testosterone. Testosterone can be converted to a dihydrotestosterone by the enzyme 5α reductase (181) or can be metabolised to 17β estradiol by the enzyme aromatase (182) .

It is important to recognize that intracellular transport of steroid substrates involved in androgen production is important with the transport of cholesterol into the mitochondrion to form pregnenolone and the transport of pregnenolone to smooth endoplasmic reticulum for the remainder of the steps in the production of testosterone.

LH, through specific receptors found on the surface of Leydig cells, controls the production and secretion of testosterone (183 , 184) . The structure of the LH receptor is that of a member of the seven transmembrane domain G protein coupled receptor super family and mutations of this receptor are the cause of familial testicular resistance and male pseudohermaphroditism (185 , 186) . Some men have constitutively activating mutations in the LH receptor and this has resulted in the onset of precocious puberty (187-189) .

The interaction of LH with its receptor initiates signalling through the cyclic AMP pathway through GTP binding proteins (190 , 191) . Signal transduction occurs through the protein kinase A pathway as its principal signal transduction mechanism. Some data suggests that intracellular calcium concentration can be induced by the action of LH by activating phospholipases in the lipoxygenase pathway (192) . In addition, the changes in calcium can also regulate adenylate cyclase through the protein kinase C pathway.

An in vivo injection or an episode of LH secretion induced by GnRH, results in stimulation of the side-chain cleavage enzyme with the subsequent release of testosterone within 30-60 minutes of LH stimulation (89) . The acute response to an injection of LH is dramatic in some species such as the rat and the ram but is much more attenuated in the human. This testosterone response lasts approximately 24-48 hours (193) . If human chorionic gonadotrophin is used as an LH substitute, the kinetics of the initial stimulation are similar to LH but a second peak of testosterone secretion is evidence with hCG and occurs 48-72 hours after the initial injection (194) . This biphasic pattern has been attributed to the observation that between 24 and 48 hours after an LH or hCG injection, the Leydig cells are refractory to further stimulation by either

hormone (195 , 196) . The second phase of testosterone secretion after hCG but not LH is associated with the longer half-life of hCG in comparison to LH. The hCG levels persist in the circulation and, following recovery from the refractoriness, testosterone levels increase. This observation has significant clinical importance since, in many men, a single weekly injection of hCG will suffice to maintain optimum testosterone responses rather than the frequent practice of giving injections of hCG two to three times per week.

It is important to recognise that LH enhances the transcription of genes that encode a range of enzymes in the steroidogenic pathway and that continued LH stimulation results in Leydig cell hypertrophy and hyperplasia (88 , 197 , 198) . In the normal male, the episodic nature of LH stimulation is likely to avoid prolonged periods of Leydig cell refractoriness to LH stimulation (199) . It is recognized that the testosterone secretory capacity of the human testis declines in ageing men and this has been shown to result from a reduction in the efficacy of the ageing testis to respond to intravenous pulses of LH (200) . They showed that the estimated down-regulation of the Leydig cell achieved by exogenous LH pulses was augmented in these healthy older men making them refractory to further pulse for a longer period. Leydig cells also secrete insulin-like peptide 3 and express its receptor, RXFP2 and further studies have shown that mouse Leydig cells *in vitro* respond to INSL3 by increasing cyclic AMP and testosterone (201) .

It is well accepted that the level of production of androgens and estrogens by the testis can regulate bone mass, with decreased production causing osteoporosis. More recently, the production of osteocalcin by bone has been shown to influence testicular function (202) , reviewed in (203) . Using co-cultures of osteoblasts with testicular tissue, they showed that testosterone could be stimulated through the secretion of osteocalcin which acted through the G-protein coupled receptors (Gprc6a) (204) .

Control of Leydig Cell Function by Seminiferous Tubules

A significant body of evidence has accumulated from studies in rats to suggest that the seminiferous tubules can exert an influence on Leydig cell testosterone production (103 , 205) . This data emerges from a number of studies where changes in Leydig cell function have been demonstrated in association with temporary disruption of spermatogenesis such as the application of single episode of heat to the rodent testis (see Figure 14) (206) . The most convincing data emerges from unilateral testicular damage such as that induced by cryptorchidism or efferent duct ligation, where the Leydig cells from the testis with spermatogenic damage shows an increased capacity for testosterone biosynthesis and a decrease in LH receptor number (207 , 208) . The nature of the molecular mechanisms involved in this regulation is yet to be elucidated. While similar mechanisms are difficult to identify in the human, it is recognized that elevated LH and low to low normal testosterone concentrations, indicative of compromised Leydig cell function are found in 15-20% of men with severe seminiferous tubule failure.

Further support for the concept that the state of spermatogenesis can affect the function of the Leydig cells in men has emerged from the studies of Andersson et al (209) , who showed that lower testosterone and higher estradiol concentrations were present, and accompanied by

higher LH levels in infertile. They concluded that this may reflect an extension of testicular dysgenesis to affect steroidogenesis or alternatively may result from inter-compartment interactions in the testis. There is increasing support for the concept that environmental factors such as the phthalates are able to influence Leydig cell function (210) . They showed that in utero exposure of rats to di(n-butyl)phthalate during the masculinization programming window in fetal life, led to focal testicular dysgenesis as expressed by Leydig cell aggregation and malformed seminiferous tubules. These features were linked to impaired intra-testicular testosterone levels and a decreased ano-genital distance, an emerging marker of deficient androgen action in utero.

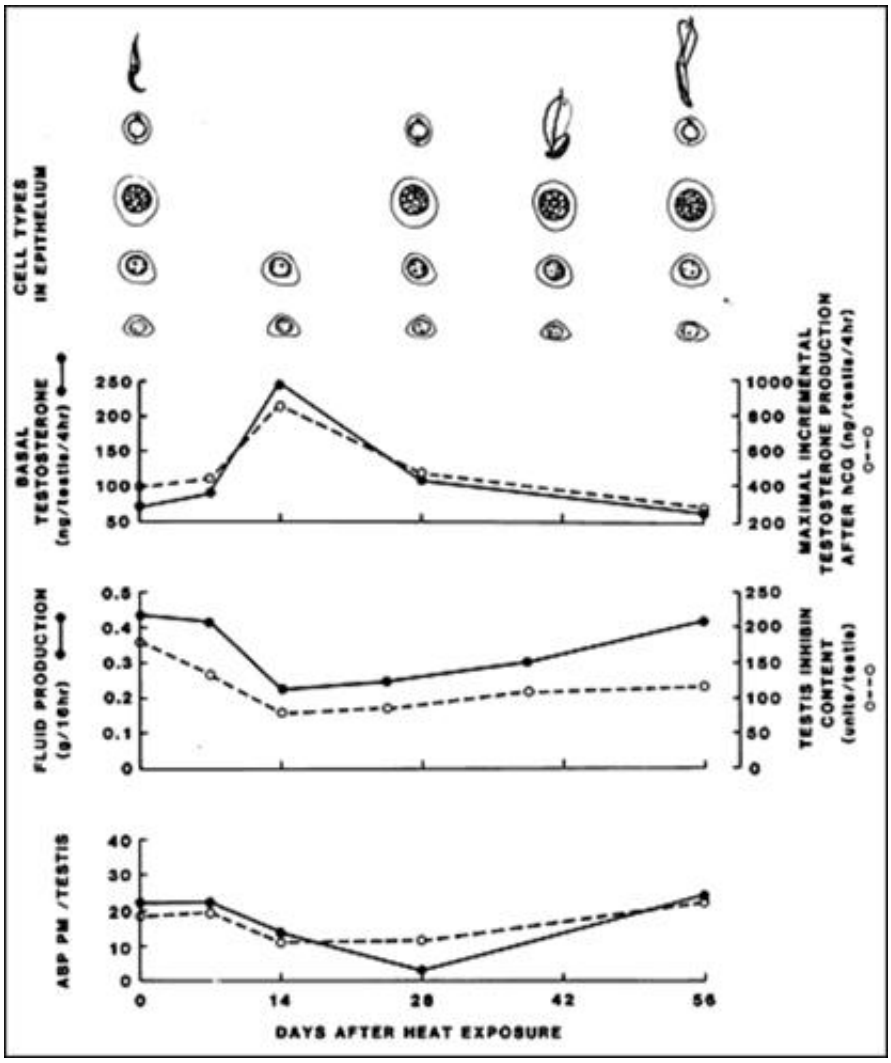

Figure 14. The changes in the seminiferous epithelium after a single episode of heat to the testis is shown. A depletion of heat sensitive germ cells is seen at 14 days and is associated with an increase in testosterone production by the testis in vitro and a decline in seminiferous tubule fluid production and androgen binding protein (ABP) levels in the testis. Data from Jégou et. al. 1984 (reference 136).

The hormonal control of spermatogenesis

The endocrine regulation of spermatogenesis has been the subject of many studies over the past four decades. Much of our knowledge of the hormonal regulation of spermatogenesis was elucidated in earlier studies involving the suppression and replacement of endocrine hormones. Modes of suppression include hypophysectomy (removal of the pituitary), suppression of pituitary gonadotropin secretion by steroid administration, and suppression of GnRH secretion by immunization or pharmacological modulation, reviewed in (211-213) . More recent studies utilized the *hpg* mouse model, which arose from a spontaneous mutation and exhibits a major deletion within the gene encoding GnRH (214) , to further define the role for androgens, estrogen and FSH in the initiation of spermatogenesis, reviewed in (211) . The advent of targeted genetic manipulation in mice has allowed the specific roles and sites of action of gonadotropins, androgens and estrogen to be even further dissected (211 , 215-217) . We refer the reader to extensive reviews on this topic (153 , 211-213 , 216 , 218 , 219) , and will briefly outline here the major roles for androgens and FSH in spermatogenesis. The role of estrogen in spermatogenesis is considered in Chapter 17.

a. Role of testosterone

The primary stimulus for the initiation of spermatogenesis is the LH-induced rise in testosterone at puberty. The absolute requirement of androgen for the initiation of spermatogenesis is demonstrated by the ability of the non-aromatisable androgen DHT to initiate complete spermatogenesis in *hpg* mice (220) , and by the observation that spermatogenesis proceeds only to meiosis in mice lacking Sertoli cell AR expression (221 , 222) . While androgens together with FSH are required for quantitatively normal spermatogenesis (see below), it is clear that androgens can initiate and support some degree of sperm production. Once spermatogenesis has been initiated during puberty, androgen alone can restore or maintain adult sperm production after experimentally-induced gonadotropin suppression, as has been demonstrated in many rodent, primate and human studies (reviewed in (211 , 213 , 216 , 218 , 223)).

By virtue of its local production in the testis, testicular concentrations of testosterone are very high, and are above those required for the initiation and maintenance of spermatogenesis. Adult spermatogenesis can be maintained by testicular testosterone levels at least 4 fold lower than normal in rats (224) , reviewed in (223) . When testicular testosterone levels are low, such as in the pubertal testis and during gonadotropin suppression, the 5α-reduction of testosterone to the more potent androgen DHT appears necessary to amplify the androgenic signal and exert its stimulatory effects on spermatogenesis (reviewed in (218)). However in the adult testis when testosterone levels are very high, it is likely that testosterone acts directly on the AR to maintain androgen-dependent functions (225) . The initiation of spermatogenesis requires a higher concentration of androgen than is required to maintain adult spermatogenesis once it is initiated, as exemplified by studies in *hpg* mice (226) . Also, the restoration of adult spermatogenesis following gonadotropin suppression occurs over a very narrow dose range, wherein small changes in testicular androgen levels can produce large changes in sperm production, reviewed in (223) . It is also worth noting that even very low levels of androgen are likely to produce a stimulatory effect on spermatogenesis. This can be illustrated by the

demonstration of low levels of sperm production in older mice lacking LH receptor expression (227) . Therefore, when considering the androgenic stimulation of adult spermatogenesis, “a little goes a long way”, and continued androgen action on AR can occur in the absence of gonadotropin stimulation, reviewed in (223) .

Within the testis, AR is expressed in Sertoli cells, peritubular myoid cells, Leydig cells and vascular endothelial cells ((108 , 228 , 229) . Germ cells are generally considered to lack AR expression, and germ cells without AR can develop normally in testes with somatic AR expression (230 , 231) . Therefore androgens must act on AR within the testicular somatic cells to support spermatogenesis. Recent studies in transgenic mice have revealed that androgen action on AR in each of the testicular somatic cell types is important for testis function. In terms of androgen action on spermatogenesis, AR expression in Sertoli cells is essential, as no sperm are produced in mice with targeted deletion of Sertoli cell AR expression (221 , 222) or in mice where the DNA binding domain of Sertoli cell AR has been deleted (232) . However AR expression in peritubular myoid cells is also important for normal spermatogenesis, an effect that may be at least partly mediated via a role for peritubular cells in supporting a normal and fully functional Sertoli cell population, which in turn supports germ cell development (78) . Ablation of AR from peritubular myoid cells also interferes with the normal development and function of the Leydig cell population (79) . The autocrine action of androgen on AR in Leydig cells is required for normal steroidogenesis and hence optimal testosterone production (231) , and AR in endothelial cells of the testicular arterioles is involved in maintaining normal fluid dynamics and vasomotion in the testis (229) . In summary, androgens act on AR in testicular somatic cells, but not germ cells, to support normal testicular function and sperm production.

Several phases of germ cell development are known to rely on androgen action. The progression of germ cell development from spermatogonia through to meiotic spermatocytes can occur in the absence of androgen, however the survival of pachytene spermatocytes during the long meiotic prophase, and entry into the final meiotic division requires androgen action, e.g. (221 , 222 , 232 , 233) . Therefore in the absence of androgen signaling, via Sertoli cells, spermatocytes cannot complete meiotic division, and no haploid round spermatids are produced. The progression of haploid spermatids through spermiogenesis also relies on androgens, and in the absence of androgen, spermiogenesis arrests at steps 7-8 due to defects in round spermatid survival and in the ability of newly elongating spermatids to adhere to Sertoli cells (234-236) . The final release of spermatids during the process of spermiation is also sensitive to androgen and/or gonadotropin inhibition, reviewed in (53) . Since germ cells do not contain AR, these actions of androgen must be mediated via AR in somatic cells. Many functions of Sertoli cells are known to be androgen-dependent, such as the maintenance of tight junction function at the blood testis barrier (237-239) and the production of androgen-responsive miRNAs (240) , and are likely to be necessary for supporting germ cell development. The mechanisms by which Sertoli cells support each androgen-dependent phase of germ cell development however, such as the signal required for the completion of meiosis (reviewed in (211)), are as yet unknown. Interestingly, the different androgen-dependent processes within germ cell development have different sensitivities to, or requirements for, androgens, reviewed in (223) . For example, the completion of meiosis requires more androgen than the completion of spermiogenesis (220) . Individual variations in the sensitivities of different spermatogenic processes to androgens may explain why a correlation between

sperm output and testicular testosterone levels has been so difficult to establish in gonadotropin-suppressed monkeys and men (241-244) .

b. Role of FSH

For many years, the relative roles of androgen vs FSH in initiating, restoring and maintaining spermatogenesis were unclear. This was in part due to the synergistic actions of these two hormones (see below), but also due to difficulties associated with investigating FSH action in a setting of complete androgen ablation. Transgenic mouse models have provided important information regarding specific roles for FSH in spermatogenesis, reviewed in (211 , 215 , 216) . FSH receptors are found only on Sertoli cells and are expressed in a stage-dependent manner (245 , 246) .

One of the most important functions of FSH is to establish a quantitatively normal adult Sertoli cell population. FSH acts as a mitogen for postnatal Sertoli cell proliferation and is required for establishing normal Sertoli cell numbers in mice, reviewed in (153 , 216) . Since Sertoli cell number determines spermatogenic output in adulthood (110) , this function of FSH is important for optimal sperm production. Observations in transgenic mice also show that FSH is needed for normal Sertoli cell morphology and for their ability to support the maximal number of germ cells, e.g. (233 , 247-249) .

FSH also plays an important role in the regulation of spermatogonia, as revealed in studies in *hpg* mice (233 , 250) and primates (251 , 252) . Numbers of type B spermatogonia correlate more closely with circulating FSH than testicular testosterone levels in gonadotropin-suppressed monkeys and humans (253 , 254) , indicating that these cells may be particularly supported by FSH. Transgenic human FSH expressed in *hpg* mice can also exert stimulatory effects on spermatocyte numbers, indicating a permissive effect on meiosis, (250) however FSH alone cannot support the completion of spermiogenesis. The acute suppression of FSH alone can also cause spermiation failure, presumably via effects on the Sertoli cell's ability to release mature spermatids (255) .

c. Co-operation between FSH and testosterone

The data reviewed above indicate that androgens and FSH have distinct roles in spermatogenesis. However, it is now well known that these hormones act co-operatively to promote maximal spermatogenic output, as reviewed elsewhere (153 , 216 , 218 , 219) . Transgenic mouse models in particular have been used to demonstrate that androgens and FSH can have synergistic, additive and redundant effects on spermatogenesis and Sertoli cell activity, e.g (233 , 250) .

Androgen and FSH can co-operate by supporting different aspects of germ cell development, for example FSH stimulation of spermatogonial populations and androgen stimulation of spermiogenesis. FSH establishes a quantitatively normal Sertoli cell population, whereas androgen initiates and maintains sperm production, thus both hormones co-operate via independent functions to enable maximal spermatogenic output.

Both testosterone and FSH facilitate normal Sertoli cell morphology and function, which are likely essential for the ability of Sertoli cells to support the maximum number of germ cells. Both hormones also promote germ cell survival, particularly of spermatocytes and round spermatids in the mid-spermatogenic stages in rodents (256) , reviewed in (153) . The fact that both hormones can prevent germ cell apoptosis explains why either hormone can maintain germ cell development, at least in the short term, following gonadotropin suppression in rodents (256) and humans (257) .

There are many examples of synergy between testosterone and FSH, reviewed in (153 , 216 , 218) . It has been demonstrated in many experimental settings that testosterone and FSH can support spermatogenesis at a lower dose when the other is present, reviewed in (218) . Testosterone and FSH likely act synergistically in the control of signaling pathways and gene expression in Sertoli cells, which in turn are important for germ cell development (216 , 219) . An example of such synergism is the demonstration that, after acute suppression of either androgen or FSH in rats, approximately 10% of mature spermatids failed to be released at spermiation, whereas suppression of both hormones resulted in 50% of spermatids failing to spermiate (255) . Both testosterone and FSH modulate the expression of many miRNA species in Sertoli cells, which likely mediate a large spectrum of proteomic changes important for Sertoli and germ cell function (240) .

It should be noted that there are species differences in the response of spermatogenesis to combined androgen and FSH suppression, reviewed in (213 , 258) . In rodents, suppression of gonadotropins causes a decline in spermatogonial populations but spermatogenesis is primarily arrested at the spermatocyte stage (259) . In monkeys and humans however, spermatogenesis is primarily arrested at spermatogonial development, however meiosis and spermiogenesis can be maintained until they undergo a gradual attrition due to the lack of spermatogonia entering meiosis (213 , 242 , 254) .

The requirement for both testosterone and FSH to support normal spermatogenesis in men was revealed in studies by Matsumoto and colleagues (260 , 261) whereby gonadotrophins were suppressed by the administration of T until suppression of spermatogenesis occurred. They then introduced injections of hCG to stimulate Leydig cell function and to restore intratesticular T concentrations which increased sperm counts but not to pre-treatment levels (Figure 15). These data suggested that, in association with undetectable FSH levels, increasing intratesticular T could partially restore sperm output (261) . Using the same model, they initiated hFSH treatment when sperm counts were suppressed and showed that, in the presence of low intratesticular T concentrations, FSH alone could partially restore sperm output (262) . The latter study strongly suggests a role for FSH which appears to be able to synergise with low T to stimulate sperm production.

In summary, it is clear that both testosterone and FSH have independent effects on spermatogenesis, however both act co-operatively and synergistically to promote maximal sperm production; both are required for quantitatively normal spermatogenesis and fertility . In rodents and primates, the combination of androgen and FSH consistently produces a better stimulatory effect on spermatogenesis than either factor alone. Conversely, the degree of androgen as well as FSH suppression, is likely important for the induction of azoospermia in a

gonadotropin-suppressed, contraceptive setting.

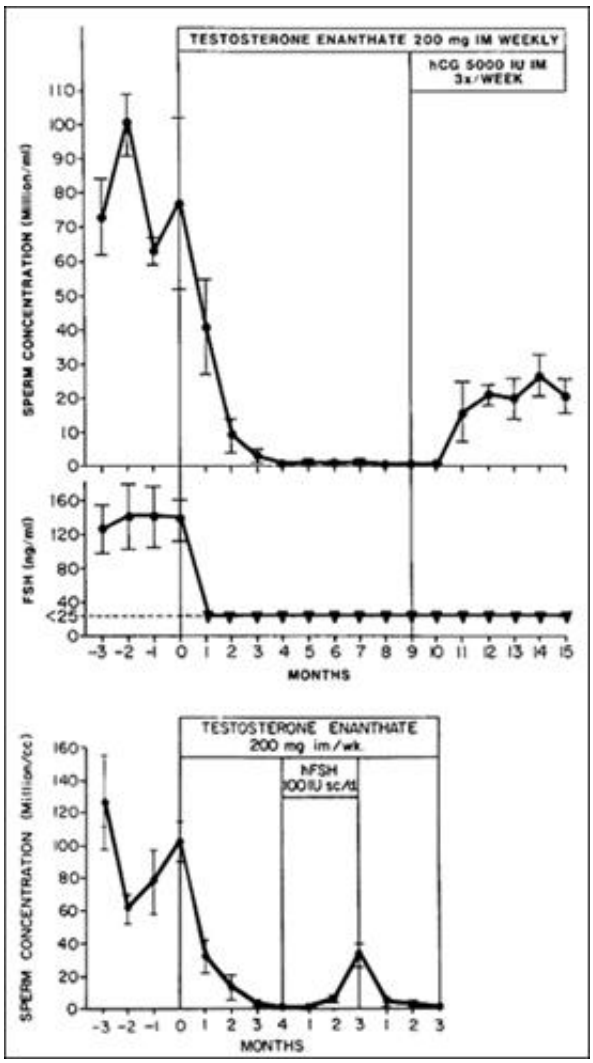

Figure 15. The response in the sperm counts from normal volunteers to a suppression of FSH and LH by testosterone injections is shown. Note the recovery in sperm counts when hCG and hFSH were introduced singly into the treatment regime. Data from Matsumoto et. al. (reference 171, 172) and Bremner et. al. (reference 172).

INTERRELATIONSHIPS BETWEEN THE HYPOTHALAMUS, PITUITARY AND TESTIS

As briefly discussed earlier in this chapter, the successful initiation of testicular function is dependent on the hypothalamic secretion of GnRH which in turn stimulates FSH and LH to act on the testis. These actions initiate spermatogenesis and testosterone production.

It is well recognised that the testis in turn, through the secretion of hormones produced in the

Sertoli and Leydig cells, exerts a negative feedback control on the production of gonadotrophins. The presence of such a negative feedback control by the testis on pituitary FSH and LH secretion is best demonstrated by the rapid rise of FSH and LH after castration. The mechanisms by which the secretion of FSH and LH increases in response castration involves a rise in the hypothalamic secretion of GnRH and also involves direct actions at the pituitary level which allow an increase in pulse amplitude. Further, the fact that LH and FSH are co-secreted by the majority of gonadotrophs raises a number of questions as to how GnRH and the inhibitory signals act on the pituitary to result in differential regulation of FSH and LH secretion.

Control of LH Secretion

There is a substantial body of evidence to indicate that the steroid hormones testosterone, estradiol and dihydrotestosterone inhibit LH secretion (263) . The demonstration that non-aromatisable androgens could inhibit LH secretion resolved the argument as to whether testosterone exerts its action directly or whether metabolism to estradiol or dihydrotestosterone was necessary (264 , 265) . From the studies by Santen and Bardin (266) , it is evident that testosterone acts at the hypothalamic level by decreasing GnRH pulse frequency without a change in pulse amplitude. However, the action of estradiol appears to be predominantly at the pituitary where it decreases LH pulse amplitude without changing pulse frequency. Further support for the action of testosterone at the hypothalamus emerged from the observation of a decrease in GnRH pulse frequency in portal blood (267) . In addition, these studies demonstrated that treatment with estradiol lowered LH levels by decreasing LH pulse amplitude without altering GnRH secretory patterns in portal blood.These conclusions have been challenged by observations that a selective aromatase inhibitor, anastrozole, caused an increase in LH pulse amplitude and pulse frequency (268) . These changes were seen in the presence of increased testosterone concentrations and were accompanied by an increase in LH and FSH. The investigators concluded that estradiol exerted a negative feedback by acting at the hypothalamus to decrease GnRH pulse frequency and at the pituitary to decrease the responsiveness to GnRH.

Control of FSH Secretion

There is a substantial body of evidence to indicate that testosterone and estradiol are capable of suppressing FSH in the male (269) . For many years, it was proposed that the action of the steroid hormones could account for the entire negative feedback exerted on FSH levels by the testis despite the existence of a hypothesis that a specific FSH feedback regulator named inhibin existed (270) .

Over the past twenty years, a substantial body of evidence has accumulated to confirm the existence of a glycoprotein hormone termed inhibin that exerts a specific negative feedback inhibition on FSH secretion at the pituitary level (271) . Two forms of inhibin have been isolated namely inhibin A and inhibin B (272-275) . These proteins represent disulphide-linked dimers of an α and β subunit. The alpha subunit is common both to inhibin A and B but the β subunit, though closely related, are different (α βA = inhibin A: aβB = inhibin B). Both inhibin A and

inhibin B have the capacity to specifically inhibit FSH secretion by pituitary cells in culture. In contrast, dimers of the β subunit, termed activins (activin A = βAβA: activin B = βBβB; activin AB = βAβB) all have the capacity to stimulate FSH secretion by pituitary cells in culture (276 , 277) . Finally, a structurally unrelated protein termed follistatin, has the capacity to suppress FSH secretion specifically by pituitary cells in culture (278-280) . This action has been demonstrated to be due to the capacity of follistatin to bind and neutralize the actions of activin thereby suppressing FSH secretion (281) .

a. Role of testosterone: In men and males from other species testosterone, when administered in an amount similar or greater to its production rate can suppress FSH as well as LH (263) . However, in most instances there was a parallel and often greater suppression of LH secretion in contrast to the actions of inhibin (269) . Further, there appears to be a difference in the response of FSH to testosterone in primates, where the actions are totally inhibitory in contrast to rats, where following an initial suppression of FSH by testosterone, higher doses caused a return of FSH levels to baseline (282 , 283) . Clear evidence for a physiological role of testosterone in the control of FSH can be shown in experiments in which the Leydig cells were destroyed by the cytotoxin ethane dimethane sulphonate (EDS). This treatment results in a rapid decline in testosterone levels and a concomitant increase in FSH concentrations to levels which were only 50% of those found in castrates (284) . Since the inhibin levels in these experiments did not change, the maintenance of FSH levels at 50% of those seen in castrate animals was likely to be due to the continuing feedback control by inhibin (285) . Further support for the dual role of testosterone and inhibin in the control of FSH emerged from the use of EDS in cryptorchid rats where baseline FSH levels were increased in association with decreased inhibin concentration. The removal of testosterone feedback in these animals with low basal inhibin levels resulted in an increase in FSH to the castrate range (286) . The observation of an increase in FSH levels in men treated with a selective aromatase inhibitor raised the possibility that estradiol exerts a negative feedback action on FSH especially since the treated men experienced a concomitant significant increase in testosterone (268) .

b. Role of inhibin, activin and follistatin: The predominant evidence indicates that in the male, inhibin is produced by the Sertoli cell and is secreted both basally across the basement membrane of the seminiferous tubule and also into the lumen (287 , 288) . Several studies have now demonstrated that the predominant form of inhibin secreted by the testis is inhibin B since the predominant mRNA was βB (289 , 290) . The levels of inhibin B in males, measured by a specific ELISA, are inversely related to the levels of FSH (291 , 292) . Several studies have indicated that FSH predominantly stimulates inhibin α subunit production and does not alter the β subunit message (287 , 293) . This action results in the testis predominantly secreting inhibin rather than activin. Further support for this concept emerges from the studies of men undergoing chemotherapy where declining inhibin B levels are associated with a rise in FSH. However, with assays that detect α subunit products, there was a clear increase in these substances under the stimulation of elevated FSH levels (294) . There is also evidence that a subunit of inhibin can be produced by Leydig cells (295) and increased LH levels result in the release of a subunit products into the circulation (296 , 297) . There is still controversy as to whether the Leydig cells can produce bioactive inhibin (295) .

In man, testosterone-induced gonadotrophin suppression reduced circulating inhibin B and pro

alpha C levels by only 25% and 50%, respectively, indicating that their secretion is not fully gonadotrophin-dependent (241) . In that model, exogenous FSH and LH both restored pro alpha C levels supporting the view that Sertoli and Leydig cell are the origins of alpha subunit peptides, respectively, but only FSH restored inhibin B presumably reflecting Sertoli cell ßB synthesis.

While there is evidence that the Sertoli cells, Leydig cells and peritubular myoid cells can produce activin, castration does not result in a decrease in circulating activin A levels (76 , 298-300) . Unfortunately, due to the lack of a suitable assay to measure activin B, there is no data available concerning the behaviour of this substance after castration. There is considerable evidence that activin exerts local actions within the testis such as the stimulation of spermatogonial mitosis (301) Further, activin A is responsible for the stimulation of Sertoli cell mitosis for a period during the development of the testis in both rats and mice (111 , 112 , 302-304) . Additionally, receptors for activin are present on primary spermatocytes, round spermatids and Sertoli cells (305) .

Follistatin is also produced in the Sertoli cells, spermatogonia, primary spermatocytes and round spermatids in the testis (306 , 307) . However, castration does not result in a net decrease in follistatin levels in the circulation suggesting that the testis does not contribute significantly to circulating levels of follistatin (308) . In fact, in these studies follistatin levels rose but the rise was also found in the sham operated rams indicating that the follistatin response was part of the acute phase response to surgery, further supported by the demonstration that IL1β could also cause such an increase (309) .

The failure of activin and follistatin to change after castration whereas the inhibin levels in the circulation decreased to undetectable levels, strongly suggest that the gonadal feedback signal on FSH secretion is inhibin. This view has been further supported by the studies of Plant and colleagues in primates where they showed that in arcuate nucleus-lesioned monkeys maintained on a constant GnRH pulse regime, testosterone could prevent the post-castration rise in LH but not FSH (310) . Further, in several species the infusion or injection of recombinant human inhibin caused a specific fall in FSH secretion commencing some six hours following administration (311-313) . In further studies, inhibin A, sufficient to restore circulating levels in castrate rams to normal, suppressed FSH levels into the normal range in the absence of testosterone (314) .

The role of these proteins appears to be more complex since substantial evidence exists that activin and follistatin can exert a paracrine role directly in the pituitary gland. The α and β subunit mRNAs are present in gonadotrophs within the pituitary gland (315) . The studies of Corrigan et al (316) strongly suggest that these substances exert a local action on FSH secretion since the inhibition of the action of activin B by the use of a specific monoclonal antibody when added to pituitary cells in culture, caused a suppression of endogenous FSH secretion. Follistatin mRNAs are also present in a number of different pituitary cell types including the folliculo-stellate cells (315 , 317) . This local production of follistatin also has the capacity to regulate the actions of activin (318) . Additionally, the studies of Bilizekian et al have demonstrated that GnRH and the sex steroids estradiol and testosterone can modulate the local production within the pituitary of α, βA, βB and follistatin mRNAs (319 , 320) . Clearly

these interactions are complex and no clear answer can be given as to the relative roles of paracrine and endocrine actions of these glycoprotein hormones.

Some correlative evidence supporting the action of inhibin on FSH secretion is the decrease in production of inhibin by Sertoli cells in parallel with the rise of FSH in a number of models of spermatogenic damage such as cryptorchidism and intra-testicular glycerol treatment (321 , 322) . The levels of circulating inhibin B appear to be inversely related to the levels of FSH to the levels of FSH following testicular damage in a number of studies (291 , 292 , 323) . Further, even in studies of large numbers of normal men, there is an inverse relationship between serum inhibin B levels and FSH (323) . It is therefore likely that the actions of inhibin are predominantly exerted through secretion from the testis and transport via the peripheral circulation whereas the actions of activins and follistatin on FSH secretion occur through paracrine actions at the level of the pituitary gland. Further evidence supporting the stimulation of FSH by activin secretion emerges from the decline in FSH levels in mice with targeted disruption of the activin type II receptor gene (324) .

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