Part 2: Male Reproductive System

Normal Physiology and Structure

Testis

Function, physiology and regulation
The testis has two major functions: 1) producing sperm from stem cell spermatogonia (spermatogenesis) and 2) producing androgens, to maintain and regulate androgen mediated functions throughout the body.

Spermatogenesis
Spermatogenesis occurs in the seminiferous tubules, of which there are 10-20 in each rat testis. Spermatogenesis is the process whereby primitive, diploid, stem cell spermatogonia give rise to highly differentiated, haploid spermatozoa (sperm).
The process comprises a series of mitotic divisions of the spermatogonia, the final one of which gives rise to the spermatocyte. The spermatocyte is the cell which undergoes the long process of meiosis beginning with duplication of its DNA during preleptotene, pairing and condensing of the chromosomes during pachytene and finally culminating in two reductive divisions to produce the haploid spermatid. The spermatid begins life as a simple round cell but rapidly undergoes a series of complex morphological changes. The nuclear DNA becomes highly condensed and elongated into a head region which is covered by a glycoprotein acrosome coat while the cytoplasm becomes a whip-like tail enclosing a flagellum and tightly-packed mitochondria. The sequential morphological steps in the differentiation of the spermatid (19 steps of spermiogenesis) provide the basis for the identification of the stages of the spermatogenic cycle in the rat.

In a cross section of a seminiferous tubule, the germ cells are arranged in discrete layers. Spermatogonia lie on the basal lamina, spermatocytes are arranged above them and then one or two layers of spermatids above them. In any given normal tubule, four generations of cells develop simultaneously and in precise synchrony with each other. As each generation develops, it moves up through the epithelium, continuously supported by Sertoli cells, until the fully formed sperm are released into the tubular lumen (spermiation). The synchrony of the development between the 4 generations of cells is such that each successive stage of development of the spermatogonium is found with its characteristic spermatocyte and spermatids.

Germ cells lie in discrete layers within the seminiferous tubule supported by the cytoplasmic processes of the Sertoli cell (SC). Spermatogonia (Sg) lie on the basal lamina, spermatocytes (Sp) lie mid way in the epithelium, round spermatids (Sd) lie in an adluminal position and the elongating spermatids lie at the luminal surface with their heads embedded in Sertoli cell cytoplasmic invaginations and the tails extending into the lumen. In each tubule there are 4 generations of germ cells developing in total synchrony with one another.
The synchronous development of the 4 generations of cells results in the repetitive appearance of specific cell associations which are referred to as stages of the spermatogenic cycle. 14 such cell associations have been described in the rat and are referred to as stages I-XIV of the spermatogenic cycle.

Normal appearance and cell types in a stage VII tubule (left) and a stage XII tubule (right)

The morphological appearance of tubules in the first half of the cycle (stages I-VIII) is different from those in the second half of the cycle (stages IX-XIV). Placing the tubules into the first (early) or second (late) half of the cycle is the first step in identifying the precise stage of spermatogenesis. This can be done at low power on the microscope.

Early stage tubules have two generations of spermatids: round spermatids and mature, elongating spermatids whereas the second half of the cycle only has one generation of spermatids which are in the early phase of elongation.

In the above stage VII tubule note the layers of round spermatids plus the adlumenal layer of elongate spermatids. Also note the single layer of small pachytene spermatocytes lying beneath the round spermatids. The few small dark staining cells at the base of the tubule are preleptotene spermatocytes.

In the late stage (XII) tubule there is only one generation of spermatids and these are elongating. The other major cell types consist of multiple layers (representing one generation) of large pachytene spermatocytes (compare with the size and appearance of the pachytene spermatocytes in the early stage tubule). The dark staining cells lying beneath the pachytene spermatocytes are leptotene/zygotene spermatocytes that have developed from the preleptotene spermatocytes seen in the early stage VII tubule.
The spermatogenic cycle of the rat can be thought of as a 14 frame, time-lapse film of germ cell development. Each frame, represented by a “stage” is fractionally different from the frame before, as each generation of germ cells develops with time. **It is essential for the pathologist to have a basic understanding of the spermatogenic cycle and to be familiar with the cellular makeup of the different stages of the spermatogenic cycle in order to be able to detect subtle changes in the testes, particularly those associated with endocrine disruption, since they are characteristically cell and stage specific.** It is beyond the scope of these guidelines to review the spermatogenic cycle, and how to recognize the cell associations, but the reader should refer to the following comprehensive reviews on the subject (Leblond and Clermont, 1952; Russell, 1990; Creasy, 1997; Creasy, 2002).

The spermatogenic cycle comprises 14 stages, each stage representing a snapshot in the process of germ cell development. The transition between stages involves specific cellular changes, such as the formation of an acrosomal cap and the enlargement of pachytene spermatocytes. The cycle is initiated by the release of mature spermatids into the lumen, and each new generation of spermatogonia begins to divide and displace the newly formed preleptotene spermatocytes off the basal lamina.

**Illustration of the cell associations comprising four of the fourteen stages of the spermatogenic cycle.** During the transition between stage I and VIII, the round spermatids are progressively forming an acrosomic cap, as they develop from step 1 to step 8 of spermiogenesis. The early pachytene spermatocytes (EP) enlarge as they move into mid pachytene (MP), and the intermediate spermatogonia (In) complete a number of mitotic divisions to become preleptotene spermatocytes. During stage VIII, the fully mature (step 19) elongated spermatids are released into the lumen. At this point a newly committed generation of spermatogonia (A) begin dividing and displace the newly formed preleptotene spermatocytes (PL) off the basal lamina. By stage IX, the round spermatid population has begun to elongate so that by stage XI there are step 11 spermatids that have an obvious elongated profile.

The pachytene spermatocytes have become very large and enter late pachytene (LP), and the preleptotene spermatocytes move into leptotene phase (L). During stage XIV the primary and secondary meiotic divisions take place and transform the large pachytene spermatocytes into new step 1 spermatids while zygotene spermatocytes enter early pachytene. It can be seen that the cellular makeup of the stage following meiotic division (stage I) is exactly the same as the cell association that the cycle began with, the difference being that one generation (of sperm) has been released and a new generation...
(of spermatogonia) has joined, and the rest of the cells are 14 days older and have moved up a layer.

Testosterone Biosynthesis

The major androgenic steroid testosterone is synthesized primarily in the Leydig cells and has both intratesticular effects (on spermatogenesis) and peripheral effects (on accessory sex organs as well as non-reproductive organs such as muscle, bone, skin and bone to name a few). While there is also significant testosterone synthesis in many peripheral tissues, it is beyond the scope of this review and will not be discussed further. The concentration of testosterone within the testis is very much greater than in the systemic circulation. For example, levels of the steroid in the testicular interstitial fluid can be up to 100-fold higher than in the plasma, and the concentrations in the two compartments are not directly proportional to one another. Therefore sampling plasma levels of testosterone does not provide a measure of testicular testosterone levels. Although these high intratesticular testosterone levels may be required to quantitatively maintain maximum spermatogenic potential, qualitatively normal spermatogenesis can be maintained with much lower intratesticular concentrations.

Testosterone is not stored within the Leydig cell, it is secreted into the interstitial fluid as it is synthesized. From here it is either i) taken up by the Sertoli cells and bound to androgen binding protein, which is then secreted by the Sertoli cell and transported through the seminiferous epithelium into the seminiferous tubule fluid and on into the epididymis or ii) diffuses into the interstitial capillaries where it binds quickly to albumin for transport through the body, where it has wide ranging effects on all other tissues of the body.

The major stimulus for testosterone production comes from blood levels of luteinizing hormone (LH) from the pituitary. Feedback inhibition of LH and hypothalamic gonadotrophic releasing hormone (GnRH) is mediated through circulating levels of testosterone and its metabolites, dihydrotestosterone (DHT) and oestradiol. Aromatization of testosterone to oestradiol takes place within the testis (indeed, oestradiol is critically important for normal testis function), and also in many peripheral tissues such as adipose tissue and the CNS, whereas conversion to DHT occurs largely in androgen dependent tissues such as the epididymis, prostate and seminal vesicles.

Maintenance of spermatogenesis

The main known effects of testosterone in supporting spermatogenesis are to stimulate seminiferous tubule fluid production by the Sertoli cell, regulate release of the mature spermatids from the Sertoli cell (spermiation) and to support the development of pachytene spermatocytes and later germ cell types through stage VII of the spermatogenic cycle. This spermatogenic support appears to be mediated by the secretion of several specific proteins from the Sertoli, peritubular and germ cells whose secretion is dependent, both on testosterone and a full complement of germ cells. Selective depletion of any of the different populations of cells (spermatocytes, round or elongating spermatids, but particularly the latter) from these stages will differentially alter (reduce or