increase) the secretion of each of the androgen regulated proteins. Regulation of spermatogenesis is therefore an extremely complex cascade of cell-cell interactions with the Leydig cells supporting germ cell development through the effects of testosterone on Sertoli and peritubular cell protein secretion but with the germ cells programming the response of these target cells to the testosterone. While the Leydig cells secrete several dozen other paracrine factors which are known to bind to receptors in the Sertoli cells, the functions of these neighbor-modulators are still being determined.

**Efferent Ducts and Epididymis**

**Function, physiology and regulation**

There are three major functions of the efferent ducts and epididymis: 1) reabsorption of seminiferous tubular fluid, 2) sperm modification and maturation and 3) sperm storage.

Sperm are transported from the testis in seminiferous tubular fluid that is secreted by the Sertoli cell. Over 98% of this fluid is reabsorbed as it passes through the rete, efferent ducts and initial segment of the epididymis. Oestrogen is a major regulatory factor in the resorptive process, and this function can be significantly disrupted by antioestrogens.

When sperm are released from the testis they are neither motile nor capable of fertilizing an oocyte. By the time they reach the cauda epididymis, they have acquired progressive forward motility and fertilizing ability. These properties are secreted by the epithelial cells in the caput and corpus epididymis and adsorbed onto the sperm, modifying their membrane function. The sperm also lose their cytoplasmic droplet from the maturing sperm in the cauda epididymis. Once in the cauda, the sperm are stored, immobilized and surrounded by a glutinous glycoprotein matrix (containing the secreted protein immobilin) until ejaculation occurs.

**Structure**

The efferent ducts comprise 7-13 ducts that link the rete testis with the initial segment of the epididymis. They are located in the epididymal fat pad and unfortunately, are generally discarded at necropsy. However, they are potentially an important target site for chemicals that disrupt oestrogen synthesis or block oestrogen receptors. For example, toxicity in these cells can reduce fluid resorption which increases the hydrostatic pressure in the testis, which will eventually shut down spermatogenesis. They are sometimes sampled when a gross observation is noted, such as discoloration or nodule or mass. If macroscopic observations are recorded in the epididymal fat pad of treated animals the pathologist should be aware of the potential for this to be evidence of endocrine disruption and recommend sampling of the epididymal fat pad from all animals.

The normal histological appearance of the efferent duct is characterized by a pale staining tall cuboidal epithelium which is covered by microvilli. The multiple ducts coalesce to form a single duct which leads into the initial segment of the epididymis. The epididymis comprises a single, convoluted tube which is approximately 6ft long in the rat and the cellular makeup, epithelial height, ductal diameter and sperm density of the epididymis all vary depending on location. Changes in endocrine status will have different impacts.
on different regions of the epididymis depending on the hormone (oestrogen or androgen) that is disrupted.

The function as well as the cellular make up of the efferent ducts and different parts of the epididymis vary. The efferent ducts, which are present in the epididymal fat pad, and the initial segment of the epididymis are made up of tall pale epithelial cells that reabsorb over 98% of the seminiferous fluid.

The caput epithelium (left) secretes protein that is important in sperm maturation while the cauda epithelium (right) reabsorbs protein and the cytoplasmic droplet that is shed from the sperm during epididymal transit. The endocytic clear cells (arrowed) are a prominent cell type of the distal corpus and cauda epithelium, which stain intensely with PAS and which become larger and more numerous when there is increased cell debris in the ductal lumens.
The accessory sex organs in rodents include the seminal vesicles, prostate and coagulating gland. They are located along the route of the urethra as it relays sperm from the vas deferens out through the penis. The glands secrete a variety of complex fluids that i) transport the sperm, ii) neutralize the acid environment of the female tract, iii) provide metabolic substrates for the sperm, and iv) combine to form the vaginal (copulatory) plug. Their structure is typical of active exocrine secretory glands, although the characteristics of the individual secretions are markedly different. Since the secretory activity of the accessory sex glands is extremely sensitive to androgen levels, weight change and altered secretory activity in the prostate and seminal vesicle can be used as a good, and relatively rapid, integrated indicator of altered circulating androgen levels.

**Prostate and Coagulating Gland**

The prostate forms multiple lobes around the urethra. It is a compound tubuloalveolar gland that secretes a colorless serous fluid into the urethra through a number of ducts. In the rat, a discrete pair of ventral lobes and a smaller group of dorsal and lateral lobes (dorsolateral lobes) are situated at the neck of the bladder. A pair of anterior lobes, otherwise known as the coagulating glands, is situated closely adjacent to and running up the medial aspect of the seminal vesicle. The glandular acini are lined by a simple columnar epithelium. The prostatic fluid secretion constitutes 15–30% of the ejaculate. It is a colorless fluid rich in proteolytic enzymes (e.g., acid phosphatase). The fluid also contains relatively high levels of zinc, inositol, transferrin, and citric acid.

The comparative histopathological structure of the various parts of the prostate varies slightly with respect to staining properties of the secretions and the degree of papillary infolding of the acinar epithelium.

Increased levels of oestrogen result in acute inflammation of the acini of the dorsal prostate and this provides an important endpoint for detection of oestrogenic compounds. The ventral lobes constitute the major part of the prostate and are the lobes that are most sensitive to circulating androgen levels.
Seminal Vesicle

The seminal vesicles are paired elongated hollow organs filled with a yellowish-white viscous fluid. They are situated distal to the ampulla of the vas deferens and empty via the ejaculatory duct into the urethra. The mucosa has a honeycombed structure formed by complex folding to produce irregular anastomosing channels that communicate with the central cavity; thin primary folds of the mucosa also extend out into the vesicle lumen. The epithelium is composed of pseudostratified columnar cells in the mouse and simple columnar epithelium in the rat. The seminal vesicle fluid is a viscous secretion constituting 50–80% of the ejaculate. The fluid is alkaline, which is thought to neutralize the acid pH of the vagina; it contains citric acid as the major component, as well as fructose and lactoferrin. Lactoferrin is one of the sperm-coating antigens and, as its name suggests, is also involved in iron binding.
Hormonal Regulation of Reproductive Tissues

Regulation of spermatogenesis relies not only on the classical endocrine control involving the hypothalamic - pituitary - testicular axis, but also on the complex autocrine and paracrine interactions involving the Sertoli cells, germ cells, Leydig cells, peritubular cells, interstitial macrophages and the endothelial cells of the interstitial vasculature. This is a rapidly advancing area of research which has important and pivotal implications for mechanistic investigations of male reproductive toxicity.

There are different levels of hormonal regulation of the reproductive tissues. Most people are familiar with classic endocrine regulation, involving the hypothalamic-pituitary-gonadal axis but it is important to be aware that there is another tier of regulation involving paracrine interactions between neighboring cells and autocrine regulation of a cell by itself. This is particularly prevalent in the testis where regulatory peptides and growth factors, secreted by the Leydig cells, Sertoli cells, germ cells and peritubular cells, are believed to mediate local control of cellular function between the various cells or within the cell that is secreting the factor.

TG407 is largely concerned with detecting disturbances in endocrine signaling and the basic pathways involved in regulation are shown in the following diagram.

Basic endocrine pathways of the hypothalamic pituitary testis axis. GnRH is released from the hypothalamus and travels to the pituitary via the hypothalamophyseal tract where it stimulates FSH and LH release into the peripheral circulation. FSH acts on the Sertoli cells and modulates spermatogenesis while LH acts on the Leydig cell to stimulate testosterone (T) biosynthesis. Negative feedback by testosterone and inhibin (secreted by the Sertoli cell in response to FSH) down regulates LH and GnRH release from the pituitary and hypothalamus. In addition to endocrine regulation, the various cells of the testis regulate one another through paracrine pathways. This involves secretion of a multitude of peptides and growth factors which provide local control of cellular function between Sertoli cells (SC), germ cells (GC), peritubular myoid cells (M), Leydig cells (LC) and endothelial cells of the blood vessels (BV).