# INTRODUCTION

Oligozoospermia is most commonly considered to be the result of deficient spermatogenesis. Nevertheless, the existence of obstructive oligozoospermia "whether partial or unilateral" must be always put in mind ***(Belmonte et al., 1998)***.

Partial obstruction is roughly defined as the presence of oligozoospermia with normal or nearly normal sperm production in the seminiferous tubules ***(Belmonte et al., 1998)***. The obstruction can occur at any site along the male genital tract. Broadly speaking, diagnosis of obstructive oligozoospermia could be suggested by some findings in history (e.g. of trauma or infection), in examination &/or investigations (e.g. epididymal swelling or marked discrepancy between testicular size and previous sperm counts) ***(Hendry et al., 1983)*** but most strongly, by the lack of correlation between score of mature spermatids (Sc + d) in the testicular biopsy and the sperm concentration per milliliter in the semen analysis ***(Silber and Rodriguez, 1981; Nistal el al., 1987)***.

The frequency of obstructive oligozoospermia is roughly estimated to be as high as 20% among patients with sperm concentrations of less than 5 million sperm/ml ***(Schoysman and Steward, 1980)*** and about 10 % among patients with sperm concentrations of less than 10 million sperm/ml ***(Silber and Rodriguez, 1981)***.

Spermatogenesis is a complex developmental process that depends primarily on pituitary gonadotrophins and testosterone. These hormones exert an indirect effect on spermatogenic cells through locally produced autocrine, paracrine and juxtacrine factors. These factors -in turn- have been shown to mediate complex interactions between Leydig cells, peritubular myoid cells, Sertoli cells and spermatogenic cells as a prerequisite of maintenance and control of spermatogenesis. However, the exact physiological role of many of these factors in the testis remain to be clearly delineated ***(Glander et al., 1996)***.

Among the molecules that are believed to be involved in the development of germ cells are insulin-like growth factor-I (IGF-I) and $\alpha_2$-macroglobulin ($\alpha$2-M). These are found both in plasma and tissue fluid ***(Glander et al., 1996)***.

Testicular IGF-I is mainly produced in Sertoli cells under the stimulation of FSH (Follicular Stimulating Hormone) ***(Ritzen, 1983)***.

Seminal IGF-1 concentrations have been shown to be significantly correlated with the percentage of morphologically normal spermatozoa and sperm concentration. It is also found that the concentration of IGF-1 is reduced in seminal plasma after vasectomy ***(Glander et al., 1996)***.

On the other hand, ($\alpha_2$-M) is a high-molecular-weight tetrameric protein. It is also synthesized in Sertoli cells under the control of FSH ***(Feldman et al., 1985)***.

Total α2 macroglobulin in seminal plasma has been also shown to be significantly correlated with the sperm count and percentage of progressively motile spermatozoa in the same previous study of ***Glander et al. (1996)***. The study could show that the concentrations of both IGF-1 and $\alpha_2$ macroglobulin in human seminal plasma were correlated to semen quality ***Glander et al. (1996)***.

Testicular biopsy is most commonly examined by the clinicians in a descriptive, non-quantitative way. This has severely limited its usefulness and led to many errors in its interpretation ***(Nelson, 1953; Mannion and Cottrell, 1961)***. For that same reason, testicular biopsy has not found much use in men with oligozoospermia ***(Albert, 1961)***.

***Heller and Clermont (1964)*** first described the histological characteristics and kinetics of the spermatogenesis in the human. They determined through radioactive tracer studies that the rate of spermatogenesis in human is always constant even when sperm output is reduced. Therefore, the amount of the sperm being produced by the testicle should be reflected by what is seen in a fixed specimen of testis biopsy.

***Tjioe et al. (1967)*** then developed a method of quantitative interpretation of the testicle biopsy. Unfortunately, the number of their patients was too small to make a precise correlation with sperm count. Furthermore, their technique was elaborate and time-consuming.

***In 1978, Zukerman et al.*** working with Steinberger, counted all components of spermatogenesis and found a superb correlation with sperm count.

***Silber and Rodriguez (1981)*** then described a simplified modification of Steinberger‘s quantitative method. Sertoli cells were not counted, while the number of mature spermatids in a minimum of 20 tubules was simply counted and divided by the number of tubules.

# Aim of the Work

To assess the value of measuring levels of IGF-1 and α2-macroglobulin in predicting testicular sperm output of the testicles as judged from quantitative analysis of testicular biopsy in oligozoospermic cases.

# Chapter (1)
# Anatomy and Histology of Male Genital Tract

The scrotum is a cutaneous pouch divided into two sacs by a partial median septum. Each sac contains the testis, epididymis and lower part of the spermatic cord. The scrotal skin is thin, pigmented, and rugose and bears a pigmented midline raphe. The skin contains numerous fine hairs and sebaceous glands. From outwards to inwards, the scrotal wall consists of skin, subcutaneous fat, dartos muscle, external spermatic fascia, cremastric muscle, internal spermatic fascia and inner lining of parietal tunica vaginalis. Contraction of the dartos muscle produces the characteristic wrinkling of the scrotal skin ***(Cho et al., 1994)***.

The normal adult testes are paired organs, ovoid in shape, measuring 4-5 cm in length, 2.5 cm in width and 3 cm in anteroposterior diameter. The left testicle usually lies lower than the right testis. Each testis is covered by a visceral layer of tunica vaginalis except for a strip that extends from the upper to the lower pole of the posterior surface leaving the region of the mediastinum testis uncovered. Each testis is enclosed by a white fibrous sheath, the tunica albuginea, which represents the testicular capsule. Each testis is divided into 200-300 lobules

separated from each other by fibrous septa. The septa extend from the mediastinum to the tunica albuginea. Each lobule contains 1-3 seminiferous tubules. Each tubule measures about 60cm in length and is coiled upon itself. Posteriorly, the tubules converge to form a plexus called the rete testis, from which emerge about 12 efferent ducts. They pierce the tunica albuginea at the upper pole of the testis to form the head of the epididymis. The efferent ducts are then fused to form a single, extremely convoluted tube that forms the body and tail of the epididymis (Fig. 1) ***(Cho et al., 1994; Ellis and Mahadevan, 2013)***.

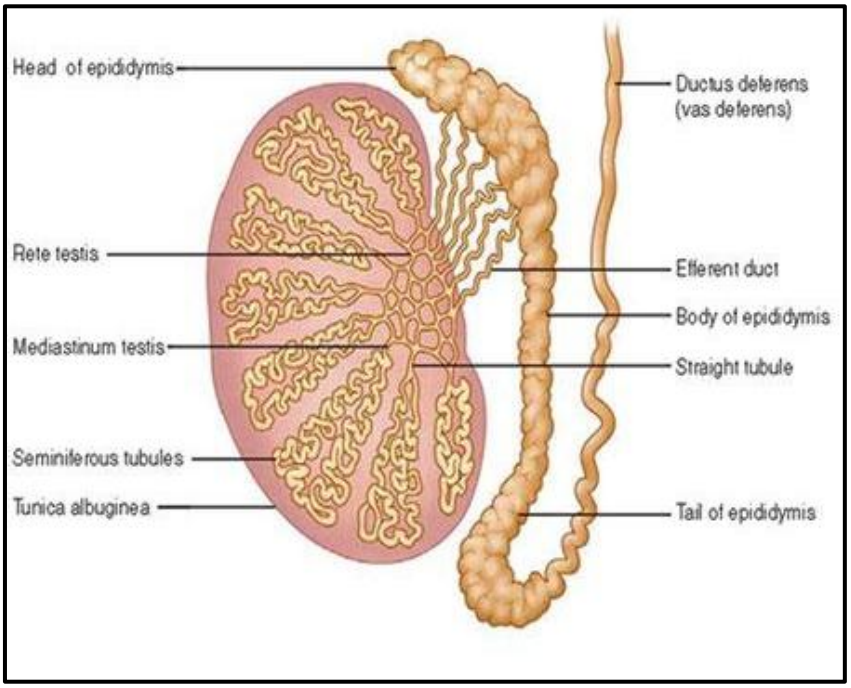


**Fig. (1):** Longitudinal section of testis, epididymis and ductus deferens ***(Berny and Ulbright, 2015).***

The testes receive their blood supply from the testicular arteries, cremastric arteries and deferential arteries. The bilateral testicular arteries are the primary blood supply of the testes that arise from abdominal aorta just inferior to the origin of the renal arteries. The cremastric arteries "external spermatic arteries" are branches of inferior epigastric artery. The deferential arteries originate from the superior vesical arteries which in turn arise from the internal iliac artery that represents the main blood supply to the pelvis. Although the testicular arteries are the main blood supply of the testis, there is sufficient anastomotic communication among all three arteries so that one artery can often be ligated without loss of adequate blood supply to the testis. The venous return of the testis is an important feature of the male reproductive tract. The veins emerge from the testis to form dense intercommunicating network known as pampiniform plexus which extends into the scrotum then through the spermatic cord (Fig. 2) ***(Roberts and Pryor, 1997)***.

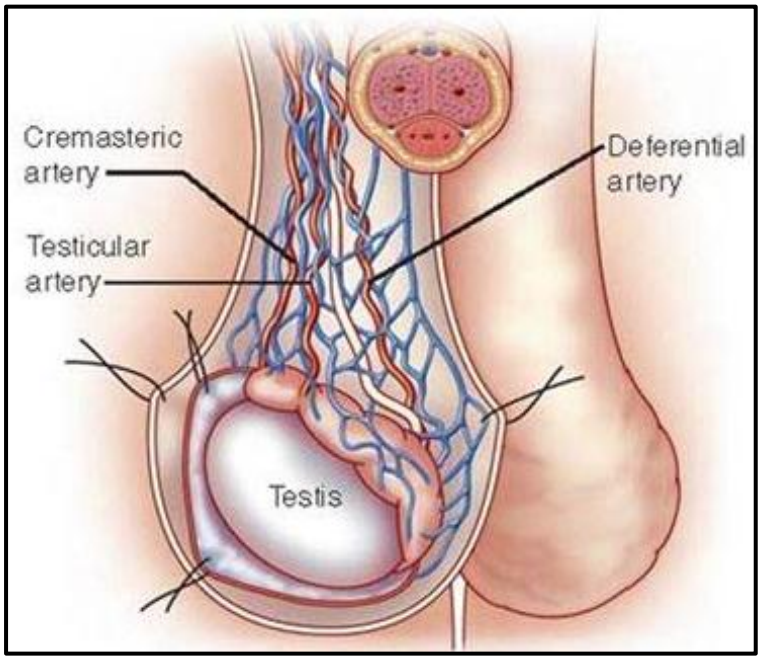


**Fig. (2):** Testicular blood supply ***(Berny and Ulbright, 2015)***.

The seminiferous tubule is made up of Sertoli cells, germ cells and peritubular myoid cells. The Sertoli cell is a non-dividing somatic cell of epithelial origin that forms the wall of the tubule. It extends from the basement membrane toward the lumen of seminiferous tubule. It has an ovoid to triangular shaped nucleus with a punched-out red nucleolus. There are tight junctions between adjacent Sertoli cells that lead to the formation of an effective barrier against the passage of macromolecules called blood testicular barrier (BTB). So, tight junctions divide the seminiferous tubules into 2 separate compartments:

a) Adluminal compartment; the portion of seminiferous tubule internal to the tight junctions.

b) Basal compartment; the portion of seminiferous tubule external to the tight junctions (Fig 3) ***(Roberts and Pryor, 1997)***.

The Sertoli cell has several distinct functions in the support of spermatogenesis; first, it provides the physical support for the germ cells providing a scaffold upon which the germ cells develop and move towards the lumen of the tubule. Second, the Sertoli cells form the BTB. Third, the Sertoli cells define the environment in which the germ cell mature by supplying nutritional factors for germ cell metabolism (e.g., lactate), secreting proteins and other factors required for germ cell maturation (e.g., transferrin) and secreting paracrine factors that mediate cell-to-cell signaling between germ cells and Sertoli cells (e.g., activin). Fourth, Sertoli cells phagocytose the excess germ cell cytoplasm that is sloughed in the form of a residual body before spermiation ***(Roberts and Pryor, 1997)***.

BTB provides the specialized switch for meiosis and spermiation by;

1- acting as a defense wall through masking the sperm antigen from the immune system.

2- functioning as an extensive barrier in governing the transit of nutrients, ions, electrolytes, hormonal and autocrine factors.

3- permitting the entry of selected drugs with the help of specific drug transporters

4- functioning as a unique testis- specific ultrastructure that undergoes an extensive remodeling to facilitate the transport of preleptotene spermatocytes across BTB ***(Cheng and Mruk, 2012)***.

The germ cell development from primary spermatocyte till spermatozoa takes place in the adluminal compartment of the seminiferous tubules beyond the tight junctional complexes. The composition of the luminal fluid in this compartment is composed primarily of Setroli cell secretions. So, the Sertoli cells are considered "the nurse cells" of the tubules facilitating the maturation of meiotic and postmeiotic germ cells ***(Roberts and Pryor, 1997)***.

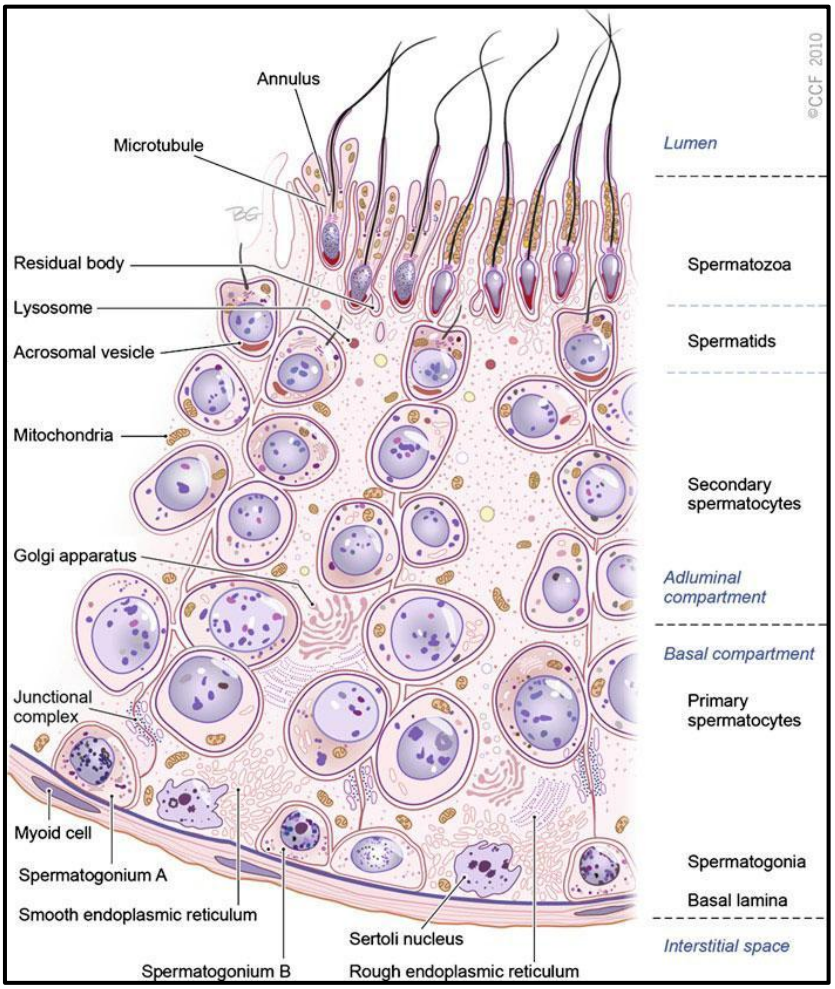


**Fig. (3)**: Germinal epithelium in the seminiferous tubule ***(Sharma and Agarwal, 2011)***.

The germ cells are arranged in a highly ordered sequence from the basement membrane to the lumen (Fig. 3). Spermatogonia lie directly on basement membrane, followed by primary spermatocytes, secondary spermatocytes and spermatids as well as sperms as they progress toward the tubule lumen. They undergo a series of proliferation and differentiation within the wall of the seminiferous tubule to give rise to spermatozoa. Each germ

cell develops in an intimate association within the Sertoli cell. Spermatogonia are large round cells with a pale staining round or ovoid nucleus. They have dark and light nuclei depending on subtle nuclear characteristics. Primary spermatocytes have the biggest nucleus in which the condensed chromosomes can form elongated filamentous structures. Secondary spermatocytes are rarely identified because the second meiotic division occurs very rapidly. The early spermatid is a small rounded cell with a hyperchromatic nucleus and featureless chromatin. Later, the spermatid becomes more conical with a flagellum and even denser nucleus to become a spermatozoan. Peritubular myoid cells surround the wall of the seminiferous tubule and are responsible for its peristaltic movements and secretion of paracrine factors. The interstitial tissue comprises fibrocytes, blood and lymphatic vessels and significant number of leucocytes (mainly macrophages and to a less extent T-lymphocytes and mast cells) as well as Leydig cells. The Leydig cells often accumulate in small groups around blood vessels. These cells are rich in smooth endoplasmic reticulum. They have abundant pink cytoplasm with lipid, lipochrome pigment, Reinke crystalloids (hexagonal prisms by electron microscopy) as well as round nuclei with distinct nucleoli. Scattered Leydig cells are also found in the spermatic cord and tunica albuginea. They synthesize testosterone as the main androgen and many protinergic factors such as growth factors, neuropeptides and cytokines ***(Roberts and Pryor, 1997; Meinhardt, 2006; Berny and Ulbright, 2015)***.

The epididymis is the first part of the efferent route from the testis and lies along its posterolateral aspect (Fig. 1). It is the site responsible for maturation and storage of the sperm. It is 6-7 cm in length, but it contains a tortuous canal of 6 meters long. It is divided into three parts; head, body and the tail. The head of the epididymis (globus major or caput epididymis) is rounded or triangular and is located on the upper pole of the testis. The efferent ductules become enlarged and exceedingly convoluted to form a series of conical masses which together form the epididymal head. All ductules in the head open into one duct named the ductus epididymis forming the body of the epididymis. The epididymal duct is slightly enlarged and thickened at the lower pole of the testis to form the tail (globus minor). From this point the epididymis ascends superiomedially to become the ductus deferens. There are three primary functions of the epididymis; maturation of the sperm, peristaltic conduit for the active transport of the sperm from the testis to the vas and storage site for mature sperm. The transit time of the sperm in human epididymis takes about 12 days but it is highly variable with some sperms progressing through the epididymis within only 2 days ***(Cho et al., 1994; Roberts and Pryor, 1997)***.

The ductus epididymis is lined by pseudostratified stereociliated columnar epithelium which consists of ciliated tall columnar (principal cells), narrow darker staining columnar cells, basal cells, clear cells and

occasionally intraepithelial lymphocytes. Tight junctions between neighboring epithelial cells form a diffusion barrier. Proximally, the tubules of the epididymis have a thin muscular coat that becomes gradually more apparent in the corpus and particularly in the vicinity of the vas deferens ***(Meinhardt, 2006)***.

The vas deferens is a thick muscular tube that carries the sperm from the cauda epididymis to the ejaculatory duct in the prostate (Fig. 1) ***(Roberts and Pryor, 1997)***.

The first part of the vas, originating at the cauda epididymis, is tortuous named "the convolued portion". Then, the vas deferens courses superiorly, along the posterior surface of the epididymis, passing through the inguinal canal with the testicular artery and veins and enters the pelvis at the internal inguinal ring. From this point, the vas deferens courses posteriomedially over the junction of the ureters with the bladder, passing behind the bladder from above and entering the prostate in an inferiomedial direction. Before the vas enters the prostate at its dorsal surface, it enlarges to form an ampulla and is joined by the duct of the seminal vesicle to form the ejaculatory duct. The latter passes through the prostate and opens into the prostatic urethra close to the orifice of the prostatic utricle ***(Roberts and Pryor, 1997; Meinhardt, 2006)***.