

## Microscopic Evaluation of The Testis in Azoospermic Patients with Reference to Androgen Receptors in Sertoli Cells

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### ABSTRACT

**Background:** The reproductive ability of sexually mature males is dependent on the capacity of the testes tubules to produce large numbers of highly viable sperms and the interstitial cells to produce adequate levels of androgens to ensure maturation of the reproductive system. Azoospermia, defined as the complete absence of spermatozoa in the ejaculate, invariably results in infertility but does not necessarily imply sterility. It is identified in approximately 1% of all men and in 10 to 15% of infertile males.

**Patients and methods:** The use of biopsies had been approved by the ethics committee of the Faculty of Medicine, Al Azhar University. All testicular biopsies were taken after written informed consent at the Department of Clinical Andrology, International Islamic Center for Population and Research Studies. Eleven patients with idiopathic infertility, were enrolled in this study and were assessed and diagnosed by a complete examination and specific investigations.

**Results:** This study included 11 patients. They were divided into two groups obstructive azoospermia (OA) and Non-obstructive azoospermia. Non-obstructive azoospermia is subdivided into 4 groups: Early maturation arrest, late maturation arrest, Sertoli cell only (Sco) and Klinefelter syndrome.

**Conclusion:** Spermatogenesis and maintenance of reproductive functions are controlled by androgens which are steroid hormones. Androgens exert most of their effects through genomic actions, which involve their binding to the androgen receptor (AR). AR plays important roles during later stages of sperm formation and maturation by influencing the four major cell types: Sertoli, Leydig, peritubular myoid, and germ cells. Except for maturation arrest non-obstructive azoospermia, AR positive staining was not detected in germ cells. The highest number of Sertoli cell androgen receptors was in sections of testis from obstructive azoospermia and non-obstructive late maturation arrest patients, Testicular sections from patients with Klinefelter syndrome presented the lowest number of androgen receptor positive cells. It was concluded that the present study revealed that demonstration of androgen receptors in testicular sections is a good indicator of spermatogenic activity. Such information is valuable for the decision of obtaining spermatozoa from the testis for ICSI.

**Keywords:** Infertility, Azoospermia, spermatogenesis, androgen receptors, Sertoli cells.

### INTRODUCTION

The AR is a member of a large family of ligand-activated nuclear receptors and is highly expressed in the testes <sup>(1)</sup>.

Azoospermia, defined as the complete absence of spermatozoa in the ejaculate, invariably results in infertility but does not necessarily imply sterility <sup>(2)</sup>. It is identified in approximately 1% of all men and in 10 to 15% of infertile males <sup>(3)</sup>.

Testicular biopsy remains the key investigation for all testicular causes of infertility. It is not the only parameter for determining the testicular histopathology pattern but apparently the strongest indicator to foresee the possibility of finding sperms in the testis for therapeutic sperm retrieval in assisted reproductive techniques <sup>(4)</sup>.

### AIM OF THE STUDY

The aim of this study is microscopic evaluation of testis in azoospermic patients for precise establishment of the disease etiology,

Androgen receptor of Sertoli cells in azoospermic patients will be studied.

### SUBJECTS AND METHODS

#### Selection of cases

Eleven patients with idiopathic infertility, were enrolled in this study and were assessed and diagnosed by a complete examination and specific investigations. The use of biopsies had been approved by the ethics committee of the Faculty of Medicine, Al Azhar University. All testicular biopsies were taken after written informed consent at the Department of Clinical Andrology, International Islamic Center for Population and Research Studies.

#### Inclusion criteria

1. Age from 28 to 35 years.
2. Infertility for more than one year.
3. Willingness to participate and signing of consent after the study had been explained.

### **Exclusion criteria**

1. Patients receiving hormonal treatment in the previous 3 months.
2. Patients with systemic organ failure.
3. Patients with previous chemotherapy or radiotherapy.

### **Samples preparation**

#### **Testicular sperm extraction technique**

Eleven patients, aged 28-35 years, underwent TESE from 12 testicular quadrants in both testes (all of them underwent color duplex mapping of the testis and TESE and were biopsied either from right only, left only, or both testes).

### **Diagnosis of the patients**

Patients were subjected to the following:

Thorough history taking (personal, infertility, sexual, medical, surgical, and drug history), general and local (penoscrotal and prostatic) examinations, and conventional semen analysis.

The diagnosis of azoospermia was made based on the analysis of at least two semen samples collected at different times, and two replicates of each sample were centrifuged at 3000g for 15 min. All the patients were subjected to assessments including a detailed personal history and physical examination.

Patients were divided into two groups as follows:

**Group I (obstructive azospermia):** This group included 3 patients.

**Group II (Non-obstructive azospermia):** This group included 8 patients.

Group II was further subdivided into 4 subgroups:

- a) *Maturation arrest*: included 1 patient.
- b) *Sertoli cell only (SCO)*: included 5 patients.
- c) *Klienfiker Syndrome*: included 1 patient.
- d) *Late Maturation arrest*: This group included 1 patient.

Specimens for the histopathological examination of testicular biopsies were processed using histological and immunohistochemical techniques.

### **Histological Methods**

Testicular biopsies were fixed by immersion in Bouin's solution and embedded in paraffin. 5  $\mu$ m thick sections were stained with hematoxylin and eosin, Mallory's trichrome stain and periodic acid-

Schiff (PAS) <sup>(5)</sup>. Micrographs of sections of different groups were examined.

### **Immunohistochemical Method**

Formalin-fixed paraffin-embedded testicular sections were mounted on positively charged glass slides. The paraffin sections were deparaffinized, hydrated, and then placed in 10% H<sub>2</sub>O<sub>2</sub> to block endogenous peroxidase activity.

Unmasking of antigenic sites was carried out by transferring sections into a jar containing 0.001 mol/l citrate buffer (pH 6).

The sections were then incubated with 1.5% nonimmunized goat serum for 30 min at room temperature, and then incubated with the primary antibody specific for each immunostain and washed three times with phosphate-buffered saline for 30 min. Thereafter, the sections were incubated with the appropriate biotinylated secondary antibodies (ABC kit, 1:200; Amersham Pharmacia Biotech, Santacruzbiotechnolgy, Inc CA, USA) for 30 min. Freshly prepared 3,3'-diaminobenzidine (DAB) was used as a chromogen. Sections were incubated with DAB for 10 min and then washed with tap water, counterstained with hematoxylin, dehydrated, and mounted. For the negative control, the primary antibody was replaced by phosphate-buffered saline.

### **Quantitative measurement**

200X digital micrographs of immuno-stained androgen receptors in testis sections representing all studied groups were subjected to quantitative evaluation. The number of positively stained cells per field were recorded using Image Pro Plus version 4.5 image analysis software (Media Cybernetics USA) at the Histology Department, Faculty of Medicine, Al-Azhar University. At least 10 equal fields from each of 5 slides were counted.

### **Data Management and analysis**

The collected data was analyzed and graphed using Microsoft Excel 10 software. The following statistical analysis were calculated and tabulated:

- 1- The mean  $\pm$  standard deviation.
- 2- Student (t) test to assess the statistical significance of the difference between each two of the study group means.

The means  $\pm$  standard deviation was graphed in column form.

**RESULTS**

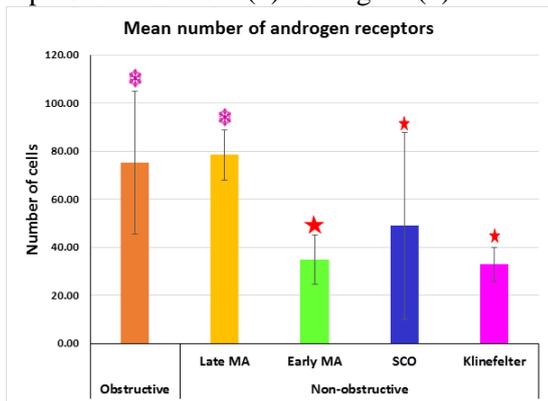
**1) Quantitative Results**

**Table (1):** Mean number of cells with positively stained androgen receptor.

	Obstructive	Non-obstructive			
		Late MA	Early MA	SCO	Klinefelter
Mean	75.21 <sup>ψ</sup>	78.5 <sup>ψ</sup>	34.86*	48.94*	32.86*
StDev	±29.60	±10.52	±10.41	±38.78	±7.0
p (Obstr)≤		0.79	0.000012	0.003	0.0007
p (Klinef)≤	0.0007	0.0000015	0.65	0.29	

\*The mean number of cells is significantly ( $p \leq 0.05$ ) different from that of obstructive group. <sup>ψ</sup>The mean number of cells is significantly ( $p \leq 0.05$ ) different from that of Klinefelter group.

Androgen receptors were demonstrated by immune-histochemical method. The number of cells with positive immune-stained receptors were counted in a fixed area. The data obtained from all studied groups is represented in table (1) and figure (1).



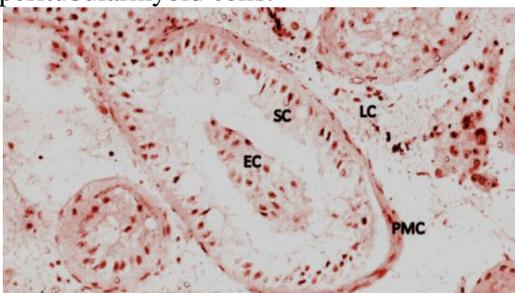
**Figure (1):** Mean number of cells with positively stained androgen receptor. Bars represent the  $\pm$  values of the standard deviation. ★ The mean number of cells is significantly ( $p \leq 0.05$ ) different from that of obstructive group. \* The mean number of cells is significantly ( $p \leq 0.05$ ) different from that of Klinefelter group.

**2) Immuno-histochemical Results**

(3)

**I. Obstructive azoospermia**

Androgen receptors are represented in the nuclei of Sertoli cells in the seminiferous tubules (Figure 2). Leydig (interstitial) cells are also positively stained. Positively stained receptors are also demonstrated in peritubularmyoid cells.

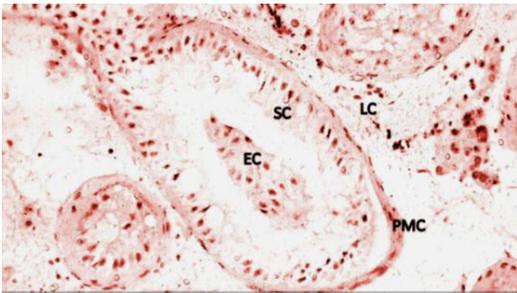


**Figure (2):** Androgen receptors in testicular cells of obstructive azoospermia patient. Positively stained antigen is demonstrated in Sertoli cells (SC) and Leydig cells (LC) or interstitial cells (PM) peritubularmyoid cells. (Immuno-histochemical stain x250)

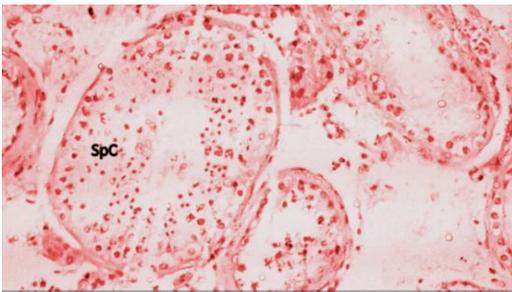
**II. Non-obstructive azoospermia**

**A. Late maturation arrest**

In this subgroup, The positively stained antigen displays a palisade shape very close Sertoli cells. Exfoliated cells in the lumen of the seminiferous tubule also demonstrate the positive staining. Some seminiferous tubules appear with very small diameter but contain positively stained cells. Leydig cells and peritubularmyoid cell nuclei are also positive (Figure 3). The seminiferous tubules that present spermatogenesis, positively stained receptors are demonstrated in different spermatogenic cells (Figure 4)



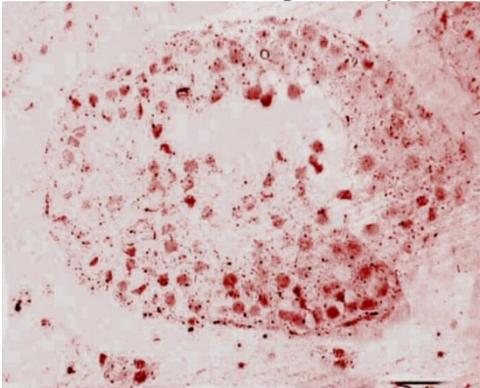
**Figure (3):** Androgen receptors are demonstrated in the nuclei of Sertoli cells (SC), intraluminal exfoliated cells (ES), Leydig cells and peritubularmyoid cells (PMC). (immuno-histochemical AGR stain x250).



**Figure (4):** In the non-obstructive late maturation arrest azoospermia patient, the different spermatogenic cells have the positive stain in their nuclei. (immunohistochemical stain x250)

### **B. Early maturation arrest**

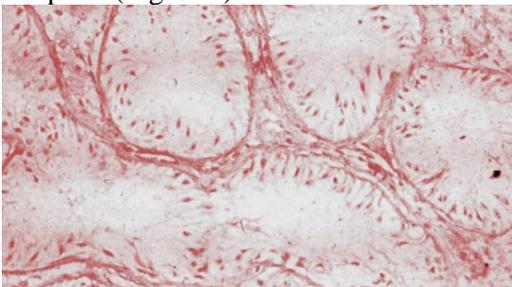
In sections of the testis of non-obstructive early maturation arrest azoospermia, all the cells lining the seminiferous tubule are positively stained (Figure 5)



**Figure (5):** In early maturation arrest in non-obstructive azoospermia, the positively stained receptors are found in all the cells of the seminiferous tubule (immune histochemical stain x250)

### **C. Sertoli Cell only (SCO)**

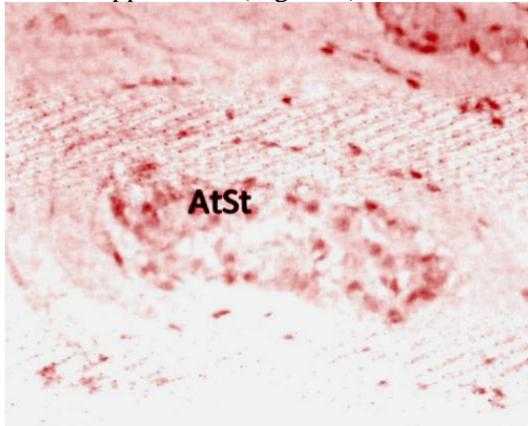
In the testis of this group, The Sertoli cells lining the seminiferous tubules show the positively stained receptors (Figure 6).



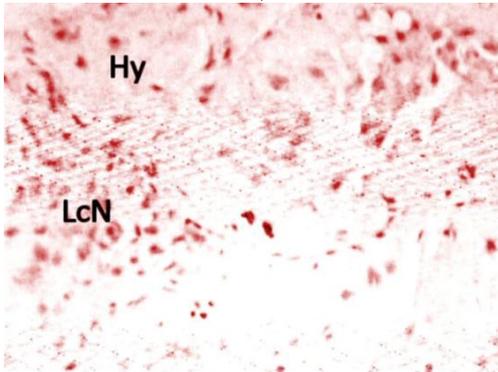
**Figure (6):** Androgen receptor stained seminiferous tubules demonstrate the positive antigen in the nuclei of Sertoli cells. Sertoli cells are the only cells detected in the tubules.

#### D. Klinefelter Syndrome

In this group the seminiferous tubules show different stages of atresia (Figure 7). The normal arrangement of the wall of the seminiferous tubule is not represented. The positively stained nuclei are the peritubularmyoid cells. Cells with large nuclei fill the lumen of the tubule. Complete atresia reformation of hyalinized tubules are evident. The androgen receptors are presented in Leydig cells which aggregate forming a nodular appearance (Figure 8)



**Figure (7):**Atretic seminiferous tubule from the testis of patient with Klinefelter syndrome. AtSt: atretic seminiferous tubule. (Immunohistochemical stain x 250)

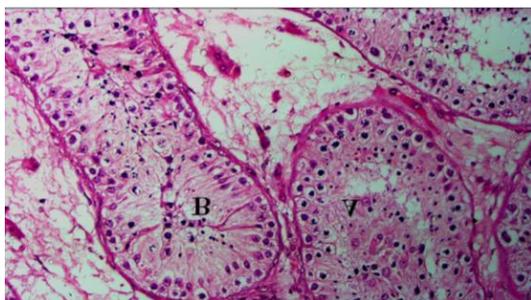


**Figure (8):** A section of testis from a patient with Klinefelter syndrome. Hy: hyalinised seminiferous tubule, LcN Leydig cell nodule (immunohistochemical staining x250)

## 2) Histological and histochemical Results

### I. Obstructive azoospermia

In sections of the testis from obstructive azoospermia patient, The wall of the seminiferous tubules show different phases of spermatogenesis. However, the lumen which in normal testis contains spermatozoa, is occupied by exfoliated cells. Other tubules have mainly spermatogonia and Sertoli cells and very few locations have Sertoli cell attached spermatozoa (Figures 9 and 10).



**Figure (9):** Histological structure of testis of obstructive azoospermia patient. Notice the exfoliated cells in the lumen of the active seminiferous tubule (A) while the other (B) have the Sertoli cells and the spermatogonia. Spermatozoa can be observed in very few locations (arrow) (Hx, E x250).

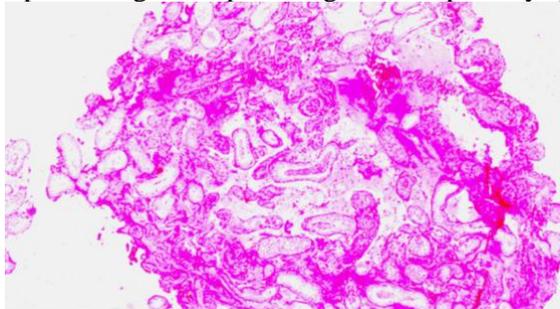


**Figure (10):** High power section of the part represented by rectangle in figure 10. In seminiferous tubule A, All stages of spermatogenesis are represented. Sc; Sertoli cell, SpG: spermatogonia, PSp: primary spermatocytes, SpTd: spermatids. In the lumen, there are exfoliated cells some of them with pyknotic nucleus others have vesicular nucleus. In seminiferous tubule B very few spermatozoa (Sz) are formed (Hx,E x500).

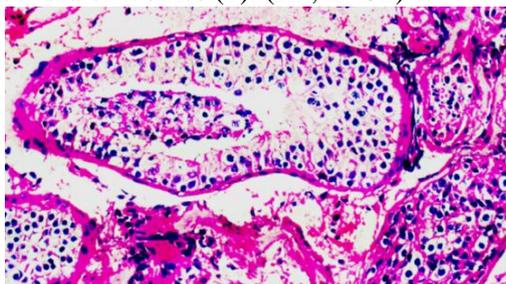
## II. Non-obstructive azoospermia

### A. Late maturation arrest

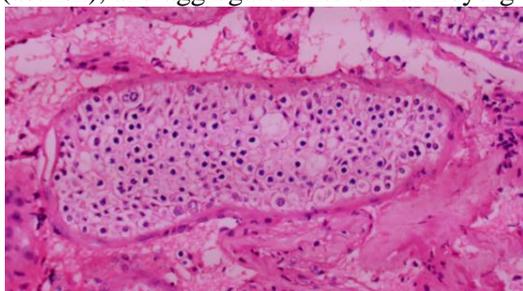
The seminiferous tubules of the testis of patients with late maturation arrest azoospermia have different sizes and shapes. Some tubules have large diameter with intra luminal exfoliated cells, others have small diameter. Some testicular areas are devoid of seminiferous tubules (Figure 11). The spermatogonia as well as primary spermatocytes and other spermatogenic cells can be observed in large tubules but not in the small tubules (Figure 12). Some tubules contain spermatogonia cells that show signs of degeneration while others are completely hyalinized (Figure 13). Cell degeneration is represented by clear cells, multinuclear giant cells representing both spermatogonia and primary spermatocytes and karyolysis of spermatogonia (Figure 14).



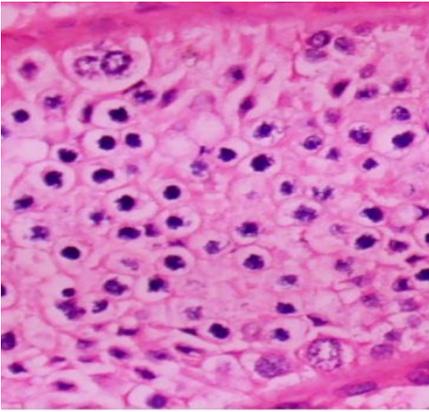
**Figure (11):** Section in the testis of late arrest non-obstructive azoospermia patient. Some tubules have a normal size (arrow), some areas are occupied by small diameter tubules (\*) while other areas are completely devoid of tubules (Ψ). (Hx, E x32).



**Figure (12):** Seminiferous tubule with intraluminal exfoliated cells (EC). Some small diameter tubules (astrisk), and aggregated interstitial-Leydig cells (LC) (Hx,Ex 250).



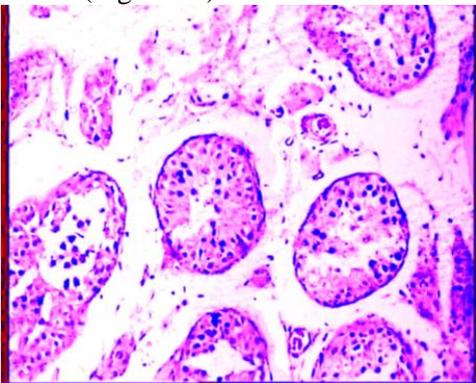
**Figure (13):** Seminiferous tubule. The lumen is obliterated by germ cells. Signs of spermatogenic cell degeneration are evident (arrow). Some tubules show degeneration (D) and others show hyalinization (H). Aggregated Leydig cells appear in interstitial area (LC) (Hx, E x250).



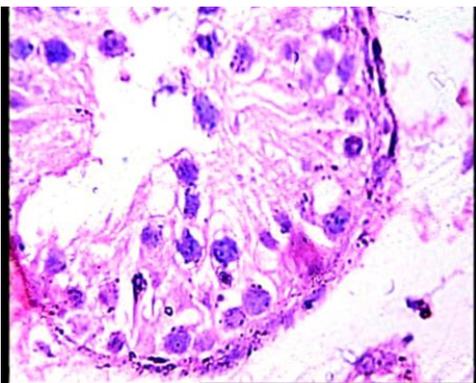
**Figure (14):** A higher magnification of the part of seminiferous tubule indicated in figure 12 by the rectangle. Degenerated multinucleated giant cells (arrow), degenerating spermatogonium (arrow head).

### **B. Early maturation arrest**

In specimens of this group, the seminiferous tubules are small in diameter and dispersed in the section (Figure 15). Only spermatogonia and primary spermatocytes are represented in the wall of seminiferous tubule (Figure 16).



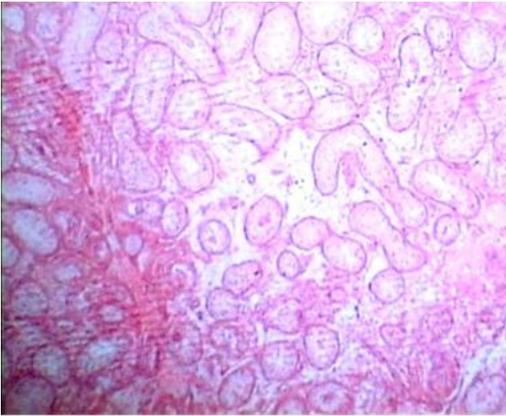
**Figure (15):** Section of testis from a patient with non obstructive early maturation arrest bazoospermia. The tubules have small diameter and dispersed. Leydig cells are found in the interstitium (HxnE x100).



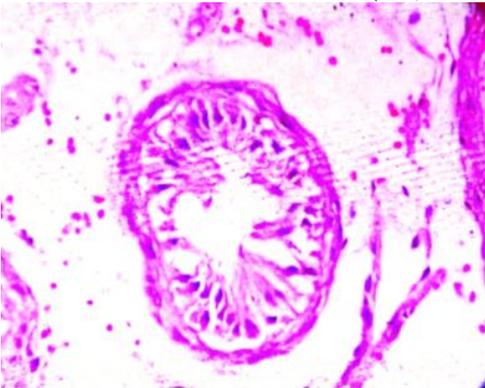
**Figure (16):** The seminiferous tubule from this group has spermatogonia and spermatocytes only. (Hx,E X400).

### **C. Sertoli Cell only (SCO)**

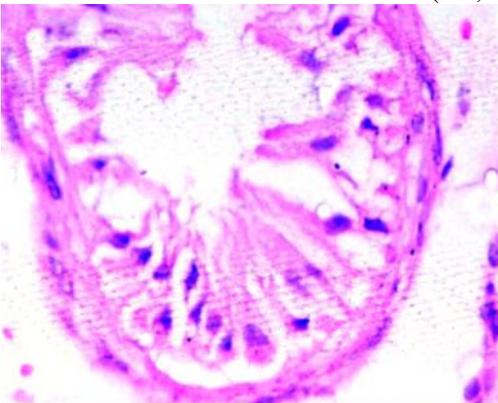
In sections of testis of this group, the wall of the seminiferous has a palisade arranged Sertoli cells. No spermatogenic cells can be detected. The nuclei of the peritubularmyoid cells and fibroblasts are associated with relatively thick basement membrane (Figures 17,18,19,20).



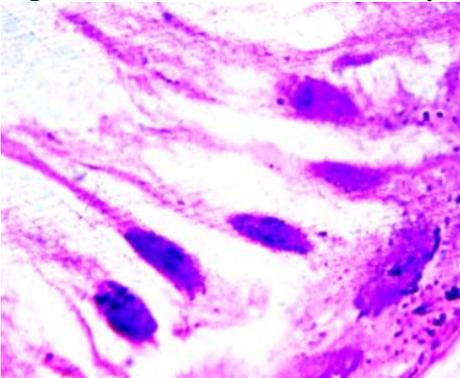
**Figure (17):** Section in the testis of a patient with Sertoli cell only azoospermia. The seminiferous tubules are distributed all over the section (Hx,E x30).



**Figure (18):** The wall of seminiferous tubule is occupied with Sertolimcells. Peritubular cells are found as well as a thick basement membrane (Hx,E x200).



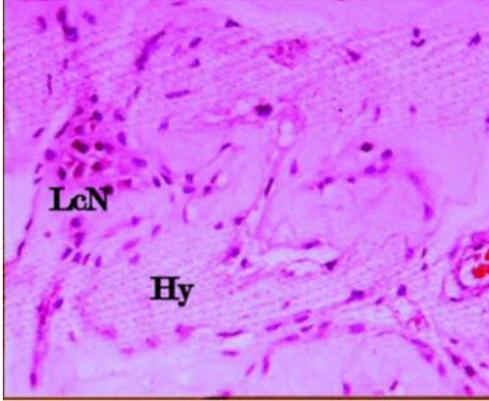
**Figure (19):**Sertoli cells are the only cells in the wall of seminiferous tubule (HxnE x400).



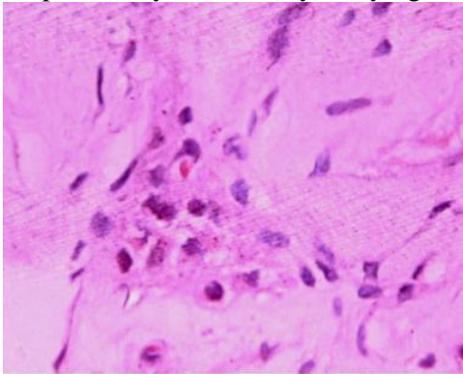
**Figure (20):** At high power, Sertoli cells are lining the tubules while nuclei of the peritubularmyoid cells are evident associated with the cell membrane (Hx,E x1000).

D. Klinefelter Syndrome

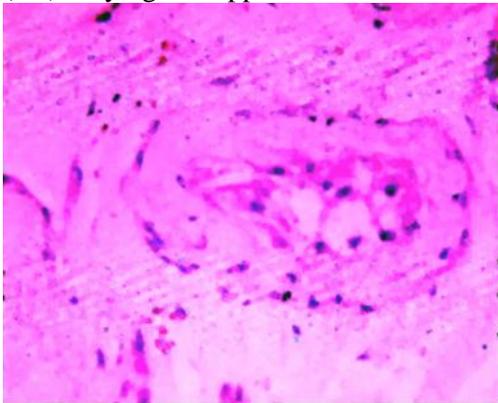
In testis of Klinefelter syndrome, many seminiferous tubules are hyalinized with abnormal appearance (Figure 21). Leydig cells are numerous and aggregated in the form of nodules (Figure 22). Some atrophied tubules appear in the sections (Figure 23).



**Figure (21):** Section in the testis of a Klinefelter syndrome patient. The seminiferous tubule is of irregular shape with hyalinized (Hy). Leydig cells are aggregated in the form of nodule (LcN). (Hx,E x 200).



**Figure (22):** Leydig cells form a nodule (LcN). Peritubular myoepithelial cells surround the hyalinized tubule (PC). Leydig cell appear in the tubular wall (Sc). No spermatogenic cells can be observed. (Hx,E x400).



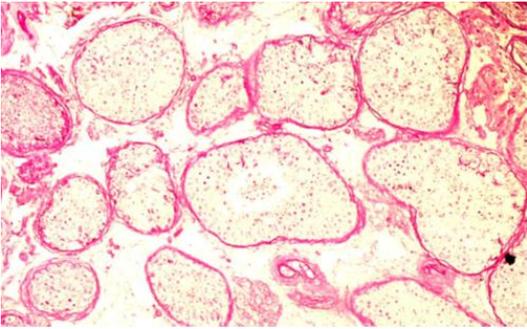
**Figure (23):** Atrophied seminiferous tubule with intratubular Sertoli cells. (Hx,E x 200).

#### 4) Histochemical results

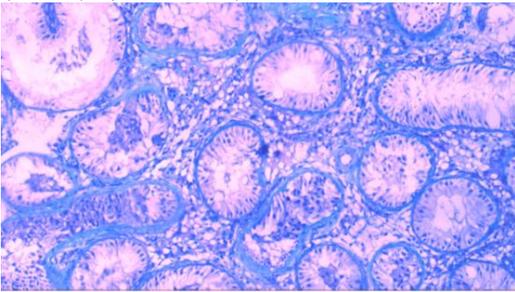
##### I. Obstructive azoospermia

In PAS stained sections of obstructive azoospermia patients, small diameter seminiferous tubules have slightly thickened basement membrane. PAS positive mucopolysaccharides are also found in the interstitial area (Figure 24).

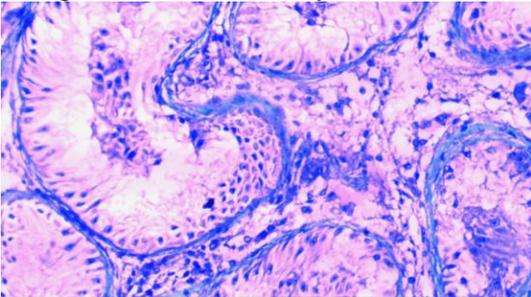
In Mallory trichrome stained sections, the interstitial area appears highly fibrillar where collagen fibers are abundant. The basement membrane of some seminiferous tubules is thicker than the others (Figure 25). Numerous Leydig cells almost completely fill the interstitium between some tubules (Figure 26, 27).



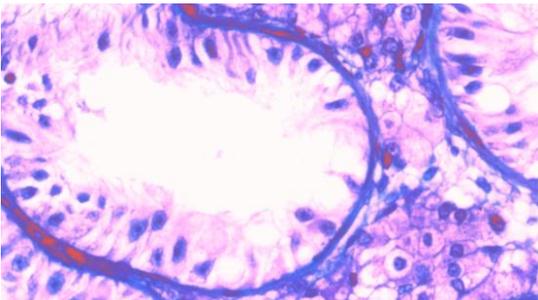
**Figure (24):** PAS postivemucoproteins in the basement membrane of the testicular seminiferous tubules of obstructive azoospermia patient. Some seminiferous tubules are small with thick basement membrane (arrow). (PAS x250).



**Figure (25):** Section in the testis of a patient with obstructive azoospermia. the interstitial area show abundant collagenous fibers (Mallory trichrome stain x125).



**Figure (26):** Section in the testis of a patient with obstructive azoospermia. The basement membrane of some seminiferous tubules is thickened. Numerous Leydig cells are found in the interstitial area. Collagen is distributed in the interstitial area and the basement membrane (Mallory trichrome stain x 250).

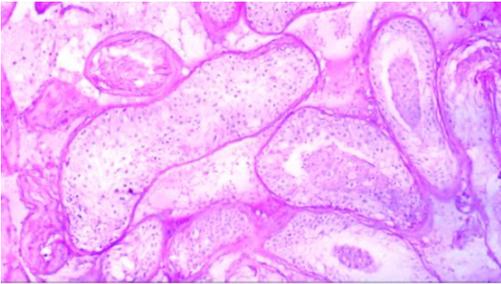


**Figure (27):** At higher magnification the Leidig cells are abundant in the interstitial area (Mallory trichrome stain x500).

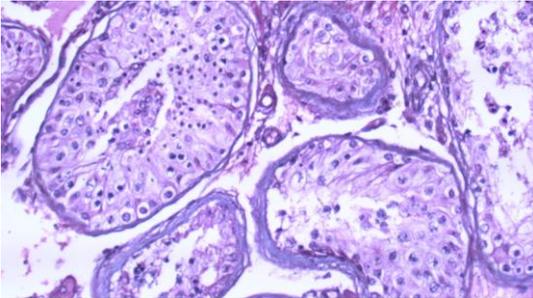
## II. Non-obstructive azoospermia

### A. Late maturation arrest

In non-obstructive late maturation arrest azoospermia patients, the basement membrane and the interstitial area are rich in PAS positive mucopolysaccharides (Figure 28) and collagenous fibers (Figure 29).



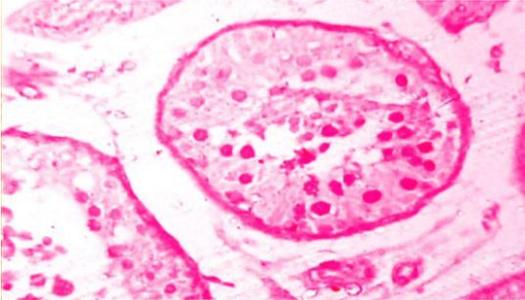
**Figure (28):** A section in the testis of non-obstructive late maturation arrest azoospermia patient, the mucopolysaccharides demonstrates in the basement membrane suggest its regular thickness. The interstitial area contains abundant mucopolysaccharides (PASx 250).



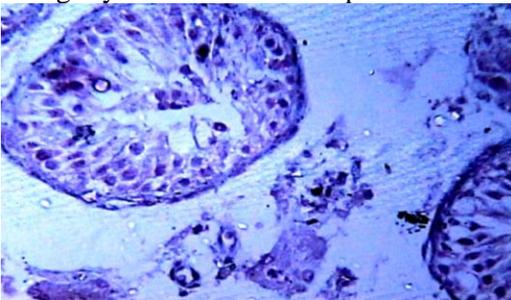
**Figure (29):** In this section, the interstitial area and the basement membrane contain the collagen fibers. Different spermatogenic cells up to the spermatids are found in the tubules. (Mallory trichrome x250).

#### B. Early maturation arrest

In the sections of testis of this group, the PAS positive staining suggest moderately thickened basement membrane (Figure 30). Collagen fibers are found both in the basement membrane and interstitial area (Figure 31). In the wall of the tubules, large nucleated cells suggest the arrest at the primary spermatocyte stage.



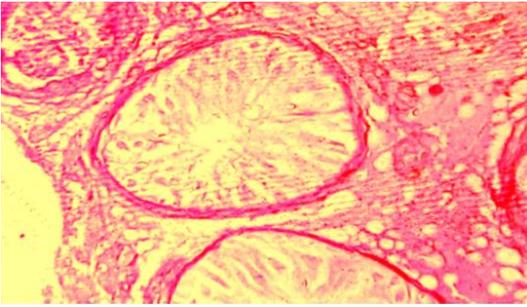
**Figure (30):** Section in the testis of non-obstructive early maturation arrest patient. The basement membrane is slightly thickened and PAS positive mucopolysaccharides are found in the interstitium (PAS x n250).



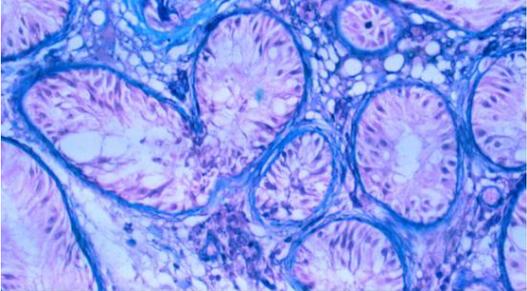
**Figure (31):** Collagen fibers are demonstrated in the basement membrane of the seminiferous tubule of non-obstructive early maturation arrest azoospermia patient. Interstitial collagen is also found. Only primary spermatocyte stage can be noticed. (Mallory trichrome stain x250).

#### C. Sertoli Cell only (SCO)

In sections of testis from non-obstructive Sertoli cell only patients, the Basement membrane of seminiferous tubules is evidently thickened. The interstitial area is rich in PAS positive mucopolysaccharides (Figure 32) and collagen fibers (Figure 33).



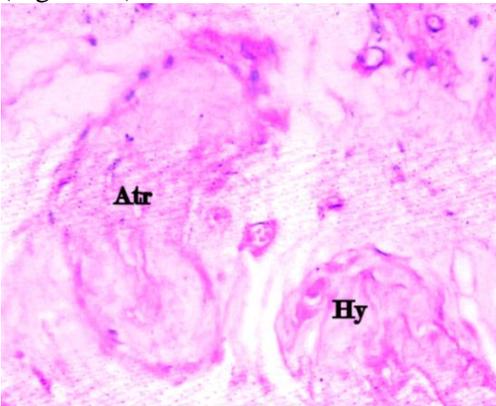
**Figure (32):** Pas positive mucopolysaccharides are abundant in the interstitial area and basement membrane of Sertoli cell only azoospermia patient testis. (PAS x250).



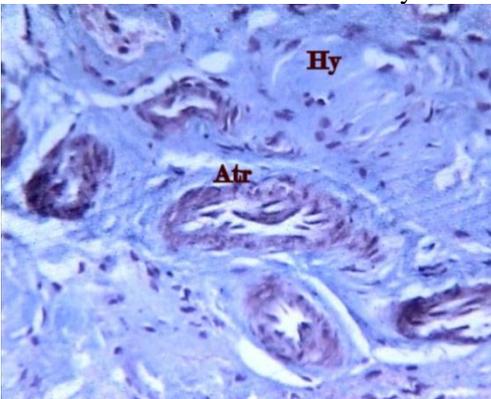
**Figure (33):** Abundant collagen is represented in the interstitial area of Sertoli cell only azoospermia patients (Mallory trichrome stain x250).

#### D. Klinefelter Syndrome Atrophied tubules

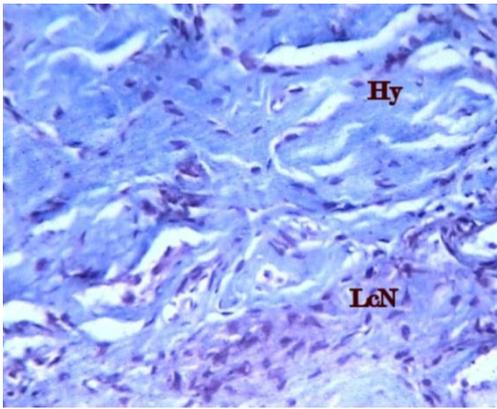
In the testis of Klinefelter syndrome patients, dysgeneration and atrophy in the seminiferous tubules is clearly evident. Hyalinized tubules are positively stained by PAS (Figure 34). Atrophied tubules (Figure 35), and an interstitial area rich in collagen fibers and Leydig cells that form nodules characteristic of this group (Figure 36).



**Figure (34):** Section in the testis of a patient with Klinefelter syndrome. Degeneration of seminiferous tubules is evident as tubules are hyalinized (Hy) or atrophied (Atr) (PASx250).



**Figure (35):** In testis of Klinefelter syndrome, the seminiferous tubules are atrophied and the interstitium is highly fibrous and hyalinized tubules (Mallory trichrome x200).



**Figure (36):** Hyalinized tubules (Hy) and Leydig cell nodules (LcN) characterize sections in the testis from Klinefelter syndrome patients (Mallory trichrome x200).

## DISCUSSION

Infertility is a common reproductive disorder and male factors account for about 40% of infertility in the world<sup>(6)</sup>. Azoospermia, defined as complete absence of sperm in the ejaculate, has been recognized as one of the most fascinating topics in male infertility. It is present in about 6–10% of all infertile men<sup>(7)</sup>.

Two major breakthroughs revolutionized the field of male infertility in the last three decades. The first was the development of intracytoplasmic sperm injection (ICSI) for the treatment of male factor infertility, and the second was application of ICSI to azoospermic males, with the demonstration that spermatozoa derived from either the epididymis or the testis were capable of normal fertilization and pregnancy. Azoospermia, therefore, results in infertility but does not necessarily imply sterility<sup>(2)</sup>.

Azoospermia is primarily classified as being a result of obstructive or non-obstructive causes. Obstructive azoospermia (OA) is caused by the obstruction of the reproductive duct. OA patients usually have normal spermatogenesis. Men with obstructive etiologies may have other cost-effective options for treatment, such as microsurgical reconstruction of the reproductive tract<sup>(8)</sup>. In contrast, non-obstructive azoospermia (NOA) is diagnosed as the dysfunction of spermatogenesis, which accounts for 60% of azoospermic men. The testicular histopathology associated with non-obstructive azoospermia (NOA) varies from markedly reduced spermatogenesis, with few normally appearing spermatozoa, a condition termed hypospermatogenesis (HP), through spermatogenic maturation arrest (MA) and the total absence of germ cells or their products, called Sertoli-cell-only (SCO) syndrome<sup>(1,9)</sup>. Androgen binding leads to AR-transactivation, that in turn, results in the modulation of AR downstream gene

expressions. Understanding the relationship between androgen receptors and azoospermia-related spermatogenic defects may lead to the development of therapeutic strategies similar to the treatment of male hypogonadism (AR agonists) and prostate diseases (AR antagonists)<sup>(1)</sup>.

In the present study, testicular biopsies were taken from 11 azoospermic male patients and processed for histological, histochemical & immunohistochemical examination. Eight of these biopsies (72.7%) were diagnosed as Non-obstructive azoospermia while four of them (27.3%) were diagnosed as Obstructive azoospermia.

In the present study Androgen receptors were detected mainly in the nuclei of Sertoli cells, Leydig cells and peritubular myoid cells. Several experimental studies suggest that AR plays important roles during later stages of sperm formation and maturation by influencing the four major cell types: Sertoli, Leydig, peritubular myoid, and germ cells<sup>(11,12)</sup>. However, except for maturation arrest non-obstructive azoospermia, AR positive staining was not detected in germ cells. Experimental germ cell Ar deletion was reported to have no effect on germ cell development<sup>(13)</sup>.

The highest number of Sertoli cell androgen receptors was in sections of testis from obstructive azoospermia and non-obstructive late maturation arrest patients. The number of androgen receptor positive cells in the later was numerically although not statistically higher.

One of the most noticed histopathological features in sections of obstructive azoospermia is the impaired spermatogenesis. Small diameter seminiferous tubules, abnormal Leydig cells and sloughing of germ cells.

The trigger of impaired spermatogenesis was suggested to be due to the high hydrostatic pressure caused by obstruction of the seminiferous

tubules<sup>(14)</sup>. The fact that Sertoli cells are responsible for the maintenance of all germ cells in the ad-luminal compartment suggests that the Sertoli cells are the target cell. The latter undergoes major ultrastructural changes, especially in the apical pole<sup>(15)</sup>.

Under normal conditions Sertoli cells carry fluids and different substances from the basal pole to the tubule lumen, and their secretory functions are regionalized. When the blockage of the spermatic ducts determines an increase in intraluminal pressure, the adluminal cytoplasm is selectively damaged, leaving the functions performed there blocked. Among these functions are those related to the adhesion between Sertoli cells and germ cells. Normally in the cytoplasm of Sertoli cells, there is an accumulation of actin filaments that are attached to the plasma membrane by an adhesive protein, vinculin. A defect in the vinculin synthesis or action alters intercellular adhesion and could release immature germ cells<sup>(16)</sup>. Other studies reported structural disorders in the Sertoli cells in case of obstruction<sup>(17)</sup>, that, beside the other structural changes, could explain the partial loss of androgen receptors.

Maturation arrest was reported as a consequence of testicular cell apoptosis<sup>(18)</sup>. Degeneration and sloughing of spermatogenic cells was also observed in the present study. These changes may be due to defective Sertoli cells<sup>(15)</sup>. The lower value of androgen receptors in the early maturation arrest group may be due to apoptosis of both spermatic cells and Sertoli cells<sup>(19)</sup>.

Sections of the Sertoli cell only azoospermia has a value of androgen receptor positive cells lower than that of late maturation arrest but higher than early maturation arrest. Sections in the testis from Sertoli cell only azoospermia patients are completely devoid of spermatogenic cells. This could be due to gene-related disruption of GATA4 transcription factor. Neonatal mice with a targeted disruption of Gata4 in Sertoli cells displayed a loss of the establishment and maintenance of the spermatogenic stem cell-pool and apoptosis of both gonocyte-derived differentiating spermatogonia and meiotic spermatocytes. Thus, progressive germ cell depletion and a Sertoli-cell-only syndrome were observed as early as the first wave of murine spermatogenesis<sup>(20)</sup>.

Testicular sections from patients with Klinefelter syndrome presented the lowest number of androgen receptor positive cells. In these sections, seminiferous tubules were either completely replaced by hyalinized structures or

appear completely atretic without any of the regular cells.

It has been proposed that the supernumerary X chromosome is the underlying etiology of testicular failure<sup>(21)</sup>. During the late stages of fetal development, when germ cells in the developing ovary of females with paired X chromosomes, undergo X chromosome reactivation and initiate meiosis, germ cells in the (XY) testis complete mitotic proliferation and enter a period of mitotic arrest. This G0 arrest persists until several days after birth, when mitotic proliferation of the germ cells resumes in the juvenile testis.

In the XXY testis, the prenatal mitotic proliferation of the gonocytes is impaired and they gradually disappear from the seminiferous tubules during the period of G0 arrest<sup>(22)</sup>. The presence of two active X chromosomes in germ cells in the testis is, therefore, incompatible with their continued survival<sup>(23)</sup>.

## CONCLUSION

Azoospermia, is present in about 6–10% of all infertile men. Azoospermia is due to dysfunction of spermatogenesis. Spermatogenesis is supported by testosterone signaling of androgen receptors on Sertoli cells.

A precise diagnosis of azoospermia and systematic evaluation of the patient to establish the disease etiology are needed to guide appropriate management options and to determine the associated cost benefits, risks and prognosis for treatment success.

The present study was, therefore, designed to study the quantitative relationship between androgen receptor expression and different types of azoospermia expressing different degrees of spermatogenic development.

Eleven patients with idiopathic infertility, were enrolled in this study and were assessed and diagnosed by a complete examination and specific investigations. They were selected from the outpatient clinics of Dermatology and Andrology department of Al-Azhar University hospitals and the International Islamic Center for Population Studies and Researches (IICPSR) from May 2013 to March 2017.

Paraffin sections from testicular biopsies were used for immune-histochemical demonstration of androgen receptors. Light micrographs were subjected to quantitative evaluation of the number of androgen receptor-positive cells using computerized image analysis followed by statistical analysis. Other sections were stained with hematoxylin and eosin, Mallory trichrome stain

and periodic acid-Schiff (PAS). Micrographs of sections of different groups were examined.

Analysis of quantitative immuno-histochemical, histochemical and histological results revealed a direct proportionality between the number of cells with positive androgen receptors and the stages of spermatogenesis and testicular development.

It was concluded that the present study revealed that demonstration of androgen receptors in testicular sections is a good indicator of spermatogenic activity. Such information is valuable for the decision of obtaining spermatozoa from the testis for ICSI.

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