Patterns Of Testicular Histopathology In Men With Primary Infertility
M Rashed, N Ragab, A Shalaby, W Ragab

Citation

Abstract
Male infertility contributes to more than half of all cases of childlessness; yet, it is a reproductive health problem that is poorly studied and understood. The present study aims at the histopathological re-evaluation of the archived testicular biopsy specimen in order to delineate the pathological causes of primary infertility in Egyptian men and to compare our results with others from different nations. Fifty primary male infertility cases were studied. Histologically; 24% were classified as obstructive azospermia; on the other side, 76% were classified as non-obstructive azospermia; these were further classified as: hypospermatogenesis; 8%, spermatocytic arrest; 28%, Sertoli cell only; 34%, and finally tubular fibrosis; 6%. In conclusion; this study confirms that testicular biopsy is an important tool in the investigation and the assessment of male infertility as it provides some light on the etiology as well as providing essential prognostic information of azospermic men in Egypt.

INTRODUCTION
Infertility is defined as the inability to achieve pregnancy after one year of unprotected intercourse [1,2]. Historically, the workup for the infertile couple focused primarily on conditions of the female. However, conditions of the male alone are now estimated to account for nearly 30% of infertile couples, and conditions of both the female and the male account for another 20% [3,4]. Conditions of the male that affect fertility are still generally under diagnosed and under treated. Testicular failure affects approximately 1% of the male populations and 10% of men who seek fertility evaluation [5].

The diagnosis of a man with azospermia includes semen analysis (volume, pH, fructose), careful inspection of the semen sediment obtained by ultracentrifugation of seminal plasma, and clinical examination for the presence of vasa deferentia [6]. The next step, differentiation between obstructive azospermia and non-obstructive azospermia (NOA), is almost unachievable without testicular biopsy, although other indirect tests such as endocrine evaluation may provide clues [7]. There are mixed views on the approach to testing. Some feel that if there is no sperm in the ejaculate, a vasography or vesiculogram should be done first to see if there is an obstruction. Others suggest performing a biopsy first and if sperm are found a vasography should be used to locate the obstruction [8].

The knowledge of the complicated process of spermatogenesis is the prerequisite to develop concepts for therapy of male infertility or handle germ cells in the management of assisted reproduction [9]. It was previously thought that sperm must transverse the male reproductive tract before acquiring the ability to normally fertilize an egg. Spermatogenesis is the process by which a complex, interdependent population of germ cells produces spermatozoa; spermatogenesis begins at puberty after a long preparatory period of “prespermatogenesis” in the fetus and the infant. Three major stages can be distinguished: spermatogonialgenesis, maturation of spermatocytes and spermatogenesis which is the cytodifferentiation of spermatids [10]. Male germ cells develop in the seminiferous tubules throughout life from puberty to old age. The complete process of germ cell development is called spermatogenesis [11]. The understanding of spermatogenesis needs detailed information about the organization of the germinal epithelium, the structure and function of different types of germ cells [12, 13]. The seminiferous tubules consist of the germinal epithelium and the peritubular tissue (Lamina propria). The germinal epithelium consists of cells that include different developmental stages of germ cells, namely spermatogonia, primary and secondary spermatocytes and spermatids; these are located within invaginations of the sertoli cells [13]. The peri-tubular tissue consists of myofibroblasts with connective tissue ground
substance, the myofibroblasts cause peristaltic contractions of the seminiferous tubules. The inter tubular space of the human testis contains the microvasculature, the endocrine, the endocrine Leydig cells, nerve fibers, macrophages, fibroblasts, further connective tissue cells compartmentalizing in part this space and lymph vessels [12].

Diagnostic testicular biopsy is one parameter for determining the testicular histopathology pattern and apparently it is the strongest indicator to foresee the possibility of finding sperms in the testis [16]. Identification of the border line between normal and disturbed spermatogenesis substantiate the diagnosis of impaired male fertility [12]. In the past, testicular biopsy was reserved for azospermia patients with a normal-sized testis and normal findings on hormonal studies to evaluate for ductal obstruction. Azospermic men with testicular failure (non-obstructive azospermia) have either sertoli cell only pattern, maturation arrest or hypospermatogenesis on testis biopsy. Until recently it was assumed that men with non-obstructive azospermia were untreatable. The discovery that azospermic men with germinal failure often have minute foci of spermatogenesis was observed in the early studies of quantitative analysis of spermatogenesis [17]. However, testicular biopsy is now also an invaluable procedure for further workup of the infertile male and for therapeutic sperm retrieval in assisted reproductive techniques [4]

AIM OF THE STUDY

The present study aims at the histopthological revaluation of the archived testicular biopsy specimen in order to delineate the pathological causes of primary infertility in Egyptian men and to compare our results with others from different nations.

PATIENTS & METHODS

The present study was a retrospective study performed between January 2004 and December 2006. We studied 50 male cases with primary infertility. Clinical data were received from medical records. All patients as recorded underwent bilateral testicular biopsies (one from each side) under local anesthesia. Biopsy samples were obtained with small curved scissors through a small scrotal window and immediately placed into freshly prepared Bouins solution. Specimens were processed by the original methods as paraffin blocks. All paraffin blocks were cut at 3-5 micron sections on albuminized glass slides for each case one slide was stained by conventional Haematoxylin and eosin.

The slides were all reviewed in a quantitative fashion. The specimens were studied histopathologically for reevaluations of the following criteria:

- General architecture
- Number of seminiferous tubules in specimen
- Seminiferous tubules Pattern
- Germ cell/ Sertoli cell ratio
- The basement membrane
- Interstitial tissue
- Leydig cells
- Tubular Hyalinization
- Tunica alburneina
- Epididymis ( if present in biopsy )

According to the histopathology criteria; testicular biopsy specimens were classified histological as:

- Normal Histology
- Hypospermatogenesis
- Sertolocytic arrest
- Sertoli cell only
- Tubular fibrosis

The seminiferous tubules were graded according to the Johnsen score [18]. In this system of classification, all tubular sections in each section of the testicular biopsy are evaluated systematically and each is given a score from 1 to 10. Complete spermatogenesis with many spermatozoa present is evaluated as score 10 [18].

SCORE HISTOLOGICAL CRITERIA (ACCORDING TO THE MODIFIED JOHNSEN SCORING) []

- (Score -10) Full spermatogenesis
- (Score - 9 ) Slightly impaired spermatogenesis, many late spermatids, disorganized epithelium
- (Score - 8 ) Less than five spermatozoa per tubule, few late spermatids
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- (Score -7 ) No spermatozoa, no late spermatids, many early spermatids
- (Score - 6) No spermatozoa, no late spermatids, few early spermatids
- (Score - 5) No spermatozoa or spermatids, many spermatocytes
- (Score - 4) No spermatozoa or spermatids, few spermatocytes
- (Score - 3) Spermatogonia only
- (Score - 2) No germinal cells, Sertoli cells only
- (Score - 1) No seminiferous epithelium

RESULTS
Fifty primary male infertility cases were studied; all were presented with azospermia. The age incidence ranged from 23-44 years; with a marriage duration ranging from 2-10 years.

The average number of semineferous tubules studied from both sides ranged from 63-100 tubules. Histologically; 12 cases; 24% were classified as obstructive azospermia and 38 cases; 76% were classified as non-obstructive azospermia.

The cases were further classified according to histopathological criteria as presented in (Table: 1). All cases were ranked according to the modified Johensen scoring system (Table: 2)

Figure 1
Table 1: Histological classification of testicular biopsies

<table>
<thead>
<tr>
<th>Histological classification</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Histology</td>
<td>12</td>
<td>24%</td>
</tr>
<tr>
<td>Hypospermatogenesis</td>
<td>4</td>
<td>8%</td>
</tr>
<tr>
<td>Spermatocytic arrest</td>
<td>14</td>
<td>28%</td>
</tr>
<tr>
<td>Sertoli cell only</td>
<td>17</td>
<td>34%</td>
</tr>
<tr>
<td>Tubular fibrosis</td>
<td>3</td>
<td>6%</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100%</td>
</tr>
</tbody>
</table>

Figure 2
Table 2: Ranking of testicular biopsies according to the modified Johensen scoring system

<table>
<thead>
<tr>
<th>Johensen scoring</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Score -10)</td>
<td>5</td>
<td>10%</td>
</tr>
<tr>
<td>(Score -9)</td>
<td>7</td>
<td>14%</td>
</tr>
<tr>
<td>(Score -8)</td>
<td>4</td>
<td>8%</td>
</tr>
<tr>
<td>(Score -7)</td>
<td>5</td>
<td>10%</td>
</tr>
<tr>
<td>(Score -6)</td>
<td>4</td>
<td>8%</td>
</tr>
<tr>
<td>(Score -5)</td>
<td>1</td>
<td>2%</td>
</tr>
<tr>
<td>(Score -4)</td>
<td>2</td>
<td>4%</td>
</tr>
<tr>
<td>(Score -3)</td>
<td>2</td>
<td>4%</td>
</tr>
<tr>
<td>(Score -2)</td>
<td>17</td>
<td>34%</td>
</tr>
<tr>
<td>(Score -1)</td>
<td>3</td>
<td>6%</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100%</td>
</tr>
</tbody>
</table>

Obstructive azospermia 12 cases; 24%; these were presenting tubules with thin basement membrane and tunica propria; as well as normal germinal epithelium showing orderly progression from spermatogonia to spermatocytes with groups of spermatids and mature spermatozoa. There was a distinct lumen in 2 cases; 4%. Sertoli cells were compressed between the germinal cells and were not easily seen. The remaining cases; 10 cases; 20% were presented with sloughing the epithelium was cellular with all stages of spermatogenesis, including mature sperms, but without the normal orderly arrangement. The central lumen was lost. In places there was a loose network of precursor cells. Elsewhere the central area of the tubule appeared more cellular than the periphery owing to the jumbled desquamated cells (Figure: 1).

In the hypospermatogenesis cases: the cellularity of germinal epithelium was reduced at all stages, the numbers of all types of germ cells (spermatogonia, spermatocytes, and spermatids) were reduced. All had normal Leydig cells (Figure: 2).

Maturation arrest histopathological description of the interruption of normal germ cell maturation at the level of a specific cell type leading form spermatogonia to spermatids (spermatogonia arrest, spermatocyte arrest, spermatid arrest) (Figure: 3,4,5 & 6).

Sertoli cell only, or complete germ cell aphasia, in most cases, the tunica propria and basement membranes are not
thickened appreciably, and the tubules are normal or slightly decreased in diameter, and contain only Sertoli cells but no other cells involved in spermatogenesis. The interstitium contains normal numbers of Leydig cells in most cases only 2 cases; 4% presented ledig cell hyperplasia (Figure: 7).

Tubular hyalinization, 3 cases; 10% the tubules were smaller in diameter with a much thickened basement membrane and tubular collagenization. The germinal epithelium was lost. Leydig cells were decreased in 2 case; 4%, increased in one case, (2%) (Figure: 8).

**Figure 3**
Figure 1: OBSTRUCTIVE AZOSPERMIA

**Figure 4**
Figure 2: HYPOSPERMATOGENESIE

**Figure 5**
Figure 3: SPERMATOGONIA ARREST

**Figure 6**
Figure 4: PRIMARY SPERMATOCYTE ARREST
DISCUSSION

Worldwide, male infertility contributes to more than half of all cases of childlessness; yet, it is a reproductive health problem that is poorly studied and understood. It is argued that male infertility may be particularly problematic for Middle Eastern men in their ponytails societies; there, both virility and fertility are typically tied to manhood. Thus, male infertility is a potentially emasculating condition, surrounded by secrecy and stigma [19].

Patterns of male infertility vary greatly among regions and even within regions. A combination of social habits, environmental conditions, and genetics is suspected to contribute to this variation the highest reported fertility rates are in Finland, while Great Britain has a low fertility rate. In...
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Egypt: extrapolation of incidence rate for male infertility to the total population is: 559,686 male infertility whereas the Total population is: 76,117,421

In the present study obstructive azospermia represented 24% of cases; 10% were (score 10) and 14% were (score: 9). Wong et al [23] recorded a similar result (25%) also Colgan et al [22], reported obstructive azospermia with normal histology in 20% of their cases. Brannen & Roth [21] Reported a higher incidence of obstructive azospermia 35%, the same was reported by Al-Rayess et al [19], (31%). Thomas J [20] study in Nigeria reported an incidence 38% for obstructive azospermia with normal histology. But on the other side there were studies that reported low incidences; Meinhard et al [22] reported 5% for obstructive azospermia, also Haddad et al [23]; a study at Jordon reported 11.2% for obstructive azospermia. Men with obstructive azospermia can now simply have their sperm retrieved from the epididymis or the testicle, frozen and saved for future procedures [3].

Hypospermatogenesis represented 8% of all cases studied and all were graded as (score 8). This finding is considerably low compared to others studies; Haddad et al [23] reported a high incidence for hypospermatogenesis they reported 55.8% in a jordan study of primary male infertility. Meinhard et al [22] reported also a high incidence of hypospermatogenesis, 46%, the same for Colgen et al, hypospermatogenesis was 49%. A study by Thomas JO [20] of the Nigerian male infertility, he reported 19% for hypospermatogenesis, for Wong et al [22] and Brannen & Roth [21] it was 23% and 27% consequently. On the other side; Al-Rayess et al [19] study of saudian primary male infertility, hypospermatogenesis represented 13% of their studied cases. Hypospermatogenesis provides good chances for isolating viable, intact spermatozoa with fertilizing capacity. The difficulties occur whenever mature spermatozoa are absent [4]. In our study the low incidence of hypospermatogenesis can be emphasized on the culture among most Egyptian male patients as to perform testicular biopsy only in cases of absolute azospermia; and as hypospermatogenesis patients can be oligospermic and so they usually avoid performing testicular biopsy.

Spermatogenic arrest is not a specific diagnosis for primary exocrine testicular failure, but a histopathological description of the interruption of normal germ cell maturation at the level of a specific cell type leading form spermatagonia to spermatids; the arrest may be caused by genetics or by secondary influences. Genetic aetiologies include trisomy, balanced-autosomal anomalies (translocations, inversions) or deletions in the Y chromosome (Yq11). Secondary factors for spermatogenerating arrest are toxic substances (radiotherapy, chemotherapy, antibiotics), heat or general diseases (liver or kidney insufficiency, sickle cell anaemia) [5]. In the present study the incidence of the spermatocytic maturation arrest was 28%, this incidence was high compared to Brannen & Roth [21] (12.5%), Golgen (11%), Al-Rayess et al [19] (11%) and Thomas JO [20](5%). Haddad et al [23] reported a very low incidence of spermatocytic maturation arrest (1.7%); but on the other side, Glina et al [4] reported a higher incidence compared to others (37.5%).

Sertoli cell only syndrome (SCOS), represented the highest incidental finding in our study (34%), all were evaluated as (score 2), this result is very high compared with others studies of different nations as it represent 9% of Thomas JO [20] studied cases, 8% in Wong et al [22] study, 12.5% in Brannen & Roth [21] study and 11% in Colgan et al [22] study. Haddad et al [23] recorded a very low incidence for SCOS, 2.9%; but the saudian study by Al-Rayess et al [19] reported in their study 23.5% for SCOS and 16% for SCOS with focal spermatogenesis. SCOS can result from numerous causes, such as cryptorchidism, cytotoxic drugs, or irradiation. However, in many cases the etiology is unknown. The absence of germ cells may be due to factors present during fetal life [4]. In our study the relatively high incidence of SCOS may be referred to the increased frequency of consanguinity in our community [3]. Studies reported that; a high proportion of parental consanguinity indicates that the disorder may be caused by a recessive mutation. Recently a genetic region controlling spermatogenesis in human beings - human azospermia factor (AZF) - has been localized to the long arm of the Y chromosome, and disturbed spermatogenesis and azospermia have been recorded in men with deletions corresponding to the AZF region, [1]. The explanation of the high incidence of germinal cell aplasia (Sertoli cell only syndrome) is not clear, but could be related to the high incidence of undetected cryptorchidism and orchitis or both in middle eastern communities [1].

Tubular atrophy and hyalinization, in the present study represented (6%) of the studied cases, these cases were (score 1), same results were reported in Meinhard et al [22] study and Al-Rayess et al [19] study; but on the other side a
high incidence was reported in Haddad et al [26] study (28.4%), also Thomas JO [27] study (23%); as orchitis is the most common cause of male infertility in most of the published Nigerian studies.

The distinction between obstructive and non-obstructive azoospermia is important. Men with obstructive azoospermia may have other cost-effective options for treatment, such as microsurgical reconstruction of the reproductive tract. Also, a real risk of failure to retrieve spermatozoa exists in men with non-obstructive azoospermia and couples must be apprised of this risk before attempting assisted reproduction. Testicular biopsy is an important tool in the investigation and the assessment of male infertility. It may shed some light on the etiology as well as providing essential prognostic information. The histological patterns in the testicular biopsies are classified according to the presence and amount of spermatogenesis, maturation of germinal cells and presence of associated tubular atrophy, interstitial fibrosis and Leydig cell hyperplasia or both [28].

CONCLUSION

This study confirms that testicular biopsy is an important tool in the investigation and the assessment of male infertility as it provides some light on the etiology as well as providing essential prognostic information of azospermic men in Egypt. The most common finding in this series was that of normal testis denoting obstruction (24%), while among cases of functional azospermia, Sertoli cell only (34%) and spermatogenic arrest (28%) was the most frequent.

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