

Histologic pictures of male infertility in Yemeni patients

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ABSTRACT

الأهداف: تحديد أنماط وشكل نسيج الخصية في المرضى اليمنيين المصابين بعقم الذكورة، مع تبين فرص وجود بؤر إنطاف منوي عند المرضى المصابين بانعدام النطاف اللانسدادية.

الطريقة: أجريت دراسة وصفية تعتمد على سجلات 485 مريض يماني مصاب بعقم الذكورة أُخذ منهم خزعة (خزعات) خصوية، حيث قاموا بمراجعة مركز المساعدة للإخصاب عبر أطفال الأنابيب - صنعاء - اليمن، خلال الفترة ما بين عام 2000م وحتى عام 2007م، وقد صنفت صور الخصية النسيجية إلى خمسة أصناف وبني هذا التصنيف على الشكل المجهرى لخزعات الخصية، كما وتم تحديد بؤر الإنطاف المنوي في الخزعات المأخوذة من المرضى المصابين بانعدام النطاف اللانسدادية.

النتائج: من بين الـ 458 حالة التي أُخذ منها خزعات، شوهدت 164 (33.8%) حالة بلا تنسج في خلايا المنتشة مع إنطاف موضعي في 50 حالة. كان هناك 116 (23.9%) حالة توقف في نضوج الخلايا المنتشة مع إنطاف موضعي في 21 حالة، كما أظهرت الدراسة تليف أنابيب وخلية الخصية في 93 (19.2%) حالة مع إنطاف موضعي في 34 (36.6%).

خاتمة: أظهرت الدراسة وجود جميع الأنماط النسيجية لمرضى عقم الذكورة، وبنسبة عالية في حالات بلا تنسج الخلايا المنتشة. كما بينت وجود بؤر إنطاف طبيعي في جميع أنماط انعدام النطاف اللانسدادية وبنسبة أعلى في حالات تليف أنابيب وخلايا الخصية.

Objectives: To define subgroups of male infertility and frequency of focal normal spermatogenesis in multiple testicular biopsies of non-obstructive azoospermia in Yemeni patients.

Methods: A descriptive record based study of 485 cases of male infertility was carried out in the in vitro fertilization (IVF) center in Sana'a, Yemen during the period from 1st August 2000 to 30th December 2007. During histological analysis the sample was evaluated regarding the size of seminiferous tubules, the thickness of tubules, the relative number and types of germ cells, the degree of fibrosis, and presence of focal normal spermatogenesis. The results were

commonly classified according to the used clinical practical classification.

Results: Out of a total of 485 testicular biopsies, 164 (33.8%) cases showed germ cell aplasia. The fibrosis represented the lowest frequency being 93 (19.2%) cases. Obstructive azoospermia was present in 99 (20.4%) cases, and maturation arrest in 116 (23.9%) cases. Focal normal spermatogenesis was found in 106 (27.5%) out of 385 cases of non-obstructive azoospermia with higher frequency in fibrosis cases (36.6%).

Conclusion: We have shown in this study the different histopathologic patterns in our local patients, which correlate with some studies and shows discrepancies with others. Therefore, these findings are informative for concerned IVF centers to help the patients who are suffering from primary infertility.

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Male infertility represents an important factor as a cause of infertility among infertile couples. Azoospermia is a common presentation of male infertility to all recently registered visitors to the In Vitro Fertilization (IVF) and Assisted Reproduction Centers.¹ Approximately 55% of infertility among Yemenis is found in males.² The evaluation of the infertile male includes a thorough clinical history and examination, semen analysis, hormonal assay, and testicular biopsy.³ The latter are particularly useful in the individual with azoospermia and normal endocrine findings.⁴⁻⁶ Biopsy specimens from infertile men with azoospermia usually show different histological conditions.⁴ The other role

of testicular biopsy is in the management of patients with non-obstructive azoospermias who are being considered candidates for sperm retrieval and IVF. In this situation, a testicular biopsy may be performed either to obtain prognostic information or to harvest sperm for cryopreservation.⁷ The interpretations of testicular biopsies are subjective and suffer from a lack of uniformity of the system of classification. Objective methods to quantify spermatogenesis are reproducible, but rarely add to the clinical management and are used primarily in research studies.⁸ The most commonly employed classification patterns are based on the appearance of spermatogenesis ranging from normal spermatogenesis (obstructive azoospermia) to Sertoli cell only syndrome with maturation arrest, and generalized fibrosis (end stage testis).⁸ This study aims in depth analysis of testicular histology and by forming defined subgroups of male infertility in Yemeni patients, and frequency of focal normal spermatogenesis in multiple testicular biopsies of non-obstructive azoospermia.

Methods. A descriptive record based study of 485 cases of male infertility was carried out in the In Vitro Fertilization Center (IVFC: the only available center dealing with assessment of fertilization in Sana'a, Yemen) during the period of 1st August 2000 to 30th December 2007. All the patients attending the IVFC suffering from primary infertility were subjected to full clinical examination, seminal analysis, hormonal assay, and bilateral testicular multiple biopsies for assessment of infertility and therapy. The testicular biopsies were carried out only for those patients with azoospermia in 2 consecutive seminal analyses. Azoospermia was reported when no spermatozoa were seen either alive or dead or deformed shape. The biopsies from both testis were taken and promptly fixed in Bouin's acid fixative for 18-24 hours at room temperature and subsequently fixed in 10% formalin solution before being processed by manual and automatic tissue processor (Shadon Southern product, Cheshire, England). After embedding in paraffin blocks, several thin sections of 2-3 micrometer thickness from each block were cut. The sections were stained with hematoxylin and eosin stain for routine histological diagnosis. Multiple testicular biopsies were taken from each testis, maximum of 3 biopsies from upper, middle, and lower pole from each testis, and these techniques increase the chance of detection of focal normal spermatogenesis.

During histological analysis, the sample was evaluated regarding the size and number of seminiferous tubules, the thickness of tubules, basement membrane, the relative number and types of germ cells, presence of focal normal spermatogenesis within the tubules, the degree of fibrosis and hyalinization of seminiferous

tubules and interstitium, and state of Leydig cells. The result of the study was classified according to the commonly used clinical practical classification.⁷ The germ cell aplasia cases show seminiferous tubules lined by Sertoli cells only with mild thickening and hyalinization of basement membrane. The maturation arrest cases show seminiferous tubules lined by germ cells with maturation arrest at the level of primary spermatocytes or at the level of spermatids. Cases of fibrosis and hyalinization show hyalinization and fibrosis of seminiferous tubules with obliteration of lumen with fibrosis of stroma and mostly show foci of Leydig cells hyperplasia. In obstructive azoospermia cases, normal spermatogenesis was found. Ethical approval was obtained from the Ethics Committee of the Faculty of Medicine and Health Sciences of Sana'a University. Statistical analysis was carried out using a calculator to compute percentages.

Results. A total of 485 testicular biopsies were taken. The study showed germ cell aplasia (Sertoli cell only syndrome) in 164 (33.8%) cases, and represents the highest frequency. While fibrosis and hyalinization (end stage testis) represents the lowest frequency in 93 (19.2%) cases. Obstructive azoospermia with morphology of normal spermatogenesis and hypospermatogenesis represents 99 (20.4%) cases. Maturation arrest at the stage of primary spermatocytes and at the stage of spermatids with round and elongated forms represents 116 (23.9%) cases. In 2.5% of cases, there was sufficient overlap of the histological pictures in both testes or in one, and in these cases, none of them fulfilled the classification, and they were labeled as heterogenous groups or unclassifiable. One case shows histological features similar to that found in fibrosis and hyalinization, and was diagnosed clinically and in karyotyping as Klinefelter's syndrome. Comparison between our findings and other studies are shown in Table 1. Focal normal spermatogenesis appeared positive in 106 (27.5%) out of 385 cases of non-obstructive azoospermia with very high frequency in fibrosis and 36.6% hyalinization type. The various histological patterns with focal normal spermatogenesis are shown in Table 2.

Discussion. At present, testicular biopsies are taken to obtain prognostic information or to confirm the presence of spermatozoa in order to harvest sperm for cryopreservation^{1,7} and IVF management of patients with non-obstructive azoospermia. The most commonly employed classification patterns are based on the appearance of spermatogenesis ranging from normal spermatogenesis (obstructive azoospermia) to germ cell aplasia with maturation arrest and generalized

Table 1 - Comparison of histologic pattern of testicular biopsies in infertile males.

Type	Current study (n=485) %	Saudi study ¹² (n=230) %	US* study ¹⁰ (n=48) %	Nigerian study ¹¹ (n=152) %
Germ cell aplasia	33.8	39	12.5	9
Normal spermatogenesis	20.4	31	35	38
Hypospermatogenesis	--	13	27	19
Maturation arrest	23.9	11	12.5	5
Fibrosis	19.2	5	--	23
Unclassifiable	2.5	--	--	--
Klinefelter's syndrome	0.2	0.4	12.5	5

*United States

Table 2 - Frequency and percentage of focal normal spermatogenesis in non-obstructive azoospermia.

Type	No. of cases	Cases with focal normal spermatogenesis n (%)
Germ cell aplasia	164	50 (30.5)
Maturation arrest	116	21 (18.0)
Fibrosis and Hyalinization	93	34 (36.6)
Unclassifiable	12	1 (8.3)
Total	385	106 (27.5)

fibrosis.^{8,9} Our results show a higher frequency of germ cells aplasia followed by maturation arrest, then obstructive azoospermia and fibrosis and hyalinization with some cases unclassifiable in 2.5%. The results showed agreement with some international studies and discrepancies with others. Our results showed low frequency of normal spermatogenesis and the absence of hypospermatogenesis category. The studies carried out in United States,¹⁰ Nigeria,¹¹ and Saudi Arabia¹² showed higher incidence rates of normal spermatogenesis and hypospermatogenesis. The possible explanation for the discrepancies between our study and others is the criteria used in selecting patients for biopsies. In our study, we include only those patients showing azoospermia in seminal analysis, while other studies include those patients who showed azoospermia and oligospermia.

The frequency of germ cell aplasia (33.8%) found in our study, is comparable with the study carried out in Saudia Arabia¹² and much higher than the findings from United States¹⁰ and Nigeria.¹¹ The possible explanation for this variation is that in many centers, patients with azoospermia, small testis, and high follicular stimulating hormone were not subjected to testicular biopsies.¹³ However, we followed the recent advances

in intracytoplasmic sperm injection (ICSI), which tries to obtain a biopsy from these patients in order to obtain at least a few sperms to carry out the insemination.¹⁴ The Nigerian and United States studies were carried out to the ICSI advances.

Fibrosis and hyalinization category in our results are comparable to that observed by Thomas¹¹ in Nigeria and Jamal et al¹⁵ in Saudi Arabia.¹⁵ The variations observed between different international literature may shed light on the differences in the etiology of male infertility and the effect of environmental factors as mentioned by some observers.¹² Many patients with non-obstructive azoospermia might have limited numbers of spermatozoa that may be harvested from testis and used in vitro to fertilize eggs.¹⁶ In our findings, focal normal spermatogenesis revealed the higher frequency in all types of non-obstructive azoospermia with higher percentage in fibrosis and hyalinization of testis (36.6%). Thus, we can conclude that this frequency is higher in the small sized atrophic testicles. This result appears reasonable as bilateral and multiple testicular biopsies increase the chance for the presence of sperms in various histological types of male infertility.¹⁷ Some investigators recommended that at least 3 open testicular biopsies from each testis are needed, in order to reach the most accurate diagnosis in cases of absolute testicular failure.¹⁸ We found in the present study, that the frequency of focal normal spermatogenesis is increased in the bilateral and multiple testicular biopsies and in those testis showing small size and atrophy. Previous studies compared the results of single versus multiple testicular biopsies in the same histopathologic groups, they found significant improvement in the percentage of successful testicular sperm extraction with multiple biopsies.^{1,19} This was clear when the patients with previously negative biopsies were subjected to repeat biopsies.²⁰ The ability to achieve pregnancy with only a single testicular sperm has broadened the indications for testicular biopsy to virtually all azoospermic men, and has turned biopsy into a potentially therapeutic as well as diagnostic procedure.²⁰ The present study was not focused upon the personal data since the researcher's interest focused on histological changes of the testis. The study was targeted at patients attending IVF service only, and excluded other infertile males who sought the care from other health facilities. Thus, our group could describes only a section of the community that are able to seek care at these expensive IVF centers.

We have shown in this retrospective study the different histopathologic patterns of male infertility in our local patients, which correlates well with some studies and shows discrepancies with others. The study showed higher frequency of focal normal spermatogenesis with multiple testicular biopsies in

non-obstructive azoospermia, with a higher percentage in the hyalinization and fibrosis type. So, these findings are informative for those IVF centers to help the patients who were suffering from primary infertility and seeking assistance for their long time problems.

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