

Evaluation of histopathological patterns and testicular biopsies in infertile male and its relationship with some hormones in Kirkuk/Iraq

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Abstract

Infertility affects between 15% to 20% of couples worldwide, making it a major concern. Testing abnormalities may result in non-obstructive, permanent azoospermia. Infertility affects between 15% to 20% of couples worldwide, making it a common cause for worry. Testing abnormalities may result in non-obstructive, permanent azoospermia. Testicular biopsy is the only procedure available for diagnosing azoospermia. The current study included a cohort of 30 people who had been clinically diagnosed with azoospermia. Additionally, a cohort of 20 healthy male volunteers was enlisted from Kirkuk, Iraq's private laboratories and recruited from Azadi Teaching Hospital. The findings of this study indicate that Sertoli-cell-only-syndrome (39.996%) displayed the highest prevalence in testicular biopsies and it categorized Johnson score2, Testicular atrophy emerged as the second most frequently encountered condition (23.331%) it categorized as the Johnson scoring 1. Followed by germ cell maturation arrest (16.665%) as Johnson score8-3, followed by hypospermatogenesis (9.999%) as Johnson scoring 7, followed by Seminiferous tubule hyalinization (6.666%) as Johnson scoring 1, Subsequently, there was an observed occurrence of normal spermatogenesis, amounting to 3.333%. The study's results indicated that within our particular geographic region, the Sertoli-cell-only syndrome (SCOS) exhibited the highest prevalence among the 30 specimens examined, which were testicular biopsies obtained from infertile males. Furthermore, it was observed that individuals diagnosed with azoospermia showed a statistically significant increase in serum luteinizing hormone levels, while experiencing a statistically significant decrease in testosterone levels.

Introduction

Infertility is a frequently reported occurrence marked by the incapacity to achieve pregnancy after engaging in unprotected sexual intercourse for a duration of one year [1].

The assessment of the male partner's role is becoming increasingly crucial and significant, particularly in light of the present condition of spermatogenesis [2,3]. The regions with the highest prevalence rates include Africa and Central and Eastern Europe; in these regions, as much as 12% of males may have fertility issues [4]. This increase can be ascribed to a number of things, including such are the effects of war, way of life, increased stress, smoking, work-related variables, food patterns, behavioral variables, and genetic susceptibility [5]. The estimated prevalence of azoospermia, an illness characterized by a lack of spermatozoa that ejaculate, among males who have infertility ranges from 10% to 20% [6]. Pre-testicular failure, arising from either testicular or endocrine dysfunction, is the underlying cause of non-obstructive azoospermia (NOA). In contrast, post-testicular failure, primarily responsible for obstructive azoospermia (OA), manifests as obstructed genitalia and ejaculatory dysfunction [7].

The histopathological observations derived from testicular biopsies play a crucial role in the identification and selection process of appropriate candidates for intracytoplasmic sperm injection (ICSI) within the population diagnosed with non-obstructive azoospermia. The performance of a testicular biopsy is of utmost importance in the classification of individuals presenting with azoospermia, as it provides valuable information and guidance for further medical interventions [8].

Spermatogenic failure in the testicles can arise from congenital, acquired, or idiopathic etiologies. Congenital testicular defects can arise from various factors, such as anorchia, testicular dysgenesis (also known as cryptorchidism), Y chromosome deletions, germ cell hyperplasia (also known as Sertoli cell-only syndrome), and spermatogenic arrest (also known as maturation arrest) are chromosomal disorders. The reasons of acquired testicular tissue include a variety of things, including trauma, torsion, infection (specifically mumps orchitis), tumors, medication usage, irradiation, surgical interventions that may compromise testicular vascularization, systemic disorders such as cirrhosis and renal failure, and the presence of varicocele [9].

Materials and Methods

The research was conducted from the month of August 2022 to the month of April 2023. The present research utilized a group of 20 male participants who were considered to be in optimal health to serve as the study's control group. In addition, a group of thirty male patients with azoospermia, a condition in which there are no sperm present in the semen, were recruited from the Azadi Teaching Hospital-Infertility Consultant Center in Kirkuk, Iraq, as well as from private laboratories in the area. Participants in the data collection were as old as 20 years old and as young as 50 years old. The surgical procedure comprises the extraction of biopsies, which are then transported to the laboratory for further examination. The specimens were preserved by submerging them in a fixative solution that contained formalin at a concentration of 10%. After that, Bouin's solution was used as a fixative, and then the typical steps for processing and staining with hematoxylin and (H and E) were carried out. [10,11].

Sampling:

A total volume of 5 milliliters of blood was obtained from the participants of the study. The blood samples were then transferred into gel tubes and left undisturbed for a duration

of 20 minutes to allow for clot formation. Subsequently, the gel tubes underwent centrifugation at a velocity of 4,000 revolutions per minute for a duration of 15 minutes to facilitate the segregation of serum from other constituents of the blood. The samples were subsequently brought to room temperature before conducting the assays. This was done after storing the serum in two Eppendorf tubes and preserving it in a deep freezer set at a temperature of -20 °C. Subsequently, upon collection, biopsy samples were promptly immersed in Bouins fixative and dispatched to the private laboratories in Kirkuk. In these laboratories, all testicular biopsies underwent standard processing procedures, including staining with hematoxylin and eosin (H and E), followed by histological examination using light microscopy.

Statistical analysis:

The hormonal results were subjected to statistical analysis using the Minitab program, with a specific focus on employing the analysis of variance t-test was employed to assess the significance of the arithmetic means. The statistical analysis revealed a level of significance below 0.05 for both the group of individuals with illnesses and the group of those without illnesses. SPSS version 22 was used to statistically analyse the results of testicular biopsies. The data were presented as the mean \pm SD.

Histological classification and tabulation (Table 1) were performed for all testicular samples, resulting in their categorization into six distinct categories as outlined below:

1. Normal Spermatogenesis
2. Hyalinization of the seminiferous tubules
3. The process of hypospermatogenesis
4. Stoppage of the development of germ cells
5. Testicular atrophy
6. Sertoli cell only syndrome

Normal spermatogenesis: The tubules of the seminiferous cords have a slender basement membrane and tunica propria. The germinal epithelium, which lines the tubules, showcases a sequential progression from spermatogonia (immature sperm cells) through spermatocytes (meiotic sperm cells) to spermatids (haploid sperm cells) and finally mature spermatozoa (Figure 1).

Hypo spermatogenesis: The germinal epithelium, which lines the seminiferous tubules of the testes, showed a reduction in cellularity. This means that there were fewer germ cells, which are the cells that develop into sperm. The various stages of germ cell development, specifically spermatogonia (immature sperm cells), spermatocytes (meiotic sperm cells), and spermatids (haploid sperm cells), were observed, however their overall number was decreased (Figure 2).

Seminiferous tubule hyalinization: A decrease in tubule diameter was seen, concomitant with a significant thickening in the basement membrane and an augmentation in tubular collagenization. In these particular cases, the germinal epithelium was seen to be lacking. [12], as shown in figure 3.

Germ cell maturation arrest (GCMA): A biological process called spermatogenesis frequently comes to a cellular standstill, particularly at the primary or secondary

spermatocyte stage. Due to the absence of spermatids, the observed pattern distinguishes itself from hypo spermatogenesis [13,14], as illustrated in figure 4.

Testicular atrophy: The seminiferous epithelium undergoes complete atrophy, resulting in the presence of only a thickened lamina propria [15,16], as shown in figure 5.

Sertoli cell only syndrome: The basement membrane and tunica propria did not exhibit any discernible thickening in the aforementioned situations. The tubules showed either normal or slightly smaller-than-normal diameters. These tubules were exclusively occupied by Sertoli cells, with no presence of any other cells involved in the process of spermatogenesis. In most instances, the interstitial region displayed a characteristic abundance of Leydig cells [17,18], as shown in figure 6.

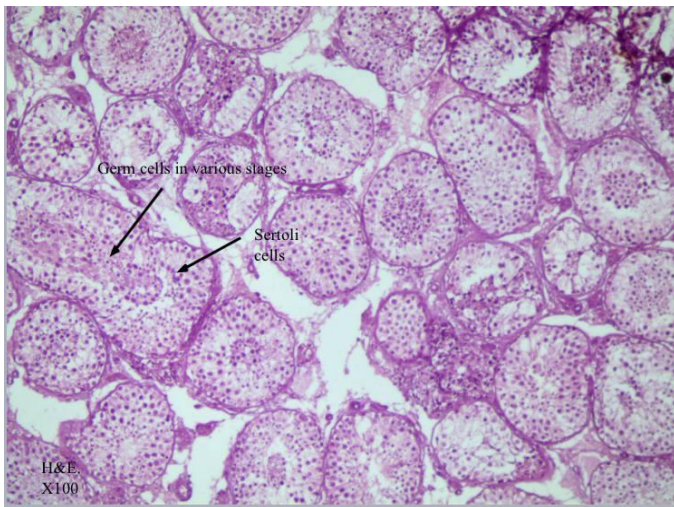


Fig 1. Normal Spermatogenesis (H & E x 100)

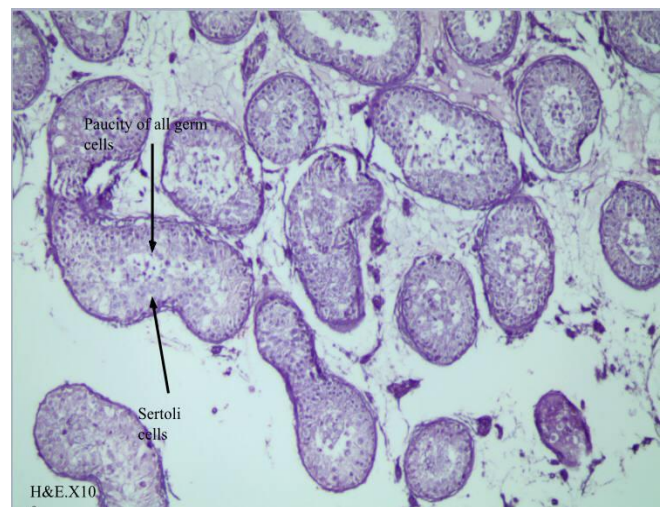


Fig 2. Hypo spermatogenesis(H & E x 100)

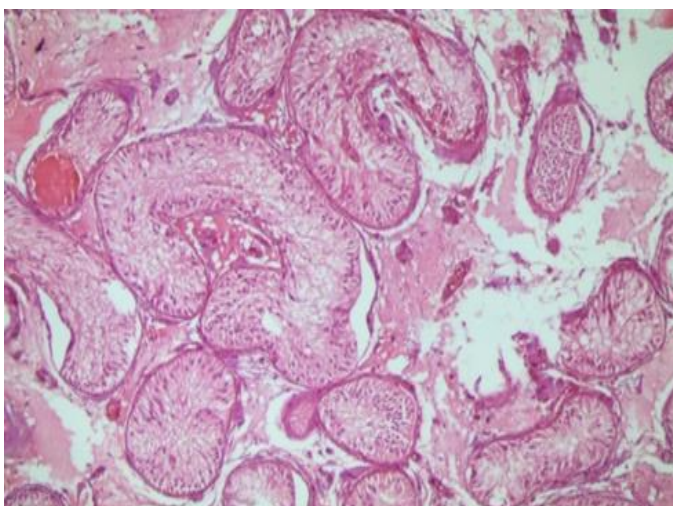


Fig. 3. Seminiferous tubule hyalinization (H & E x 400)

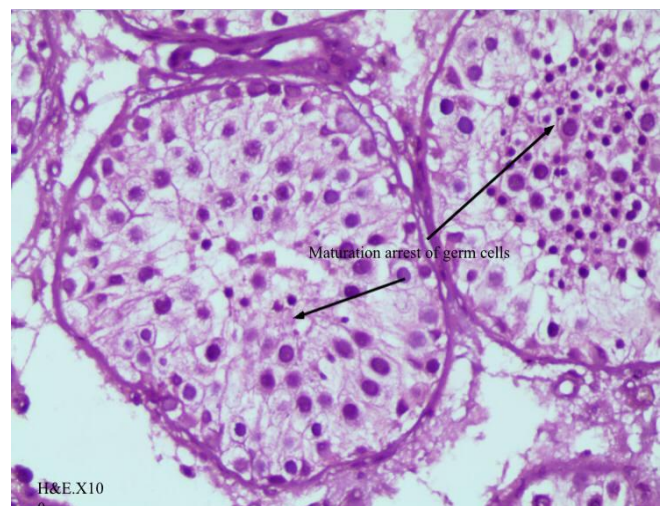


Fig 4. Arrest of germ cell maturation (H&E x 400)

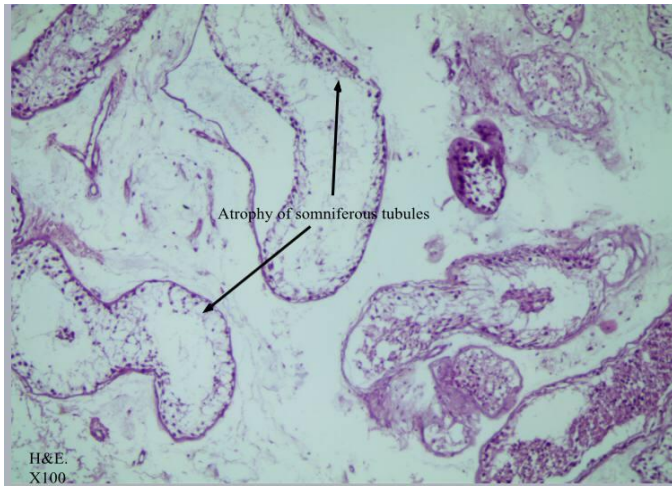


Fig 5. testicular atrophy (H & E x 100)

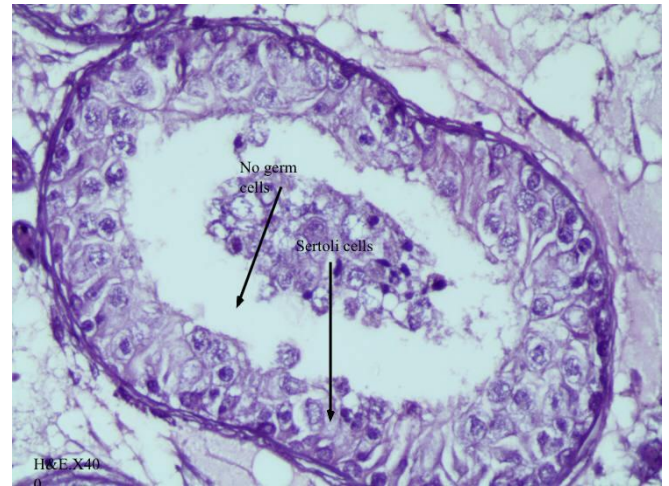


Fig 6. Sertoli cell only syndrome(H & E x 400)

Observations

It is important to mention that all of 30 testicular specimens were taken from facilities that were privately owned in the city of Kirkuk in Iraq. In the course of the routine examination of the patients' sperm, it was discovered that all of the patients had azoospermia, which is evidence of non-obstructive azoospermia. The diagnosis of testicular atrophy was assigned to a total of seven cases, which represented 23.331% of the cases that were reported. Moreover, a total of five cases, comprising 16.665% of the reported cases, were classified as GCMA, with a prominent impact on primary spermatocytes. The study revealed that the occurrence of instances with Hypo spermatogenesis was determined to be 3, which constituted around 9.999% of the overall cases (Table 1 and Table 2).

Furthermore, the study included patients aged 20 to 50 years, with the age group of 31 to 40 years being the most commonly observed. The histopathological patterns that were discovered can be classified into the following categories: A collective sum of ten cases, constituting approximately 39.996% of the reported cases, were determined to be instances of Sertoli cell only syndrome.

Furthermore, the presence of seminiferous tubule hyalinization types was noted in two instances, accounting for 6.666% of the overall cases. One case, accounting for 3.333% of the total, demonstrated normal spermatogenesis. Based on the scoring technique employed, the testicular samples were evaluated further and numbered 1–10. Johnson scoring is defined as follows:

Score 10: Spermatogenesis is a complete process.

Score 9: The process of spermatogenesis exhibits incomplete progression, characterized by the presence of numerous late-stage spermatids.

Score 8: The number of spermatozoa per tubule is less than 5, and there is a limited presence of late spermatids.

Score 7: A multitude of early spermatids are present, while the absence of spermatozoa or late spermatids is notable.

Score 6: A limited number of early spermatids are present, while the absence of spermatozoa or late spermatids is observed.

Score 5: A multitude of spermatocytes are present, yet spermatozoa or spermatids are notably absent.

Score 4: The presence of spermatocytes is observed, while the absence of spermatozoa or spermatids is noted.

Score 3: There exists solely spermatogonia within this context.

Score 2: The observed state had just Sertoli cells and no germinal epithelial cells.

Score 1: The presence of seminiferous epithelium is absent. The findings were documented on a standardized form in all instances.

Table 1: Testicular biopsies from infertile men were histopathologically classified.

Histopathological classification	No of cases-30	%
Normal spermatogenesis	1	3.333
Hypospermatogenesis	3	9.999
Seminiferous tubule hyalinization	2	6.666
Germ cell maturation arrest	5	16.665
testicular atrophy	7	23.331
Sertoli cell only syndrome	12	39.996
Total	30	100

Table 2: Categorization of testicular biopsies according to Johnson scoring system.

The Johnson scoring system		
Johnson scoring	No of cases-30	%
10	1	3.333
9	0	0
8	5	16.665
7	3	9.999
6	0	0
5	0	0
4	0	0
3	2	6.666
2	10	33.330
1	9	29.997
Total	30	100

Study enrollment procedures:

The data collected for this study encompassed socio-demographic characteristics of the study participants, specifically pertaining to their age and total sperm count, as recorded in their medical records.

Ethical consent: Following the acquisition of institutional ethical approval and written informed consent from each patient, they were able to participate in the current study. This research has been granted approval by the Central Scientific Research Ethics Committee at Tikrit University.

Inclusion criteria: These were based on Infertile male with primary or secondary infertility. patients who agreed to participate in this study.

Exclusion criteria: Participants who meet any of the following requirements will not be considered for this study:

1. Male infertility with a known etiology, such as obstructive azoospermia, testicular, trauma, genetic abnormalities, cancer treatment, or treatment for cancer.
2. Patients having female factors for infertility. Normal fertile male participants, cases where the consent was refused.

Evaluation of LH (luteinizing hormone) And Testosterone serum concentrations:

Enzyme-Linked Immunosorbent Assay (ELISA) technique was employed to assess the concentrations of luteinizing hormone (LH) and testosterone in serum samples, utilizing commercially available kits (BIOTECH, China). The confirmation of infertility was established by gathering the patients' medical history and conducting various diagnostic tests, including semen analyses and hormone tests.

Results

This study shows that azoospermia patients had lower testosterone levels. Additionally, it reveals that patients with azoospermia exhibit elevated levels of serum luteinizing hormone compared to other cohorts in previous studies, with statistical significance established at a threshold of $P < 0.05$. This information is visually represented in Table 3 and Figures 7, 8.

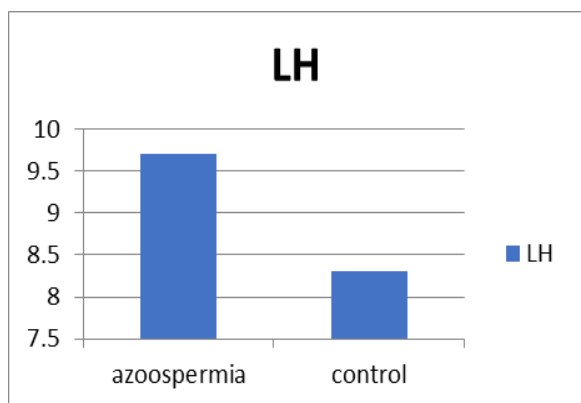


Figure 7: Assessment Serum LH (mIU/L) in studied groups.

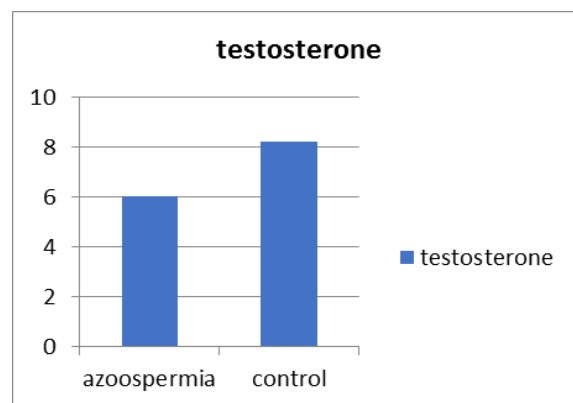


Figure 8: Assessment Serum Testosterone (ng/ml) in studied groups

Table 3: LH and Testosterone Mean and Standard Deviation

NO	Group	N	LH		Testosterone	
			Mean	standard deviation ±	Mean	standard deviation ±
1	Zero-Sperm	30	9.700 a	3.997	6.045 b	1.148
2	Control	20	8.377 b	3.029	8.216 a	2.573
P-Value			0.05*		0.05*	

Discussion

The present study's results were consistent with the findings of Huijben et al [18]. The individual who documented the observation of an increase in LH levels in response to decreased sperm quality, as compared to the corresponding parameters in fertile individuals, suggested that this finding signifies a compensatory decrease in Leydig cell function. Furthermore, it has been proposed that there may be a correlation between LH and spermatogenesis in patients with azoospermia [19, 20].

Furthermore, the results of this experiment were in line with the findings of Ibrahim et al. [21]. The information presented by the source indicates that a decrease in testosterone levels, in comparison to the levels found in typically viable males, is connected to testicular spermatogenesis regulation decline. This study found that men with testosterone levels below 8.295 ng/dL had inferior sperm morphology. [21, 22].

Sertoli cell only syndrome (SCOS) dominated the study with 10 patients (39.996%). In testicular biopsies, germ cell aplasia is characterized by exclusively Sertoli cells in seminiferous tubules. Even though most boys with Sertoli cell-only syndrome (SCOS) have normal chromosomes, earlier research has linked it to Klinefelter syndrome. Endocrine systems and signaling networks may contribute to Sertoli cell-only syndrome (SCOS) in addition to genetic abnormalities. Sertoli cell-only syndrome (SCOS) treatment includes sperm retrieval and intracytoplasmic sperm injection. However, SCOS patients may have trouble getting therapeutic interventions that might help them become biological parents. [22]. The study conducted by the researchers observed a similar pattern, although it was found to have a higher proportion in the result of Al-Jebouri et al [23]. According to a source from Tikrit, there was a reported prevalence of 35% of cases. In contrast to the findings of other previous studies, which reported a lower prevalence rate of 18.75% in the result of Gune et al [24] from Kolhapur. In individuals diagnosed with Sertoli cell-only syndrome (SCOS) [25].

In the sample of 30 cases under investigation, a notable finding was the presence of testicular atrophy in 7 cases, representing approximately 23.331% of the whole sample. Previous investigations conducted by Al-Jebouri et al [23] in Tikrit and Emokpae et al [25] in India have indicated a substantially greater prevalence of testicular atrophy, with a relative proportion of 30% [26,27].

Pathological testicular atrophy causes male reproductive organs to shrink and perhaps malfunction. notably the testes situated in the scrotum of individuals. The observed augmentation in testicular dimensions can be attributed to this particular medical condition. This does not suggest temporary alterations, such as those caused by cold temperatures. Testicular shrinkage can be ascribed to a range of reasons, including hormonal imbalances, the administration of drugs, or the existence of disorders such as varicocele [28].

This study identified a phenomena referred to as "germ cell maturation arrest (GCMA)" in five cases, which constituted 16.665% of the overall sample. The phenomenon of maturation arrest was observed to manifest at several levels, however none of the studied cases progressed to the level of spermatid maturity. The cases examined in this investigation demonstrated maturation arrest at a level lower than 5 spermatozoa per tubule, accompanied by a limited presence of late spermatids and exclusively maturation of spermatogonia. Sayed et al [29] and Thang et al [30] showed a greater prevalence of maturation arrest, with relative proportions of 20% and 18% respectively. The persons in question are of Northern Indian and Vietnamese heritage, respectively. However, prior studies in Bosnia and Herzegovina found a lower maturation arrest rate. Spahovic et al. [31] found 15%, while Philip et al. [32] found 0.4%.

Hypo spermatogenesis was found in 9.999% of patients. Abdullah et al. [1] in Saudi Arabia found a similar trend at 29%. The documented incidence rate of 2.96% was significantly below average. Sayed et al. [29] studied Northern India.

The prevalence of seminiferous tubule hyalinization was 6.666%. Gune et al. [24] reported 34.375%. A low incidence rate of 2.22% was recorded in Northern India by Sayed et al [29].

A variety of testicular biopsies performed for infertility have shown spermatogenesis within the specified range. These often indicate post-testicular problems such obstruction [33,34,35]. Only one case (3.333% of the sample) showed normal spermatogenesis. Al-Jebouri et al. [36] found a similar tendency in Tikrit, but at 5%. The phenomenon under study has been observed in several studies. In Pakistan, Manzoor et al. [37] found 19.72 incidence. Southwest Nigeria had a 0.9% proportion in previous investigations. Adesoji et al. [5] documented their investigation.

Conclusions

According to the study's findings, testicular biopsies taken from infertile males in our particular geographic area showed the highest prevalence of the Sertoli-cell-only syndrome (SCOS) among the 30 specimens examined.

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تقييم الأنماط النسيجية المرضية وخزعات الخصية في الذكور المصابين بالعقم وعلاقتها ببعض الهرمونات في كركوك / العراق

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البحث مستل من اطروحة دكتوراه الباحث الاول

الخلاصة:

العقم هو مصدر قلق سائد على نطاق عالمي، ويؤثر على ما يقرب من 15-20٪ من الأزواج. اضطرابات الخصية لديها القدرة على التسبب في فقد النطاف غير الانسدادي الذي لا رجعة فيه. في حالات فقد النطاف، خزعة الخصية هي الطريقة الوحيدة للتشخيص، وقد شملت الدراسة الحالية مجموعة من 30 فردًا تم تشخيصهم إكلينيكيًا بمرض فقد النطاف، علاوة على ذلك، تم تجنيد مجموعة من 20 متطوعًا من الذكور الذين كانوا بصحة جيدة من مستشفى آزادي التعليمي وتحديدًا من المعامل الخاصة الموجودة في كركوك، العراق. تشير نتائج هذه الدراسة إلى أن متلازمة خلايا سيرتولي فقط (39.996٪) أظهرت أعلى معدل انتشار في خزعات الخصية وصنفت درجة جونسون 2، وظهر ضمور الخصية كثاني أكثر الحالات التي يتم مواجهتها (23.331٪) التي صنفتها على أنها جونسون 1. يليه توقف نضج الخلايا الجرثومية (16.665٪) حيث سجل جونسون 8-3، يليه نقص النطاف (9.999٪) حيث سجل جونسون 7، يليه تحلل الأنابيب المنوية (6.666٪) حيث سجل جونسون 1، يليه (3.333٪). حدوث تكوين طبيعي للحيوانات المنوية، وخلصت نتائج الدراسة إلى أنه من بين العينات الثلاثين التي تم تحليلها، أظهرت متلازمة خلايا سيرتولي (SCOS) أعلى معدل انتشار في خزعات الخصية التي تم جمعها من الذكور المصابين بالعقم في منطقتنا الجغرافية المحددة ومصل الهرمون اللوتيني وجد ارتفاعًا ذا دلالة إحصائية في حين أظهر هرمون التستوستيرون انخفاضًا ذا دلالة إحصائية في الأفراد المصابين بفقدان النطاف.

معلومات البحث:

تاريخ الاستلام: 2023/07/23

تاريخ التعديل : 2023/09/11

تاريخ القبول: 2023/09/15

تاريخ النشر: 2024/03/30

الكلمات المفتاحية:

عقم الرجال في كركوك ، تعديل درجة

جونسون ، الهرمون اللوتيني، هرمون

التستوستيرون.

معلومات المؤلف

الايمل:

الموبايل: