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Assessment of Inhibin-B hormone level and its relationship with some gonadal hormones in males having idiopathic infertility

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ABSTRACT

he aim of the current study to determine levels of Inhibin-B hormone and its relationship with some gonadal hormones, including follicle-stimulating hormone (FSH), luteinizing hormone (LH) prolactin, and testosterone hormones in some males with idiopathic infertility in Shirqat city.

Blood and seminal fluid samples from (60) idiopathic male infertile and (30) healthful individuals as (control group) aged (18 to 60 years) were collected from private clinics. Enzyme-linked immunosorbent assay (ELISA) was used to estimate serum Inhibin B, FSH, LH, prolactin, and testosterone hormones.

According to types of sperm count, patients have divided onto two groups 49 persons as azoospermia group (zero /ml) and 11 persons as oligospermia group less than 20 million/ml. Inhibin B and testosterone levels were significantly decreased ($P \le 0.01$) in the infertile men compared with the control group. While the FSH level, LH, and prolactin were significantly higher ($P \le 0.01$) in the infertile men than in the control group, the results of Inhibin B were non-significant.

In contrast, levels of FSH, LH, and prolactin were higher significant ($P \le 0.01$), except testosterone level was significant differences ($P \le 0.05$) in the age groups <20-30 year, 31–40 year, and ≥ 40 years. There were significant differences ($P \le 0.01$) in levels of FSH and prolactin, but the levels of Inhibin B and LH were non-significant in the infertility period ≤ 10 year, 11-20 years, ≥ 20 years. Smoker infertile men have low levels ($P \le 0.01$) in the prolactin while had high levels in the Inhibin B and LH hormones compared with control. Patients with a family history showed a significant difference ($P \le 0.05$) in the levels of Inhibin B, testosterone, LH, and prolactin.

In conclusion, this study revealed a significant decrease in the Inhibin-B levels of the Azoospermic and oligospermia infertile men. The negative correlation between inhibin B and testosterone and weak correlation with other gonadal hormones. Therefore, Inhibin-B has an important role in diagnosing idiopathic male infertility, and it's one of the important markers in the diagnosis of normal spermatogenesis.

Introduction Human infertility

Human infertility is considered as a major health problem; it induces the incapability of marriages who are unfertilized from unprotected sexual correlation at least after 12 months of marriage, the impact of infertility on both male and female, referring to the word infertility, in females is a complete lack of pregnancy ability [1]. About 20-35% and 20-30%

cases of infertility that either female and male factors can describe, respectively, while 25-40% of patients may be due to a concern with both partners. In 10-20 % of instances, there may be no recognized cause[2]. Like female infertility, male infertility has always been a heterogeneous disorder that can be based on congenital and genetic influences in 15-30 % of cases, acquired impacts in approximately 20%, and

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idiopathic infertility in. Male infertility is a severe disease involving environmental as well as genetic factors. Abnormal sperm production or activity is probably related to various acquired or congenital factors that act on the pretesticular, post-testicular, or directly testicular level [3]. Genetic disorders, such as chromosomal abnormalities and single-gene mutations, could occur in about 15 percent of men's infertility cases [4,5]. Due to acquired or congenital urogenital deformities, tumours, infections of the urogenital tract, elevated scrotal temperature (e.g., in the case of varicocele), endocrine disorders, genetic mutations, and immunological status can also be decreased male fertility[6] . The infertility of males can be categorized by hormonal investigation and seminal fluid analysis using the light microscopy. The total lack of sperms in semen is termed azoospermia, but if it is less than 20 million / ml, termed oligospermia, whereas if the total number is 20 million / ml, termed Asthenospermia[7]. Human Inhibin B is a glycoprotein hormone (32 kilo Daltons) and a heterodimeric glycoprotein consisting of one alpha-subunit and one beta-subunit; the independent subunits typically have no physiological effects. Thus, the inhibin's bioactivity relies on the development of a dimeric alpha-beta structure. Inhibin B is a dimeric polypeptide gonadal hormone that controls FSH synthesis and releases of a negative feedback system. Inhibin B plays an essential role in hypothalamic-pituitary-gonadal maintaining the hormonal axis during human male childhood and puberty development[8] . Measurement of inhibin B is a useful marker of infertile state than LH and FSH. In patients with infertility, the inhibin B level could provide useful spermatogenesis information and may serve as a specific spermatogenesis marker. Sertoli cells produce inhibin B, which creates negative responses on FSH releasing, and it could also be a good indicator for the function of the seminiferous tubules [9]. FSH enhances inhibin B production from the testis; on the other hand, the inhibit B inhibits FSH secretion. Clinical results showed a significant inverse relationship between FSH and inhibin B in healthful males and males with the disorder in testicular function [10]. Additionally, except for LHtestosterone, the manufacture of Inhibin B doesn't depend solely on FSH post-puberty. The central control unit for inhibin B in the availability of FSH is spermatogenesis, and inhibin Levels manufacturing is directly related the spermatogenesis's quantity, even though demonstrated by a direct relation between sperm count and serum inhibin B[8]. As in testicular irradiation or Sertoli cell-only syndrome, damage of spermatogenesis leads to falling the Inhibin B and elevation FSH. In these situations, Sertoli-cells alter the function, even though the germ cells' lack prevents Sertoli cells from fulfilling one's roles. In every event, even if FSH stimulates the secretion of inhibin B in normal male[11]. Previous studies

investigated the role of inhibin B in the diagnosis of men's infertility 90 samples of semen were collected for analysis, and serological assay Antisperm antibodies (ASAs) and 90 serum samples were collected for serological assay (ASA), which used micro agglutination and hormonal assay tests and showed a significantly low level of inhibin B for infertile men compared with the fertile control group[12]. In other study the average age (24 to 45 years) in 30 patients (infertile men) and 14 healthy controls (fertile men). The two groups of patients were divided into oligospermia and azoospermia. They showed a significant decrease in testosterone and inhibin B levels in infertile men than in the control group[13].

In Iraq Clinical diagnostic and hormone levels features related to the infertile patients' male infertility factor (n=75) and known fertile men were studied as a control (normozoospermic ones) (n=25) and concluded from this study that low levels of LH, inhibin B, and Testosterone, but the high level of FSH hormone were showed in the asthenozoospermia group as an example of infertile men and raised in LH levels, inhibin B, and Testosterone with a reduced level of FSH was showed in normozoospermic as the control group[14]. specimen of 80 adults' men aged 25-55 years were studied and showed significantly low levels of testosterone and inhibin B in the azoospermic patients group compared with the control group, while significantly increased LH and FSH levels in the azoospermic patient's group compared with the control group[15].

This study is aimed to determine the Inhibin-B hormone levels and their relationship with some gonadal hormones such as FSH, LH, prolactin, and testosterone in males with idiopathic infertility in Shirqat city.

Materials and Methods

The sample included 90 men, 60 of them were infertile and 30 healthful individuals as a control group from private clinics in Shirqat city in the province of Salahuddin from the beginning of October to the end of December 2019. Patients obtained the information were ranged from 18 to 60 years old and divided onto three categories (under 20-30 years, 31-40 years, and more than 40 years). The period of infertility was recorded after marriage, and the patients were classified in to detect the effects period of infertility in three categories (less than 10 years, 11-20 years, and over 20 years). The impact of family medical history investigated. The patients are classified into two categories based on this factor (10 men have a family history with infertility and 50 men do not.). The impact of smoking was investigated. The patients were classified into two groups (15 men smokers and 45 men non-smokers). According to types of sperm count, patients have divided into two groups 49 persons as azoospermia group and 11 persons as oligospermia.

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Inhibin B and gonadal hormones such as FSH, LH, testosterone, and prolactin were measures using the ELISA method according to the manufacturer's instructions (Sunlong Biotech company).

Statistical Analysis

Statistical analyses were performed using SPSS version 20 computer software. The mean, standard deviation (SD), and p-value of hormones parameters were calculate using the student's t-test and ANOVA test (which considered significant when p < 0.05 and

highly significant when p<0.01) for the patients and healthy group.

Results

Results of table 1 indicated a significant decrease $(P \le 0.01)$ in the levels of Inhibin B and testosterone compared with control group. In addition, there was a significant increase $(P \le 0.01)$ in the level of FSH, LH) and prolactin compared with control group.

Table 1 Comparison between hormonal levels of the infertile and fertile males.

Hormones	infertile (No. 60)	Control (No. 30)	
	Mean ± SD	Mean ± SD	P value
Inhibin B pg/ml	21.103±9.504	55.914±10.202	≤ 0.01 **
Testosterone (ng/ml)	2.5658±0.331	3.960±0.274	≤ 0.01 **
FSH (mIU/ml)	14.620±4.143	4.565±2.325	≤ 0.01 **
LH (mIU/ml)	8.869±2.646	4.632±1.110	≤ 0.01 **
Prolactin (ng/ml)	14.872±2.834	9.303±2.429	≤ 0.01 **

(*Significant, p < 0.05, ** High significant, p < 0.01). Color indicates the lowest level.

Results in the table -2 showed a significant decrease ($P \le 0.01$) in the levels of Inhibin B and testosterone in the azoospermic and oligospermic group compared

with the control group. However, there was a significant increase ($P \le 0.01$) in the level of. LH and Prolactin compared with control group.

Table 2: Hormonal levels of the infertile males according to infertility type

Hormones	Azoospermia (No. 49)	Oligospermia (No. 11)	Control (No. 30)	P value
	Mean ± SD	Mean ± SD	Mean ± SD	
Inhibin B pg/ml	21.097±5.294	21.1291±4.931	55.914±10.202	≤ 0.01 **
Testosterone (ng/ml)	2.610±0.350	2.368±0.244	3.960±0.274	≤ 0.01 **
FSH (mIU/ml)	14.929±1.055	13.248±2.472	4.565±2.325	≤ 0.01 **
LH (mIU/ml)	9.424±2.313	6.394±2.237	4.632±1.110	≤ 0.01 **
Prolactin (ng/ml)	15.924±2.463	10.722±1.953	9.303±2.429	≤ 0.01 **

(*Significant, p<0.05, ** High significant, p<0.01). Color indicates the lowest level.

no significant differences were detected (P>0.05) in the level of the inhibin according to age group<20-30 Year, 31 – 40 Year, \geq 40 Years (table 3), The current study indicated significant differences (P<0.05) in the level of the Testosterone according to age

group<20-30 Year, 31 - 40 Year, ≥ 40 Years. The study also, showed significant differences ($P \leq 0.01$) in the levels of FSH, LH and Prolactin according to age group<20-30 Years, 31 - 40 Year, ≥ 40 Years.

Table 3: Hormonal levels of the infertile males according to Age

Hormones	<20-30 Year (No. 19)	31 – 40 Year (No. 25)	≥ 40 Year (No. 16)	P value
Tior mones	Mean ± SD	Mean ± SD	Mean ± SD	1 value
Inhibin B pg/ml	22.620±4.872	20.504±3.190	20.236±3.966	0.706
Testosterone (ng/ml)	2.788±0.356	2.71±0.461	2.075±0.202	0.018 *
FSH (mIU/ml)	15.490±4.045	11.758±4.593	18.061±3.495	≤ 0.01 **
LH (mIU/ml)	8.776±2.302	7.648 ± 2.600	10.887±1.375	≤ 0.01 **
Prolactin (ng/ml)	17.876±3.338	15.427±3.614	10.806±2.490	≤ 0.01 **

(*Significant at p \le 0.05, ** High significant at p \le 0.01). Color indicates the lowest level.

This study also showed anon-significantly differences (P>0.05) in the level of the inhibin B pg/ml 20.785±4.949, 22.382±3.460, 19.63±2.631. LH (mIU/ml) 8.632±1.445, 9.375±1.818, 8.86±1.317 respectively according to infertility period \leq 10 Year,11 – 20 Year, \geq 20 Years (table 4). On the other hand, there was a significant difference (P<0.01) in

the levels of Testosterone (ng/ml) 2.709 \pm 0.573, 2.536 \pm 0.607, 1.896 \pm 0.437. FSH (mIU/ml) 14.216 \pm 2.177, 13.075 \pm 1.937, 20.455 \pm 2.577. Prolactin (ng/ml) 16.794 \pm 1.797, 12.664 \pm 1.495, 10.351 \pm 0.981. respectively according to infertility period \leq 10 Year, 11 – 20 Year, \geq 20 Year.

Table 4 Hormonal levels of the infertile males according to infertility period

Hormones	≤10 Year	11 – 20 Year	≥ 20 Year	
	(No. 36)	(No. 17)	(No. 7)	P value
	Mean ± SD	Mean ± SD	Mean ± SD	
Inhibin B pg/ml	20.785±4.949	22.382±3.460	19.63±2.631	0.303
Testosterone (ng/ml)	2.709±0.573	2.536±0.607	1.896±0.437	≤ 0.01 **
FSH (mIU/ml)	14.216±2.177	13.075±1.937	20.455±2.577	≤ 0.01 **
LH (mIU/ml)	8.632±1.445	9.375±1.818	8.86±1.317	0.796
Prolactin (ng/ml)	16.794±1.797	12.664±1.495	10.351±0.981	≤ 0.01 **

(*Significant at p \le 0.05, ** High significant at p \le 0.01) Color indicates the lowest level.

As shown in table (5) there is a remarkable difference (P<0.05) in the level of inhibin B pg/ml 23.269±4.037, 20.381±4.566 respectively according to smokers and non-smokers. Also, we showed a significant difference (P<0.01) in the levels of LH (mIU/ml) 11.368±2.144, 8.036±2.137. Prolactin (ng/ml) 13.308±1.841, 15.394±2.215. respectively

according to smokers and non-smokers. While we showed a non-significant difference (P >0.05) in the levels of Testosterone (ng/ml) 2.635 ± 0.559 , 2.542 ± 0.532 . FSH (mIU/ml) 14.741 ± 2.477 , 14.580 ± 2.399 . respectively according to smokers and non-smokers.

Table 5: Hormonal levels of the infertile males according to smoking

	Smokers (No. 15)	Non-Smokers (No. 45)	
Hormones	Mean ± SD	Mean ± SD	P value
Inhibin B pg/ml	23.269±4.037	20.381±4.566	0.033 *
Testosterone (ng/ml)	2.635±0.559	2.542±0.532	0.565
FSH (mIU/ml)	14.741±2.477	14.580±2.399	0.886
LH (mIU/ml)	11.368±2.144	8.036±2.137	≤ 0.01 **
Prolactin (ng/ml)	13.308±1.841	15.394±2.215	≤ 0.01 **

(*Significant at p \le 0.05, ** High significant at p \le 0.01). Color indicates the lowest level.

Results in table (6) showed significantly differences (P \leq 0.01) in the levels of Inhibin B pg/ml18.608 \pm 1.757, 21.582 \pm 2.269. FSH (mIU/ml) 19.825 \pm 2.543, 13.58 \pm 2.073. LH (mIU/ml) 7.414 \pm 1.993, 9.160 \pm 1.714 respectively according to Have family history and Don't have family history.

Also, we showed significantly differences (P<0.05) in the levels of Testosterone (ng/ml)2.357±0.376, 2.607±0.323. Prolactin (ng/ml) 12.18±2.851, 15.411±3.767. respectively according to Have family history and Don't have family history.

Table 6: Hormonal levels of the infertile males according to family history

Hormones	Have family history (No. 10)	Don't have family history (No. 50)	P value
	Mean ± SD	Mean ± SD	
Inhibin B pg/ml	18.608±1.757	21.582±2.269	≤ 0.01 **
Testosterone (ng/ml)	2.357±0.376	2.607±0.323	0.034 *
FSH (mIU/ml)	19.825±2.543	13.58±2.073	≤ 0.01 **
LH (mIU/ml)	7.414±1.993	9.160±1.714	≤ 0.01 **
Prolactin (ng/ml)	12.18±2.851	15.411±3.767	0.021 *

(*Significant at p≤0.05, ** High significant at p≤0.01). Color indicates the lowest level.

The results in the table (7) show non-significant differences between Inhibin B and FSH, LH, prolactin and testosterone. and non-significant weak correlation between Inhibin B and FSH, LH, and

prolactin R factor 0.015, 0.08, 0.092 respectively. While negative correlation between Inhibin B and Testosterone R factor-0.135.

Table 7: Correlation between Inhibin-B and hormonal levels of the infertile males

Parameter	Hormones	R factor	P value
Inhibin-B	FSH (mIU/ml)	0.015	0.909
	LH (mIU/ml)	0.08	0.543
	Prolactin (ng/ml)	0.092	0.484
	Testosterone (ng/ml)	-0.135	0.303

^{*}Significant at p \le 0.05. (- indicates negative correlation)



Discussion

In this study we observed that the LH, FSH and prolactin in infertile men were significantly ($P \le 0.01$) increased compared with controls. Conversely, the Total testosterone had also been significantly lower in infertile men compared to control. The cause of these results are the signalling of negative feedback system. In agreement with [16] in the Egyptian that the serum FSH level in infertile men has increased significantly compared with The fertile group and level of serum testosterone decreased significantly while no statistically significant relationship with serum LH was recognised.

In general, increased FSH levels are a good predictor of germinal epithelial destruction and are commonly associated with azoospermia or oligospermia, and lower Total testosterone levels are markers of hypothalamic or pituitary origin hypogonadism. [17] In the Iranian population, discovered that the FSH serum level in infertile men was greater than in control and the Luteinizing hormone and Testosterone serum levels in sperm count were lower than the control group, but the difference did not reach statistical significance among two groups (P=0.08 and P=0.06).[18] found that in theoligozoospermia subgroup the connection with significantly elevated FSH (P=0.01) and extremely was Inhibin B (*P*=0.046) significant. low Interestingly, meta-analysis from across Estonian idiopathic infertility group as well as the Baltic male cohort achieved statistically significant correlation with not only the reduced serum Inhibin B (*P*=0.037) as well as with decreases testosterone level (P=0.034).

Hyperprolactinemia diminishes men fertility when suppressed Synthesis. testosterone is hyperprolactinemia is well known to decrease libido cause reduce sperm production, and hyperprolactinemia may, on the other hand, be caused by a number of pathological factors, such as hypothalamic disorders, adenoma of pituitary gland, hypothyroidism, and hypogonadism and this may imply some disturbances in the spermatogenesis phases in infertile patients. The results of this study are compatible with Al-Nahi survey results [19] significant statistical are present differences $(P \le 0.05)$ in the FSH, prolactin and testosterone hormone averages in infertile men compared with the group.[20] demonstrated fertile men control significant improvements in LH, FSH testosterone levels total as well as showed no significant differences in prolactin between both the infertile men and fertile men control groups.

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The hypothalamus naturally secretes gonadotropin-releasing hormone (GNRH) which controls pituitary gonadotropin, LH and FSH secretion as well as dopamine hormone, a neurotransmitter, which also controls the release of prolactin. GnRH releasing is pulsatile and is therefore, in turn, those of LH and FSH. Even though, only one releasing hormone exists, FSH and LH secretion often, does not occur at the same time and may well be changed by the feedback from circulating gonadal androgen ,and or estrogen concentrations [21].

[22] found and showed an increase in the levels of FSH with no increase in the levels of LH and testosterone and invers correlation among levels of FSH and concentration of sperm. [23] investigated that patients with both semen and hormone disorders showed an increase in serum FSH, a decrease in testosterone serum with decrease in sperm count & motility of active sperm.[24] proposed which the particular connection of testosterone with semen characteristics (enhanced sperm motility) and connection among both LH and semen parameters (reduced sperm composition, morphology are related to disturbances motility) compensatory mechanism in the hypothalamo_ pituitarygonadal axis. FSH enhances the production of inhibin B from the testis and the inhibin B in turn inhibits FSH secretion, Clinical results showed a significant inverse relationship among FSH and inhibin B in healthful male and male with disorder in Testicular function [10]. As mentioned, above the non -significance grades of comparative analysis of gonadal hormones could also be attributed to the cascading requirements of the inhibin B and gonadal hormones, which could be interpreted as the significant roles of inhibin B within the assessed correlations which show up to become the physiological negative feedback signal for FSH through spermatogenesis. Our study agrees with the study of [25] determined serum levels of FSH, LH. testosterone and inhibin B in patients infertile men and healthy fertile men.

We concluded from the current study that, there were a significant decreased in the level of Inhibin B for the Azoospermic and oligospermic infertile men with a significant increase in the level of FSH which improve the inverse relationship between FSH and inhibin B hormone. Negative correlation between inhibin B and testosterone was shown as well as a weak correlation with other gonadal hormones. Therefore, Inhibin B has an important role in the diagnosis of idiopathic male infertility and as markers in the diagnosis of normal spermatogenesis.

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تقييم مستوى هرمون Inhibin-B وعلاقته بهرمونات الغدد التناسلية الأخرى عند الذكور المصابين بالعقم مجهول السبب

فخري غزال محمد ، عدنان فاضل العزاوي قسم علوم الحياة ، كلية العلوم ، جامعة تكريت ، تكريت ، العراق

الملخص

هدف هذه الدراسة تحديد مستوى هرمون ال Inhibin-B وعلاقته مع بعض هرمونات الغدد التناسلية مثل هرمون محفز الجريبات (FSH) ، الهرمون اللوتيني (LH) ، هرمون الحليب والتستوستيرون لبعض الذكور الذين يعانون من العقم مجهول السبب في مدينة الشرقاط.

جمعت عينات الدم والسائل المنوي من (60) من الرجال العقيمين مجهولين السبب ومن (30) من الرجال الاصحاء (مجموعة سيطرة) تتراوحت العمارهم بين (18 إلى 60 سنة) من العيادات الخاصة. استخدمت تقنية أختبار الأمتزاز المناعي للأنزيم المرتبط -Enzyme linked immune أعمارهم بين (18 إلى 60 سنة) من العيادات الخاصة. استخدمت تقنية أختبار الأمتزاز المناعي للأنزيم المرتبط (Sorbent assay ELISA) هرمون الحليب (FSH)، الهرمون اللوتيني (LH) ، هرمون الحليب والتستوستيرون.

حسب نوع اعداد الحيوانات المنوية تم تقسيم المرضى الى مجموعتين الاولى 49 شخص كمجموعة انعدام الانطاف (صفر/ مل) والثانية 11 شخصا كمجموعة قلة النطاف أقل من 20 مليون/ مل. انخفضت مستويات BInhibin B والتستوستيرون بشكل ملحوظ ($P \le 0.01$) في الرجال العقيمين مقارنة مع مجموعة السيطرة. في حين كان مستويات هرمون محفز الجريبات ($P \le 0.01$)، الهرمون اللوتيني ($P \le 0.01$) في الرجال العقيمين مقارنة مع مجموعة السيطرة. كانت نتائج هرمون ال B المالما غير معنوية, بينما كانت مستويات هرمون بكثير ($P \le 0.01$) باستثناء مستوى التستوستيرون كانت عند محفز الجريبات ($P \le 0.01$)، الهرمون اللوتيني ($P \le 0.01$) وهرمون الحليب أعلى عند مستوى معنوية ($P \le 0.01$) باستثناء مستوى التستوستيرون كانت عند مستوى ($P \le 0.01$) في مستويات الهرمون محفز الجريبات ($P \ge 0.01$) في مستويات ال عمرية حالي في المنات العمرية حاليب، لكن مستويات ال B المالمات على عند مستويات هورمون الحليب، لكن مستويات العرمون الحليب بينما كانت مستويات هرمونات B المرمون اللوتيني السيطرة. أظهر المرضى الذين لديهم مستويات منخفضة ($P \ge 0.01$) من هرمون الحليب بينما كانت مستويات ال B المالمات، التستوستيرون، الهرمون اللوتيني السيطرة. أظهر المرضى الذين لديهم تاريخ عائلي اختلافات معنوية ($P \ge 0.01$) في مستويات ال B المستوستيرون، الهرمون اللوتيني السيطرة. أظهر المرضى الذين لديهم تاريخ عائلي اختلافات معنوية ($P \ge 0.01$) في مستويات ال B المالمون الحليب.

في الختام، كشفت هذه الدراسة انخفاض كبير في مستويات إينهيبين-B لدى الرجال العقيمين من نوع عديمي وقليلي النطف. هناك ارتباط سلبي بين ال inhibin B والتستوستيرون وارتباط ضعيف مع هرمونات الغدد التناسلية الأخرى. لذلك، فإن ال Inhibin-B له دور مهم في تشخيص عقم الرجال مجهول السبب وهو واحد من المؤشرات المهمة في تشخيص تكوين الحيوانات المنوية الطبيعية.