

EVALUATION OF SERUM FSH, LH AND TESTOSTERONE LEVELS IN INFERTILE PATIENTS AFFECTED WITH DIFFERENT MALE INFERTILITY FACTORS AFTER IUI TECHNIQUE

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ABSTRACT:

The present study was designed to evaluate the serum FSH, LH and testosterone levels in 75 infertile male affected with different male infertility factors. In this study, one hundred infertile males were involved and according to cause of infertility were divided into 25 normozoospermia, 25 oligozoospermia, and 25 asthenozoospermia infertile couples. From each male, semen samples were collected and sperm parameters including sperm concentration, sperm motility, progressive sperm motility, and normal sperm morphology were evaluated according to standard World Health Organization (WHO) criteria. For semen processing, sperm prepared using conventional layering technique through incubation for 30 minute in 5% CO₂ at 37°C. The results of the present study demonstrated that serum FSH and LH levels significantly ($P < 0.05$) increase in infertile couples with normozoospermia, oligozoospermia, and decrease in asthenozoospermia as compare with fertile control group (No. =25). In contract, clearly significant decrease in serum testosterone levels ($P < 0.05$) in infertile men with normozoospermia and oligozoospermia and non significant increase ($P > 0.05$) in asthenozoospermia when compare with healthy fertile male. From the results of the present study, it was concluded that evaluation of serum FSH, LH, and Testosterone are diagnostic and prognostic tools in management of male infertility and acts as major control device of germ cell development. Further studies are recommended to assess the effect of elevated levels of FSH and LH and low levels of testosterone on DNA damage and embryo quality after in vitro fertilization and embryo transfer (IVF-ET).

INTRODUCTION:

Infertility is an important medical and social problem in the world as regards 15% of couples are infertile and 40% are infertile because of male factor infertility and 40% are because of female factor infertility and in the remainder both factors are associated (1). It is already common knowledge that an appropriate endocrine milieu is necessary for the sexual differentiation, normal potency as

well as for spermiogenesis maturation (2). As a consequence of the complex anatomical and functional integration of the reproductive system, both spermiogenesis in the germinal epithelium and regulative role of hypothalamo-hypophyseal-testicular axis are very sensitive. Their alterations become apparent also in the deterioration of fertility (3). The impact of spermiogenesis is encountered if the germinal epithelium

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and the Sertoli cells are in the appropriate androgenic environment. For this environment, the LH hormone of the adenohypophysis is responsible (4). This glycoprotein regulates the testosterone synthesis of the extratubular Leydig cells. The other gonadotropic hormone, FSH controls spermiocytogenesis and spermiogenesis by affecting both the germinal epithelium and Sertoli cells (5). However, LH secretion is regulated by the negative feedback of the testosterone in the vascular system. The serum LH concentration reflects the function of Leydig cells; it is an important factor in the differential diagnosis between primary orchopathy and hypothalamo-hypophyseal hormone deficient (6). Moreover, FSH secretion is regulated by the negative feedback of the testosterone and inhibin, a protein-type substance produced in Sertoli cells. The actual inhibin production reflects the extent of the alteration of spermiogenesis; a considerable oligozoospermia results in decreased inhibin synthesis, which leads to an increased FSH production. Therefore, inhibin may be used as FSH marker even in therapeutical practice. As well as, determination of FSH is of considerable value in the examination of the epithelial function of the seminiferous tubules (7). The successful and complete male germ cell development is dependent on hypothalamic balanced endocrine interplay, pituitary and testes (8). Gonadotrophin releasing hormone (GnRH) secreted by hypothalamus elicits the release of gonadotropins such as FSH and LH from the pituitary gland. Both FSH and LH are secreted in a pulsatile manner with rapid fluctuations over the normal range. The pulsatility of FSH is less pronounced than that of LH. The release of both FSH and LH is under negative feedback control by the gonads (9). Previously a hormone accomplishes its physiological function, its rate of secretion is prevented from increasing further and

at times is even decreased, and this is caused by negative feedback phenomenon to be important in nervous control systems (10). The FSH, LH and testosterone are prime regulators of germ cell development and useful in management of male infertility. The quantitative production of spermatozoa generally requires the presence of FSH, LH and testosterone. FSH binds with receptors in the Sertoli cells and stimulates spermatogenesis, while LH stimulates the production of testosterone in Leydig cells, which in turn may act on Sertoli and peritubular cells of the seminiferous tubules and stimulates spermatogenesis. The failure of pituitary to secrete FSH and LH will result in disruption of testicular function leading to infertility (11).

2. Materials & Methods

2.1. Subjects:

This study was carried out on various male infertility factors and semen samples were obtained from IVF Institute of Embryo Research and Infertility Treatment/Al-Nahrain University and collected by masturbation after 3-5 days abstinence. The total number of infertile couples enrolled in this study was 75 men with control group as healthy fertile men (No. =25). The study extended from October, 2007 to October, 2008.

The criteria applied for patient's selection was based on proper history taking, physical examination, and serum reproductive hormones levels assessment. From each male, semen samples were collected and sperm parameters including sperm concentration, sperm motility, progressive sperm motility, and normal sperm morphology were evaluated according to standard World Health Organization (WHO) criteria. For sperm processing, the sperm prepared using conventional layering technique through incubation for 30 minute in 5% CO₂ at

37°C.

2.2. Clinical and laboratory investigation:

2.2.1. Seminal fluid collection and analysis:

Semen samples were collected by masturbation after 3-5 days abstinence into a dry, clean, and sterile Petri-dish labeled with name and age of patient, period of abstinence and time of collection. The specimens were placed in the incubator at 37°C to allow the semen liquefaction. WHO criteria for normal semen values were applied (WHO, 1999).

2.2.2. Blood sampling and hormonal assessment:

The blood samples (3-5 ml fresh blood) was drawn and collected in a clean, disposable plastic tube from anterior cubital vein under aseptic condition for hormonal analysis. Concentrations of serum FSH, LH and serum testosterone were measured using MiniVIDAS apparatus (VIDAS 12, 1992, Biomerieux Company, France) through an enzyme linked fluorescent assay (ELFA) technique.

2.2.3. Semen processing technique:

The semen was prepared using conventional layering technique. However, 1ml of prepared IVF culture medium (Medi-Cult Company, Jyllinge, Denmark) was added to the test tube, and then 1ml of the liquefied semen was layered beneath a culture medium. After incubation for 30 minute in 5% CO₂ at 37°C, 10µl. of the mixture was aspirated by pasture pipette and examined under light microscope at 400X magnification for assessment parameters of sperm function test.

2.2.4. Timing of IUI and controlled ovarian hyper-stimulation syndrome:

Sonographic examination of follicular size was starting beginning 16 day from expected menses. Intrauterine insemination (IUI) was performed by threading a very thin flexible rubber

catheter through the cervix and injected washed sperm into the uterus and female were given clomiphene citrate (50 mg) two times daily for 5 days (cyclic day; 2-6 day), then recombinant FSH (Gonal-F; 75IU; Serono; Italy) for another 5 days (cyclic day; 7-11). The vaginal ultrasounographic demonstration was performed for four times (7, 9, 11 and 13 day). At least, when one ovarian follicle reaches ≥ 18 mm average diameters associated with a serum LH of at least 200pg/ml, Human chorionic gonadotropin (hCG; 10000IU; Profasi; Serono; Rome; Italy) was injected, and later IUI was done after 36 hours and no more than 48 hours from the initiation of LH surge and 12-24 hours from the peak.

3. Statistical analysis of the data

Statistical analysis was performed with the SPSS version 12.00 by Statistical Package for Social Sciences Software. The data analysis was done using paired sample t-test to assess statistical differences in results of sperm function. Mean (\pm S.E.M) obtained from crude data to compare between seminal parameters. P-value < 0.05 was used as a level of statistically significant.

4. RESULTS

Seventy-five infertile couples with male factors infertility with mean age 31.35 ± 0.66 years old with range from 18-49 and duration of infertility 5.66 ± 0.33 years with range from 2-16 years.

The results of the present study demonstrated that serum FSH and LH levels significantly ($P < 0.05$) increase in infertile couples with normozoospermia, oligozoospermia, and decrease in infertile patients in asthenozoospermia as compare with fertile control group (No. =25). In contract, clearly significant decrease in serum testosterone levels ($P < 0.05$) in infertile men with normozoospermia and oligozoospermia and non significant increase ($P > 0.05$) in infertile patients with asthenozoospermia when compare with

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healthy fertile male (Table1). It was worthwhile, in this study; the 16 clinical pregnancies were achieved for infertile couples and distributed as a following (7) for fertile control group, (5) for normozoospermic men, (1) for oligozoospermic men, and (3) for asthenozoospermic men as show in figure (4). Therefore, male partner with oligozoospermia of 25 infertile couples give low clinical pregnancy rates in contract to men healthy fertile group.

DISCUSSION

The FSH, LH and testosterone evaluation is useful in the management of male infertility. FSH is necessary for initiation of spermatogenesis and maturation of spermatozoa (12). In infertile men, higher concentration of FSH is considered to be a reliable indicator of germinal epithelial damage, and was shown to be associated with azoospermia and severe oligozoospermia (13). De-Kretser et al. (14) reported that elevated levels of serum FSH with increasing severity of seminiferous epithelial destruction. Babu et al. (15) indicated that gonadotropin (FSH and LH) levels were significantly elevated in infertile males when compared with the levels in proven fertile controls. However, Sulthan et al. (16) and Zabul et al. (17), showed elevated levels of both serum FSH and LH in infertile males. However, FSH acts directly on the seminiferous tubules whereas LH stimulates spermatogenesis indirectly via testosterone. FSH plays a key role in stimulating mitotic and meiotic DNA synthesis in spermatogonia (18). The increase in serum levels of gonadotropins might have disrupted the spermatogenic process leading to the decline in the sperm count and infertility (19). In the present study, elevated serum levels of FSH and LH were observed in oligozoospermic and asthenozoospermic males when compared with normozoospermic men (20). The low

levels of serum testosterone was observed in normozoospermic males and directly associated with infertility due to loss of germinal epithelium and disturbance of seminiferous tubules function probably due to deficient secretion of inhibin and sex steroids, but Leydig cells of testes remain intact (21). It has been demonstrated that low levels of serum FSH, LH and testosterone are found in men with oligoasthenozoospermia (22). While, elevated FSH level may represent a hormone of reduced biologic activity or an imbalance in gonadal-pituitary feedback mechanism and not irreversible germinal epithelial damage. Similarly, isolated elevation of LH level may suggest the presence of a cross reacting substance such as hCG (23). The primary role of LH in the male is to stimulate the production of testosterone by the Leydig cells and FSH regulates spermatogenesis in the Sertoli cells of the seminiferous tubules of the testes (24). Testosterone exerts a negative feed back on the release of LH. In male, LH and FSH regulate spermatogenesis (25). The Sertoli cells also play a role for the germinal epithelium and the Sertoli cells have been shown to be the exclusive target cells for FSH in seminiferous tubules, and since FSH is required for normal spermatogenesis, it has also been suggested that it influences the spermatogenic process, and it is of interest to note that all the steroid metabolic activities of the Sertoli cells are stimulated by FSH (26). Many recent studies reported that deficiency of LH and FSH prevents the gonads from either producing sperms or sufficient quality of testosterone (27). Also, decreased secretions of FSH and LH in oligozoospermic and asthenozoospermic men are due to prolonged half life of LH, reduced bio-active LH secretary disintegrate amplitude, and lower immunoactive ratio for LH burst amplitude and reduced bioactive-immunoactive ratio in the mass of LH

secreted per burst and decreased coordinated release of bioactive LH and testosterone (28). The suppression of testosterone secretion could be due to deficiency of hypothalamic GnRH, resulting in impairment of gonadotropin secretion from pituitary (29). It has also been demonstrated that hyper activity of the LH axis, leading to male infertility could be due to mutation of LH receptor gene (30). A close negative correlation has been observed in serum levels of FSH and inhibin B among infertile men with elevated FSH concentrations (31). It has been reported that, in addition to FSH

secreting pituitary adenomas or testicular failure, hyperactivity of the FSH axis, could also be due to mutations of FSH receptor (32). While, the increased estradiol level inhibits FSH and LH secretion from pituitary, which results in reduced FSH and LH stimulation of Sertoli and Leydig cells in the testes and a reduction in testosterone synthesis and sperm production (33). Decreased level of testosterone was observed in subfertile males, as sometimes there is a loss of germinal epithelium, but Leydig cells of testes remain intact (34).

TABLES and FIGURES:

Table (1): Serum FSH, LH and Testosterone levels in fertile and infertile men groups

Fertile and Infertile groups	Numbers	Hormonal levels Mean \pm S.E.M		
		FSH (mIU/ml)	LH (mIU/ml)	Testosterone (ng/ml)
Fertile control	25	7.34 \pm 4.29	6.33 \pm 2.53	4.02 \pm 1.62
Normozoospermia	25	08.50 \pm 5.46	12.96 \pm 9.36	06.23 \pm 1.08
Oligozoospermia	25	06.42 \pm 1.03a	11.64 \pm 2.82a	04.53 \pm 1.13b
Asthenozoospermia	25	16.32 \pm 1.05a	12.43 \pm 1.08b	15.20 \pm 1.03a

Values are Mean \pm S.E.M

Normal levels of Testosterone = 3.0 -10.6 ng/ml, FSH= 1.70 -12.0 mIU/ml; LH = 1.1-7.0 mIU/ml

a = Non-significant (P>0.05), b = Significant (P<0.001)

Total No. of infertile patients=75

Mean of age for infertile patients (31.35 \pm 0.66 years)

Mean of duration of infertility for infertile patients (5.66 \pm 0.33 years)

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Figure (1): Frequency distribution for serum FSH levels in fertile and infertile men groups

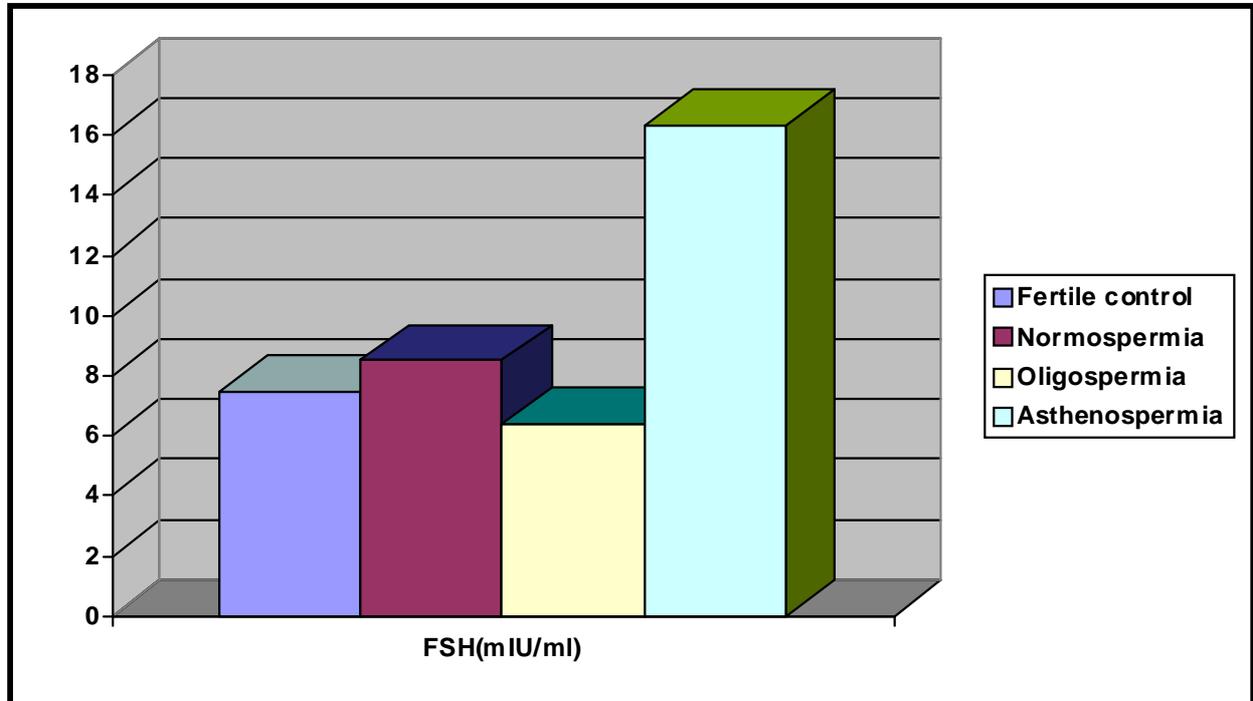


Figure (2): Frequency distribution for serum LH levels in fertile and infertile men groups

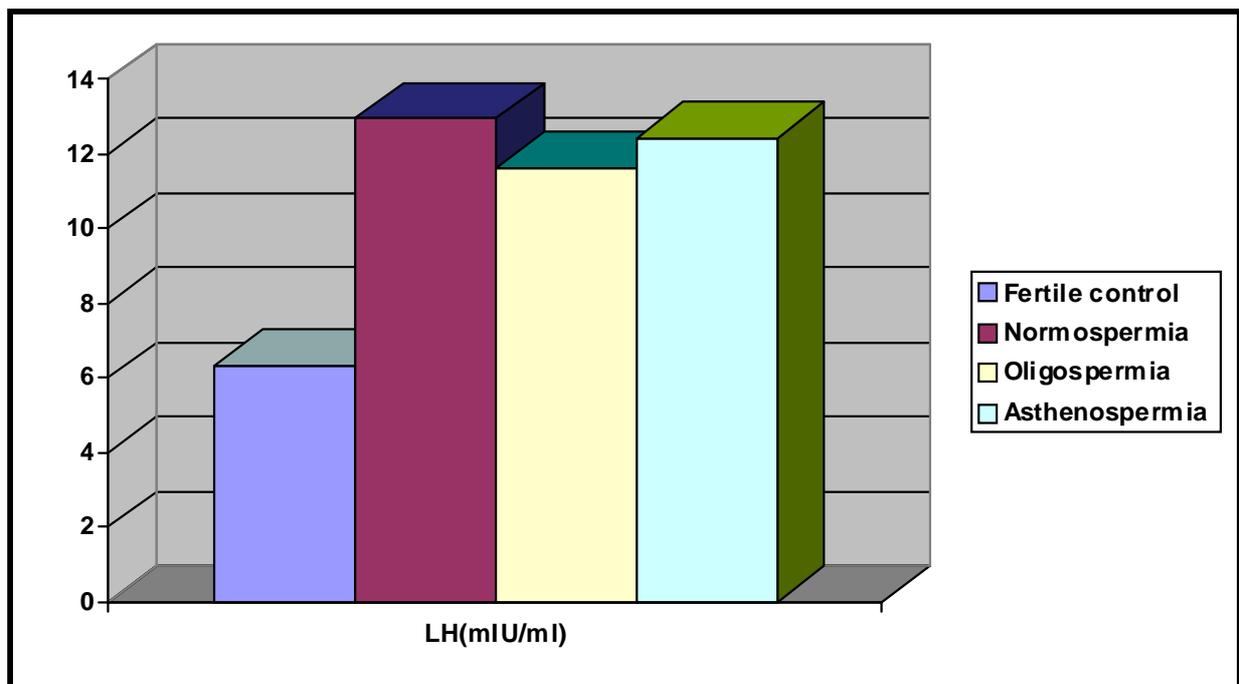


Figure (3): Frequency distribution for Testosterone levels in fertile and infertile men groups

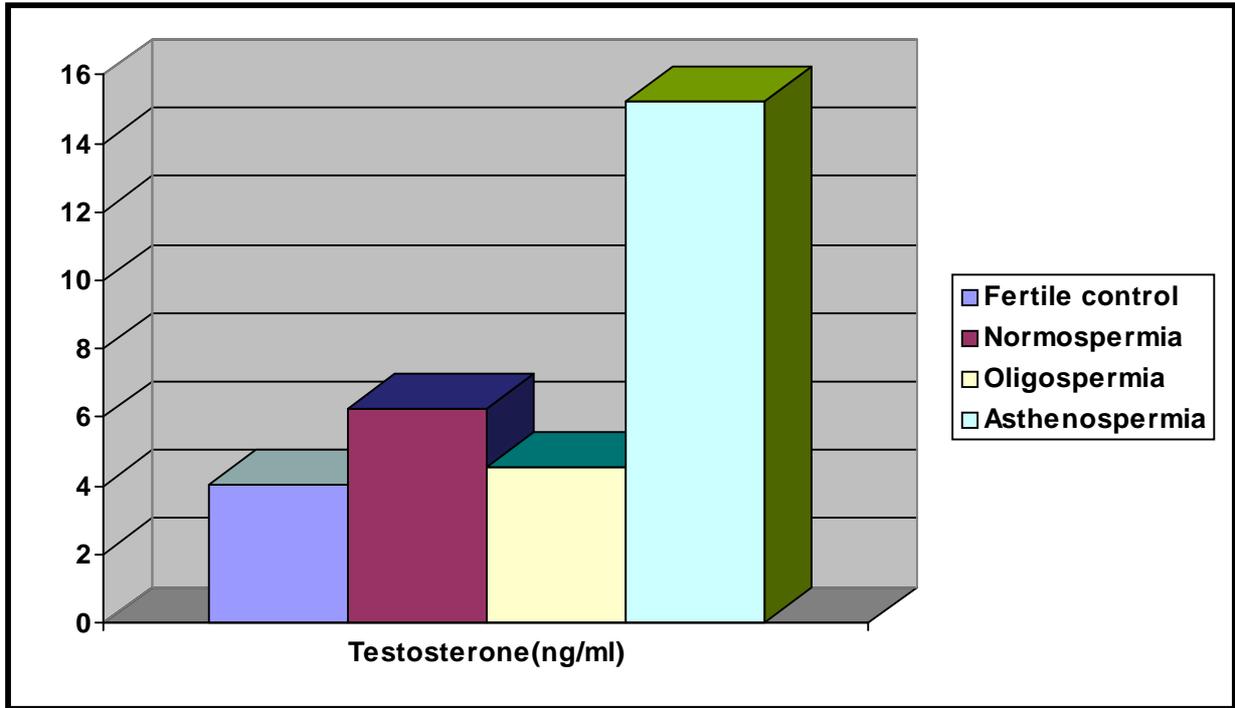
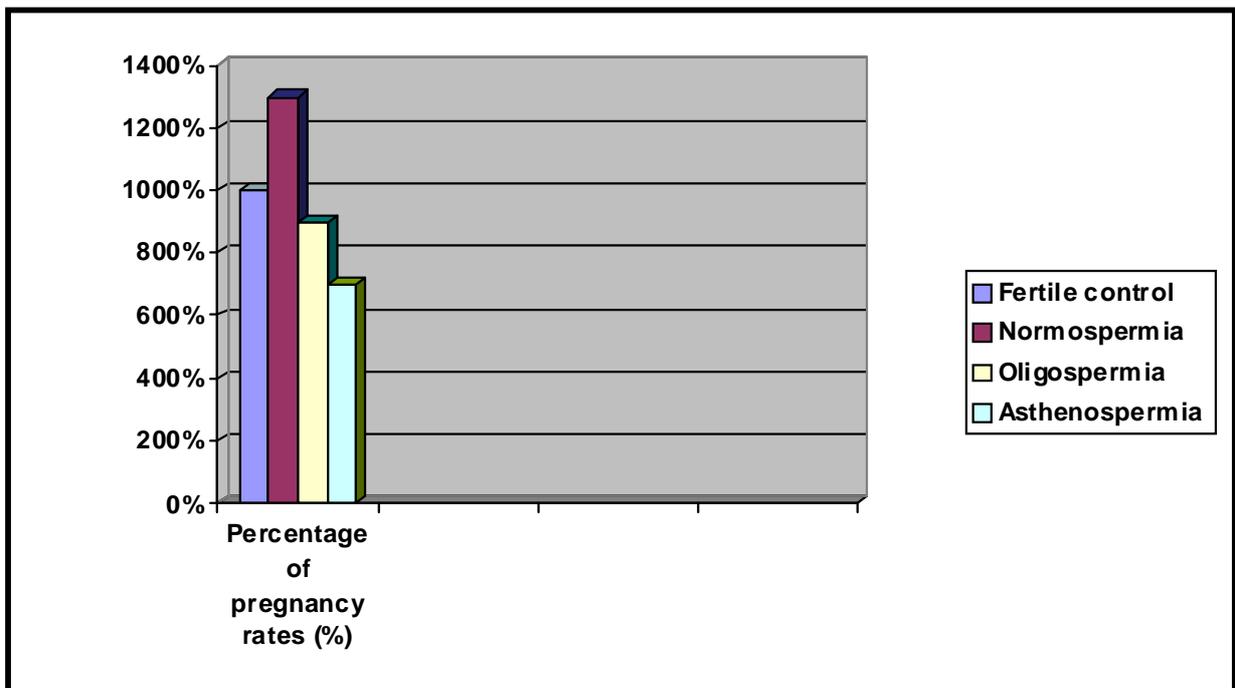


Figure (4): Frequency distribution for percentage of pregnancy rates enrolled in this study



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تقييم مستوى الهرمون المحفز للجريبات المبيضية والهرمون المصفر و مستوى الهرمون الخصوي لمرضى العقم المصابين بعوامل مختلفة للعقم الذكري بعد اجراء عملية التلقيح الاصطناعي داخل الرحم

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الخلاصة:

صممت الدراسة الحالية الى تقييم مستوى الهرمون المحفز للجريبات المبيضية والهرمون المصفر و مستوى الهرمون الخصوي لمرضى العقم الذين يعانون من عوامل مختلفة للعقم الذكري. اشترك في هذه الدراسة مائة شخص يعاني من العقم تم تقسيمهم اعتماداً على سبب العقم الى ثلاث مجاميع: الاولى مرضى يعانون من العقم ولكن متغيرات تحليل السائل المنوي طبيعية (العدد: ٢٥) و الثانية مرضى يعانون من قلة تركيز النطف (العدد: ٢٥) والثالثة مرضى يعانون من قلة حركة النطف (العدد: ٢٥)، كذلك تم اخذ مجموعة رابعة لمرضى اصحاء كمجموعة سيطرة (العدد: ٢٥) تم الحصول على عينة السائل المنوي وتقييم متغيرات النطف والتي شملت تركيز النطف والنسب المئوية لحركة النطف والحركة التقدمية للنطف والشكل الظاهري للنطف السوية اعتماداً على متغيرات منظمة الصحة العالمية (WHO) القياسية. تم تحضير نطف مرضى العقم باستخدام التقنية الطباقية البسيطة مباشرة من خلال الحضن لفترة ٣٠ دقيقة في ٥% ثنائي أوكسيد الكربون وبدرجة ٣٧ م°. أثبتت نتائج الدراسة الحالية ان مستوى الهرمون المحفز للجريبات المبيضية والهرمون المصفر اعطى زيادة معنوية لمرضى العقم الذين يعانون من سبب العقم الغير معروف ومرضى العقم الذين يعانون من قلة تركيز العقم ونقصان واضح في مرضى العقم الذين يعانون من قلة حركة النطف مقارنة بمجموعة السيطرة للمرضى القادرين على الاخصاب. هذا فضلاً عن الانخفاض المعنوي الواضح في مستوى الهرمون الخصوي لمرضى العقم الذين يعانون من سبب العقم الغير معروف ومرضى العقم الذين يعانون من قلة تركيز العقم، تم تقييم فروقات غير معنوية في مستوى الهرمون الخصوي لمرضى العقم الذين يعانون من قلة حركة النطف مقارنة بمجموعة السيطرة. نستنتج من نتائج هذه الدراسة ان ارتفاع مستوى هرموني المحفز للجريبات المبيضية والهرمون المصفر وانخفاض مستوى الهرمون الخصوي يؤثر بشكل كبير على مستوى كفاءة خلايا لايدل والخصائص الجنسية الثانوية الذكرية لمرضى العقم. نوصي باجراء دراسات لاحقة لتقييم تأثير ارتفاع مستوى هرموني المحفز للجريبات المبيضية والهرمون المصفر وانخفاض مستوى الهرمون الخصوي على تضرر الـ دي-ان-اي (DNA) ونوعية الأجنة بعد اجراء عمليات الاخصاب الخارجي ونقل الأجنة (IVF-ET).

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