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Serological Evaluation Of The Presence Of Anti-Spermatozoa Antibody In Infertile Males In Thi-Qar Governorate

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Abstract

A case control study was conducted in Thi-Qar governorate, south of Iraq, included a total 154 individuals 104 of theme were infertile patients while the other 50 males were fertile healthy control males, who attended the infertility center in A Al- Hussein Teaching +0hospital, from 20/10/ 2016 to 16/1/ 2018. aged between (l8-5l) years with mean of age was (29.95±0.68) and (50) healthy controls males aged between (23-37) years with mean age was (27.58±0.51). All patient submitted full reports about their medical status history, in addition to their infertility type (primary or secondary). Seminal fluid samples were collected by masturbation after three days of sexual avoidance. All patients and healthy control males were tested for presence of anti-spermatozoa antibodies in their seminal fluid. seminal fluid analysis in this study showed there were significant differences ($p < 0.05$) between fertile control group and infertile males (patients) in (The seminal fluid viscosity, Liquefaction time, Semen volume, Sperm motility and total sperm account while the results revealed there were no significant differences in the seminal fluid parameters between infertile patients and fertile control group in semen color, pH and sperm morphology . The overall prevalence of ASA in seminal plasma among studied papulation was 16.23% (25/l54). Among healthy control group anti-sperm antibody in seminal plasma were positive in 6% (3 /50) and negative in 94% (47/50) with mean concentration of anti-sperm antibody was 24.97±3.7 U/ml (normal range 0 – 60 U/ml). Among infertile patients anti-sperm antibody was positive in 21.15% (22/104) whereas negative in 78.84 % (82/104). The mean concentration of anti-sperm antibody was 40.71±2.32 U/ml. The statistical analysis revealed highly significant differences ($P <0.05$) in presence of ASA in seminal plasma of infertile

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patients compered to normal fertile males, also concentrations of ASA in seminal plasma was significantly higher in patients compered to healthy control males. In conclusion ASA was proportionally high among patient with infertility compered to healthy fertile control group.

Introduction

It is estimated that more than 48.5 million couples that have unprotected intercourse suffer from infertility worldwide (Ombelet *et al.*, 2008). With prevalence between 5.0% and 25.7% (Fertility, 2013). The percentage of infertile males varied from 2.5-12% globally, largest percentage of male infertility occurred in Central and Eastern Europe (8% to 12%) and Australia (8% to 9%). North America reveals rates of male infertility 4.5-6% (Ombelet *et al.*, 2008).According the result of meta-analysis, at least 30 million men worldwide are infertile with the highest rates in Africa and Eastern Europe (Mascarenhas *et al.*, 2012). over-all, 50% of sterility cases are due to a woman factor, pure man factor reported as 20-30% of the problem, and the rest 20-30% is caused by a combining of both female and male factors (Sharlip *et al.*, 2002). Data from a WHO study conducted from 1994-2000 revealed that, North and West Africa had the high rates of infertility, which ranged from 4.24%-6.35%. Central and East Asia had the lowest rates of infertility, with 2.05%-3.07% of infertility cases due to male factor alone (Mascarenhas *et al.*, 2012). A reduction in the ability of male fertility may be caused by congenital or acquired factors such as urogenital abnormalities, varicocele, infections of the genital tract, genetic abnormalities, hormonal abnormalities, testicular failure, immunologic problems, cancer, systemic diseases, altered lifestyle, and exposure to gonadotoxic factors (Dohle et al., 2005), (Jungwirth et al., 2012). The cause of fertility disability cannot be determined in many cases, in spite of the advances in diagnostic tests. Infertility of unknown cause, it is a situation in which fertility defect occurs spontaneously or caused by an obscure or unknown cause. Infertility of unknown cause accounts about 37–58% (Moghissi & Wallach, 1983), (Jungwirth et al., 2012), (Rowe, Comhaire, Hargreave, Mellows, & Organization, 1993). The category ‘unexplained male infertility’ (UMI) is reserved for infertile men with infertility of unknown origin with normal semen parameters and have normal findings on clinical examination and hormonal laboratory testing and in which female infertility factors have been ruled out (Jungwirth et al., 2012).

The initial assessment of sub-fertile men includes medical history, physical examination, and at least two semen analyses after 12 months of unprotected intercourse (Pacey & Eiser, 2011). In approximately half of the patients, the initial assessment will identify the cause of infertility,

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whereas many other patients will need to go through several complementary tests to find its cause (Wiser, Sandlow, & Köhler, 2012).

Immunologic factors are regarded as significant cause for infertility (Bohring, Krause, Habermann, & Krause, 2001). The first immunological correlation with male infertility was reported in 1954 by Wilson and Rumke with the identification of anti-sperm antibodies (Zhao, Zhao, Zhang, & Zhang, 2015), (Vazquez- Levin, et al. 2014).The prevalence of anti-sperm antibodies in infertile men varies from 9%-36% (Jiang et al., 2016) The main cause being the loss of the blood-testicular barrier and the association with chronic inflammation (Garcia, Rubio, & Pereira, 2007). Immune infertility has been shown to be found in 15% of patients with varicocele (Rossato, Galeazzi, Ferigo, & Foresta, 2004). Anti-sperm antibodies are found in cervical mucus, seminal fluid of men, Presence of anti-sperm antibodies in seminal fluid considered risk factor for infertility (Bohring et al., 2001). Despite the fact that millions of sperm cells are present within seminal fluid, only hundreds to tens found at any one within the fallopian tube (Esteves, Schneider, & Verza Jr, 2007). Immune response to sperm able to induce male infertility; antibodies that formed against spermatozoa did not necessarily impair male fertility unless the circulating antibodies are also found within the reproductive tract and on the living spermatozoa surface. Anti-sperm antibodies are produced in autoimmune infertility. Germ cells are normally seq uestrated from the immune system by the blood–testis barrier formed by Sertoli cells (Hjort, 1999). situations that cause damage of this barrier, like trauma, testicular surgery, varicocele, infection and inflammation as orchitis, may cause exposure of the germ cells to the immune system and production of anti-spermatozoa antibodies (Esteves et al., 2007), (Francavilla, Santucci, Barbonetti, & Francavilla, 2007), (Francavilla et al., 2007). The body treat the spermatozoa as foreign body. To clarify this, three hypothesizes of explanation. The first claim that spermatozoa are not display at the time of embryological development during which the immune system establishes tolerance to self-antigens (Francavilla et al., 2007). The second explanation that spermatozoa are haploid and have not the same chromosomal make-up from the somatic cells (Francavilla et al., 2007). The third hypothesis, named ‘immunosuppression theory’, suggests that T-suppressor lymphocytes, which suppress immune response, are activated by small amounts of spermatozoal antigens continuously leaked from the genital tract (Francavilla et al., 2007). Immediately when sperms formed during puberty, the immune system consider them as foreign cells, so they should be completely isolated from the immune system (Kayama, 2005). This isolation occurs within the testis, one of the immunologically privileged sites, by the blood–testis barrier. In other

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regions of the male genital tract, the epithelial lining, probably supplemented by a local immunosuppressive barrier, is responsible for this isolation (Kayama, 2005). In spite of its immune-privileged condition, the testis is clearly able of mounting inflammatory responses, as proved by its effective cellular and humoral defense against infections (Kipersztok et al., 2003), (DIEKMAN et al., 2000). It is well documented that the presence of anti-sperm antibodies can interfere with fertilization related to harmful effects on embryonic development and implantation.6,7(Verón et al., 2016), (Cui et al., 2015) . It has also been Together cellular and humoral immunity have been involved in the cause of immune infertility disease (Ombelet et al., 2003). Anti-spermatozoa antibodies could be defined as immunoglobulins of the IgG, IgA or IgM isotype that are formed against various parts of the sperm (head, tail, mid-piece or all of them) (Yeh, Acosta, Seltman, & Doncel, 1995). Immunoglobulin subclasses IgA and IgG can be found in the ejaculates of males with anti-sperm autoimmunity. However, IgM looks to have no clinical effect, it is rarely detected alone or combined with IgA or IgG (Ombelet et al., 2003),(Yeh et al., 1995).

Aim of the study:

- To identify the relation between presence of anti-sperm antibodies and male fertility alteration in Thi-Qar governorate.
- To estimate the prevalence of the presence of anti-sperm antibody in Thi-Qar governorate.
- To evaluate the association between presence of ASA in idiopathic infertile male

Material and methods: The study was conducted in Thi-Qar governorate, south of Iraq. A total 154 specimens of seminal fluid were collected for study. A case-control study involve fifty (50) healthy fertile males as control group and one hundred four (104) males with fertility disorders who attending infertility center / Al- Hussein Teaching hospital, from 20/10/ 2016 to 16/1/ 2018. aged between (l8-5l) years with mean of age was (29.95±0.68) and (50) healthy controls males aged between (23-37) years with mean age was (27.58±0.51). All patient submitted full reports about their medical status history, in addition to their infertility type (primary or secondary). Seminal fluid samples were collected by masturbation after three days of sexual avoidance. All patients and healthy control males were tested for presence of anti-spermatozoa antibodies in their seminal fluid.

Ethical and Official Approval: The procedure of this research were approved and granted from the ethical research approval committee of college of medicine / university of basrah. All participants were given verbal approvals to involvement in this study.

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Seminal fluid samples were collected in a private room adjacent the laboratory, in order to limit the exposure of semen to fluctuations in temperature and to control the time between collection and analysis. The samples were collected after a minimum of two days and a maximum of seven days of sexual avoidance according to WHO 2010 criteria. The man informed by clear spoken instructions about the collection of seminal fluid sample to ensure that seminal fluid sample be complete and not loss of any fraction of the sample. The following information was recorded on the report form the man's name, age and personal code number, the period of abstinence, the date and time of collection, the completeness of the sample, any difficulties in producing the sample, and the interval between collection and the start of the semen analysis (WHO standards 2010) . Anti-Spermatozoa Antibody ELISA test used for the determination antibodies directed against human spermatozoa. This test is designed for the use with seminal plasma.

Results

1. Sorting of age in this study

In the current study the total number of males included in this study was (n=154), lo4 of them were infertile patients while the other 50 males were fertile healthy control group. with mean age (29.95±o.68) and (27.58±0.51) years respectively, also the age distributed in to three age groups and the age intervals as shown in (table 1). In the infertile patients, the highest percentage of studied patients was with in age interval 26-35 was 56 patients (53.84%). followed by age interval 15-25 was 27 patients (25.96%) and then the age interval 36-51 was 21 patients (20.19%) which represents the lowest percentage. In the fertile control group, the highest percentage within age interval 26-35 was 76% (38 patients). Followed by age interval 15-25 was 18% (9 patients) and the age group 36-51 which showed the lowest percentage 6% (3 patients) as shown in table (1). The statistical analysis revealed there was significant association ($P < 0.05$) among age intervals of patients and control group.

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(Table 1) Distribution of patients and control according to age groups

Age groups(year)		Patients		Healthy control	
	Total	No.	%	No.	%
l5-25	36 (23.37%)	27	25.96	9	18
26-35	94 (61.03%)	56	53.84	38	76
36-5l	24 (15.58%)	21	20.19	3	6
Total number	154 (100%)	104	100	50	100
Mean ± SE		29.95± 0.68		27.58±o.5l	
X^2		33.510*		63.06*	
P value		<0.00001		<0.00001	

2. **Seminal fluid parameters and types of infertility**

The presented results of seminal fluid analysis in this study showed there were significant differences ($p < 0.05$) between fertile control group and infertile males (patients) in seminal fluid parameters as revealed in table (2). The seminal fluid viscosity was normal in 98% of fertile healthy males in comparison to 82.69% of infertile patients with normal viscosity while 17.3% of patients have abnormal result. Liquefaction time it was normal (within 30-60 min after collection of the sample) in 100% fertile healthy males while it was 74.03% of infertile males . Semen volume was normal in 100% and 90.38% of fertile healty males and infertile males respectively. Sperm motility was normal in 100% and 75.96% of fertile healty males and infertile males respectively while it was abnormal in 24.04% of infertile patients. Sperm vitality was normal in 100% of fertile healty males while it was normal in 91.43% of infertile males. The mean of total sperm count was 259.98 ± 1.16 million sperm/ ejaculate and 180.61 ± 0.77 million sperm / ejaculate of fertile healty males and infertile males respectively. The mean of sperm concentration was 62.73 ± 0.08 million sperm/ ml for fertile healty males while it was 54.32 ± 0.13 million sperm/ ml for infertile males. The other seminal fluid parameters as semen color, seminal fluid pH and percentage of sperm normal morphology were revealed non-significant differences ($P > 0.05$) as shown in table (2).

Regarding the type of infertility, the primary type of infertility was the more frequent type (83.65%) compared to secondary type of infertility (16.34%), and according to

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statistical analysis the difference was significant (P < 0.05) between two types of infertility as shown in (table 2).

Table (2) Seminal fluid parameters and types of infertility in patients and control.

Semen Parameters	Fertile males (healthy control) (50)	Infertile males (104)	T-test	X^2	P value	Normal WHO ranges
Viscosity	(49/50) 98% within WHO normal range	(86/104) 82.69%		7.316	0.007	Abnormal viscosity when the thread exceeds 2 cm
liquefaction	(50/50) 100% within (30-60)min	(77/104) 74.03%normal and (27/104) 25.96% take more than 60 min		15.740	<0.0 0001	Normal within (15-60 min) when take more than 60 min. must be recorded
Color	(50/50) 100% Gray-opalescent	(104/104) 100% Gray- opalescent		0	1	Gray-opalescent
Semen Volume (S.V.)	(50/50) 100% with normal semen volume Mean vol.± SD (4.l39 ± 0.0l8) ml	(94/104) 90.38% normal S.Vol. while 9.62% with S. Vol. <l.5 with mean vol.± SD (3.228 ± 0.01) ml		43.102	<0.0 000l	normal semen volume >1.5 ml
pH	(50/50) 100% alkaline	(l02/104) 98.07% alkaline while l.93% (2/104) acidic		1.014	o.314	Alkaline
Total sperm count.	259.48 ± l.l6 million sperm	180.6l ± 0.77 million sperm	56.355		<0.0 000l	WHO (LRL) 39 × 10^6 spermatozoa per ejaculate
Sperm count per ml	62.73 ± 0.08 million sperm	54.32 ± 0.l3 million sperm	53.466		<0.0 0001	WHO (LRL) 15 × 10^6 spermatozoa per ml
No. Males within	(50/50) 100% within normal range >4% with mean	(104/104) 100% within normal range >4% with mean percentage of normality (53.l9 ± 0.05%)		0	1	The lower reference

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WHO range of normal % of sperm morphology	percentage of normality (60.86 ± 0.06 %)				limit for normal forms is 4%
Sperm motility	(50/50) 100% have PR >32% and (PR+NP) >40%	(79/104) 75.96% have PR >32% and (PR+NP) >40%	l4.349	<0.0 0001	PR >32% and (PR+NP) >40%
Sperm Vitality	(50/50) 100% normal vitality percentage >58%	9l.34% (95/lo4) with normal sperms vitality >58%	4.595	0.032	>58%
Type of infertility	------	83.65% (87/104) with primary infertility while 16.34% (17/104) was with secondary type of infertility	92.480	<0.0 0001	

3. **Detection of anti-spermatozoa antibody in seminal plasma by ELISA test.**

overall prevalence of ASA in seminal plasma among studied papulation was 16.23% (25/l54). Among healthy control group, anti-sperm antibody in seminal plasma were positive in 6% (3 /50) and negative in 94% (47/50) with mean concentration of anti-sperm antibody was 24.97±3.7 U/ml (normal range 0 – 60 U/ml). Among infertile patients anti-sperm antibody was positive in 21.15% (22/104) whereas negative in 78.84 % (82/104). The mean concentration of anti-sperm antibody was 40.71±2.32 U/ml. The statistical analysis revealed highly significant differences (P <0.05) in presence of ASA in seminal plasma of infertile patients compered to normal fertile males, also concentrations of ASA in seminal plasma was significantly higher in patients compered to healthy control males and as shown in (3).

Table (3) presence of anti-sperm antibody in the seminal plasma in the healthy control and patients as estimated by ELISA.

ASA test result	**Total number & %**	**Fertile males (Healthy control)**		**Infertility males**		**T test**	**P value**
		N	%	N	%		
Positive	25 (16.23%)	3	6	22	21.15		
Negative	129 (83.76%)	47	94	82	78.84		
Total	154 (100%)	50	104	104	100		
Mean conc. ±SE		24.97±3.7		40.71±2.32		3.742*	<0.0000l
X^2		5.702*					
P value		0.017					

4. Distribution of anti-sperm antibody in relation to age groups of infertile patients and fertile males.

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The ASA was detected in 21.15% of infertile male and in 6% of normal healthy control. The majority of infertile males (50%) with positive ASA in their seminal plasma was found in age interval of 26-35 y old. followed by 27.27% in age interval 36-51 y old and then 22.72% in age interval l5-25 y old as shown in (table 4). This difference was statistically not significant (p> 0.05) among age groups.

Age distribution in fertile healthy control shows that, the highest percentage of ASA was 66.66% (2/3) detected in age interval (26-35). followed by 33.33% (1/3) in age interval (15-25). This difference was statistically not- significant (p>0.05) as shown in (table 4).

Table (4) distribution of anti-sperm antibody in relation to age groups infertile males and fertile males

Age groups	Total number	Infertility				fertility			
		negative		Positive		Negative		Positive	
		N	%	N	%	N	%	N	%
l5-25	36(23.37)	22	26.82	5	22.72	8	17.02	1	33.33
26-35	94(61.03)	45	54.87	11	5o	36	76.59	2	66.66
36-5l	24(15.58)	15	l8.29	6	27.27	3	6.38	0	0
Total	154(100)	82	53.24	22	14.28	47	30.51	3	1.94
Mean conc.±SE		32.69±1.84		77.26±2.32		19.91±1.94		104.72±29.78	
X2		7.758							
P value		0.256							

5. Anti-spermatozoa antibody in relation to type of infertility.

Primary infertility accounted to 83.65% of patients while secondary type of infertility was (16.34%). According to the type of infertility, the majority of patients with positive ASA suffering from primary type of infertility (86.36%) but only 13.63% with secondary type of infertility (table 5). The difference was statistically significant (P<0.05). However, the mean concentration of ASA of infertile was 77.26 IU/ml compared to that among control group (32.69 IU/ml).

(Table 5.) Anti-sperm antibody in relation to types of infertility.

Infertility type	Total number& %	ASA test result				T value	P value
		Negative		Positive			
		N	%	N	%		
Primary	87(83.65%)	68	82.92	19	86.36		
secondary	17(16.34%)	14	17.07	3	13.63		
Total	104(100%)	82	100	22	100		
Mean conc.±SE		32.69±l.84		77.26±2.32		10.858*	<0.00001
X^2		71.122*		23.273*			
P value		<0.00001		<0.00001			

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Discussion:

Patients with primary infertility represent the more frequent visitors to the fertility clinics (84%) in this study which is almost consistent with other studies in Iraq from Al-Nahrain University, (80.9%) (Al-Dujaily, Chakir, & Hantoosh, 2012) and Dohok (77.2%) (Razzak & Wais, 2002)at the north of Iraq. However, in another study from India reported a lower rate (62%) of primary infertility type (Samal, Dhadwe, Gupta, & Gupta, 2012).The findings from studies above revealed that, the primary infertility seems to be higher than secondary infertility and this will support the result found in this study.

Because the body treat the sperms as foreign body. Autoimmune infertility has long been postulated as one of the causes of subfertility (Bohring et al., 2001), (Esteves et al., 2007) and The prevalence of anti-sperm antibodies in infertile men varies from 9%-36% (Jiang et al., 2016).

Rumke and Wilson first documented that, the presence of anti-spermatozoa antibodies in infertile males, and suggest the possible potential effect of ASA in causes of infertility in males (Wilson, 1954),

In our study there was significant difference ($p < 0.05$) in the presence of anti-sperm antibody in seminal fluid between fertile healthy control group (6%). and infertile patients (21.15%). However, some studies reported ASA rates lower than the figure in this study (Hossain, Islam, Aryal, & Madanes, 2007). While another study in kirkuk university almost in agreement with our finding (26.6%) of infertile patients who shows ASA in their seminal fluids plasma ($p< 0.05$) and considered statistically significance (Hussein, 2012). The results from this study showed that the majority of infertile patients with ASA was in age group of 25-35 years of age (50%) which is comparable to (Arora, SUDHAN, & Sharma, 1999) study which revealed that, 64% of sub-fertile patients were in the age group of 30-35. There were only 3% patients between the ages of 20 and 25 years and only 2% between the ages of 40-50 years (Arora et al., 1999)

Also this study revealed that, the positive ASA results were distributed to 86.36% with primary type of infertility and 13.63% with secondary type of infertility which is consistent with other studies reported by (Foresta et al. 2015) and in an Indian study (Khatoon, Chaudhari, Singh, & Prajapati, 2011)which included 109 infertile couples revealed that, out of which 71 were suffering from primary infertility & 38 were of secondary infertility, among those with primary infertility couples 52.12% were positive for ASA and in secondary infertility couples the incidence of ASA was 39.47% (Khatoon et al., 2011) which is almost consistent with

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the finding in this study. However, another study performed by (Damianova, Dimitrova-Dikanarova, Kalaĭdzhiev, & Vatev, 1999), revealed that no significant difference in the incidence of anti-sperm antibodies among primary and secondary infertility and the highest incidence of anti-sperm antibodies amongst patients with primary unexplained infertility (Damianova et al., 1999) that in agreement with this study where there was non-significant difference in the percentage of ASA in primary and secondary infertility .

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التقييم السيرولوجي لوجود اضداد النطف في الرجال العقيمين في محافظة ذي قار

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الخلاصة:

أجريت هذه الدراسة (case control study) في محافظة ذي قار، جنوب العراق، شملت ما مجموعه ١٥٤ شخصاً (males) (منهم ١٠٤ مرضى يعانون من العقم، في حين كان مجموعة السيطرة تتكون من ٥٠ من الرجال الخصبين)، كان المرضى من الرجال الذين يراجعون مركز العقم في مستشفى الامام الحسين التعليمي من ٢٠١٦/١٠/٢٠ إلى ٢٠١٨/١/١٦ ،والذين تتراوح أعمارهم بين (١٨-٥١) سنة مع متوسط العمر (٢٩,٩٥ ± ٠,٦٨) واما بالنسبة الى (٥٠) رجل الاخرين الذين يمثلون مجموعة السيطرة فكان معدل أعمارهم تتراوح بين (٢٣-٣٧) سنة مع متوسط العمر كان (٢٧,٥٨ ± ٠,٥١). قدَم جميع المرضى تقارير كاملة عن تاريخ حالتهم الطبية، بالإضافة إلى نوع العقم (الابتدائي أو الثانوي). تم جمع عينات السائل المنوي بعد ثلاثة أيام من الامتناع الجنسي. تم فحص جميع المرضى ومجموعة السيطرة بطريقة (seminal fluid analysis) و حسب ضوابط كتاب منظمة الصحة العالمية في اجراء فحص السائل المنوي لسنة ٢٠١٠ الطبعة الخامسة. بالإضافة الى ذلك تم فحص كلتا المجموعتين بطريقة ELISA للتحري عن وجود الأجسام المضادة للنطف (ASA) في السائل المنوي

. أظهر نتائج الفحص العام للسائل المنوي أن هناك اختلافات كبيرة (P< ٠,٠٥) بين مجموعة السيطرة والمرضى في (لزوجة السائل المنوي، وقت التسييل، حجم السائل المنوي، حركية الحيوانات المنوية وعدد الحيوانات المنوية الكلي في حين

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كشفت النتائج أنه لم تكن هناك اختلافات ذات قيمة إحصائية في صفات السائل المنوية (لون السائل المنوي، ودرجة الحموضة(pH) وشكل الحيوانات المنوية.

في حين أظهرت نتائج الفحص السيورولوجي بطريقة ELISA ,للتحري عن الحيوانات المنوية ما يلي

ان الانتشار العام ASA في البلازما المنوية في ١٥٤ رجل (كلتا المجموعتين التي دخل الدراسة من الرجال) كانت ١٦,٢٣٪ (١٥٤/٢٥). وقد كانت نتيجة فحص وجود الجسام المضادة للحيوانات المنوية في بلازما السائل المنوي في مجموعة السيطرة إيجابية في ٦٪ (٣ /٥٠) وسلبية في ٩٤٪ (٥٠/٤٧) مع متوسط تركيز (ASA ٢٤,٩٧ ± ٣,٧ IU / مل (المدى الطبيعي ٠ - ٦٠ IU / مل). اما بالنسبة الى المرضى الذين يعانون من العقم فقد كان فحص ASA إيجابي في ٢١,١٥٪ (١٠٤/٢٢) و سلبي في ٧٨,٨٤٪ (١٠٤/٨٢). وكان متوسط تركيز الأجسام المضادة للنطف ٤٠,٧١ ± ٢,٣٢ IU / مل. وكشف التحليل الإحصائي عن اختلافات كبيرة (P <0.05) في وجود ASA في البلازما المنوية من المرضى الذين يعانون من العقم بالمقارنة إلى الذكور الخصبين، كما كانت تركيزات ASA في البلازما المنوية أعلى بكثير في المرضى. الملخص ان تركيز ASA كان عالي نسبيا بين المرضى الذين يعانون من العقم بالمقارنة إلى مجموعة السيطرة.