------ Raf. J. Sci., Vol. 24, No.3 pp. 1-16, 2013 ------

Detection of Antisperm Antibodies in Relation to Inhibin B in Infertile Men

Ruaa E. Shaya **Nawfal Y. Al-Dabbagh**
Department of Microbiology
College of Medicine
University of Mosul

(Received 14 / 8 / 2012 ; Accepted 26 / 11 / 2012)

ABSTRACT

Antisperm antibodies (ASAs) was considered as an immunological cause of male infertility. Serum inhbin B has a strong relationship with spermatogenesis and can be considered as a cause of infertility. The aims of the study are to: 1- Detect the presence of antisperm antibodies in both sera and seminal plasma of infertile patients and control group. 2- Find the effect of antisperm antibodies in seminal fluid parameters. 3- Exclude other male sex hormones as a cause of infertility. 4- Estimate serum level of inhbin B in the studied groups. 5- Determine the relationship between serum inhbin B and total sperm count in infertile males.

A total number of (90) individual, in which 70 infertile male patients with period of infertility more than one year and 20 fertile males as control group. The study was carried out on patients attending infertility care in a period of one year from June 2011 to June 2012, 90 semen samples were studied for semen analysis and for serological (ASAs) test and 90 serum samples for serological (ASA) study by using micro agglutination test and for hormonal assay (Latinizing hormone (LH), Follicular stimulating hormone (FSH), testosterone, prolactin) and inhibin B by using Enzyme Linked Immunosorbent Assay (ELISA). There was no significant difference between patients and controls according to the serum level of (LH, FSH ,testosterone, and prolactin), antisperm antibodies found positive in sera and semen of 15 (21.42%) of patients with titer 1/32 on semen and 1/64 in serum. The remainder 55 (78.57%) patients, showed negative results for (ASAs). There was a significant difference between mean % of total motility, mean % of normal sperm morphology and mean of total sperm counts of patients with positive ASAs results when compared to its mean in the control group. Mean concentration of serum inhibin B in patients with negative (ASAs) result was (32.24) pg/ml, while of control group was (73.55) pg/ml. The difference was considered highly significant. There was significant correlation between total sperm count of patients with negative ASAs results and mean concentration of serum inhibin B. This study suggests that the detection of ASAs in semen and sera of patients decrease in normal seminal fluid parameters especially the motility and (ASAs) can be considered as a cause of infertility in those patients, while low serum inhibin B in the reminder patients can be considered as a cause of infertility, then inhibin B can be considered as a marker for spermatogenesis.

Keywords: Male infertility, Antisperm antibodies, Inhibin B hormone.

الكشف عن الأجسام المضادة للحيامن وعلاقتها بهرمون انهبين ب في الذكور غير الخصبين

الملخص

تهدف الدراسة إلى:1- الكشف عن الأجسام المضادة للحيامن في السائل المنوي والبلازما المنوية لمجموعتي المرض والسيطرة. 2- دراسة تأثير الأجسام المضادة هذه على مقاييس السائل المنوي لدى الرجال العقيمين.3- استبعاد أي خلل هرموني بوصفه سبباً للعقم. 4- قياس مستوى البلازما لهرمون انهبين ب لدى مجموعتي المرض والسيطرة. 5- تحديد العلاقة بين مستوى البلازما لهرمون انهبين ب وانخفاض عدد الحيامن في السائل المنوي لدى الرجال غير الخصبين. اما تصميم الدراسة فتشمل مقارنة الحالات المرضية مع حالات السيطرة. تم جمع العينات من 70 رجل مصاب بالعقم من مركز العقم في أربيل للفترة من الأول من حزيران 2011 لغاية الأول من حزيران 2012 وكذلك من 20 رجل غير عقيم، كما تم تسجيل البيانات الديموغرافية وفترة العقم للمرضى. حيث تم أخذ 90 عينة من السائل المنوي لدراسة خصائص السائل المنوي ولإيجاد أجسام مضادة للنطف، كما تم أخذ 90 عينة دم للتحري عن الأجسام المضادة للنطف في البلازما ولقياس مستوى الهرمونات (الهرمون المنبه للجريب FSH هرمون ملوتن LH وهرمون برولاكتين .Prol وهرمون تستوستيرون .Testo) وانهبين ب وذلك باستخدام مقايسة الممتز المناعي المرتبط بالإنزيم. إن النسبة المئوية للأجسام المضادة للنطف في الدراسة كانت 21.24% للرجال العقيمين الذين لديهم فحوص إيجابية للأجسام المضادة للنطف، أما النسبة المئوية للرجال العقيمين التي جاءت نتائجهم سلبية فهي 78.57%، وان وجود هذه الأجسام المضادة يوثر على خصائص السائل المنوي للرجال العقيمين. إن الفحص الهرموني لكل من مجموعة المرض والسيطرة أظهر عدم وجود خلل هرموني لكل من الهرمونات (Lh, Fsh, Testosteron, Prolactin) إلا أن هناك فرقاً واضحاً في مستوى انهبين ب، إذ انه أعلى لدى مجموعة السيطرة (72.55 pg/ml) واقل عند الرجال العقيمين (32.24 pg/ml). هناك علاقة واضحة بين انخفاض مستوى انهبين ب وانخفاض عدد الحيامن في السائل المنوي للرجال العقيمين. وأخيرا تبين أن وجود الأجسام المضادة للحيامن لها تأثير على مقاييس السائل المنوي وان الهرمونات الجنسية الذكرية ليست السبب في العقم، ويمكن عد انهبين ب وسيلة لمعرفة قدرة الخصية لإنتاج الحيامن (النطف).

INTRODUCTION

Infertility is defined as the inability to achieve pregnancy after one year of unprotected intercourse. An estimated 15% of couples meet this criterion and are considered infertile, with approximately 35% due to female factors alone, 30% due to male factors alone, 20% due to combination of female and male factors, and 15% unexplained. Conditions of the male that affect fertility are still generally underdiagnosed and undertreated (Brugh and Lipshultz , 2004).

A male factor alone is the cause of infertility in up to 20% of infertile couples and a contributing factor in another 30% to 40% of all couples presenting for infertility

evaluations (American Urological Association [AUA] and American Society for Reproductive Medicine (ASRM), 2004).

The major constituents of semen are; spermatozoa; fructose, which is produced by seminal vesicles, provides a source of energy for sperm motility; clotting and anti-coagulated factors (Kacsoh, 2000; Guyton and hall, 2006). Causes of infertility in men can be explained by deficiencies in sperm formation, concentration (eg, oligospermia [too few sperm], azoospermia [no sperm in the ejaculate]), or transportation. The presence of antisperm antibodies (ASAs) was considered as one for the main causes of male infertility. (Hinting and Mahmoud, 1996). The production of ASAs is closely related to the stimulation of sperm antigen.

The initial evaluation of the male patient should be rapid, noninvasive, and cost-effective, starting with semen analysis, the human sperm cell is paddle- shaped with a rounded head (4-5) microns long and 2-3 microns in width and a long tail of flagellum about 50 microns which contain a central contractile unit called as the axoneme (Rao *et al.,* 2004). Treatment options are based on the underlying etiology and range from optimizing semen production and transportation with medical therapy or surgical procedures to complex assisted reproduction techniques (Ghanem *et al*., 2010).

Treatments of ASAs include: (i) condom use; (ii) washing, enzymatic treatment; (iii) split ejaculate, depletion; (iv) steroids; and (v) insemination, (IVF). Each of these therapies has its advocates (Francavilla *et al*., 2007).

Inhibin B is a glycoprotein hormone of gonadal origin, consisting of 2 dissimilar disulfide-linked subunits (α and either βA or βB), that has an inhibitory effect on pituitary gonadotropin production of FSH and LH (Illingworth *et al*., 1996).

Serum inhibin B is considered a marker of spermatogenesis. One major finding is that inhibin B is the physiologically important form of inhibin in the male. Inhbin B can cause male infertility, in which its serum level in infertile male is lower than fertile male.

MATERIAL AND METHODS

In this case -control study, the serum and semen from (70) infertile male patients attending infertility care in Erbil city and (20) fertile male as control group were collected. The study was carried out from June 2011 to June 2012. The sample size is determined by statistical program of Epi info version 6.

The total number of samples was 90 in which the serum from (70) infertile male patients and (20) fertile male as control group were collected. A total 5ml of blood was obtained by vein puncture, serum was separated by centrifugation and stored under (-20^{o}C) until analyzed (for serological tests).

A semen specimen was collected after 3days of abstinence period, in a wide –mouthed, clean and sterile container. Each patient had been told to urinate before collection to decrease contamination of semen from debris or leukocytes (Eliasson,1986), carefully instructed to avoid inner contamination of container and wash the hands and glans penis with soap and water prior to masturbation. It is important that the entire specimen was collected. Semen was incubated for 30 minutes at 37 $^{\circ}$C for liquefaction. Name, time of ejaculation and period of abstinence were mentioned (Chernecky and Berger, 1997). Part of these samples were used for semen analysis and the remaining of the seminal fluid was centrifuged at (3000) rpm for 15 minutes and the seminal plasma was freezed and stored

at (-20^{o}C) to be used later for the detection of ASAs. 90 semen samples studied for semen analysis and for serological (ASAs) test and 90 serum samples for serological (ASA) study and for hormonal assay (LH,FSH, testoesteron, prolactin) and inhibin B done by using ELISA.

Normal seminal fluid sample from healthy fertile male having two kids was also, used as donor for sperm antigen. The ASAs in both sera and semen of studied group were done by using Tray agglutination test (TAT) or micro agglutination test (MAT).

Suitable inferential statistics were used to analyze the results which include:

1. Pearson Chi-squared test (χ^2).
2. Fisher exact test.
3. t-test which was used to find significant difference between the means of different groups.

A P-value less than 0.05 was considered statistically significant (S), and less than 0.01 considered highly significant (HS), P-value greater than 0.05 considered non significant (NS).

RESULTS

The highest number and percentage of patients were in age range (24-28)year and (39-43) year with 20 (28.57%) and 18 (25.71%) respectively as Table (1) showed, and most frequent duration of infertility was (1-5) year with 60% (Table 2).

The present results showed that there was no significant difference between mean concentration of prolactin, FSH, and LH in sera of patients compared to control group, $p>0.05$, and although there was a significant difference in mean concentration of serum testosterone of patients compared to control group but it was still within normal range as the data presented in Table (3).

From the 70 samples of semen and serum of patients, only 15 (21.42%) showed positive results of ASAs as shown in Table (4). The remainder 55 (78.57%), showed negative results.

The highest number and percentage of patients with positive (+ve) results of ASAs with Head-Head agglutination was 11 (73.33%) (Table 5).

The data represented in table (6) showed that the highest number and percentage (%) of Normozoospermia was in patients with negative (-ve) ASAs 43 (47.7%), while the whole control group was normozoospermia with 20 (22.22%). The highest number and percentage of oligozoospermia and azoospermia patients were those with negative ASAs with 6 (6.6%) for both, while only 2 (2.2%) and 4 (4.4%) respectively showed oligospermia, azoospermia in patients with positive ASAs.

Table (7) showed that there was a significant difference between mean total sperm count $*10^6$ of control group (92.60) when compared to mean total sperm count in patients with –ve ASA and +ve ASA (47.30) and (38.53) respectively.

Table (8)showed the highest number and percentage of patients with +ve ASA had <40% total motility (PR+NP) 11 (12.22%), compared to –ve ASA patients who had 8 (8.88%). All control group were having 40% total motility(PR+NP). The highest number and % of patients with zero total motility were in –ve ASA is patients 6 (6.66 %) compared to +ve group who had only 2 (2.22%).

Table (9) showed that there was a significant difference between mean % of total motility of control group which was (47.15) when compared to its mean in –ve and +ve ASA patients (27.78), (21.33) respectively.

Table (10) showed that all control group 20 (22.22%) had 30% normal morphology, the highest number and % of < 30% normal morphology presented in –ve ASA patients with 9 (10%) compared to +ve ASA patients who had 2 (2.22%). The highest number and % of zero % of normal morphology was in –ve ASA patients with 6 (6.66%) compared to +ve ASA patients who had 2 (2.22%).

Table (11) showed that there was significant difference between mean % of normal morphology of control group which was (67.3)% when compared to its mean in –ve and +ve ASA patients (48.18)%, (44.00) % respectively.

Table (12) illustrated that the highest number and % of patients with positive ASAs results was in age range group (29-33) years, which represent 7 (10%).

The data represented in Table (13) showed that mean concentration of serum inhibin B in patients with negative ASAs result was (32.24) pg/ml, while of control group was (73. 55) pg/ml, p <0.0001 so the difference was considered highly significant.

Fig. (1) showed that there was a significant correlation between mean serum inhibin B (pg/ml) and mean total sperm count $*10^6$/ml in patients with negative ASAs.

Table 1: Distribution of infertile patients according to age groups (year)

Age range roups (year)	Frequency	percentage
19-23	5	7.14
24-28	20	28.57
29-33	16	22.85
34-38	11	15.73
39-43	18	25.71
Total	70	100%

Table 2: Distribution of patients according to duration of infertility in year

Duration of infertility (years)	No. of patients	percentage
1-5 6-10 11-15 16-20	42 22 4 2	60 31.42 5.72 2.85
Total	70	100%

Table 3: Mean conc. of prolactin (ng/ml), Testosterone (ng/ml), LH(m.I.U/ml) and FSH (m.I.U/ml) in sera of study groups

Study groups	NO.	Mean conc. of testosterone (ng/ml)		Mean conc. of prolactin (ng/ml)	Mean conc. of LH (m.I.U/ml)	Mean conc. of FSH (m.I.U/ml)	Statistical study
patients	70	3.2	S.	8.8	8.55	7.8	N.S.
controls	20	3.6		8.2	8.64	9.6	
P < 0. p <0.001highly significant***, p<0.05significant(S.) ** , p<0.01highly significant***, p>0.05 Non sig Non significant (N.S.)*.							

Table 4: Distribution of patients according to result of ASAs in serum and seminal plasma

Results of ASA	number	percentage	Titer of ASA
In semen			
-ve ASA	55	78.57	--------
+ve ASA	15	21.42	1/32
In sera			
-ve ASA	55	78.57	--------
+ veASA	15	21.42	1/64

Table 5: Types of sperm agglutination in semen of patients with + result for ASAs

Types of sperm agglutination	Number of patients +ve ASA	percentage
Head-head	11	73.33
Head-tail	2	13.33
Tail-tail	2	13.33
Total	15	100

Table 6: Distribution of patients and control group according to WHO criteria (2010) regarding total sperm count *10^6/ml:

Study group	sperm count*10^6/ml						Total	
	Normozoospermia		oligozoospermia		azoospermia			
	NO.	%	NO.	%	NO.	%	No.	%
ASAs+ve patient(15)	9	10	4	4.4	2	2.2	15	16.2
ASAs-ve patient(55)	43	47.7	6	6.6	6	6.6	55	60.7
Controls(20)	20	22.22	0	0	0	0	20	22.2
Total	72	79.92	10	11	8	8.8	90	100

Table 7: Mean total sperm count*10^6/ml in samples of study groups

Study group	Mean total sperm count *10^6/ml				
	No.	Range	mean	S.D.	Statistical analysis
+ve ASA patients(a)	15	0-60	38.53	27.77	a-b a-c*
-ve ASA patients(b)	55	0-80	47.30	30.64	b-a b-c*
Controls(c)	20	90-105	92.60	7.21	c-a* c-b*
Total	90	----------	----------	----------	----------

*The mean difference significant at the 0.05 level according to LSD.

Table 8: Distribution of patients and control group according to WHO criteria (2010) regarding % total motility (progressive+Non progressive)

Study group	% of Total motility (PR+NP)						Total	
	>40%		<40%		zero			
	NO.	%	NO.	%	NO.	%	No.	%
ASAs+ patient(15)	2	2.22	11	12.22	2	2.22	15	16.66
ASAs- patient(55)	41	45.55	8	8.88	6	6.66	55	61.22
Controls(20)	20	22.22	0	0	0	0	20	22.22
Total	63	69.99	19	21.10	8	8.88	90	100%

Table 9: Mean percentage of total motility (PR+NP) in study groups

Study group	Mean % of total motility(PR+NP)			
	No.	mean	S.D.	Statistical study
+ve ASA patients(a)	15	21.33	16.08	a-b a-c*
-ve ASA patients(b)	55	27.78	19.63	b-a b-c*
Controls(c)	20	47.15	8.05	c-a* c-b*
Total	90	---------	---------	----------

Table 10: Distribution of patients and control group according to WHO criteria (2010)* regarding % of normal morphology

Study group	% of normal morphology						Total	
	normal morphology 30%		normal morphology < 30%		Normal morphology % zero			
	NO.	%	NO.	%	NO.	%	NO.	%
ASAs+ patient(15)	11	12.22	2	2.22	2	2.22	15	16.66
ASAs- patient(55)	48	53.33	9	10	6	6.66	55	61.22
Controls(20)	20	22.22	0	0	0	0	20	22.22
Total	79	87.77	11	12.22	8	8.88	90	100

- According to the WHO criteria max. reference of normal morphology is 30%.

Table 11: Mean percentage of normal morphology in study group

Study group	mean % of normal morphology			
	No.	mean	S.D.	Statistical analysis
+ve ASA patients(a)	15	44.00	19.19	a-b a-c*
-ve ASA patients(b)	55	48.18	21.88	b-a b-c*
Controls(c)	20	67.3	8.16	c-a* c-b*
Total	90	----------	-------	--------

Table 12: Distribution of +ve ASAs patients according to age groups

Age range groups (year)	+ve ASAs in both semen and serum	
	Number of patients	%
19-23	0	0
24-28	2	2.8
29-33	7	10
34-38	4	5.7
39-43	2	2.8
Total	15	21.42

Table 13: Relationship between mean conc. of serum inhbin B in patients with negative result for ASAs and mean of controls

Study groups	Range of serum conc. of inhbin B (pg/ml)	Mean con. of inhbin B pg/ml	SD	S.S
Patients (55) (-ve ASAs)	20-75	32.24	13.75	Highly significant
Controls(20)	40-135	73.55	20.78	

P<0.0001 at (2-Tailed).

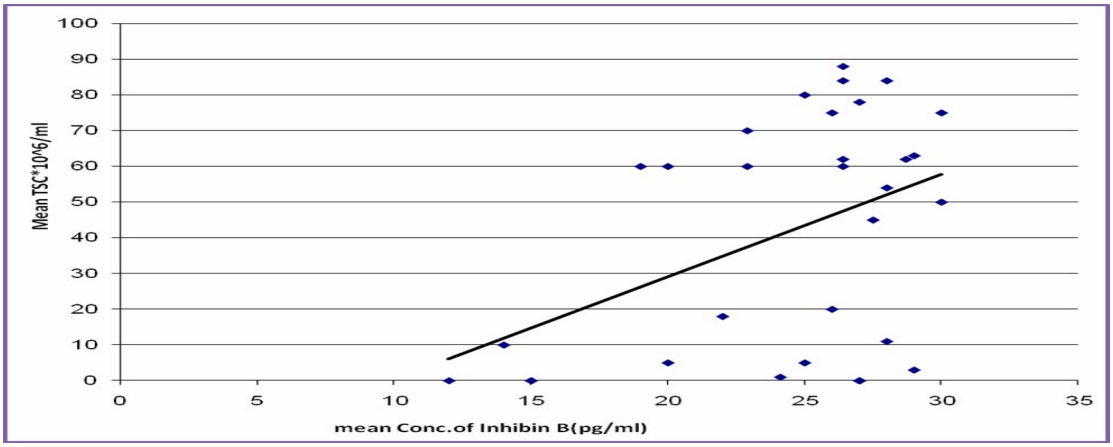

Fig. 1: Correlation between mean conc. of inhibin B in pg/ml and mean total sperm count* 10^6/ml in patients with negative results for antisperm antibodies

DISCUSSION

This study stated that most patients were in the age range groups of (24-28) years and (39-43) years, this finding agreed with the finding of Gardi (2005) who reported that most common age presentation of male infertility is around (25-31) years. It has been suggested that the aging male has reduced fertility that begins in his late thirties and early forties (Bayer *et al.*, 2007).

Gnoth *et al*., (2005) found that in general cumulative probabilities of conception decline with age. Sharon *et al*., (2001) concluded that increased male age is associated with a decline in semen volume, sperm motility and sperm morphology. The highest percentages of patients have duration of infertility about (1-5) years in this study and this agreed with Abdullah (2009) who observed the highest percentage of infertile male patients in duration between (1-4) years.

This short duration may be due to the awareness of the public about the reproductive techniques and this is due to media attention.

Allow (1999) stated that the introduction of IVF, which increased the possibilities for effective treatment for infertile couples is partially due to the media attention, paid to these techniques. The result of this study disagreed with one study that stated the highest % of infertile patients were having duration of infertility between (1-8) years (Gardi, 2005).

Table (3) revealed that there was no significant difference between mean concentration of prolactine in sera of patients and control group,($p>0.5$).

The result of this study are supported by studies of Beltran *et al*., (2008) who demonstrated that mean concentration of prolactin in sera of adult men was (7.8) ng/ml, and Greenspan *et al*., (2003) who found that up to 15 ng /ml of serum prolactin is normal in men.

The present results showed that there was a significant difference between mean concentration of testosterone in sera of patients (3.2) ng/ml when compared to its mean in sera of control group (3.6) ng/ml ($p>0.05$). Testosterone is necessary for normal sperm development. It activates genes in Sertoli cells, which promote differentiation of spermatogonia (Mehta *et al*., 2008).This agree with the results of Fischbach (1998) who found that the normal value of adult testosterone is between (3-10) ng/ml, this result proved that patients and control group had normal serum testosterone. The data also showed that the mean concentration of LH in sera of patients was (8.5) m.I.U./ml, while of control group was (8.6) m.I.U./ml, so the difference was not significant, $p > 0.05$.

The results above agree with Greenspan *et al*., (2003) who found that the normal value of LH in adult male was between (2-14) m.I.U/ml.

The data represented in table(3) showed that there was no significant difference between mean concentration of FSH in sera of patients when compared to its mean in control group, the range of serum concentration of FSH of control group was between (5-14) m.I.U/ml .

The results agree with Greenspan *et al*., (2003) who found that the normal serum FSH ranges between (1-12) m.I.U/ml.

A number of reports described the association between ASAs with the occurrence of infertility in either men or women, although usually the unexplained infertility has been the point of emphasis (Shulman *et al*., 2005).

Antisperm antibodies can be defined as immunoglobulines of the IgG, IgA and/or IgM classes that are directed to various aspects of spermatozoa (head, tail, midpiece or combination).

The sperm antibodies in men are polycolonal that is directed to more than one sperm antigen. The possible effects of immunologic reaction to fertility are:

1. Disordered spermatogenesis resulting in oligozoospemia and azoospermia.
2. Binding of antibodies to post testicular spermatozoa inhibiting their effective transport in male reproductive tract.
3. Induction of sperm immunity in the female. (Bennet, 1997; Mazumdar and Levine, 1998; Karaki *et al*., 2000).

Table (4) showed that the distribution of ASAs which was detected by using MAT in the sera of patients and control group sera and seminal plasma was (21.42%).This result is in agreement with that of Al-Khayat (2004) who observed (22.3%) positive ASAs in male serum of 130 cases studied by (TAT) method.

A study in Kirkuk governorate by Marbeen *et al.*, (2007) found that by using ELISA, ASA was detected in the seminal fluid of (25.8%) infertile. Abdullah (2009) found that positive ASAs in semen and serum was (29.94%), (21.01%) respectively in 157 infertile couples in Erbil city. Ameen (2007) found that 6.03% of 223 infertile male in Kurdistan have ASAs in seminal plasma by using TAT.

Agglutination means adhering spermatozoa without other cells or debris. Small aggregates of dead spermatozoa often found in semen from normal men (Kuist and Bjorndahi, 2002).

Table (5) showed that the highest number and % of patients 11(73.33%) had sperm agglutination of Head-Head type.

The ASAs directed against the sperm head were of primary importance, whereas those the tail were involved in poor motility. Moreover, ASA-coated sperm may be more vulnerable to phagocytosis in the female reproductive tract. (Lombardo *et al*., 2001).
Modification of sperm motility is the main cause of infertility in some patients, resulting in blockage of transport to, and union with, the oocyte (Rooney *et al*., 1993).

It is very difficult to tell an infertile man how many sperms needed for conception. In this study, the chamber method for the evaluation of sperm count used is due to its accuracy and reliability in keeping with several articles (Sarkar and Henry,1996).

Table (6) showed that control group was all normozoospermia, while there was oligospermia and azoospermia in +ve ASAs patients with 4(4.4%), 2(2.2%) respectively.

This result goes with Abdullah (2009) who delineated that Abnormal seminal fluid parameters which include sperm count related to the presence of antisperm antibodies in semen of infertile patients. Mardar *et al.*,(2002) found that antisperm autoimmunity appears to play a significant role in impairment of spermatogenesis.

Table (7) showed that the mean total sperm count of control group was 92.60*10^6/ml which was higher than the mean of +ve and –ve ASAs infertile patients.

These results were in agreement the with finding of Shen *et al*., (1999); Chia *et al*., (2000); Okonofua *et al.*, (2005) and Ameen (2007) who recorded that sperm concentration and total sperm count from healthy fertile men are higher than infertile men. Khosrowbeygi *et al*., (2004) found that sperm concentration is higher in fertile group men than in asthenozoospermic, asthenoteratozoospermic, and ligoasthenoteratozoospermic patients.

Regarding motility and According to WHO (2010) criteria and as table (8) showed that all control group having >40% total motility, while 8(8.88%) of –ve ASA and 11(12.2%)of +ve ASAs patients had <40% total motility.

This result is in agreement also with the studies of Chia *et al*., (2000); Okonofua *et al*., (2005); and Ameen (2007). They observed that sperm motility in infertile men are lower than that of fertile men.

Table (9) showed that there was a significant difference between mean % of total motility (PR+NP) of +ve ASA patients when compared to its mean in control group.
The above data agree with Abdullah (2009) who found that ASAs in semen affect sperm motility in infertile male. ASAs can negatively affect sperm motility, (Bohring *et al.*, 2004).

Another study stated that abnormalities in motility and quality of movements can arise from the presence of ASAs (Seaman *et al*., 1994).

Sperm motility is an important factor in the fertilization potential of the sperm, and motility has been shown to correlate closely with the fertilization rates of human oocyte *In vitro* (Schultel *et al*., 2008).

Table (10) showed that the control group had 30% normal sperm morphology, while the -ve ASA patients groups had 9 (10%) of <30% normal sperm morphology and 6(6.6%) of zero % of normal sperm morphology.

The result of this study is in agreement with the finding by Ameen (2007) who reported a high normal sperm morphology in normozoospermic group compared to other groups like oligoasthenozoospermic group. Khosrowbeygi *et al*., (2004), showed that normozoospermic fertile men have higher normal sperm morphology than oligoasthenoteratozoospermic, asthenozoospermic, and asthenoteratozoospermic infertile men.

Table (11) showed that mean % of normal sperm morphology in control group was higher than its mean in +ve ASAs patients, and there was a significant difference between the two. $p>0.05$

The presence of ASAs affect the morphology of sperm in semen of infertile patients (Abdullah, 2009).

The current study delineates that there is a significant relation between abnormal variable of SFA and the presence of ASA in semen.

In this study, there is a significant relation between ASAs in male serum and semen with abnormal seminal fluid parameters and this is supported by studies of Devin *et al*., (1993); Kipersztok *et al*., (2003); Brandy *et al*., (2003). WHO found that positive semen samples had a significant abnormal sperm count, low motility, and morphology (WHO, 2010).

This study showed that positive ASAs were associated with abnormal variable of seminal fluid. Marbeen *et al*., (2007) found that the highest percentage of positive ASAs was associated with abnormal seminal fluid parameters.

Garcia *et al*., (2007) intended to detect the presence of ASA and their incidence in men with unexplained infertility indicated that ASA are involved in reduced infertility and a correlation between infertility and altered seminal parameters reinforce the ASA participation in this pathology.

Experimental studies indicate that infertility may have an immune basis, resulting from spontaneous or induced autoimmune diseases that target the testis, ovary, or the spermatozoa (Tung, 1998). Two immunological mechanisms may be operateve in these

diseases; first, sperm antibodies which incapacitate spermatozoa motility or viability by complement-dependant cytotoxity, interferance with their transport in the female genital tract, or blockage of cellular interaction in the fertilization process. Second, T-cell mediated inflammation may result in atrophy of the gonads associated with loss of germ cells (Rose *et al*., 2002).

The study indicated that highest percentage of serum and semen ASAs was in the age group (29-33) years as illustrated in Table (12).

This study goes also with Abdullah (2009) who indicated that the highest percentage of serum ASAs was in the age group (25-34) years in Erbil city and with that of Hossain *et al*., (2007) who indicated that the age related variations in the incidence of ASAs might suggest that the vulnerability of different age groups to immunological imbalances is not the same.

A study by Collins *et al*., (1993) and Heidenreich *et al*., (1994) found that ASAs, in both sexes, increased with age, this probably suggests that age may be a contributing factor in induction of ASAs.

The study showed a significant difference between mean serum conc. of inhibin B of control group and its mean in –ve ASA patients as shown in table (13) $p<0.0001$. This result agree with which is found that serum concentration of inhibin B in infertile male was lower than serum concentration in fertile control group (Gavin *et al.*, 2009), and with study that stated that serum inhibin B concentration were significantly higher in fertile control group than serum concentration of inhibin B in patients with primary testicular failure (Bradford *et al*., 2000).

Mean serum inhibin B in pg/ml was found to be lower in infertile males than normal control group in the study of Kumanovp *et al*., (2006).

As data illustrated in Fig. (1) there was significant correlation between concentration of inhbin B in sera of –ve ASAs patients and total sperm count of those patients, which agrees with the study that concluded that serum inhbin B concentration an interesting marker for spermatogenesis (Pierik, 1998).

Inhbin B level correlated positively with total sperm concentration as found by Gavin (2009) and this goes with result of this study.

The sperm count may be affected by many factors such as hormonal. Some causes of azoospermia related to hormonal imbalance, (Kondoh *et al*., 1999).

Anderson (2004) found that inhibin-B levels decreased consistently with the decrease in spermatogenesis. Therefore, spermatogenesis partially determines inhibin-B levels. A study of (Gavin, 2009) stated that inhbin B correlated positively with sperm count, but the predictive power is best when inhbin B is low.

There are some studies confirm that serum inhbin B level reflects testicular function and more precisely sertoli function (Anawalt *et al.*, 1996).

There was a study done by Frank *et al*.,(1998) demonstrated a significant correlation between sperm concentration, sperm count, and testicular volume on the one hand, and serum inhibin B levels on the other.

These results provide a strong evidence that inhibin B is an important marker of the competence of sertoli cells and spermatogenesis in the human, which is in accordance with the few reports on inhibin B and quality of spermatogenesis up to now (*Illingworth et al.*,1996; Anawalt *et al*.,1996).

CONCLUSIONS

Antisperm antibodies are present in both serum and semen of patients and this affecting normal seminal fluid parameters of these patients. Serum inhibin B (pg/ml) lower in infertile male than fertile male and inhbin B has an effect on total sperm count and can cause male infertility. Also serum inhbin B was considered as marker for spermatogenesis.

ACKNOWLEDGEMENT

I would like to express my deepest gratitude and respect to Dr. Nabeel E. Fatouhi assistant prof. in mmunology in medical college in Erbil University for his help and advice, meticulous supervision, and guidance throughout the execution of this study. Also I am very grateful to Dr. Honar Abdul- Kader and Mr. Mwafaq who work in infertility clinic in Erbil city.

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