DETECTION AND LOCALIZATION OF ANTI-SPERM ANTIBODIES IN INFERTILE MALES IN DUHOK CITY

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ABSTRACT

Background and objectives Anti-sperm antibodies are the main cause of immunological infertility, as they impair sperm function by binding to the sperm membrane. The aim of the study is to assess the relationship between the presence of anti-sperm antibodies in sera of normozoospermia infertile patient with the localization of anti-sperm antibodies on sperm surface by using ELISA and IIF tests respectively. The current study is an attempt to determine the rate of anti-sperm antibodies in sera and surfaces of sperm among a population of infertile patients in Duhok province which is the first study done in all Kurdistan region/Iraq.

Methods The study was started at the beginning of April 2007 and finished at the end of December 2007. A total of 480 semen samples were collected from infertile males suffering from primary and secondary infertility attending infertility clinic/Azadi teaching hospital. The patients were categorized into 4 age groups ranging from 23-42 years. All semen samples were examined macroscopically and microscopically. The information of each semen samples were recorded in a special form.

Results It was found that only 105 (21.8%) males were normozoospermia, while 375 (78.2%) males with abnormal semen characteristics according to the criteria of World Health Organization. The results of ELISA test revealed that 18 (17.1%) sera samples of infertile males were positive for anti-sperm antibodies, while 22 (20.9%)were equivocal and65 (61.9%) were negative for the presence of anti-sperm antibodies. The results of indirect immuno-fluorescent test revealed that all the 15 ELISA positive samples were positive for anti-sperm antibodies, whereas the other 15 negative samples were negative for antisperm antibodies.

Conclusion The presence of anti-sperm antibodies was high among infertile males in Duhok province. ELISA and indirect immuno-fluorescent tests were found to be sensitive and rapid for detection of anti-sperm antibodies in infertile males compared with other tests.

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Key words: Anti-sperm antibodies, ELISA, Indirect immuno-fluorescent test

The term immune induced infertility is defined as spontaneously occurring antibodies binding to antigens of the gametes impairing sperm-oocyte interaction. Antisperm antibodies (ASAs) are far more frequent than oocyte antibodies.[1] In the male the presence of ASAs is an autoimmune disease causing 'immune infertility'. ASAs have involved virtually all components of sperm and diminished sperm–oocyte binding and faulty zona pellucida penetration. Antisperm antibodies have also been implicated in decreasing sperm motility, cervical mucus penetration, sperm survival and blocking the initiation of embryo

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cleavage.[2] The most common male immunologic concern in infertility, however, is antisperm antibodies. It has been shown that both males and females can make antibodies that react with human sperm. In male, for example, ASAs can be detected in seminal plasma and serum, and are also located on the surface of sperm, which cannot be detected in a routine semen analysis. The female may produce ASAs, which may be found in circulating blood, or produced in the cervical mucus.[3]

There are sufficient evidences that ASAs impair fertility in couples with unexplained infertility. A number of different methodologies are available, which may be used in their detection. However, in many cases, test interpretation is subjective. Although there is no enough evidence to support systemic treatment for ASAs, application of a variety of assisted reproductive technologies improves outcome.[2] Yeh et al[4] found that two males antisperm Immunoglobulin isotypes significantly impaired fertilization rates. Immunoglobulin A exerted its impact only when high level of binding was detected on the head of sperm, on the other hand Immunoglobulin M, present in 44% of the males, was the Ig isotype that most significantly affected fertilization rates when localized both at the head and at the tail tip level of sperm.

The aim of the study is to assess the relationship between the presence of ASAs in sera of normozoospermia infertile patient with the localization of ASAs on sperm surface by using ELISA and IIF tests respectively.

METHODS

This study was started at the beginning of April 2007 and finished at the end of December 2007, and conducted in Dohuk city-Kurdistan Region –North of Iraq. Fresh semen samples were collected from 480 infertile patients suffering from primary or secondary infertility.

The patients were attending infertility clinic at Azadi teaching hospital. Some of them were attending Private clinics and laboratories. The age of patients ranged between (23- 42) years. A total of 480 semen samples were collected and examined both macroscopically and microscopically according to the World Health Organization criteria.[5] Blood samples (3ml) were collected from some patients who are suffering from primary and secondary infertility but with normal sperm count. A total of 105 blood samples were collected for detecting the antisperm antibodies by using enzyme linked immunosorbant assay (ELISA).

Blood samples were collected by using a sterile syringe and put in a sterile clean glass test tube without anticoagulant. The blood left to clot for 30 minutes and then centrifuged at 3000 rpm. for 5 minutes. Then serum sample was collected in plane tube and labeled for each patient. All serum samples were frozen at -20c° until the time of ELISA test and Indirect immunofluorescent test (IIFT). The presence of antisperm antibodies in 105 selected sera of patients suffering from infertility were assessed by using ELISA test. The commercial kit used in this study was obtained from VARELISA ANTIBODIES, Germany. The results were interpreted as shown in table 1.

The ASAs were also assessed on sperm surfaces of 30 patients by IIFT. The commercial kit used in this study was obtained from EUROIMMUN LABORDIGNODTIC, Germany.

The serum samples were selected as 15 samples positive for ASA by ELISA test and 15 samples negative for ASA by the same test and the results were interpreted as shown in table 2.

RESULTS

Of the 480 semen samples analyzed macroscopically and microscopically, it was found that only 105 (21.8 %) persons have normal characteristics and parameters

of seminal fluid samples according to the WHO criteria.

On the other hand, 375 (78.2 %) persons with abnormal parameters, most of them have had oligospermia, azoospermia, asthenozoospermia, teratozoospermia or long liquification time and presence of large number of pus cells. These results are summarized in table 3. The semen samples with abnormal characteristics were excluded from the immunologic study.

The results revealed that 18 (17.1%) sera of infertile men were positive for the presence of ASAs by ELISA test, while 22 (20.9%), 65 (61.9%) were equivocal and negative for ASAs respectively.

In this study the IIF test has been used for the detection of ASAs location on the surface of sperm. A total of 15 positive serum samples tested by ELISA, were selected and screened by IIF were positive when tested by IIF test while a total of 15 negative serum samples when using ELISA, were negative when tested by IIF test. The positive result of IIF test showed that ASAs stained by fluorescent stain and appeared as shiny green color spots on sperm surface.

The intensity of color depends on the titre of antibodies (Figures 1 A and B, 2 A and B, 3, and 4), showing the location of antibodies on the sperm surface. The exact distribution of Ag-Ab complexes on the sperm surface and sperm antibodies load was estimated (Figures 1 B and 2 B). The head of fixed spermatozoa with high fluorescence intensity was uniformly labeled and a spotted type of fluorescence was detected on the tail. The pattern of the localization of ASAs on the head and tail of sperms incubated with sera (ASAs +) of normozoospermia infertile patients was unform (Figure 1 A and B). On the surface of spermatozoa, which incubated with positive control ASAs sera, the high level of fluorescence intensity was not entirely uniform. (Figures 2 A and B).

The fluorescence intensity of the spermatozoa was much lower after their incubation with ASA-negative serua of the patients and the control negative serum (Figures 3 and 4).

The distribution of presence of ASAs according to age groups in this study is listed in (table 4). The highest number of the presence of ASAs was seen in the age group between 28-32 year.

Table 1. Interpretation of Elisa results

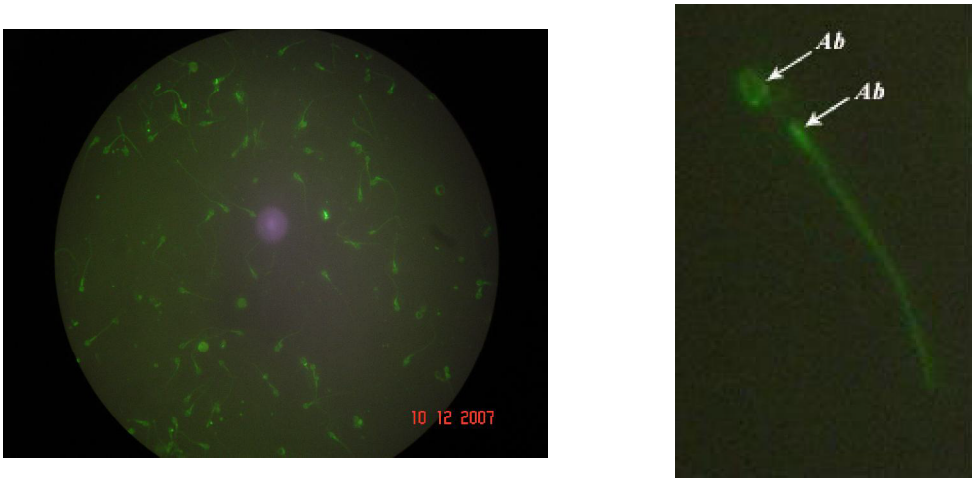
Assessment	Semiquantitative evaluation	Qualitative evaluation
Negative	< 14 U/ml	Ratio < 1.0
Equivocal	14 - 20 U/ml	Ratio 1.0 - 1.4
Positive	> 20 U/ml	Ratio > 1.4

Table 2. Interpretation of spermatozoa reactivity

Reactivity of sprmatozoa	Evaluation
No reaction at 1:10	Negative. No IgG, IgA and IgM class antibodies against human sperms detected in the patient samples.
Positive reaction at 1:10	Positive. Possible existance of an autoimmune or alloimmune genesis in unclear fertility disorders.

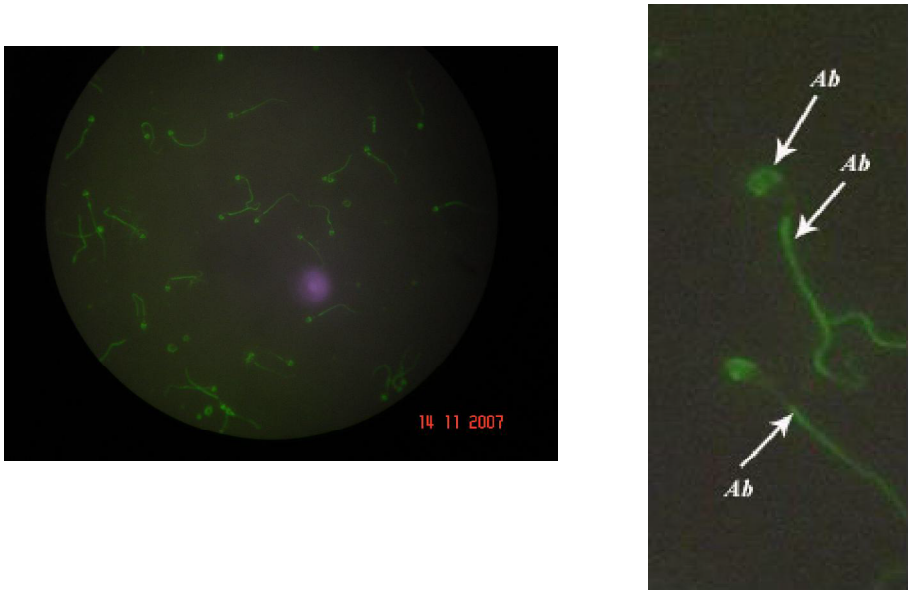
Table 3. Presence of ASA in sera of infertile men examined by ELISA test

No. of examined sera	No. (%) of ASAs positive sera	No. (%) of ASAs equivocal sera	No. (%) of ASAs negative sera
105	18 (17.1%)	22 (20.9%)	65 (61.9 %)



(A B)

Figure 1. (A) Serum of an infertile patient with positive ASAs by IIF test (100X). (B) Single sperm with spots of ASAs(400X)



(A) (B)

Figure 2. (A) positive control of ASAs by IIF test (100X). (B) Tow sperms with greenish spots of ASAs (400X)

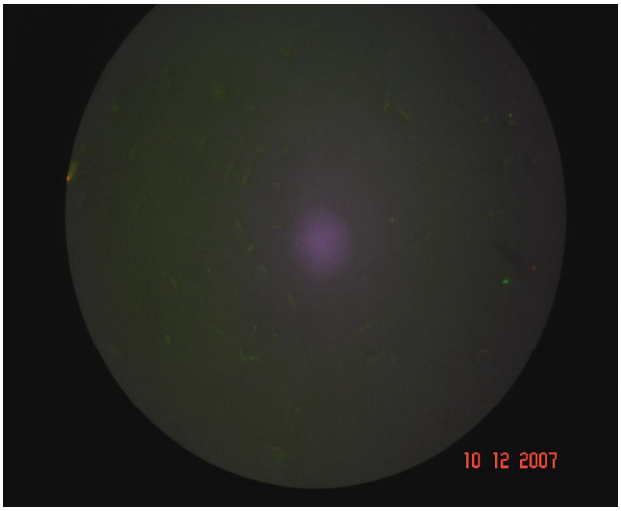


Figure 3. Fluorescence microscopy of normal spermatozoa after their incubation with ASA-negative sera (100X). (Note the low fluorescence intensity pattern on sperm surface

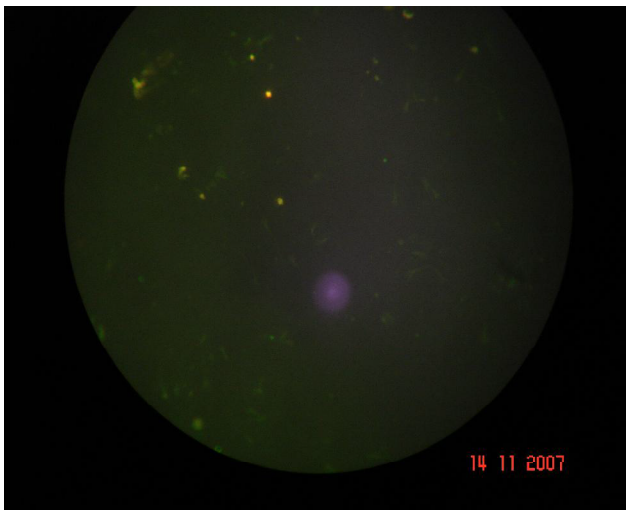


Figure 4. Fluorescence microscopy of normal spermatozoa after their incubation with negative Control of ASAs by IIF test (100X). (Note the low fluorescence intensity pattern on sperm surface

Table 4. Distribution of ASAs by ELISA test according to age groups

Age group	No.	Presence of ASAs No. (%)	Absence of ASAs No. (%)
23-27	40	3 (7.5)	27 (67.5)
28-32	36	9 (50)	21 (58.3)
33-37	23	3 (13)	14 (60.8)
38-42	6	3 (25)	3 (50)
Total	105	18	65

DISCUSSION

This is the first study done in Kurdistan Region, Iraq, using immunhistofluorescent detection of antisperm-antbodies (ASAs) on the surface of sperms, it is an attempt to determine the rate of anti sperm antibodies (ASAs) in the sera and surfaces of sperms among a population of infertile patient in Duhok province.

In our community till now there are little information about immunological infertility cases. The present study was the first trial for determining the sides of this problem since the number of infertile couples are increasing in the last years. The percentage of positive cases for ASAs among sera of 105 infertile men was 17.1% examined by ELISA test. This percentage is considered to be very high compared with other studies done elsewhere such as in Iran (8%),[6] while it is almost close to our results in other studies. The incidence of immunity to sperm in infertile couples worldwide was estimated to be (9-36 %).[2] In contrast the prevalence of ASAs in the general population was approximately (0-2%) in UK.[7] Numerous investigations have reported the identification of specific antigens using sera from immune infertile men. Serum antibodies were adsorbed onto normal motile spermatozoa and subsequently eluted from the sperm membrane[8]. The presence of sperm-antibodies (SpAb) may reduce fertility by affecting sperm motility and acrosomal reaction. The presence of the SpAb was shown to prevent sperm penetration of cervical mucus, inhibit sperm-zona pellucida interaction, and interfere with the sperm-egg fusion. The antigens were identified by bound SpAb, the sources of which were seminal plasma samples of infertile patients or of patients following vasectomy. This research was done in Germany[1] using IIF. Also, Cross and Moore[9] showed that antibodies bound to both head and tail.

In term of serum ASAs, the percentage of equivocal results in the tested cases in the present study was 19.1%. this means that percentage of ASAs cases among infertile men, may be more or less than 17.1% because the equivocal results may turn to positive or negative regarding the presence of ASAs. Only 2 patients were suffering from secondary infertility while 16 patients were suffering from primary infertility. This result indicated that immunologic infertility due to presence of ASAs mainly found in those patients with primary infertility.

The percentage of patients suffering from abnormal semen parameters was high (78.2%) among the total number of 480 semen samples . The results of the current study indicated that the rate of immune induced infertile cases were very high among the screened population. Some of these conditions may be due to reversible causes such as ductal obstruction and hypogonadotropic hypogonadism. Other conditions may not be reversible such as bilateral testicular atrophy. Similar high cases of ASAs (30.3%) among infertile men was reported[10] in U.K. by using sperm agglutination technique.

In a study conducted by Andolz et al[11] in Spain on 178 infertile men, the percentage of ASAs was high 35%, 21% by using tray agglutination test (TAT) and Immunobead test (IBT) techniques respectively.

Among American infertile men, a study was performed on the presence of ASAs. This study was done by Menge and Beitner[12] on 377 infertile men and found that relatively high percentage (19%) of cases were detected positive for ASAs in their serum samples by using sperm agglutination test.

In France, Mirilas and Almeida[13] reported very high number of positive cases of ASAs when they tested 183 infertile men, their ages ranging between 21-47 years. This study found that 39% of patients were positive for ASAs. Those results showed that presence of ASAs in sera were disagreeing with many other

studies that were conducted in different countries of the world. One of these studies was done on 400 Italian infertile men.[14] They found a low percentage (13%) of ASAs (IgG &IgM) positive cases when examined their sera by sperm agglutination test. The same study found that 5.7% of ASAs positive cases when examined their semen (IgA) was examined by the same test. On the other hand, Munuce et al[15] in Argentina reported low percentage of ASAs positive cases (15%) when examined 144 infertile patients. A lower percentage of positive cases of ASAs were recorded in Canada.[16] It was found that 38 (8.1%) sera to be positive of males for ASAs by using tray agglutination test on 471 Canadian couples. In Italy also Gandini et al[17] recorded that the number of patients with a clinically significant positivity to direct immunobead test(d-IBT) was 134 (20.1%) male infertility cases from a total of 667 patients. In Bulgaria Fichorova and Boulanov[18] recorded that 72 (18%) of infertile males with normal semen analysis were positive for ASAs in their sera by ELISA test. In USA, another study was done by Devine et al.[19] The authors found that 88(16.7%) of a total of 520 infertile men were positive to the presence of ASAs by examining their sera with mixed agglutination reaction test (MART) and immunobead tests (IBT). Fertilizing and non-fertilizing sperm populations were not different with respect to the percentage of motility, the normal morphology, the multiple anomalies index, the acrosome abnormalities, and the spontaneous or induced acrosome reaction. If the proportion of spermatozoa coated with either immunoglobulin (Ig)A or IgG antibodies was similar in the two groups, their localization was often different: antibodies were mainly on the sperm heads in the cases of fertilization failure.[20]

The presence of ASAs was high among infertile males in Duhok province. ELISA and IIF tests proved to be sensitive and rapid for detection of ASAs in infertile males compared with other tests. ELISA and IIF tests may be used as routine tests in laboratories for detection of ASAs in infertile couples. For more precise results other more developed diagnostic tests like PCR for detection of ASAs are recommended. Further studies are needed to assess the role of ASAs and its frequent distribution among infertile couples.

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پوختە

دیارکرنا و دە ستنیشانکرنا جهین دژوکین سبیرمیت زەلامیت نەزوك ل باژیرێ دهوکێ

پێشەکی و ئارمانج: Intrauterineدژەکان – دژی سپێرم Antisperm Antibodies دهێتە هژمارتن ئەگەرێ سەرەکی بو نەزاینا مناعی Immunological Infertility چونکی ئەو کارێ سپێرمێ تێك ددەت بگرێدان ب پەردا سپێرمێ. خاندنا نهو دهێتە هەلسەنگاندن پەیوەندیا دژەکان – دژی سپێرمێ د مصولا هەردوو نەزوکا – بتنێ (سپێرما) normozoospermia و ئەنجامێت (خوجهکرن) Localization یێت دژەکان لسەر روێ (سپێرمێ) ب بکار ئینانا (الاقزاز المناعی) – یێ گرێدای ب ئەنزیمێقە و رێکا پیك کرنا مناعی یا نە ئێکسەر Indirect Immunofluorescent Assay ل دیف ئێك. خاندنا نو هەولەکە بو ژبو دەست نیشانکرنا هەبونا دژەکان– دژی سپێرمێ ل مەصلدا و لسەر روێ سپێرمێ دناف بەرا ئاکنجێت نەزوك ل باژێرێ دهوك، ئەڤە خاندنا ئێکێ یە ل هەرێما کوردستانێ.

ریکێن ڤەکولینێ: ل نیسانا سالا ٢٠٠٧ دەست بڤێ خاندنێ هاتە کرن و ل دوماهیا تشرینا دوێ ل وێ سالێ تمام بو و چەند نمونە بژمارا ٤٨٠ نمونە هاتنە کوم کرن ژ ئافا زەلامی ژ وان کەسێت نەزوك یێت توشی نەزوکا یەکەم و دووەم یێت سەرەدانا کلینیکا دختورا ددەن کریە و هاتنە دابەشکرن بو چار پشکا ژ تەمەنی دنافبەرا ٢٣– ٤٢ سال ئەڤ نمومنە هاتنە کوم کرن و هاتنە تاقیکرن بچاڤ و میکرسکوب و پێژانین هاتنە کوم کرن ل لیستەکێ تایبەت.

ئەنجام: دیاربوو کە ١٠٥ (٢١,٨ ٪) ژ زەلامێت نەزوك بتنێ خودان ساخلەتێت مەنی بێت وەك هەڤن بەلێ ٣٧٥ (٧٨,٢ ٪) د ساخلەتێت مەنی بێت نە وەك یەك ل دویف ساخلەتێت رێکخراوا تەندروستیا جیهانی. ئەو نموونێت مەنی بێت خودان ساخلەتێت نە وەك هەڤ ژ ڤێ خاندنێ هاتە دویر ئێخستن، مەصلا ١٠٥ زەلامێت نەزوك بێت وەك یەك ژ ئافێ هاتنە کوم کرن ژبو دیار کرنا دژێت ئافا زەلامی ژ مەصلێت وان ئەوژی بکارئینانا تاقیکرنا ELISA . ئەنجامێت تاقیکرنا ELISA دیاربوو کە ١٨ (١٧,١ ٪) مەصلا زەلامێت نەزوك بێت پوزێتڤ بوون ژبەر هەبوونا دژەکان بوو سپێرمێ بەلێ ٢٢ (٢,٩ ٪) ، ٦٥ (٦١,٩ ٪) یا شێلی بوو Equivocal و نێگەتڤ بوو ژبەر هەبوونا دژەکان بوو سپێرمێ لدویف ئێك. ئافا زەلامی هاتە پاراستن ژ بێت وان بێت وەك هەڤ ئەوژی بکارئینانا تاقیکرنا IIF وهاتە شلوڤەکرن ب میکروسکوبا فلورسنتی وهەر وەسا ب تاقیگەها مجهەری یا فلورسنتی ژبو دیارکرنا نموونێ دیارکری ورێك وپێك ژبەر دژواریا Intensity پەیدابوونا ئاریشا ل سەر سپێرمێ. ژ ئەنجامێت التألق المناعی یا نە ئێکسەر دیار بوو کە هەموو نموونێت ١٥ بێت پوزێتڤ ب تاقیکرنا ELISA یا پوزێتیڤ بوو ب تاقیکرنا IIF و ١٥ نموونێت نێگێتڤ ب تاقیکرنا IIF دەرچوو نێگەتڤ ل دویف کوما تەمەنی ودیار بوو کە بلندترین رێژە ٢٥ ٪ ژبەر هەڤبوونا ASAs و بوو کومەلا تەمەنی ٢٨ – ٣٢ کە هاتیە پێگیرکرن ل دویف رێژا سەدی (١٣ ٪) بو کومەلا تەمەنی (٣٣ – ٣٧).

دەرئەنجام: هەبوونا دژەکان – دژی سپێرمێ یا بلند بوو لجەم زەلامێن تووشی نەزوکیێ بووین. وەسا دیار بوو کە تاقیکرنا ELISA و IIF زور د حەساس بوون ژ بوو دیارکرنا ژ دژەکان – دژی سپێرمێ ل زەلامێت نەزوك.

الخلاصة

الكشف عن وتموضع الأجسام المضاده للنطف لدى الذكور العقيمين في مدينة دهوك

خلفية و اهداف البحث: تُعَدْ الأضداد – المضادة للنطفة Antisperm Antibodies السبب الرئيسي للعقم المناعي Immunological Infertility لأنها تفسد وظيفة النطفة بالارتباط بغشاء النطفة. تُقَيّم الدراسة علاقة وجود أضداد النطفة في مصول العقيمين – سوّى النطاف normozoospermia ونتائج تموضع Localization الاضداد على سطح النطف باستخدام الاقزاز المناعي – المرتبط بالانزيم ELISA وطريقة التألق المناعي غير المباشرة Indirect Immunofluorescent Assay على التوالي. الدراسة الجارية هي محاولة لتحديد وجود اضداد النطفة في المصل وعلى سطح النطفة بين الأشخاص العقيمين في مدينة دهوك، تعد هذه الدراسة هي الأولى في اقليم كوردستان العراق.

طرق البحث: تم البدء بالدراسة في مستهل نيسان ٢٠٠٧ وأُنهيت في نهاية تشرين الثاني لنفس السنة ٢٠٠٧. جُمعت عينات المني بعدد كلي (٤٨٠ عينة) من ذكور عقيمين يُعانون من العقم الأولي والثانوي المراجعين لعيادات العقم. تم تقسيم المرضى الى أربعة مجاميع عمرية تتراوح بين ٢٣ – ٤٢ سنة. جميع عينات المني فحصت عيانياً ومجهرياً وتمّ تسجيل معلومات كل عينة مني في استمارة خاصة. لقد وجد أنه ١٠٥ (٢١.٨ %) ذكر عقيم فقط بميزات منّى سوية بينما ٣٧٥ (٧٨.٢ %) بميزات منّى غير سويّة وفقاً لمواصفات منظمة الصحة العالمية. أُستُبْعِدَت عينات المنى ذات الصفات غير السويّة من هذه الدراسة. جمعت مصول ١٠٥ رجل عقيم سوّيي النطاف للكشف عن أضداد النطف في مصولهم باستخدام اختبار ELISA.

النتائج: أظهرت نتائج اختبار ELISA أن ١٨ (١٧.١ %) مصل لذكور عقيمين كانت موجبة لوجود أضداد النطفة بينما ٢٢ (٢٠.٩ %) ، ٦٥ (٦١.٩ %) كانت ملتبسة (مشبوهة) Equivocal وسالبة على التوالي. قد تم حضن نطاف رجال سوّيي النطاف (مجهزة تجارياً) مع ١٥ مصل (+ve ELISA) و ١٥ مصل (−ve ELISA) لمرضى عقيمين سوّى النطاف باستخدام اختبار IIF وحللت بالمجهر الخاص و بالفحص المجهري الفلورسنتي للكشف عن النمط المبقع تناسقياً لشدة التألق المعلم على سطح النطفة. ظهرت نتائج التألق المناعي غير المباشر أنه جميع النماذج الخمسة عشرة الموجبة باختبار ELISA كانت أيضاً موجبة باختبار IIF بينما نماذج ١٥ السالبة باختبار IIF كانت سالبة قياساً بالمجموعة العمرية وُجِدَ أن أعلى نسبة (٢٥ %) لوجود أضداد النطف كانت للمجموعة العمرية (٢٨ – ٣٢) والتي أُتبعت بالنسبة المئوية (١٣ %) للمجموعة العمرية (٣٣ – ٣٧). لقد وجد أن اختبار ELISA و IIF كانتا حساستين في الكشف عن الاضداد – المضادة للنطفة في الذكور العقيمين.

الاستنتاج: وجود أضداد النطف كانت عالية بين الذكورالعقيمين في محافظة دهوك. استخدام الاقزاز المناعي – المرتبط بالانزيم ELISA وطريقة التألق المناعي غير المباشرة Indirect Immunofluorescent Assay وجدت بانها سريعة و حساسة للكشف عن أضداد النطف بين الذكورالعقيمين مقارنة بالطرق الاخرى.