

## Determination some of complement components in infertility women with antisperm antibodies.

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### Abstract

**Background:** Classical activation of complement by antigen and antibody complex leads to formation of membrane attack complex (MAC) that leads to formation holes on the spermatozoa ending in their destruction.

**Objective:** To determine the complements levels and antisperm antibodies in the sera of infertile women of unknown etiology.

**Patients and methods:** Study group consisted of 45 infertile women consulting Kammal El-Sammarei Hospital for Infertility and In Vitro Fertilization from Jun -2008 to June-2009. Twenty-four (53.3%) patients had primary infertility and the rest had secondary infertility. Control group: consisted of thirty fertile women. Blood samples were collected from them and anti sperm antibodies in the serum were detected by indirect immunofluorescence test (EURO IMMUNE –GERMENY). In addition to that serum were tested for complement levels (C3 and C4) using single

radial immune diffusions test (BINDARID) KIT BIRMINGHAM .UK.

**Results:** Detections of antisperm antibodies in the serum of infertile women were (64.4%) which is significantly ( $p<0.05$ ) higher from control group using indirect immunofluorescence test. There was a significant ( $p=0.000$ ) difference in the complements levels among infertile women who had ASA positive and ASA negative and control group.

**Conclusions:** These higher levels of complement components may be due to activation of classical pathway by ASA that directed against sperm antigens ending in defect in function and motility of the sperms.

**Key words:** Infertility, antisperm antibody, complement.

IRAQI J MED SCI, 2010; VOL.8 (2):18-23

### Introduction

Complement (C) system is an enzymatic cascade of proteins that forms a vital part of the innate immune system and the end products of complement activation is pores formation by membrane attack complex<sup>(1)</sup>. They present in low concentrations in the serum and once it was activated by any pathways (classical, alternative and Lectin), its levels were increased<sup>(2)</sup>. The presence of antisperm antibodies in the reproductive tracts of some infertile individuals, and presence of complement in cervical and ovarian

follicular fluid, suggests that complement-mediated damage of spermatozoa is involved in some cases of infertility. Furthermore, deposition of maternal IgG and complement in the extra fetal tissues indicates that complement activation occurs within the fetoplacental unit<sup>(3)</sup>. Complement and its regulation is important in reproduction, Donev *etal* 2008 reported CD59b was significantly expressed only in testis and played a role in sperm acrosome activation and motility<sup>(4)</sup>. There was no evidence of antibody or complement fixation by viable spermatozoa. It had been found that antibodies present in the serum of women that bind to nonviable spermatozoa(ASA) belong to the IgG and IgM class then Complement fixation occurred via the classical (antibody-mediated) and alternative pathway. This indicated that viable

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Received: 9<sup>th</sup> September 2009, Accepted: 21<sup>st</sup> February 2010.

spermatozoa may possess antigenic properties different from nonviable spermatozoa. This leads to lack of immunological reaction of women to viable spermatozoa<sup>(5)</sup>. Anti sperm antibodies could inactivate human sperm motility in the presence of complement, showing that complement-dependent inactivation of sperm motility might be the biological mechanism of female infertility<sup>(6)</sup>, because incubation of motile sperm with complement-fixing immune sera resulted in a significant loss (43-87%) of motility, then activation of (C5b-9) induced alterations in sperm morphology leading to sperm lyses<sup>(7)</sup>.

In this study, we tried to determine the presence of ASA in the sera of infertile women and measure the main complement components (C3 and C4) in the sera of same patients.

#### **Patients and methods**

Patients group: consisted of 45 infertile women consulting Kammal El-Sammarei Hospital for Infertility and In Vitro Fertilization from Jun - 2008 to June-2009. The exclusion criteria was women with congenital abnormalities in the uterus, tubes and ovaries, women who ages were more than 45 and less than 20 years, women with defect in ovulation, hormonal disturbances and tube occlusion were excluded. Thus, study group included only women with unknown cause of infertility.

Control group: consisted of thirty healthy fertile women.

Blood was collected from two groups and anti sperm antibodies (ASA) were detected in their serum by indirect immunofluorescence test using kit from EURO IMMUNE – GERMENY. The sites (head, neck and tail) where ASAs directed were also recorded.

Anti-nuclear antibodies were done by indirect immunofluorescence test using kit from EURO IMMUNE –

GERMENY for those who had positive antisperm antibodies to sperm head to get rid from cross-reactions.

Serum of both groups were tested for complement levels (C3 and C4) using single radial immune diffusion test (BINDARID) KIT BIRMINGHAM .UK. These tests were done in Immunological department-central public Health.

The study was approved by the Ethical Committee of the Al-Kindi College of Medicine- Baghdad University, Kammal El-Sammarei Hospital for Infertility and Central Public Health. All samples were obtained with informed consent in accordance with Kammal El-Sammarei Hospital for Infertility Declaration. This study was carried out with the approval of the Ministry of Health and District Health Authority Ethical Committee in Baghdad-Al-Resaffa.

#### **Statistical analysis**

Student's t-test and ANOVA test used in analysis data statistically by MiniTab statistical software program 13.20. A P- value  $\leq 0.05$  was considered to be significant.

#### **Results**

The patients group consisted from forty-five female patients, their ages ranged from (22-45 years), (median=33). They were complaining from infertility, Twenty-four (53.3%) patients had primary infertility, their ages ranged from (22-40 years) (median =29.9) and the rest (No. =21, 46.7%) had secondary infertility, their ages ranged from (24-45 years) (median =31). The control group their ages were ranged between (17-39 years), median= 30.6.

Detections of antisperm antibodies in the serum of infertile women were (64.4%) which is significantly ( $P<0.001$ ) higher from control group (3.3%) using indirect immunofluorescence test. The highest percentage of antibodies was directed

towards neck (31.3%) as shown in table-1, 2-. Antinuclear antibodies could not be detected in these women.

Complement levels (C3 and C4) in the serum of infertile women as shown

in table-3- , figure-1-2-, there were significant (p=0.000) difference between infertile women who had ASA + and ASA- and control group.

**Table 1: Percentages of antisperm antibodies in the serum of infertile women and control group using Indirect Immunofluorescence test.**

Indirect immunofluorescence Test Control group Number =30		Indirect immunofluorescence Test Infertile women Number =45		Titer		Isotype
No.	%	No.	%	No.	%	
1	3.3	29	64.4	<u>1:10</u> 15	51.7%	IgG IgM IgA mix
	P<0.001			<u>1:100</u> 14	48.2%	

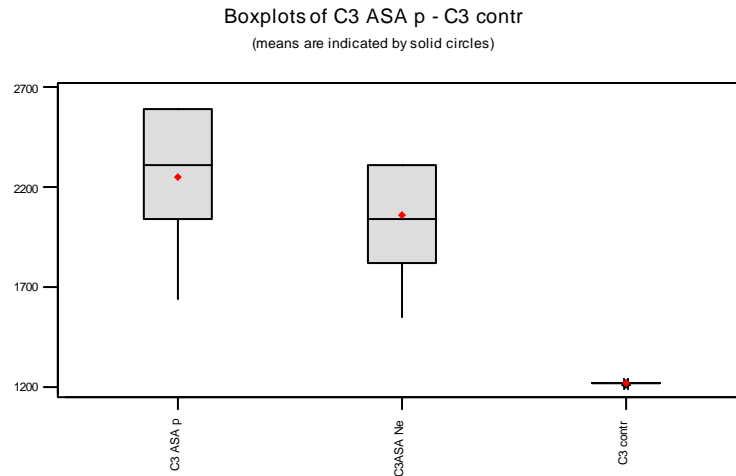
**Table 2: Detection sites of antisperm antibodies using Indirect Immunofluorescence test.**

Antisperm antibodies directed against											
Head		Neck		Tail		Head+ Neck		Neck+ tail		Head +neck+ tail	
No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
4	13.7	9	31.03	0	0	7	24.1	7	24.1	2	6.8

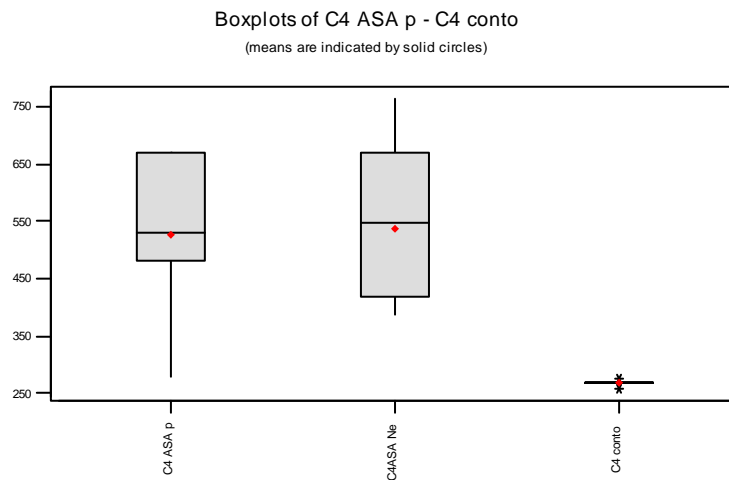
**Table 3: Serum levels of complement components in infertile women with and without antisperm antibodies compared with control group.**

Complement Levels (mg/L)	Infertile women with positive antisperm antibodies	Infertile women with negative antisperm antibodies	Control fertile women
	Number=29 Means ± SE X ± SEM	Number=16 X ± SEM	Number =30 X ± SEM
C3	2249.3± 57.5	2059.4±69.7	1216±0.2
C4	526.6±25.2	538.7±34.1	268±0.38

P value = 0.000 using ANOVA test  
SE= standard error



**Figure 1: Serum levels of complement components C3 in infertile women with and without antisperm antibodies compared with control group**



**Figure 2: Serum levels of complement components C4 in infertile women with and without antisperm antibodies compared with control group**

### Discussion

Spermatozoa are cells that must survive transplantation into a foreign host in order to perform their physiological role to reach the oocyte and penetrate it. The biggest hurdle to overcome is innate immune defense that will target the invaders in the female genital tract.

The human immune system is trained during the early postnatal period. In women when become sexually active, their immune system will inevitably contact sperm antigens

after coitus. Therefore, once sperm, as an autoantigen, activates the human immune system, an autoimmune response against human sperm will occur and leads to formation of ASA against sperm antigens. This leads to complement activation and complement is a major player in innate immunity. Spermatozoa must therefore evade complement attack if they are wanted to reach their goal.

In order to complement activation needs antibodies and the antibodies in

this study were ASA and were detected in higher percentages in the infertile women 64.4% that is in agreement with other study <sup>(8)</sup>. The highest percentage was directed against neck of the sperm. Those ASA that directed against head will affect penetration of ova and ASA directed against tail will affect movement of the sperms <sup>(9)</sup>. These directed against neck when there were complement activation will lead to pores formation and damage the sperms especially when the isotype was IgG because IgM produced for only short period about two weeks <sup>(10)</sup>. We found in this study a higher titer of ASA (1:100) in 48.2% of infertile women. IgG ASAs were capable of activating complement and depositing MC5b-9 on human sperm. Meanwhile, the concomitant detection of sperm-bound IgG and the initial (C3d) and terminal (C5b-9) complement components on the surface of human sperm could be confirmed using a flow cytometric assay <sup>(11)</sup>. In addition to that, the deposition of activated C3 fragments, the assembly of terminal membrane attack complexes (C5b-9) and oxygen radicals could lead to C3-mediated sperm binding to neutrophils or C5b-9-mediated sperm-motility loss <sup>(12)</sup>. This show the way to complement activation; we found significant higher levels of C3 and C4 (main components of complements) in the serum of infertile women with ASA. Complement evasion is achieved by the presence of complement regulators both in seminal plasma and on the spermatozoa <sup>(13)</sup>. Women who have generated an anti-sperm antibody (ASA) response may be particularly at risk because C activation will be enhanced with subsequent spermatozoal damage and destruction and perhaps also inflammatory damage to the female reproductive tract <sup>(14,15)</sup> and this was in agreement with our results. As a result, impairments of

complement components might predispose women to infections and autoimmune diseases that affect fertility <sup>(16,17)</sup>.

The message of this study is that C and C regulation play important though poorly defined roles in several components. Defects in C regulation may contribute to infertility and manipulation of C at this site may be of benefit either for improving fertility or for contraception.

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