Evaluation of Anti sperm Antibodies in Relevance to Progesterone Levels in Serum and Seminal Plasma in Infertile Men

Maryam H. Khaleefah, Hayder A. L. Mossa*, Mohammad O. Selman

High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al- Nahrain University, Baghdad, Iraq.

ABSTRACT

Background: Anti-sperm antibody (ASA) and hormonal oscillation have been considered as two systems in close association that affect each other. ASA influence the capability of insemination of spermatozoa. In addition, ASA is present in approximately 10% of infertile male patients and can cause infertility. Progesterone is a steroid hormone known to control lymphocyte proliferation during cytokine action and contributes to humoral immune responses by formation of antibody. Objectives: To study relationship between ASA positive or negative with level of progesterone in both serum and seminal plasma accordingly to sperm function parameters in different groups of infertile patients. Patients, Materials and Methods: The study consists of 80 volunteers, 20 of whom are normozoospermic, 20 Asthenozoospermic, 20 Oligozoospermic, and 20 Azoospermic subjects. The anti-sperm antibody and progesterone hormone were measured in both serum and seminal plasma by using Enzyme-Linked Immunosorbent Assay (ELISA). Results: The study showed (12.5%) out of 80 volunteers with positive ASA in all groups. The result was considered positive if the ratio was ≥60 RU/ml. On these bases and screening criteria, the positive ASA group showed the association with the high levels of progesterone in both types of the samples (serum and seminal plasma). These results exhibited a highly significant difference when compared with negative ASA group; ASA positive and ASA negative groups in sperm function parameters were not significantly different. Conclusion: Determination of the relevance of the levels of progesterone and anti-sperm antibodies in the serum and seminal plasma, which in turn is important to determine the type of infertility especially the immunological type.

Key Words: Antisperm Antibodies, Progesterone,Seminal Plasma, Serum, Infertile Men

eIJPPR 2018; 8(5):48-52

HOW TO CITE THIS ARTICLE: Maryam H. Khaleefah, Hayder A. L. Mossa, Mohammad O. Selman (2018). "Evaluation of anti sperm antibodies in relevance to progesterone levels in serum and seminal plasma in infertile men", International Journal of Pharmaceutical and Phytopharmacological Research, 8(5), pp.48-52.

INTRODUCTION

Immune infertility, especially ASA, is the cause of delayed pregnancy in about 9-36% of couples, by restricting sperm motility and thus effecting fertilization [1]. Anti-sperm antibody (ASA) is found in about 10% of the infertile male patients [2]. The circulating antibodies are also present within the reproductive tract and on the living spermatozoa surface [3]. ASA and hormonal oscillation have been considered as two systems that play in close association and affect each other [4]. Progesterone is a steroid hormone known to regulate lymphocyte proliferation through cytokine action, and participate in humoral immune responses by modulating antibody synthesis [5].

PATIENTS, MATERIALS AND METHODS:

This study was carried out in the High Institute for Infertility diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, during November 2017 to May 2018. The clinical examination was performed by a consultant urologist in charge of the male Infertility Unit in the Institute for all the men attended in this study. It included collection of seminal plasma and serum of 80 patients attended the male infertility outpatient

Corresponding author: Hayder A. L. Mossa
Address: Assistant Professor, High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al- Nahrain University, Baghdad Iraq.
E-mail: ✉ haydermossa @ gmail.com
Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest .
Received: 02 August 2018; **Revised:** 25 October 2018; **Accepted:** 26 October 2018

clinic in the institute. The total number of infertile couples included in this study was 60 as the study group (GA), which were classified into three subgroups namely Asthenozoospermia (Group A1=20), Oligozoospermia (GA2=20), Azoospermia (GA3=20), and the group B (GB) with twenty normozoospermic men (GB=20).

Blood Collection and Samples Preparation:

The blood was allowed to coagulate for 30 minutes, then centrifuged at 3000 rpm for 10 minute to separate the serum. The serum was quickly frozen and stored at -36C° until hormonal assays were performed. After liquefaction step, the seminal plasma was separated by centrifugation of seminal fluid at 3000 rpm for 10 minutes and stored at -36°C until hormonal analysis. Eighty semen samples were taken by masturbation after 3–7 days of sexual abstinence in a disposable sterile container from the patients who attended to the infertility clinic. Patients gave the specimen within special collection room performed for this purpose. Each sample collection was transported to the laboratory immediately and placed in an incubator at 37°C until complete liquefaction time. After that, the semen samples were analyzed by microscopic examination using the standardization of WHO (2010).

Hormonal and Anti-sperm antibody Assay:

The progesterone hormone and ASA value in serum and seminal plasma were measured by using ELISA (enzyme-linked immunosorbent assay).

RESULTS:

The comparison between different groups in level of progesterone:

The comparison of the different groups with respect to progesterone is shown based on the value in serum and seminal plasma of both the mean and standard error (SE) in each group, and as follows, the mean and SE of progesterone in serum for Asthenozoospermic men were significantly higher than other groups, as shown in table (1). The mean and SE of progesterone in seminal plasma for asthenozoospermic men were significantly higher than other groups, as shown in table (2).

Comparison between ASA positive and ASA negative groups in sperm functions parameters.

The mean and SE of sperm concentration, progressive motile sperm and morphologically normal sperm for ASA positive was significantly less than that of ASA negative. The mean and SE for Non-progressive motile sperm, immotile sperm, round cells and agglutination in ASA positive was significantly higher than that of ASA negative, as shown in table (3).

Comparison between ASA+ve and ASA-ve groups in levels of progesterone in serum and seminal plasma.

The mean and SE of progesterone in serum for ASA+ve were 1.005±0.11, which was significantly higher than ASA-ve (0.567±0.06), as shown in table (4), while the mean and SE of progesterone in seminal plasma for ASA+ve were 3.61±0.24, which was significantly higher than ASA-ve (2.631±0.31) as shown in table (4).

Table 1: Comparison between different groups in level of progesterone in serum:

Group	No.	Mean ± SE
Normozoospermic men	20	0.476 ± 0.03 b
Asthenozoospermic men	20	0.779 ± 0.13 a
Oligozoospermic men	20	0.635 ± 0.08 ab
Azoospermic men	20	0.558 ± 0.05 ab
LSD value	---	0.247 *
P-value	---	0.0466

* (P<0.05)

Means having with the different letters in same column differed significantly.

Table 2: Comparison between different groups in level of progesterone in seminal plasma:

Group	No.	Mean ± SE
Normozoospermic men	20	1.94 ± 0.14 c
Asthenozoospermic men	20	3.69 ± 0.27 a
Oligozoospermic men	20	2.93 ± 0.16 b
Azoosoermic men	20	2.31 ± 0.11 c
LSD value	---	0.517 **
P-value	---	0.0001

** (P<0.01)

Means having with the different letters in same column differed significantly.

Table 3: Comparison between ASA+ve and ASA-ve groups in sperm function parameters

Sperm function parameters	ASA positive (Mean ± SE)	ASA negative (Mean ± SE)	P-Value
Concentration	21.4±7.60	31.05±3.15	0.157 NS
Progressive motile sperm	13.5±3.68	22.31±2.22	0.075 NS
Non-progressive motile sperm	26±4.49	21.6±1.7	0.186 NS
Immotile sperm	40.5±7.6	33.74±2.88	0.143 NS
Round cells	7.6±1.34	6.92±0.47	0.309 NS
Morphologically normal sperm	19.1±3.31	24.89±2.16	0.138 NS
agglutination	5±2.10	2.21±0.75	0.1003 NS

Table 4: Comparison between ASA positive and ASA negative groups in levels of progesterone in serum and seminal plasma

Hormones	ASA positive (Mean ± SE)	ASA negative (Mean ± SE)	P-value
Progesterone in serum	1.005±0.11	0.567±0.05	0.0005
Progesterone in seminal plasma	3.61±0.24	2.631±0.13	0.002

ISSN (Online) 2249-6084 (Print) 2250-1029

DISCUSSION

Infertility is a multi-cause disorder. The motility of sperm is the main factor that determines the function of sperm; this factor in turn is affected mainly by the immune and hormonal factor [2]. All adrenal cortex hormones are produced from cholesterol, which turns into pregnenolone with P450scc, within mitochondria of the adrenal cortex. Progesterone hormones are produced from the cholesterol that is converted by the B-450S to the pregnenalone, which can be metabolized to 17α-hydroxypregnenolone by cytochrome P450c17, and then converted by 3β-HSD to progesterone [6]. Regarding the whole study group, the current study is consistent with regard to seminal plasma with Rangari & Shrivastav (2007), which demonstrated that higher progesterone levels were accompanied with low sperm motility when compared with samples with normal sperm motility; and the study differs with respect to the serum from Rangari & Shrivastav (2007), as they proved that there is no significant difference in the mean serum progesterone level of men with low sperm motility and normal sperm motility [4]. The current study demonstrates that progesterone in serum was non-significantly correlated with all sperm function parameters, but progesterone in seminal plasma was significantly correlated with sperm motility parameters only. In relevance to the study parameters, there are no previous researches about the mentioned parameters like sperm concentration, round cells, morphologically normal sperm and sperm agglutination. But the study of Rangari & Shrivastav (2007) mentioned the progesterone relevance to sperm motility, where there were significantly higher levels of progesterone in the sperm plasma of samples with lower sperm movement compared to samples with normal movement of sperm [4].

Infertility is caused by a defect in the resulting sperm function either from damage to the testis or from autoimmune [7, 8]. ASA has been considered as a potential causative agent in infertility by many authors. ASA was determined in the semenal plasma of only 1-2% of fertile men and about 5-15% of male infertility [9-11]. The effect of ASA lies in restricting the motility of sperm [12]. A positive score of ASA was found in this study based on a numerical ratio, where the result was positive if the ratio was ≥60 RU/ml. The proportion of men with positive ASA out of 80 men was detected as much as 10% in serum sample and 2.5% in seminal plasma sample; so the total positive ASA sample detected in all study subgroups was 12.5%. These findings were in accordance with the findings of Rangari & Shrivastav (2007), Amjad *et al* (2007), Mohammed *et al* (2016), and Fauzia *et al* (2012) [4, 13-15].

Findings of Cui *et al* (2015) and Zini *et al* (2010) are in line with the current study which compared anti-sperm antibody positive and anti-sperm antibody negative groups in sperm function parameters [16, 17]. The result of this comparison for each of sperm concentrations, progressive motile sperm, and morphologically normal sperm was within decreased values in presence of positive anti-sperm antibody compared with negative anti-sperm antibody group. While each of the non-progressive motile sperm and immotile sperm in positive anti-sperm antibody group was significantly higher than that of negative anti-sperm antibody group.

Bozhedomov *et al*. (2013) reported that immune infertility is accompanied by more expressed impairment of the sperm quality including lower concentration, motility and morphological changes [18], as listed in present study.

The sperm agglutination is a good indicator for diagnosis of immunological infertility [17]. The ASA leads to the sperm agglutination, decreases its motility, impairs the sperm penetration into cervical mucus, and hinders the fertilization of the ovum [19-23], which was similar to what has been proven in the current study

The findings of the current study were in accordance with studies of Ahmed *et al* (2017), Toshimori & Ito (2015), Cui *et al.* (2015), Wald (2005), and Bubanovic *et al* (2004), which demonstrated that the agglutination values were the highest ones within the positive anti-sperm antibody positive group [17, 24-27]. According to WHO 2010, the presence of non-sperm cells in semen refers to damage to the testes (immature germ cells), pathology of efferent channels or adenocarcinitis (white blood cells), which in turn are considered as indicators of infertility [28]. The current study showed that the round cells in positive anti-sperm antibody group were significantly higher than round cells in negative anti-sperm antibody samples. Regarding the study parameters, there are no previous studies showing the relevance between ASA and round-cell parameters.

One of the main objectives of the present study is the comparison between positive and negative anti-sperm antibody groups in level of progesterone. The study of Rangari & Shrivastav (2013) that deals with these comparisons, agreed on the one hand and differed on the other hand with the current study; in the progesterone it was agreed in regards of seminal plasma, while it was not agreed in regards of serum [29]. These results could be ascribed to the one of the roles of progesterone which is known to regulate lymphocyte proliferation through cytokine action [30], and participate in humoral immune responses by modulating antibody synthesis [4, 31].

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