



CODEN [USA]: IAJPBB

ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

<http://doi.org/10.5281/zenodo.3241507>

Available online at: <http://www.iajps.com>

Research Article

ANTISPERM ANTIBODY AND MALE INFERTILITY

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Article Received: April 2019

Accepted: May 2019

Published: June 2019

Abstract:

Introduction: Antisperm antibody (ASA) in men cause autoimmune disease, respectively, immune infertility. DNA fragmentation is the separation or breaking of DNA strands in pieces. DNA fragmentation testing is a type of male fertility test that measures the percentage of damaged DNA in a sperm sample.

Aim: The purpose of this study was to detect the presence of ASA and their incidence in male infertility with unknown etiology, as well as to evaluate the correlation between ASA and the percentage of sperm DNA fragmentation.

Methods: The study included 61 men with unknown infertility and 39 males control. Evaluation of the presence of antisperm antibody in the semen resulted in a direct mixed anti globulin (MAR) reaction and sperm DNA fragmentation with the Halosperm Halotech DNA, Madrid, Spain, test based on SCD technique, based on DNA-denatured.

Results: In the MAR test, 21.68 % of infertile men with unknown etiology were positive for ASA. Only one case was found in fertile male with positive ASA. Results of the DNA fragmentation index (DFI %) did not have a positive correlation with the ASA presence percentage in patients with positive test MAR.

Conclusions: Results show that antisperm antibody (ASA) are involved in decreased fertility in vivo conditions in patients with positive test MAR. DNA Fragmentation Index Results (% DFI %) did not show genetic damage to the spermatozoa in these cases with immune infertility.

Key words: Antisperm antibody, male infertility, Sperm DNA fragmentation (DFI), MAR test.

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Please cite this article in press Agim Shabani et al., *Antisperm Antibody And Male Infertility*, Indo Am. J. P. Sci, 2019; 06(06).

INTRODUCTION:

Antisperm antibody are antibodies produced against sperm cells antigens [1]. ASA has been considered as infertility cause in around 10–30% of infertile couples, and in males, about 12–13% [2]. All infertility diagnosed to date is linked to an immunological reason. The incidence may be higher, as the contribution to idiopathic infertility (31% of all cases) remains incomprehensible. The presence of ASA in the fertile population suggests that not all ASAs cause infertility [3]. Risk factors for the formation of antisperm antibody in men include the breakdown of the blood-testis barrier, trauma, and surgery, orchitis [4], varicocele [5], infections, prostatitis, testicular cancer [6]. In both men and women, ASA production is directed against superficial sperm antigens that may interfere with sperm movement, transport through female reproductive tract, curbing the capacity and reaction of acrosomes, damaged fertilization, impact on the process the implantation and the growth and development of the affected embryo [7]. The antibodies can damage or kill sperm. If a high number of sperm antibodies come into contact with a man's sperm, it may be hard for the sperm to fertilize an egg. This is called immunologic infertility. Antisperm antibodies directed against sperm did not necessarily impair fertility unless the circulating antibodies are also present within the reproductive tract and on the living sperm surface [8]. Such analysis is based on the fact that sperm parameters, such as sperm concentration, motility, and morphology, have been shown to be closely related to the degree of pregnancy. This because it is a cost-effective and non-invasive test has led to the widespread use of sperm analysis in the initial assessment of infertile males [9]. Criteria for normal sperm parameters vary according to the WHO lab manuals for human sperm examination and processing [10]. Sperm analysis does not give information about defects in the sperm. Men's assessment of fertility should go far beyond sperm counting and assessment of mobility and morphology. It should be supplemented by a proper clinical examination, history, hormonal analysis (fsh, lh, prol, test), genetic analysis, sperm DNA

fragmentation, etc. [11]. Formation of anti-sperm antibodies is reported in 42% of males with unexplained infertility, 10.7% of males subject to infertility estimates, 10% of males in pairs subject to IVF treatment, but only in 2% of fertile men. Moreover, sperm contains several substances that inhibit the complement system of activation elements required by immobilizing and apoptogenic ASAs [12, 13]. Therefore, finding normal sperm parameters in men with immune infertility is a common occurrence. Additional factors associated with altered DNA integrity include advanced paternal age, inadequate diet, drug abuse, tobacco use, environmental factors such as pesticide exposure or air pollution, varicocele, systemic diseases, and genital inflammation [14]. Studies have linked DNA damage with infertility, showing greater DNA damage in the sperm of infertile men than of fertile men [15, 16]. In fact, it has been suggested that sperm DNA fragmentation is one of the chief causes of reduced fertility potential. The DNA damage is reported in 5-8% of infertile normozoospermic men [17].

Purpose of the work:

The purpose of this study was to detect the presence of ASA and their incidence in male infertility with unknown etiology, as well as to evaluate the correlation between ASA and the percentage of sperm DNA fragmentation. All the participants in the study were from the Republic of Kosovo. The statistical processing of the data is done with the statistical package SPSS 22.0. The level of significance was set at $P < 0.05$.

MATERIALS AND METHODS:

The study included 61 men with unknown infertility and 39 males control. Evaluation of the presence of antisperm antibody in the semen resulted in a direct mixed antiglobulin (MAR) reaction and sperm DNA fragmentation with the Halosperm Halotech DNA, Madrid, Spain, test based on SCD technique, based on DNA-denatured.

Sperm analysis was done according to WHO 2010 recommendations, manual V.

Cut-off reference values for semen characteristics as published in consecutive WHO manuals

Semen characteristics	WHO 1980	WHO 1987	WHO 1992	WHO 1999	WHO 2010
Volume (mL)	ND	≥ 2	≥ 2	≥ 2	≥ 1.5
Sperm count (10 ⁶ /mL)	20-200	≥ 20	≥ 20	≥ 20	≥ 15
Total sperm count (10 ⁶)	ND	≥ 40	≥ 40	≥ 40	≥ 39
Total motility (%)	≥ 60	≥ 50	≥ 50	≥ 50	≥ 40
Progressive motility	≥ 2	≥ 25%	≥ 25% (a)	≥ 25% (a)	≥ 32% (a+b)
Vitality (%)	ND	≥ 50	≥ 75	≥ 75	≥ 58
Morphology (%)	80.5	≥ 50	≥ 30	(14)*	≥ 4*
Leukocyte count (10 ⁶ /mL)	< 4.7	< 1.0	< 1.0	< 1.0	< 1.0

Foto 1. Assume clinical significance (infertility due to immune reaction) if >50% of sperm have beads attached.



Foto 2. The working reagent for the Sperm DNA fragmentation assay, and how is a normal DNA fragmentation analysis in spermatozoa.

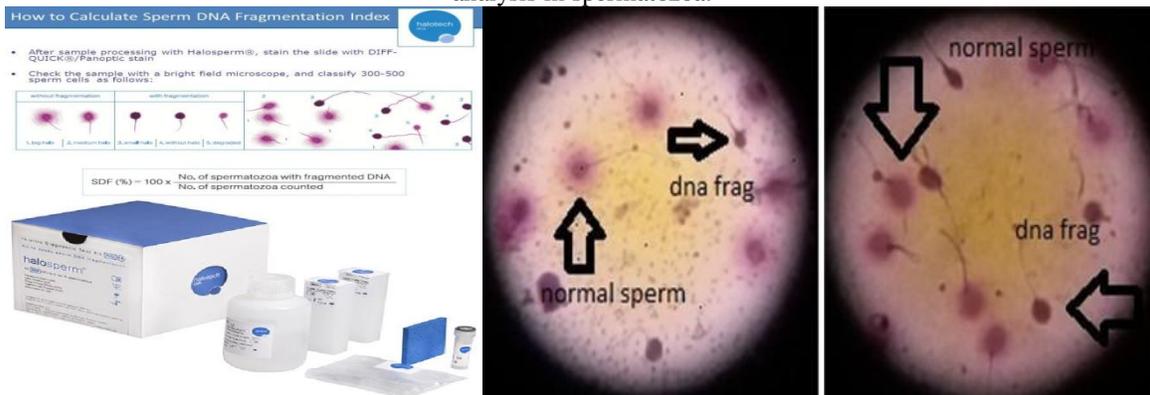


Foto 3. Analysis of DNA fragmentation in spermatozoa with positive test MAR.



Our results show that positive MAR tests did not affect DNA damage to the sperm.

RESULTS:

Table 1. Semen analyses (macroscopic and microscopic), antisperm antibodies test (MAR test) and Sperm DNA fragmentation (%) in fertile and infertile men.

In the MAR test, 21.68 % of infertile men with unknown etiology were positive for ASA. Only one case was found in fertile male with positive ASA. Results of the DNA fragmentation index (DFI %) did not have a positive correlation with the ASA presence percentage in patients with positive test MAR.

DISCUSSION:

Antisperm antibodies can affect adversely human fertility but normally may be controlled by anti-idiotypic antibodies which along with immune suppressor factors in semen prevent their induction to a significant degree. This balance between the detrimental and "beneficial" immune response to sperm may be shifted towards an antisperm antibody response by stimulatory factors such as infection [18]. Michael S Cookson et al 1995 [19], performed retrospective study to evaluate the ability to predict sperm surface antisperm antibody in patients with primary infertility on the basis of impaired sperm motility. He reviewed seminal characteristics of 70 consecutive ASA positive infertility patients & found patients with lower motilities were significantly ASA positive ($P=0.016$) as compared to patients with sperm concentrations of >20 million/ml ($P=0.002$) therefore, the impaired motility was significantly associated in ASA positive patients. So it is concluded that although the difference in the incidence of ASA in primary & secondary infertility is not statistically significant, its detection can provide a clue to the aetiological factor in such couples. Immunoglobulin's A (IgA) and G (IgG) classes are functionally important antibodies to male infertility as IgM has high molecular weight and can not penetrate the blood barrier. These antibodies are associated with sperm and decrease the ability of fertilization. Clark et al., Showed a 27% fertilization rate when $\geq 80\%$ of the sperm contained IgA and IgG-related sperm while the 72% fertilization rate was seen when $\leq 80\%$ of sperm had ASA-related spermatozoa [20]. It is not clear whether the location of the associated semen, whether the head of the sperm or the tail, is ASA, since there are contradictory reports that estimate the localization value and its correlation with the fertilization capacity [21]. ASA has the ability to damage some of the stages of the fertilization process. Filling in the cervix of the cervix can be associated with antibodies and cause sperm lysis, reducing mobility and preventing sperm's ability to penetrate cervical mucus [22, 23]. Evidence suggests that ASA affects sperm to have lower rates of spontaneous

reaction and cause acrosome damage [24, 25]. Some ASAs have also shown that they inhibit the spontaneous response of sperm capacity, and have been provided to suggest that ASA may interfere with the recognition of sperm-associated sites in the pellucid area [21]. High percentages of DNA damage to the sperm have a negative impact on the human ability to reach pregnancy naturally and relate to spontaneous loss of pregnancy [26, 27]. It has also been shown that the high rate of DNA damage in the sperm is related to the failure to fertilize intrauterine (IUI) [28], and IVF [29, 30], but not ICSI [30].

CONCLUSIONS:

Sperm routine analysis does not guarantee fertility. This data is important for all clinicians in charge of medically assisted medicine involved in the management of infertile males. Proper knowledge of the in vivo process of human fertilization and the interaction of sperm eggs in vitro is key to predicting functional changes of spermatozoa with extraordinary influence in the diagnosis and treatment of male infertility. In our study, we have not found that ASA has affected DNA fragmentation in the sperm. MAR test, a mixed antiglobulin reaction (test MAR), using red cells coated with antibody and sperm, was used to detect the presence of IgA or IgG on the surface of the sperm. Positive MAR (IgA) tests showed a very significant correlation with the presence of anti-sperm antibodies in the seminal plasma. We recommend the testing of ASA and sperm DNA fragmentation in infertile couples, where infertility causes are males.

Conflicts of interest:

The Authors declare that there are no conflicts of interest.

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