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Original Research Article

Prevalence of anti-sperm antibodies, risk factors associated and their impact on spermatobioscopy in infertile men

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ABSTRACT

Background: The first immunological correlation with male infertility was reported in 1954 by Wilson and Rumke with the identification of anti-sperm antibodies. The prevalence of anti-sperm antibodies in infertile men varies from 9%-36%, the main cause being the loss of the blood-testicular barrier and otherwise the association with chronic inflammation. It has been shown that immune infertility is found in 15% of patients with varicocele.
Methods: A transversal comparative study was carried out with 360 infertile men who were tested for anti-sperm antibodies between January 2011 and July 2018. Two groups were integrated; Group 1, infertile men with positive anti-sperm antibodies >50%, group 2, infertile men with negative anti-sperm <50%. Seminogram parameters were evaluated according to the WHO 5th edition and associated risk factors with anti-sperm antibodies.
Results: 360 infertile men were evaluated during the study, 42 were excluded because they did not meet the inclusion criteria, the prevalence of anti-sperm antibodies was 14.5%. Group 1; n=46 (14.5%) and group 2, n=272 (85.5%), the clinical characteristics and the hormonal profile were compared at study admission without significant difference. There was a significant decrease in progressive motility in group 1 (38.7±23.8) vs group 2 (50.1±18.9) p=0.03. Analyzing the risk factors, varicocele was found to be significant 23.7%, OR 2.14 (1.27-3.61) p=0.004 as well as retractable testicle 26.4%, OR 2.13 (1.23-3.70) p= 0.008.
Conclusions: The affectation of motility was confirmed, which leads to the suspect varicocele and retractable testicle as risk factors.

Keywords: Anti-sperm antibody, Chronic prostatitis, Infection, Inflammation, Male infertility, MAR test, Seminal parameters, Varicocelectomy

INTRODUCTION

The first immunological correlation with male infertility was reported in 1954 by Wilson and Rumke with the identification of anti-sperm antibodies.[1,2] The prevalence of anti-sperm antibodies in infertile men varies from 9%-36%.[3] The main cause being the loss of the blood-testicular barrier and the association with chronic inflammation.[4] Immune infertility has been shown to be found in 15% of patients with varicocele.[5] In mammals it is well documented that the presence of anti-sperm antibodies can interfere with fertilization. It has also been

related to harmful effects on embryonic development and implantation.[6,7] There is evidence that antibodies can affect sperm binding by affecting the acrosomal reaction altering the binding such as the IZUMO protein and the YLP-12E1 antigen. They are targets of the antibodies and are necessary for said reaction. It is suggested that anti-sperm antibodies affect fertility producing toxic compounds by inhibiting the synthesis of DNA and the enzyme in the mitochondrial respiratory chain coupled with a decrease in superoxide dismutase, an enzyme responsible for maintaining an oxidant balance, thereby producing oxidative stress and lipid peroxidation damaging the sperm membrane.[1]

The World Health Organization in the 5th edition of its manual (2010) recommends evaluating anti-sperm antibodies by means of two tests, the mixed antiglobulin reaction (MAR) (Figure 1) or the Immunobeads (IB) test, with a cut off value to be considered positive greater than 50% of mobile sperm with the presence of anti-sperm antibodies.[6,8]

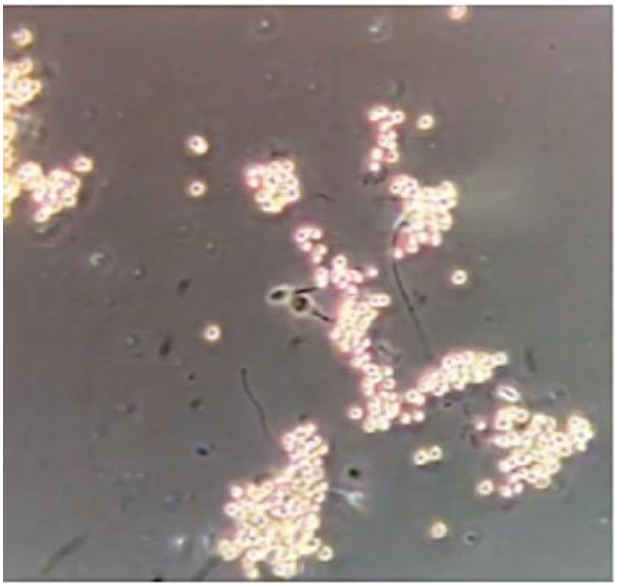

Figure 1: Photograph in phase contrast microscope with 100x objective, observing the mixed antiglobulin reaction test (MAR).

Two types of IgG, IgA antibodies are evaluated and IgM is rarely found.[2] The development time of anti-sperm antibodies is not clear, the data of a murine model in which the presence of antibodies after vasectomy was evaluated. suggest that IgM develops within 2 weeks after injury to the testicular hematogenous barrier; Immunoglobulin M titers subsequently decrease over 4-8 weeks, followed by increasing titers of immunoglobulin G between 8 to 12 weeks.[9] In previous reports, the negative effect of anti-sperm antibodies on semen parameters and natural pregnancy has been observed.[3] In the work of Abshagen et al, 1998, in which fertility was evaluated in 157 infertile couples with an anti-sperm antibody test, different cut-off points were evaluated finding that when there was a 10% presence of anti-sperm antibodies the cumulative pregnancy rate during 6 years was high, when they were greater than 10% but less than 50% was low and when anti-sperm antibodies were 50-90% the cumulative pregnancy rate at 6 years was very low.[10,11] It has been questioned whether anti-sperm antibodies could cause post-fertilization problems that result in clinical pregnancies and result in an increase in the rate of spontaneous abortion.[11,12] In evaluating this issue, a spontaneous abortion rate of 38% was found in those males with the presence of anti-sperm antibodies vs 0% in controls, Check J et al, 2017 found a spontaneous abortion rate of 14%. % in those with anti-sperm antibodies <50% versus 25% for those with >80% this problem has not been resolved to date.[13]

The objective of the study was to determine the prevalence of anti-spermatozoidetozoid antibodies, the risk factors and their effect on the parameters of spermatobioscopy in males with infertility.

METHODS

A comparative cross-sectional study was conducted, which included 360 men with infertility who attended the Clinic of Andrology, who underwent an anti-sperm antibody test between January 2011 and July 2018. Two groups were integrated; Group 1, men with infertility and positive anti-sperm antibodies, group 2, men with infertility and negative anti-sperm antibodies.

Inclusion criteria

- Men with complete seminogram according to WHO criteria (2010); SEA test (mixed antiglobulin reaction) reported as a percentage, male infertility, minimum duration of 12 months, hormonal profile and complete medical history, obtaining information showing history of scrotal surgery, testicular trauma, varicocele, testicular neoplasia, retractable testicle, infections (epididymitis and/or prostatitis).

Exclusion criteria

- Men with azoospermia or with incomplete information were eliminated.

For the analysis, the data were obtained from the clinical file and from the Andrology laboratory database. A selection of anti-sperm antibodies was made using the mixed antiglobulin reaction test (Ferti SpermMar IgG kit, Fertipro, Belgium). The reading was made in duplicate, a percentage of 50% of the mobile sperm involved in the mixed agglutination was considered positive for testing. The seminal analysis was obtained through masturbation in a period of abstinence of 3 days and no more than 7 days, all the procedures and interpretations used were subject to the criteria established by the World Health Organization 2010, the evaluation of the morphology of the sperm was performed according to the strict Kruger

criteria. The analysis included sperm concentration, mobility, morphology and the hypoosmotic swelling test (HOS).

The study variables were sperm concentration, progressive sperm motility, sperm morphology, hypoosmotic swelling test (HOS), leukocytes in semen, luteinizing hormone, follicle stimulating hormone, estradiol, total testosterone, prolactin, thyrotropin, scrotal surgery (antecedent of varicocelectomy and recanalization of the vas deferens), testicular trauma defined as direct contusion in one or both testes, the varicocele was diagnosed using the standard criteria (Jungwirth et al, 2013), including the 3 grades, testicular neoplasia defined as history of testicular cancer in the clinical history, retractable testicle defined as the mobile testicle of the inguinal canal to the scrotal pouch, infections (including epididymitis and/or prostatitis) using the consensus criteria of the National Institutes Health (NIH).

The sample size was calculated to find a prevalence of 15% positive anti-sperm antibodies with a confidence level of 95% and an accuracy or error of 5%. 196 men were required, so it was decided to enter all men in the period of study.

Statistical analysis

Descriptive statistics were used to characterize both groups, using mean and standard deviation and/or frequency and percentage for quantitative and qualitative variables, respectively. The chi-squared test was performed for the proportional differences and the Student's T for the differences in the means. 2x2 contingency tables were performed to calculate the odds ratio (OR), with a confidence interval of 95% (95% CI).

The statistical analysis was performed with the Statistical Package for Social Sciences for Windows program in its version number 24.

RESULTS

During the study period, 360 men with infertility were evaluated, 42 were excluded because they did not meet the inclusion criteria. Therefore, in the present study, 318 infertile men were analyzed with determination of anti-sperm antibodies. The prevalence of antisperm antibodies was 14.5%. Group 1 included a sample of n:46 (14.5%) and group 2, n:272 (85.5%).

Table 1 compared the clinical characteristics and the hormonal profile at study entry, the average age of the male infertile with positive anti-sperm antibodies was: 36±5.3 and in males with negative anti-sperm antibodies 36.0±6.8 years without significant differences. There were also no significant differences between the two groups in weight, height, body mass index, follicle stimulating hormone, luteinizing hormone, estradiol, total testosterone, prolactin, thyrotropin.

Table 2 compares the parameters of the seminogram in the two study groups.

Table 1: Clinical characteristics and hormonal profile upon admission to the clinic of andrology.

Characteristics	Group 1 N=46 Mean±SD	Group 2 N=272 Mean±SD	P value
Age (years)	36.0±5.3	37.3±6.8	0.22
Weight (kg)	80.8±13.2	78.8±12.8	0.32
Height (cm)	169±.07	168±.06	0.91
Body mass index (kg/m^2)	28.3±4.4	27.6±4.3	0.32
Follicle stimulating hormone (mUI/ml)	3.7±2.8	4.0±3.1	0.53
Luteinizing hormone (mUI/ml)	2.9±1.3	2.9±1.3	0.89
Estradiol (pg/ml)	41.4±15.7	40.2±13.4	0.59
Total Testosterone (nmol/L)	13.3±5.3	12.9±7.3	0.76
Prolactin (ng/ml)	10±5.8	9.3±5.1	0.41
Thyrotropin (mUI/ml)	2.8±2.0	2.9±1.1	0.49

SD:Standard Deviation

Table 2: Spermatobioscopy in both groups of males with infertility.

Parameters of spermatobioscopy	Group 1 N=46 Mean±SD	Group 2 N=272 Mean±SD	P value
Sperm concentration (10^6/ml)	47.5±38.1	64.3±41.2	0.09
Morphology (normal forms %)	2.0±1.8	2.1±1.5	0.59
Progressive mobility (A+B %)	38.7±23.8	50.1±18.9	0.03
Hypo-osmotic test (% normal)	56.4±16.9	59.3±22.9	0.40
Leukocytes (10^6/ml)	1.1±1.6	1.8±2.9	0.13

SD:Standard Deviation

Table 3: Risk factors related to the presence of anti-sperm antibodies.

Risk factors	Group 1 N=46 (%)	Group 2 N=272 (%)	OR (95% CI)	P value
Testicular trauma	7 (12.3)	50 (87.7)	0.80 (0.38-1.71)	0.570
Testicular neoplasia	1 (25.0)	4 (75.0)	1.71 (0.30-9.57)	0.550
Varicocele	22 (23.7)	71 (76.3)	2.14 (1.27-3.61)	0.004
Scrotal surgery	4 (8.3)	44 (91.7)	0.52 (0.19-1.40)	0.170
Retractile testicle	14 (26.4)	39 (73.6)	2.13 (1.23-3.70)	0.008
infection	8 (12.1)	58 (87.9)	0.78 (0.38-1.60)	0.500

There was a significant decrease in progressive mobility in group 1 (38.7±23.8) vs group 2 (50.1±18.9) p=0.03, however, these values are within the lower limit of normal according to the WHO parameters 2010.

No significant correlation was observed between anti-sperm antibodies and other parameters of ejaculation such as concentration, (p 0.09) morphology (p 0.59) and the hypoosmotic test (p 0.40), did not show significant difference in both men with positive antibodies as negative.

The concentrations of leukocytes in the semen of males with infertility are elevated in both groups without finding significant difference (p 0.13).

Subsequently, the risk factors associated with the presence of anti-sperm antibodies were analyzed (Table 3), finding the risk of varicocele 23.7%, OR 2.14 (1.27-3.61) p=0.004 and the retractable testicle 26.4%, OR as significant risk. 2.13 (1.23-3.70) p=0.008, unlike testicular trauma 12.3% OR 0.80 (0.38-1.71) p=0.57, testicular neoplasia 25% OR 1.71 (0.30-9.57) p=0.55, scrotal surgery 8.3% OR 0.52 (0.19-1.40) p=0.17, infection 12.1% OR 0.78 (0.38-1.60) p=0.50 in which no significant difference was found.

DISCUSSION

The prevalence of infertile men with positive anti-sperm antibodies was 14.5%, similar to that reported in the literature of couples with positive anti-sperm antibodies with a prevalence ranging from 8.1 to 30.3%, however, their presence has been reported also in fertile couples in a 1.2 to 19%.[2,11-14] Leushuis E et al, in his work that included 1794 infertile marriages, a positive anti-sperm antibody test was detected in 3% and Garcia PC et al, in 18.1%.[4,15] Using lower cut-off points, a small difference in the detection rate of anti-sperm antibodies could appear, however its clinical effects on patients' fertility status would probably be negligible.[16]

Endocrine disruptors and seminal infection that interfere with the synthesis, storage, release, transport of sperm responsible for the regulation of homeostasis and the process of development can lead to compromised fertility.[4] However, in present study, endocrinological characteristics were compared somatometric and infectious of both groups without finding significant difference between the groups in any of the characteristics achieving with this, 2 homogeneous groups to compare the alterations in spermatobioscopy.

In this study, it was shown that in men who are infertile with positive anti-sperm antibodies, progressive mobility (<p 0.03) is significantly reduced, suggestive of damage to the sperm membrane, as reported by Veron G et al, 2016 in your studio.[6]

Rossato et al, showed recently that the score of the Hypoosmotica test in the sperm sample of patients with autoimmune infertility was significantly lower than that observed in the spermatozoa of normozoospermic subjects negative for anti-sperm antibodies.[5] In present work, no significant difference was found in the hypoosmotic test (p 0.40) concluding that no damage was found to the spermatic membrane with this test.

In the work of Garcia P et al, studying a Latin American population, it was observed that a higher proportion of motile sperm do not have progressive movement but this finding was not significantly associated with anti-sperm antibodies, but found a correlation between positive anti-sperm antibodies and increase of white blood cells in semen as well as decrease of the hypoosmotic test.[4] Unlike the present work in which the white blood cells in semen and the Hypo- osmotic test did not show the same correlation.

Any damage to the blood-testicular barrier should be suspected as a risk factor for the development of anti-sperm antibodies, including the increase in temperature and scrotal infections, as described by Yumei J et al in their meta-analysis the rate of positive anti-sperm antibodies was higher in patients with chronic prostatitis OR 3.26 (1.86-5.71) than in healthy individuals.[3,5] In present study, this result was not replicated since authors found an OR 0.78 (0.38-1.60) for prostatitis similar to that observed by Marconi, M et al, with non-significant levels of anti-sperm antibodies in patients with chronic epididymitis and orchitis.[16]

It has been shown that immune infertility is found in 15% of patients with varicocele, it is suggested that positive anti-sperm antibodies prior to varicocelectomy is an unfavorable prognostic factor for sperm after

varicocelectomy because it worsens prognosis. recovery to fertility after varicocelectomy.[5] In this study, 22% of male infertile men positive for anti-sperm antibodies had varicocele, this being a significant risk factor, OR 2.14 (1.27-3.61) p=0.004 and scrotal surgery 8.3% OR 0.52 (0.19-1.40) p=0.17 without being significantly risky. Varicocelectomy can lead to improved sperm count and morphology, but motility is affected when it is accompanied by positive anti-sperm antibodies similar to that found in this work.[5,17]

In the work of Sinisi A et al, authors report an increase in the prevalence of anti-sperm antibodies of 28% in men with a history of cryptorchidism treated or not treated with orchidopexy. In present study, it was found in 26.4% of infertile men with positive anti-sperm antibodies and retractable testicles with an OR 2.13 (1.23-3.70) P=0.008, however in the population studied by Sinasi A, it included a prepuber population and there is a high possibility that an indefinite percentage of patients underwent orchidopexy and this is the cause of the appearance of antisperm antibodies.[18,19] In the population studied by Jiang H et al, authors reviewed a history of 48 men with cryptorchidism who underwent orchidopexy at the prepubertal age, finding 6.7% with antisperm antibodies, without being greater than 50%. Therefore, they could not demonstrate a significant level of anti-sperm antibodies in infertile men with a history of cryptorchidism.[20]

In present study, a prevalence of 14.5% males with positive anti-sperm antibodies was observed, the condition of sperm motility was confirmed, and the varicocele and retractable testicle were observed as the main associated risk factor. Among the limitations of the study was not a group of fertile men with anti-sperm antibodies with which the seminal alterations could be compared, the study does not assess the real role of anti-sperm antibodies in human fertility, in the following Investigations will be able to compare the presence of anti-sperm antibodies and the fertilization rate with different reproduction techniques.

CONCLUSION

The main risk factors associated with the presence of anti-sperm antibodies are varicocele and retractable testis. Presenting in 14.5% of males with infertility in which sperm motility is affected in the semen.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Ethics and Local Research Committee of the National Institute of Perinatology

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