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Functional deficit of sperm and fertility impairment in men with antisperm antibodies

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ABSTRACT

Autoimmune reactions against the sperm cells play an ambiguous role in fertility impairment. The objective of this study was to characterize functional deficit of sperm conditioned by antisperm immune response in normozoospermic men. This was a multi-centric, cross-sectional, case–control study. The study subjects were 1060 infertile normozoospermic men and 107 fertile men. The main outcome measures were clinical examination, semen analysis including MAR test for antisperm antibodies (ASA), computer-aided sperm analysis, acrosome reaction (AR) detected with flow cytometry, DNA fragmentation measured with sperm chromatin dispersion, reactive oxygen species (ROS) assessed using the luminol-dependent chemiluminescence method. 2% of the fertile men had MAR-IgG ≥ 50%, but all subjects with MAR-IgG > 12% were outliers; 16% infertile men had MAR-IgG ≥ 50% ($p < 0.0001$). There was a direct correlation between the infertility duration and MAR-IgG ($R = 0.3$; $p < 0.0001$). The ASA-positive infertile men had AR disorders 2.1 times more frequently ($p < 0.02$), predominantly inductivity disorders. We found signs of hyperactivation proportionate to the ASA level ($p < 0.001$). DNA fragmentation was more highly expressed and was 1.6 and 1.3 times more frequent compared with the fertile and the ASA-negative patients, respectively ($p < 0.001$ and $p < 0.05$). We found signs of oxidative stress (OS): ROS generation by washed ASA-positive spermatozoa was 3.7 times higher than in the fertile men ($p < 0.00001$) and depended on the ASA levels ($R = 0.5$; $p < 0.0001$). The ASA correlation with ROS generation in native sperm was weak ($R = 0.2$; $p < 0.001$). We concluded that autoimmune reactions against spermatozoa are accompanied by a fertility decrease in normozoospermia. This results from AR and capacitation disorders and DNA fragmentation. The pathogenesis of sperm abnormalities in immune infertility is associated with the OS of spermatozoa.

1. Introduction

The World Health Organization (WHO) defines autoimmune reactions against the sperm cells as one of the causes of infertility in men (WHO, 2000). Antisperm antibodies (ASA) have been described in 8–21% of infertile men; however, the presence of ASA has also been detected in 1.2–19% of fertile men, suggesting that not all ASA cause infertility (Krause, 2009; Francavilla and Barbonetti, 2009; Vazquez-Levin et al., 2014). Antibodies directed against spermatozoa components have been shown to exert detrimental effects on different pre- and post-fertilization events: ASA can affect cell transport and motility, gamete interaction, and also early embryonic development, implantation, and fetal development (Mazumdar & Levine, 1998; Bronson, 1999; Chiu and

Abbreviations: ALH, amplitude of lateral head displacement; AR, acrosome reaction; ASA, antisperm antibodies; CAP, capacitation; CPM, ROS counted as photons per minute; DFI, DNA fragmentation index; Ig, immunoglobulin; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; MAR, mixed antiglobulin reaction; OS, oxidative stress; ROS, reactive oxygen species; SCD, sperm chromatin dispersion; SCSA, sperm chromatin structure assay; VCL, curvilinear velocity; VSL, straight-line (rectilinear) velocity; WHO, World Health Organization.

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Chamley, 2004; Francavilla and Barbonetti, 2009; Vazquez-Levin et al., 2014; Cui et al., 2015). However, in the most recent and largest study, a highly positive IgG-MAR test (IgG-ASA ≥ 50%) revealed a reduced, albeit not significantly, possibility of conception in only 3% of 1794 patients with infertile marriages during a one-year follow-up (Leushuis et al., 2009). Some researchers believe that ASA do not influence outcome independently of sperm motility and agglutination (Tomlinson et al., 2013). Others have found no ASA effect on fertilization outcome (Pagidas et al., 1994; Yeh et al., 1995; Vujisic et al., 2005). In a meta-analysis study, no relationship was found between ASA levels in semen and pregnancy rates following IVF and ICSI (Zini et al., 2011). Thus, infertility risk and mechanisms of fertility decrease in ASA-positive men and the influence of ASA on the processes following fertilization are still unclear, which is especially important in the era of assisted reproduction technologies.

The study objective was to evaluate the sperm functional deficit resulting from antisperm immune response and to identify the pathogenesis of these disorders in normozoospermia. This paper discusses the role of IgG class ASA; IgA antibodies, whenever they occur, are always found in association with IgG, while the opposite situation is very rare (Francavilla and Barbonetti, 2009).

2. Materials and methods

The cross-sectional multi-centric study was performed at I.M. Sechenov First Moscow State Medical University, Research Center for Obstetrics, Gynecology and Perinatology of Ministry of Healthcare of the Russian Federation, and Peoples' Friendship University of Russia, from 2008 to 2014. The study is based on a retrospective analysis of the medical data of 2556 married heterosexual men, aged 20–45, with a regular sexual life and with a suspected male infertility factor. The study was approved by the Institutional Review Board; written informed consent was obtained from all subjects.

The couples were examined according to WHO recommendations (WHO, 2000). The inclusion criteria for the study group were: at least 12 months of involuntary infertility with at least one unprotected sexual intercourse taking place per week, and a female partner under 35 without confirmed female infertility factors. The exclusion criteria were: female partner infertility (amenorrhea, anovulation, bilateral tubal occlusion); ejaculation or sexual disorders that prevent the semen from penetrating the vagina; infection and inflammation of ancillary genital glands (leukocyte count more than 1 million/ml) in male subjects; reproductive tract infections; oligo-, asteno- and/or teratozoospermia; azoospermia.

The laboratory tests of semen quality included spermatozoa and leukocyte counts, the total sperm volume, the evaluation of the nature of the spermatozoa (their vitality, motility, and morphology), etc. The mixed antiglobulin reaction (MAR) test (SpermMar Kit, FertiPro, Belgium) was performed to define the percentage of spermatozoa coated with IgG (MAR-IgG) (WHO, 2010).

Following the cross-sectional study, a group was formed of the men whose ejaculate met the WHO "normozoospermia" criteria ($n = 1060$). In this group, we evaluated the number of ASA cases, established the correlation between ASA counts and the duration of involuntary infertility, test results, which characterize the functional state of the spermatozoa: motility, acrosome reaction (AR), DNA fragmentation, and intracellular oxidative stress. The case–control study was a comparative study of the groups of normospermic patients with different ASA counts: the study group consisted of the men at a high risk of immune infertility, who had MAR-IgG ≥ 50%, ($n = 166$), the comparison group with MAR-IgG = 0% was formed using the stratification method ($n = 211$); the age and standard spermogram parameters of the groups were statistically similar.

One hundred and thirty-two fertile men aged 19–45 years were examined; 107 of them met the normozoospermia criteria and were enrolled for the control group. The inclusion criteria for the control group were a spontaneous 8- to 16-week pregnancy in their female partners. The study and the control groups had similar baseline characteristics.

Sperm motility characteristics were evaluated in undiluted semen with computer-aided sperm analysis (CASA) using the sperm analyzer "MTG" (the program "medeaLAB CASA"; Medical Technology Vertriebs Gmbh, Bruckberg, Germany). Curvilinear (track) velocity (VCL, μm/s), straight-line velocity (VSL, μm/s), and amplitude of lateral head displacement (ALH, μm) were calculated.

The spontaneous and induced AR was evaluated by flow cytometry (Nikolaeva et al., 1998) with double fluorescent staining of spermatozoa using fluorescein-labeled lectin from *P. sativum* (Sigma, USA) and rhodamine-labeled lectin from *A. hypogaea* (Sigma, USA). The intensity of green and red fluorescence of each cell subpopulation was evaluated by the Facscan flow cytometer (Becton Dickinson, USA). The AR was induced by exposure of the spermatozoa to the ionophore A23187 (Sigma, USA). The AR after ionophore challenge was calculated as the study group AR minus the control group AR.

The DNA fragmentation was evaluated using the sperm chromatin dispersion method (Halosperm®; Halotech DNA, Spain) in an inert agarose gel with the visual microscopic halation assessment after DNA acid denaturation and nuclear protein lysis (Gosalvez et al., 2011). According to the test-system manufacturer's instructions, the reference level of the sperm DNA fragmentation index (DFI) was below 20%.

The generation of reactive oxygen species (ROS) was measured in sperm cells with the chemiluminescence assay using luminol (Sigma Chemical Co, USA) staining (Agarwal and Deepinder, 2009; WHO, 2010). ROS levels were determined by measuring chemiluminescence via a luminometer (Wallac Co., Finland; "Lum-5773", Moscow State University, Russia). For the ROS assessment in washed semen, the aliquots of liquefied semen were centrifuged at 300 *g* for seven minutes. The sperm pellet was washed twice with human tubal fluid, HEPES buffered (Irvine Scientific, USA). Ten microliters of 5-mM luminol (Sigma Chemical Co, USA) prepared in dimethylsulfoxide (DMSO) (Sigma, USA) was added to 400 mL of the washed sperm suspension. The test results of the fertile men in the control group were considered normal.

The data were processed using the "STATISTICA" software package (StatSoft, USA). Median, mean with standard deviation (M ± S), 25–75% percentiles, and maximum–minimum values were calculated. On the box-and-whisker graphics, "outliers" were defined as remote from the distribution center and non-typical values (probably due to an observational or other biases) and calculated as follows: higher than [upper box limit + coefficient × (upper box limit – lower box limit)] or lower than [lower box limit – coefficient × (upper box limit – lower box limit)] with the coefficient × 1.0. For "extremes," see the calculation above with the coefficient × 3.0.

The statistical significance was assessed using the Mann–Whitney test, the *t* test for independent samples, and the Chi-squared test. The correlation analysis was also performed (R-Spearman and gamma coefficients were calculated).

3. Results

The fertile men of the control group (n=107) included the subjects with the ASA: MAR-IgG>10% - 14 (13.1%; MAR-IgG ≥ 50% – 2 (1.9%); and MAR-IgG = 100% – 1 (1.0%). The distribution of the MAR test values in this group differed markedly from the normal distribution: median = 0% (mean = 5.4 ± 13.8); 50% of values (25–75%