Journal of Reproductive Immunology 112 (2015) 95–101

Contents lists available at ScienceDirect

Journal of Reproductive Immunology

journal homepage: www.elsevier.com/locate/jreprimm

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

V.A. Bozhedomov[a,c,*], M.A. Nikolaeva[b], I.V. Ushakova[b], N.A. Lipatova[c], G.E. Bozhedomova[c], G.T. Sukhikh[a,b]

[a] Department of Obstetrics, Gynecology, Perinatology and Reproduction, I.M. Sechenov First Moscow State Medical University, Oparin st. 4, 117997 Moscow, Russia
[b] Federal State Budget Institution "Research Center for Obstetrics, Gynecology and Perinatology" of Ministry of Healthcare of the Russian Federation, Oparin st. 4, 117997 Moscow, Russia
[c] Department of Clinical Andrology, Federal State Budget Institution Peoples' Friendship University of Russia, Stavropol'skaya st. 23, 109386 Moscow, Russia

ARTICLE INFO

Article history:
Received 15 July 2015
Received in revised form 11 August 2015
Accepted 17 August 2015

Keywords:
Acrosome reaction
Antisperm antibodies
Normozoospermia
Reactive oxygen species
Sperm DNA fragmentation

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.

* Corresponding author at: Department of Obstetrics, Gynecology, Perinatology and Reproduction, I.M. Sechenov First Moscow State Medical University and Department of Clinical Andrology, Federal State Budget Institution Peoples' Friendship University of Russia, Oparin st. 4, 117997 Moscow, Russia.
E-mail address: vbojedomov@mail.ru (V.A. Bozhedomov).

http://dx.doi.org/10.1016/j.jri.2015.08.002
0165-0378/

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%

Table 1A
Acrosome reaction rates in fertile and ASA-positive infertile men with normozoospermia.

Parameter	Total number of patients, n	Spontaneous AR, M ± S	Induced AR, M ± S	AR after ionophore challenge (ARIC)[a], M ± S
Fertile men	47	14 ± 11%	41 ± 13%	27 ± 16%
Infertile men[b]	67	18 ± 14%	33 ± 17%	16 ± 14%
Odds ratio	–	1.3	0.8	0.6
Student's *t* test	–	ND	$p < 0.01$	$p < 0.001$

Note: Reaction (so-called "the acrosome reaction after ionophore challenge (ARIC) test").
[a] ARIC = the test%AR minus the control%AR.
[b] ASA-positive patients with MAR-IgG ≥ 50% and normozoospermia; ND—no difference.

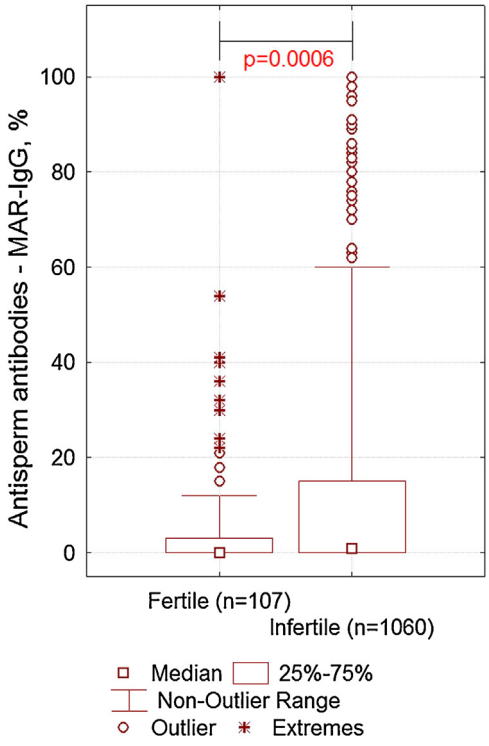

Fig. 1. The results of the direct method of identification of ASA-IgG on the spermatozoa surface in fertile and infertile men with normozoospermia.

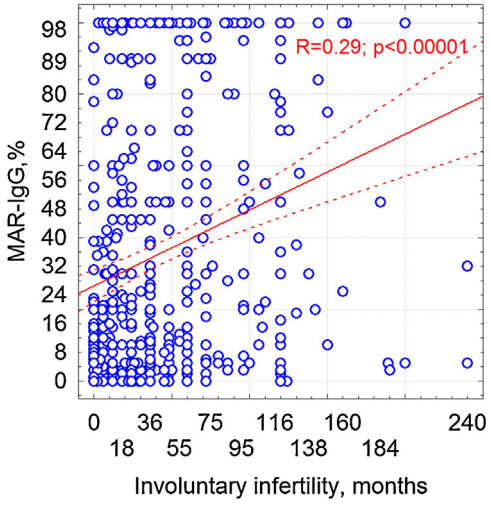

Fig. 2. The correlation between the duration of involuntary infertility with normozoospermia and MAR-IgG levels.
Note: the fertile men are included (involuntary infertility = 0).

percentiles) were in the 0–3% diapason, 95% percentile was equal to 32%. Additionally, the box-and-whisker plot shows that the outliers (the values far from the median and not typical for this study set) were MAR-IgG > 12% (Fig. 1).

In the men from the infertile couples ($n = 2556$), 41.5% were normozoospermic ($n = 1060$). They had ASA-IgG significantly more often than the fertile men: MAR ≥ 50% in 15.6% (166 of 1060) of cases (p = 0.0001), mean ASA count was higher (Fig. 1): M = 16.6% with the non-outlier range = (0–60) compared with M = 5.4% with the non-outlier range = (0–12) in fertile men (p = 0.0006).

The correlation analysis (Fig. 2) confirmed the direct correlation between the motile sperm cells coated with ASA and the duration of involuntary infertility in normozoospermic patients ($R = 0.3$; $p < 0.00001$). The regression line crosses the ordinate scale at MAR-IgG = 24% in "involuntary infertility = 0.Ṫhus, IgG-ASA on motile spermatozoa is the cause of a decrease in reproductive function even in men with normal standard spermogram parameters. The relative infertility risk for MAR ≥ 50% is 8.38, which increases starting from MAR-IgG > 25% (Fig. 2).

We evaluated the functional state of spermatozoa to clarify the mechanisms of fertility decrease in ASA-positive men with normozoospermia.

The AR evaluation showed that ASA presence was associated with a higher number of spermatozoa, which spontaneously, i.e., prematurely, lost the acrosome (Table 1A), and a lower number of spermatozoa releasing acrosome in ionophore A23187 challenge ($p < 0.01$). The percentage of patients with a normal AR in the ASA-positive patients (Table 1B) is 2.1 times lower in comparison with the fertile men (77 and 36%; p < 0.02), resulting first of all from the decrease in inductiveness (4.5 times; p < 0.02). Thus, in the presence of ASA, AR takes place before the spermatozoa enter the female reproductive tract and/or there is no reaction to the inducer that may decrease the probability of egg fertilization.

Another sign of premature capacitation is the changes in the spermatozoa motility. The computer-assisted characterization of motility (Fig. 3) showed a positive correlation between MAR-IgG and VCL ($R = 0.47$; $p < 0.001$), VSL ($R = 0.46$; $p < 0.001$), and ALH ($R = 0.43$; $p < 0.001$).

We found that ASA were associated with an increased proportion of gametes with chromosome structure defects (Table 2). The percentage of sperm cells with DNA fragmentation in the ASA-positive men was 1.3 times higher than in the infertile ASA-negative men ($p < 0.05$), and 1.6 times higher than in the fertile men ($p < 0.001$). There are differences in halo size that characterize the degree of DNA fragmentation ($p < 0.05$). The proportion of cases in which the percentage of sperm cells with DNA fragmentation exceeds the reference value (>20%) was 50% in the ASA-positive group, which is 1.4 times higher than in the infertile ASA-negative men and 2.3 times higher than in the fertile men ($p < 0.05$). These changes in the chromatin structure associated with ASA may be the factor that affects the processes of reproduction, both during and after egg fertilization.

To specify a potential pathogenic factor of spermatozoa damage, we evaluated the significant increase in ROS generation in the ASA-positive group. The washed spermatozoa of the fertile men (Fig. 4A) were characterized by low levels of ROS

Table 1B
Status of AR patterns in fertile and ASA-positive infertile men with normozoospermia.

Parameter	Total number of patients, *n* (%)	Normal AR, *n* (%)	Excessive spontaneous AR, *n* (%)	Failure of induced AR, *n* (%)	Excessive spontaneous and failed induced AR, *n* (%)
Fertile men	47 (100%)	36 (77%)	2 (4%)	3 (6%)	6 (13%)
Infertile men[a]	67 (100%)	24 (36%)	8 (12%)	18 (27%)	17 (25%)
Odds ratio	–	0.5	3.0	4.5	1.9
Chi-squared test	–	$p < 0.02$	ND	$p < 0.02$	ND

Note: normal acrosome reaction: spontaneous reaction in less than 20% of sperm cells, induced reaction in not less than 15% of sperm cells (Tesarik, 1996). The cases of AR deviation from the reference value in the fertile men are accounted for by the fact that flow cytometry was used while the reference values were developed for fluorescence optics.

[a] ASA-positive patients with MAR-IgG ≥ 50% and normozoospermia; ND—no difference.

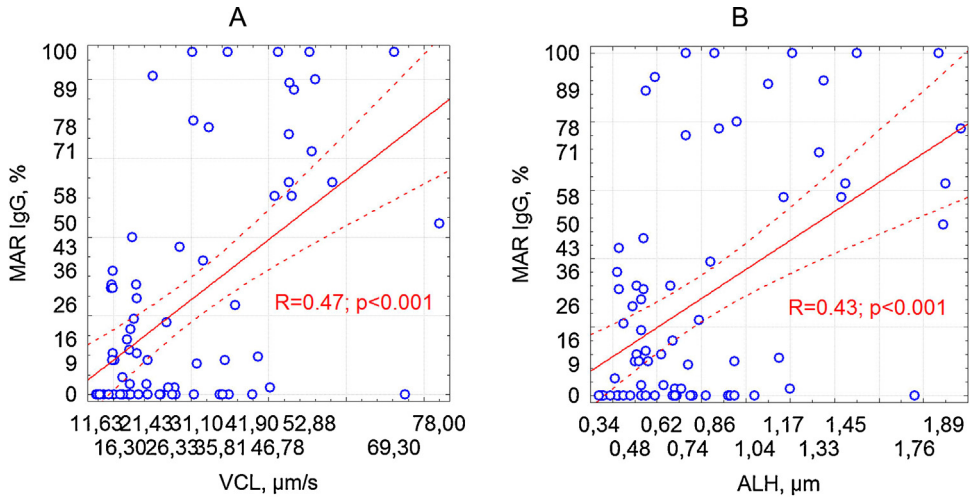

Fig. 3. The correlation between the percentage of ASA-positive sperm cells (MAR-IgG) and (A) their curvilinear (track) velocity (VCL), (B) amplitude of lateral head displacement (ALH).
Note: R—Spearman rank correlation coefficient; MAR-IgG values from 0 to 100% are included in the figure ($n = 75$).

Table 2
Sperm DNA fragmentation in fertile men, ASA-negative and ASA-positive patients from infertile couples.

Parameters	Fertile men ($n = 23$)	Subfertile patients ($n = 245$)		*p*		
		ASA-negative patients (MAR-IgG = 0%) ($n = 211$)	ASA-positive patients (MAR-IgG ≥ 50%) ($n = 34$)	Group numbers	Student's *t* test	Chi-squared test
	1	2	3			
Percentage of sperm cell with DNA fragmentation[a], M ± S	14 ± 8.8%	18 ± 13.7%	22 ± 18.2%	1–2 1–3 2–3	0.017 0.0006 0.013	
The number of cases where the percentage of sperm cell with DNA fragmentation exceeds the reference value (≥20%), *n* (%)	5/23 (22%)	79/211 (37%)	17/34 (50%)	1–2 1–3 2–3		ND 0.032 ND
Halo size, M ± S	1.5 ± 0.34	1.6 ± 0.45	1.7 ± 0.55	1–2 1–3 2–3	ND 0.016 ND	
The number of cases where the halo size exceeds the reference value (≥2), n (%)	2/23 (9%)	42/211 (20%)	8/34 (24%)	1–2 1–3 2–3		ND ND ND

Note: ND—no difference.

[a] Spermatozoa with small or without halo and degraded.

generation (0.27 ± 0.19 × 10^6 CPM; median—0.23 × 10^6 CPM; non-outlier range = 0.01–0.51). The ROS generation in the infertile group with MAR-IgG≥50% was 3.7-fold higher than in the fertile group (0.91 ± 0.66, median—0.84; non-outlier range = 0.03–2.10; $p < 0.0001$). We found a direct correlation between MAR-IgG and ROS generation in a washed semen sample ($R = 0.48$; $p < 0.0001$). The correlation between MAR-IgG and ROS production in the native sperm is significantly weaker ($R = 0.18$; p = 0.0008); the differences between mean values for the fertile and infertile MAR-IgG ≥ 50% groups are nonsignificant (Fig. 4B; $p > 0.05$). These differences reflect the ROS sources: spermatozoa in the washed sperm samples, and predominantly leukocytes in the native sperm; ROS produc-

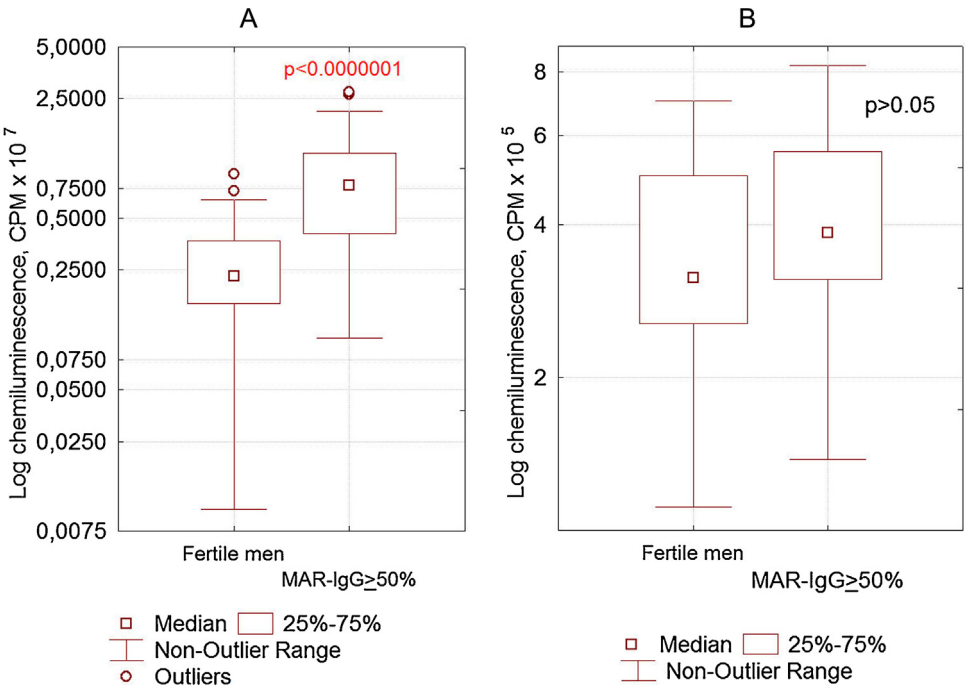

Fig. 4. Detecting spontaneous generation of reactive oxygen species by chemiluminescence assay: (A) washed spermatozoa suspension and (B) total ROS activity in neat semen in the fertile men with normozoospermia ($n = 45$) and the patients from the infertile couples with MAR-IgG ≥ 50% and normozoospermia ($n = 41$).
Note: CPM—counted as photons per minute; the outliers are excluded; Mann–Whitney U test was used for the analysis and $p < 0.05$ was considered statistically significant.

tion in the latter case is directly proportionate to sperm leukocytes ($R = 0.31$; $p < 0.0001$).

4. Discussion

The correlation between ASA and infertility is not considered from the point of view of evidence-based medicine (Mazumdar and Levine, 1998; Francavilla and Barbonetti, 2009; Leushuis et al., 2009; Vazquez-Levin et al., 2014; Cui et al., 2015; Jungwirth et al., 2015; and others). Our interest in this problem was predominantly stimulated by the results of our studies demonstrating higher ROS production in a washed semen sample in ASA-positive men (Korotkova et al., 2001; Bozhedomov et al., 2009, 2014). The role of oxidative stress (OS) in male infertility has been actively discussed recently, as it is associated with a higher risk of spermatozoa DNA fragmentation and consequent spontaneous abortions and congenital abnormalities in children (Zini and Agarwal, 2011; Lampio et al., 2012; Aitken et al., 2014).

Our study provided evidence to support the point that the autoimmune antisperm reactions are a considerable factor in fertility impairment.

We found ASA in 13% of the fertile men with normozoospermia, with 2% who had MAR-IgG ≥ 50% and 1% who had all motile spermatozoa coated with ASA. It suggests that a significant amount of ASA do not always impair fertility, as highlighted by Leushuis et al. (2009), Krause (2009), and others. Fertility impairment probably depends not only on the proportion of ASA-positive spermatozoa, but also on the ASA localization to the cells (head, neck or tail), their number on the gametes, IgG subtypes, the functional characteristics of ASA-bound antigens, the affinity of this binding, and other factors that were not assessed in this study. At the same time, we found that the upper MAR-IgG 95% percentile is 32%. The regression line (Fig. 2) crosses the ordinate scale in MAR-IgG = 24% in "involuntary infertility = 0.Äll cases of MAR-IgG > 12% are outliers for the fertile men and are therefore not typical of the general population (Fig. 1). These data give rise to the following question: are the current WHO guidelines (2000) too tolerant to the presence of increased ASA levels?

The ASA role as an independent predictor of infertility impairment was supported by the findings of the examination of the men from infertile couples. The men with normozoospermia in infertile couples have highly positive ASA (MAR-IgG ≥ 50%) 8.4 times more frequently than the fertile men. The mean and median values were substantially higher (Fig. 1). Even in the normozoospermic patients, the duration of involuntary infertility was directly in proportion to the MAR-IgG value (Fig. 2). This affects sperm parameters other than concentration, motility, and morphology, which are evaluated at the light-optic level. These data agree with the recent meta-analysis that showed that ASA-positive patients did not demonstrate changes in most standard spermogram parameters, including progressive motility and percentage of normal forms (Cui et al., 2015).

In normozoospermia, the decrease in fertility may result from the disorders in the female reproductive tract: during movement through the cervical mucosa, in capacitation and interaction with the egg (Mazumdar and Levine, 1998; Bronson, 1999; Munuce et al., 2000; Vazquez-Levin et al., 2014 and others). Our finding demonstrated that significant changes in the ASA-positive spermatozoa quality took place in the male reproductive tract. We found the association between ASA and such functional disorders as premature capacitation and AR, and DNA fragmentation.

The signs of premature capacitation are the increase in track speed and lateral fluctuation of the sperm cell head (Suarez, 2008) that were found in our (Fig. 3) and other studies (Munuce et al., 2000). Premature AR is indicated by the increase in the number of spermatozoa with AR (Table 1). It is described elsewhere (Bohring et al., 2001; Myogo et al., 2001; Peknicova et al., 2005). Our findings suggest that AR inductivity disorder might be more significant: the percentage of spermatozoa reacting on ionophore is 4.5 times lower in the ASA-positive group. Paradoxically, ionophore A23187 had a significant inhibitory effect on the spontaneous AR in 10% of autoimmune patients. These changes, both individually and in combination, result in 2.1 lower percentage of spermatozoa with

normal AR in the ASA-positive patients (Table 1). However, there is no direct relationship between ASA-positive spermatozoa and a specific AR disorder (premature AR or inhibition of induced AR); the MAR-IgG test allows prediction of its probability rather than of its type.

The percentage of sperm cells with DNA fragmentation in the ASA-positive men with normozoospermia in infertile couples was significantly higher (1.3 times) than in the infertile ASA-negative men, and in fertile men (1.6 times). There are significant differences in halo size that characterize the degree of DNA fragmentation (Table 2). This can help to explain some well-known facts, in particular, elevated DNA fragmentation and poor IVF ICSI results in men with normozoospermia when ASA levels were not considered (Avendaño and Oehninger, 2011). On the other hand, many researchers demonstrated that ASA-positive patients had lower quality embryos in the assisted reproduction programs (Mandelbaum et al., 1987; Clarke, 1988; De Almeida et al., 1989; Rajah et al., 1993; Acosta et al., 1994; Vazquez-Levin et al., 1997; Calamera et al., 2002).

At the same time, some studies showed that the incubation of ASA-negative spermatozoa in the ASA-positive environment did not result in the DNA integrity of the sperm (Evans et al., 1999) or in a decrease in the embryo quality (Calamera et al., 2002). Zini et al. (2010), however, did not find a difference in DNA fragmentation between the ASA-positive and ASA-negative groups that can be easily explained. In our study, the baseline spermogram parameters were similar in the control and the study groups; moreover, our subjects were normospermic, whereas in the study by Zini et al. the groups were heterogeneous. Mean ASA-positive and ASA-negative values differed 3.2 times in concentration and 1.7 times in motility; the standard error in the percentage of spermatozoa with DNA fragmentation exceeded the mean value (Zini et al., 2010). Moreover, a different method of DNA damage detection was used—we used the SCD test, which is more sensitive than SCSA—and the ASA-positive group included the patients with MAR% $\geq$ 40% (in our study, not less than 50%). However, we fully agree with Zini et al. (2010) that ASA cannot cause DNA fragmentation. We believe that the association between ASA and DNA fragmentation found in our study rather illustrates the binding of ASA to already damaged spermatozoa with DNA fragmentation. This means that exogenic ASA cannot damage gametes at a genome level. However, endogenic ASA can interfere with already damaged nucleoproteins, whose structural changes cause on the one hand immune tolerance and on the other, decrease the quality of a spermatozoa and consequently of an embryo.

There are many indirect forms of proof of the relationship between the spermatozoa apoptosis and ASA. Activation of the proapoptotic factors (caspase-8, -9, Bax) in testicles during autoimmune orchitis has been described (Theas et al., 2006). Other signs of apoptosis are heat-shock proteins and caspases, whose presence was indicated by the corresponding antibodies in the ASA-positive infertile men (Krause, 2009; Naz, 2011; Vazquez-Levin et al., 2014). Another sign of the initiated apoptosis is the intercellular OS caused by the mitochondrial "oxidative burst" (El-Fakahany and Sakkas, 2011; Vaithinathan et al., 2012; Aitken et al., 2014), whereby the OS is probably considered to be both a cause (exogenous ROS) and an effect of the apoptosis (intercellular ROS generation).

We believe that the pathogenetic role of the increased ROS production in the pathogenesis of the immune infertility is quite convincing. We found that in MAR-IgG $\geq$ 50% spermatozoa generate almost four times higher than the normal amount of ROS, which suggests intracellular OS (Fig. 4). The correlation between MAR-IgG and ROS generation in a washed semen sample is highly significant ($R = 0.5$). While the correlation between MAR-IgG and ROS generation in native sperm is 2.7 times lower, the mean value difference is nonsignificant (Fig. 4). Notably, OS can lead to capacitation, AR, and DNA fragmentation disorders in ASA-positive men. In fact, a low ROS rate is necessary for a normal AR, but the AR disorder may result from higher ROS levels (Morielli and O'Flaherty, 2015). Apparently, ROS may cause a higher DNA fragmentation in ASA-positive spermatozoa (Table 2). The accepted point of view indicates that OS might cause chromatin damage to the spermatozoa (Zini and Agarwal, 2011; Lampio et al., 2012; Aitken et al., 2014). At the same time, there is no correlation between MAR-IgG test data and DNA fragmentation. This means that the changes in the spermatozoa depend not only on the amount of ROS generated in the sperm, but on additional factors associated with both spermatozoa and the cellular and biochemical composition of the sperm, i.e., activity of oxidative system enzymes, heat shock proteins, topoisomerases which repair DNA breaks, and other factors.

A search for additional factors that affect fertility in the antisperm immune response should be the matter for future research.

5. Conclusion

Men who have more than a half or even all motile spermatozoa coated with IgG-ASA can be fertile, but MAR-IgG > 12% values for fertile men are outliers. The major factor of decreased fertility in the ASA-positive patients is a functional deficit of sperm. In normozoospermia, immune infertility is found in 16% of cases. The increase in the percentage of IgG-ASA-positive spermatozoa is more frequently accompanied by spontaneous and/or induced AR disorders, hyperactivation, and spermatozoa chromatin damage. The pathogenesis of sperm pathology in immune infertility is associated with the OS of the spermatozoa. Additional research is necessary to clarify the pathogenesis of immune infertility in men.

References

Acosta, A.A., van der Merwe, J.P., Doncel, G., Kruger, T.F., Sayilgan, A., Franken, D.R., et al., 1994. Fertilization efficiency of morphologically abnormal spermatozoa in assisted reproduction is further impaired by antisperm antibodies on the male partner's sperm. Fertil. Steril 62, 826–833.

Agarwal, A., Deepinder, F., 2009. Determination of seminal oxidants (reactive oxygen species). In: Lipshults, L.I., Howards, S.S., Niederberger, C.S. (Eds.), Infertility in the Male. , Fourth ed. Cambridge University Press, Cambridge, UK, pp. 618–632.

Aitken, R.J., Smith, T.B., Jobling, M.S., Baker, M.A., De Iuliis, G.N., 2014. Oxidative stress and male reproductive health. Asian J. Androl. 16, 31–38.

Avendaño, C., Oehninger, S., 2011. DNA fragmentation in morphologically normal spermatozoa: how much should we be concerned in the ICSI era? J. Androl. 32, 356–363.

Bohring, C., Skrzypek, J., Krause, W., 2001. Influence of antisperm antibodies on the acrosome reaction as determined by flow cytometry. Fertil. Steril. 76, 275–280.

Bozhedomov, V.A., Gromenko, D.S., Ushakova, I.V., Toroptseva, M.V., Aleksandrova, L.A., Sukhikh, T.G., et al., 2009. Oxidative stress of spermatozoa in pathogenesis of male infertility. Urologiia 2, 51–56, Article in Russian.

Bozhedomov, V.A., Lipatova, N.A., Rokhlikov, I.M., Alexeev, R.A., Ushakova, I.V., Sukhikh, G.T., 2014. Male fertility and varicocoele: role of immune factors. Andrology 2, 51–58.

Bronson, R.A., 1999. Antisperm antibodies: a critical evaluation and clinical guidelines. J. Reprod. Immunol. 45 (2), 159–183.

Calamera, J.C., Doncel, G.F., Brugo-Olmedo, S., Sayago, A., Acosta, A.A., 2002. Male antisperm antibodies: association with a modified sperm stress test and lipid peroxidation. Andrologia 34, 63–68.

Chiu, W.W., Chamley, L.W., 2004. Clinical associations and mechanisms of action of antisperm antibodies. Fertil. Steril. 82, 529–535.

Clarke, G.N., 1988. Sperm antibodies and human fertilization. Am. J. Reprod. Immunol. 17, 65–71.

Cui, D., Han, G., Shang, Y., Liu, C., Xia, L., Li, L., et al., 2015. Antisperm antibodies in infertile men and their effect on semen parameters: a systematic review and meta-analysis. Clin. Chim. Acta 444, 29–36.

De Almeida, M., Gazagne, I., Jeulin, C., Herry, M., Belaisch-Allart, J., Frydman, R., et al., 1989. In-vitro processing of sperm with autoantibodies and in-vitro fertilization results. Hum. Reprod. 4, 49–53.

El-Fakahany, H.M., Sakkas, D., 2011. Abortive apoptosis and sperm chromatin damage. In: Zini, A., Agarwal, A. (Eds.), Sperm Chromatin. Biological and Clinical Applications in Male Infertility and Assisted Reproduction. Springer, pp. 295–306.

Evans, M.L., Chan, P.J., Patton, W.C., King, A., 1999. Sperm artificially exposed to antisperm antibodies show altered deoxyribonucleic acid. J. Assist. Reprod. Genet. 16, 443–449.

Francavilla, F., Barbonetti, A., 2009. Male autoimmune infertility. In: Krause, W.K., Naz, R.K. (Eds.), Immune Infertility. The Impact of Immune Reaction on Human Infertility. Springer, Verlag Berlin, pp. 145–153.
Gosalvez, J., Lopez-Fernandez, C., Fernandez, J.L., 2011. Sperm chromatin dispersion test: technical aspects and clinical applications. In: Zini, A., Agarwal, A. (Eds.), Sperm Chromatin. Biological and Clinical Applications in Male Infertility and Assisted Reproduction. Springer, pp. 151–170.
Jungwirth, A. (chair), Diemer, T., Dohle, G.R., Giwercman, A., Kopa, Z., Tournaye, H. et al. 2015. Guidelines on Male Infertility. © European Association of Urology (UPDATE MARCH 2014).
Korotkova, I.V., Nikolaeva, M.A., Bozhedomov, V.A., Golubeva, E.L., Ter-Avanesov, G.V., Sukhikh, G.T., et al., 2001. Free radical generation in ejaculate samples from infertile patients. Bull. Exp. Biol. Med. 131, 555–557, Article in Russian.
Krause, W., 2009. Sperm function influenced by immune reaction. In: Krause, W.K., Naz, R.K. (Eds.), Immune Infertility. The Impact of Immune Reaction on Human Infertility. Springer, pp. 49–65.
Lampio, F., Opperman, C.J., Agarwal, A., De Plessis, S.S., 2012. Oxidative stress. In: Parekattil, S.J., Agarwal, A. (Eds.), Male Infertility: Contemporary Clinical Approaches, Andrology, ART & Antioxidants. Springer, pp. 225–235.
Leushuis, E., van der Steeg, J.W., Steures, P., Repping, S., Schöls, W., van der Veen, F., et al., 2009. Immunoglobulin G antisperm antibodies and prediction of spontaneous pregnancy. Fertil. Steril. 92, 1659–1665.
Mandelbaum, S.L., Diamond, M.P., DeCherney, A.H., 1987. Relationship of antisperm antibodies to oocyte fertilization in in vitro fertilization embryo transfer. Fertil. Steril. 47, 644–651.
Mazumdar, M.D., Levine, A.S., 1998. Antisperm antibodies: etiology, pathogenesis, diagnosis and treatment. Fertil. Steril. 70, 799–810.
Morielli, T., O'Flaherty, C., 2015. Oxidative stress impairs function and increases redox protein modifications in human spermatozoa. Reproduction 149, 113–123.
Munuce, M.J., Berta, C.L., Pauluzzi, F., Caille, A.M., 2000. Relationship between antisperm antibodies, sperm movement, and semen quality. Urol. Int. 65, 200–203.
Myogo, K., Yamano, S., Nakagawa, K., Kamada, M., Maegawa, M., Irahara, M.E., t al, 2001. Sperm-immobilizing antibodies block capacitation in human spermatozoa. Arch. Androl. 47, 135–142.
Naz, R.K., 2011. Antisperm contraceptive vaccines: where we are and where we are going? Am. J. Reprod. Immunol. 66 (1), 5–12.
Nikolaeva, M.A., Golubeva, E.L., Kulakov, V.I., Sukhikh, G.T., 1998. Evaluation of stimulus-induced acrosome reaction by two-colour flow cytometric analysis. Mol. Hum. Reprod. 4, 243–250.
Pagidas, K., Hemmings, R., Falcone, T., Miron, P., 1994. The effect of antisperm autoantibodies in male or female partners undergoing in vitro fertilization-embryo transfer. Fertil. Steril 62, 363–369.
Peknicova, J., Chladek, D., Hozak, P., 2005. Monoclonal antibodies against sperm intra-acrosomal antigens as markers for male infertility diagnostics and estimation of spermatogenesis. Am. J. Reprod. Immunol. 53, 42–49.
Rajah, S.V., Parslow, J.M., Howell, R.J., Hendry, W.F., 1993. The effects on in vitro fertilization of autoantibodies to spermatozoa in subfertile men. Hum. Reprod. 8, 1079–1082.
Suarez, S.S., 2008. Control of hyperactivation in sperm. Hum. Reprod. Update 14, 647–657.
Tesarik, J., 1996. Acrosome reaction testing. Report of the consensus workshop on advanced diagnostic andrology techniques. ESHRE, andrology special interest group. Hum. Reprod. 11, 1463–1479.
Theas, M.S., Rival, C., Jarazo-Dietrich, S., Guazzone, V.A., Lustig, L., 2006. Death receptor and mitochondrial pathways are involved in germ cell apoptosis in an experimental model of autoimmune orchitis. Hum. Reprod. 21, 1734–1742.
Tomlinson, M., Lewis, S., Morroll, D., 2013. Sperm quality and its relationship to natural and assisted conception: British fertility society guidelines for practice. Hum. Fertil. (Camb) 16, 175–193.
Vaithinathan, S., D'Cruz, S.C., Mathur, P.P., 2012. Apoptosis and male infertility. In: Parekattil, S.J., Agarwal, A. (Eds.), Male Infertility: Contemporary Clinical Approaches Andrology ART & Antioxidants. Springer, pp. 329–336.
Vazquez-Levin, M.H., Notrica, J.A., Polak de Fried, E., 1997. Male immunologic infertility: sperm performance on in vitro fertilization. Fertil. Steril. 68, 675–681.
Vazquez-Levin, M.H., Martn-Briggiler, C.I., Veaute, C., 2014. Antisperm antibodies: invaluable tools toward the identification of sperm proteins involved in fertilization. Am. J. Reprod. Immunol. 72, 206–218.
Vujisic, S., Lepej, S.Z., Jerkovic, L., Emedi, I., Sokolic, B., 2005. Antisperm antibodies in semen, sera and follicular fluids of infertile patients: relation to reproductive outcome after in vitro fertilization. Am. J. Reprod. Immunol. 54, 13–20.
WHO, 2000. Manual for the standardized investigation and diagnosis of the infertile couple. In: WHO, Third ed. Cambridge university press, 83 p.
WHO, 2010. Laboratory manual for the examination and processing of human semen. In: WHO, Fifth ed, 271 p.
Yeh, W.R., Acosta, A.A., Seltman, H.J., Doncel, G., 1995. Impact of immunoglobulin isotype and sperm surface location of antisperm antibodies on fertilization in vitro in the human. Fertil. Steril. 63, 1287–1292.
Zini, A., Agarwal, A. (Eds.), 2011. Sperm Chromatin: Biological and Clinical Application in Male Infertility and Assisted Reproduction. Springer, p. 512.
Zini, A., Fahmy, N., Belzile, E., Ciampi, A., Al-Hathal, N., Kotb, A., 2011. Antisperm antibodies are not associated with pregnancy rates after IVF and ICSI: systematic review and meta-analysis. Hum. Reprod. 26, 1288–1295.
Zini, A., Phillips, S., Lefebvre, J., Baazeem, A., Bissonnette, F., Kadoch, I.J., et al., 2010. Anti-sperm antibodies are not associated with sperm DNA damage: a prospective study of infertile men. J. Reprod. Immunol. 85, 205–208.