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Correspondence:
Vladimir A. Bozhedomov, 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, Oparina st. 4, 117997 Moscow, Russia.
E-mail: vbojedomov@mail.ru

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The role of the antisperm antibodies in male infertility assessment after microsurgical varicocelectomy

[1,2]V. A. Bozhedomov, [2]N. A. Lipatova, [2]R. A. Alexeev, [2]L. M. Alexandrova, [3]M. A. Nikolaeva, and [1,3]G. T. Sukhikh

[1]Department of Obstetrics, Gynecology, Perinatology and Reproduction, I.M. Sechenov First Moscow State Medical University, [2]Department of Clinical Andrology, The Federal State Budget Institution Peoples' Friendship University of Russia, and [3]The Federal State Budget Institution "Research Center for Obstetrics, Gynecology and Perinatology" of the Ministry of Healthcare of the Russian Federation, Moscow, Russia

SUMMARY

Antisperm antibodies (ASA) are a cause of male infertility. ASA are often found in varicocele patients. The study objective was to assess the ASA role in fertility recovery after varicocelectomy. The longitudinal study involved 99 patients with varicocele. Patients were examined according to the WHO recommendations; ASA level was measured using the direct method of Sperm MAR test: 66 patients were ASA-negative, 33 had MAR-IgG ≥ 10%. All patients underwent microsurgical varicocelectomy. Student's *t*-test, Wilcoxon test, Chi-squared test and signed rank test were used for data analysis. The retrospective analysis of all operated patients data showed that the patients without spermiogram improvement after varicocelectomy had higher ASA levels. 3 months after the surgery, the initially ASA-negative varicocele patients demonstrated 2.5 times increase in number of progressive motile spermatozoa in the ejaculate ($p < 0.001$), accompanied by 6% decrease in abnormal sperm count ($p < 0.05$); the spermiogram parameters improved in 77% of cases ($p < 0.01$). After the surgery, ASA developed in 16% of cases (Max - MAR-IgG = 12%). The patients who were initially ASA-positive demonstrated ASA decrease only in half of the cases (16 of 33; $p > 0.05$). The main outcome in this group was a favourable response to the surgery (ASA level decrease) vs. no reduction in autoimmune process. The improvement in the ASA-positive group was demonstrated in the patients with higher varicocele grade (median – 2 vs. 1; $p < 0.05$) and lower ASA level (MAR-IgG = 48% vs. 92%; $p < 0.01$). The pregnancy rate within a year after surgery was 2.8 times more frequent in couples with ASA-negative men: 39% (25 of 65) in the ASA-negative group compared to 14% (4 of 28) in the ASA-positive group ($p < 0.05$). Thus, antisperm immune response decreases the varicocelectomy efficacy for reproductive function recovery: the higher percentage of ASA and lower grade of varicocele are associated with an unfavourable prognosis.

INTRODUCTION

Varicocele is a highly prevalent condition in the infertile male population (WHO, 1992; Vital & Health Statistics, 2009; Kroese *et al.*, 2012; Miyaoka & Esteves, 2012). Controversy still remains regarding the benefit of varicocele repair to improve male fertility (Marmar *et al.*, 2007; Evers *et al.*, 2009; Nieschlag *et al.*, 2010; Jungwirth *et al.*, 2013). Evidence exists both in favour and against it, but as of now, most of specialty societies recognize that varicocele is detrimental to male reproductive health and its treatment may improve sperm function and chances of conceiving (Agarwal *et al.*, 2007; Baazeem *et al.*, 2011; Ficarra *et al.*, 2012; Kroese *et al.*, 2012; Lopushnyan & Walsh, 2012; Miyaoka & Esteves, 2012; Kim *et al.*, 2013). Despite the several different theories that aim to explain the impact of varicocele on testicular function, none can fully clarify the variable effect of varicocele on human spermatogenesis and male fertility. Proposed mechanisms include hypoxia and stasis, testicular venous hypertension, elevated testicular temperature, reflux of adrenal catecholamines, increased oxidative stress and autoimmunity (Marmar, 2001; Nagler & Grotas, 2009; Weinbauer *et al.*, 2010; Eisenberg & Lipshultz, 2011; Esteves, 2012).

The autoimmune reactions against the spermatozoa with the antisperm antibodies (ASA) production are one of the causes of infertility in men (WHO, 2000; Walsh & Turek, 2009; Nieschlag *et al.*, 2010; Wiser *et al.*, 2012). The ASA cause the sperm agglutination and decrease its motility as well as impair the sperm penetration into cervical mucus and hinder the fertilization of the ovum; the ASA can affect the fetal early development, implantation and gestation course (Mazumdar & Levine, 1998; Francavilla & Barbonetti, 2009; Krause, 2009; Walsh & Turek, 2009; Check, 2010). Recently we showed that immune infertility is found in 15% of varicocele patients. The immune infertility is accompanied by more expressed impairment of the sperm quality: lower concentration and motility, morphological changes, impaired acrosome reaction and DNA fragmentation. The possible pathogenetic mechanism of these damages are higher levels of reactive oxygen species in ASA-positive patients (Bozhedomov *et al.*, 2014).

Surgical treatment is the gold standard, and subinguinal microsurgical approach seems to offer the best results with fewer complications (Cayan *et al.*, 2009; Diegidio *et al.*, 2011; Ding *et al.*, 2012; Lopushnyan & Walsh, 2012). The reasons why fertility potential is not always improved are still obscure, and consistent data are lacking to determine prognostic factors that might help identify the best candidates for treatment (Nieschlag *et al.*, 1998; Redmon *et al.*, 2002; Nieschlag *et al.*, 2010; Miyaoka & Esteves, 2012). There is no general consensus on the efficacy of varicocele surgical treatment for male fertility recovery. There are multiple reports of varicocelectomy resulting in spermiogram improvement (Knudson *et al.*, 1994) and lower ASA titers (Mehrsai *et al.*, 2005; Djaladat *et al.*, 2006; Kendirci & Hellstrom, 2006), while several researchers challenge this (Sizyakin, 1996; Gubin *et al.*, 1998; Bonyadi *et al.*, 2013). The recent meta-analyses of Baazeem *et al.* (2011), Kroese *et al.* (2012), Kim *et al.* (2013) on the surgical treatment of patients with the male infertility factor did not consider ASA as a relevant factor in varicocelectomy.

The study objective was to evaluate ASA role in fertility recovery after varicocelectomy.

MATERIALS AND METHODS

Study population

At the initial stage of the multicentre cross-sectional study, 1639 male patients from infertile couples were examined according to the WHO (2000) recommendations: varicocele including subclinical forms was diagnosed in 524 (32.0%), ASA according to Sperm MAR test were found in 599 (36.5%). Varicocele patients underwent surgical (retroperitoneal, laparoscopic, microsurgical inguinal or subinguinal varicocelectomy) or conservative (carnitines, pentoxifylline, inosine, vitamins and antioxidant agents) treatment depending on the physician's and patient's choice. The present article includes the results of the longitudinal study of the microsurgical subinguinal varicocelectomy on 99 patients with clinically manifested varicocele. One-third of the patients had normozoospermia according to WHO (2010) criteria but underwent surgery since there were no evident signs of female infertility in their spouses and/or functional spermatozoa disorders were found (or suspected because of reactive oxygen species hyperproduction): acrosome reaction disorders, DNA fragmentation, autoimmune reaction against spermatozoa. The study procedures were approved by the Institutional Review Board; the written informed consent was obtained from all subjects.

The inclusion criteria for the study groups were as follows: the duration of involuntary infertility for at least 12 months, a regular sexual life not less than once a week without using a contraception, the age of the female partner under 35 and MAR-IgG $\geq$ 10% (more than 10% of sperm cells coated with IgG). MAR-IgG $\geq$ 50% was the criterion for 'immune infertility' diagnosis (WHO, 2010). The patients of the comparison group did not have ASA (MAR-IgG = 0%).

The exclusion criteria were as follows: evident causes of the female infertility (amenorrhoea, unovulation and bilateral tubal occlusion), ejaculation or sexual disorders that interrupt semen penetration into vagina, the infectious inflammatory processes of ancillary genital glands (leucocyte count more than 1 Mio/mL) in male subjects, the reproductive tract infections and marked oligozoospermia (sperm count less than 5 Mio/mL). Such a selection was aimed to increase the sensibility of the direct MAR test and to exclude the cases of the genetic hypogonadism.

Study design

ASA role was evaluated with two different methodological approaches:

- Retrospective analysis of pre-surgery data.

(a) In 3 months after surgery, the patients were retrospectively classified into two groups according to spermiogram changes: with (I) and without (NI) improvements, taking into the account presence and level of ASA. The improvement criterion was the increase in estimated 'sperm quality index' (the number of progressively motile spermatozoa in the ejaculate, calculated as volume (mL) $\times$ sperm concentration (Mio/mL) $\times$ proportion of progressively motile spermatozoa $\times$ proportion of morphologically normal spermatozoa) in comparison to pre-surgery values. In 3 months the increase in 'sperm quality index' was found in 75 patients (70%), decreased and unchanged index in 24 (30%). We compared baseline spermiogram parameters including ASA level in these groups.

(b) The same type of analysis was performed for the ASA-positive patients who responded to the surgery (decreased ASA level) (n = 16) and those without the autoimmune process reduction (n = 17). The evaluated baseline factors were: left-side varicocele grade, occurrence of bilateral varicocele and IgG ASA level.

- Prospective cohort study.

(a) We evaluated varicocelectomy results in the groups differing in the presence or absence of ASA-positive spermatozoa prior to the surgery: the group without ASA (n = 66) and the group with a diagnosed antisperm immune response prior to the surgery (n = 33). The observation period was 1 year, spermiogram and ASA tests were performed at the beginning of the treatment and 3 and 6 months after the surgery. The main outcome in the ASA-positive group was a favourable response to the surgery (ASA level decreased) vs. no decrease in the autoimmune process.

Methods used

The varicocele was diagnosed using the standard criteria (Jungwirth *et al.*, 2013). Based on the physical examination, the grade of the spermatic cord vein dilation was evaluated (0 – no dilation, 1+, 2+ and 3+). The backflow in the veins of spermatic cord and in pampiniform plexus was confirmed by the ultrasound examination. The subclinical forms of the disorder when the vein dilatation was not palpable or visible at rest or during Valsalva maneuver were diagnosed by a Doppler ultrasound test. The tests were performed using LOGIQ-5 and LOGIQ-9 (GE, Milwaukee, WI, USA) and Flex Focus 1202 (B-K Medical, Herlev, Denmark). The sperm evaluation was performed according to the WHO requirements (WHO, 2010).

The IgG-ASA-coated motile sperm counts were evaluated with mixed agglutination reaction test - Sperm MAR test. The direct MAR test (Hinting *et al.*, 1988) was performed on a microscope slide by mixing one drop (approximately 10 μL in volume) of fresh semen, one drop of latex particles coated with IgG and one drop of antiserum from rabbit against human IgG (SpermMar Kit, FertiPro, Belgium). The reactions were examined by phase contrast microscopy at 400×, and the percentage of motile spermatozoa carrying one or more latex particles was determined by the scoring of 100 motile spermatozoa. The results were read after 2–3 min and again after 10 min.

During the surgery, a 2.5-cm subinguinal incision was made and the testicle was delivered. Through the operating microscope at 10–20× magnification, internal spermatic veins were identified and ligated. Smaller veins were cauterized with an electrocautery. The testicular artery was identified using the microdoppler probe. We employed hydrodissection in identifying and isolating the testicular artery. The spermatic cord was then repeatedly examined until no veins other than deferential veins remained. The gubernaculum was also thinned sufficiently so that veins on both the sides could be identified and ligated. Testicular delivery was performed and external spermatic veins as well as gubernacular veins were ligated.

Statistical analysis

The data were processed with Statistica software package (StatSoft, Tulsa, OK, USA). Median, mean (M) and standard deviation (SD) were calculated; the differences' significance was assessed according with Student's *t*-test, Wilcoxon test, Chi-squared test and signed rank test.

RESULTS

There was no age difference in the group who demonstrated the increase in the number of progressively motile and morphologically normal spermatozoa in the ejaculate ($n = 75$) and in the group who did not ($n = 24$): 31.2 ± 14.3 and 32.2 ± 5.4 years, respectively ($p > 0.05$). The baseline of left-side varicocele grade was significantly higher in the group with improvements after the surgery compared to the group without improvements in the spermiogram: median – 2+, 25–75% = (1.2) and 1+, 25–75% = (1.2), respectively ($p = 0.02$), while no significant differences in the testicle size were found ($p > 0.05$). The standard spermiogram values differed only in concentration (Fig. 1; $p < 0.001$); the difference in the proportion of progressively motile and morphologically normal spermatozoa in the ejaculate was insignificant ($p > 0.05$). Mean MAR-IgG test results in the group with spermiogram improvement were 1.8 times lower: 11.1 ± 25.5% and 19.8 ± 35.3%; although the most results were outliers (median – 0%, 25–75% = (0.5) and 2%, 25–75% = (0.8), respectively), the differences were statistically significant (Fig. 1; $p = 0.03$). The proportion of patients with diagnosed ASA (MAR-IgG ≥ 10%) in the groups differed insignificantly (21% and 29%), but in the group with the spermiogram improvement after the surgery, the number of patients with active autoimmune process against the spermatozoa prior to the surgery (MAR-IgG ≥ 50% – immune infertility according to WHO (2010)) was 2.8 times less (9% and 25%, respectively; $p = 0.05$).

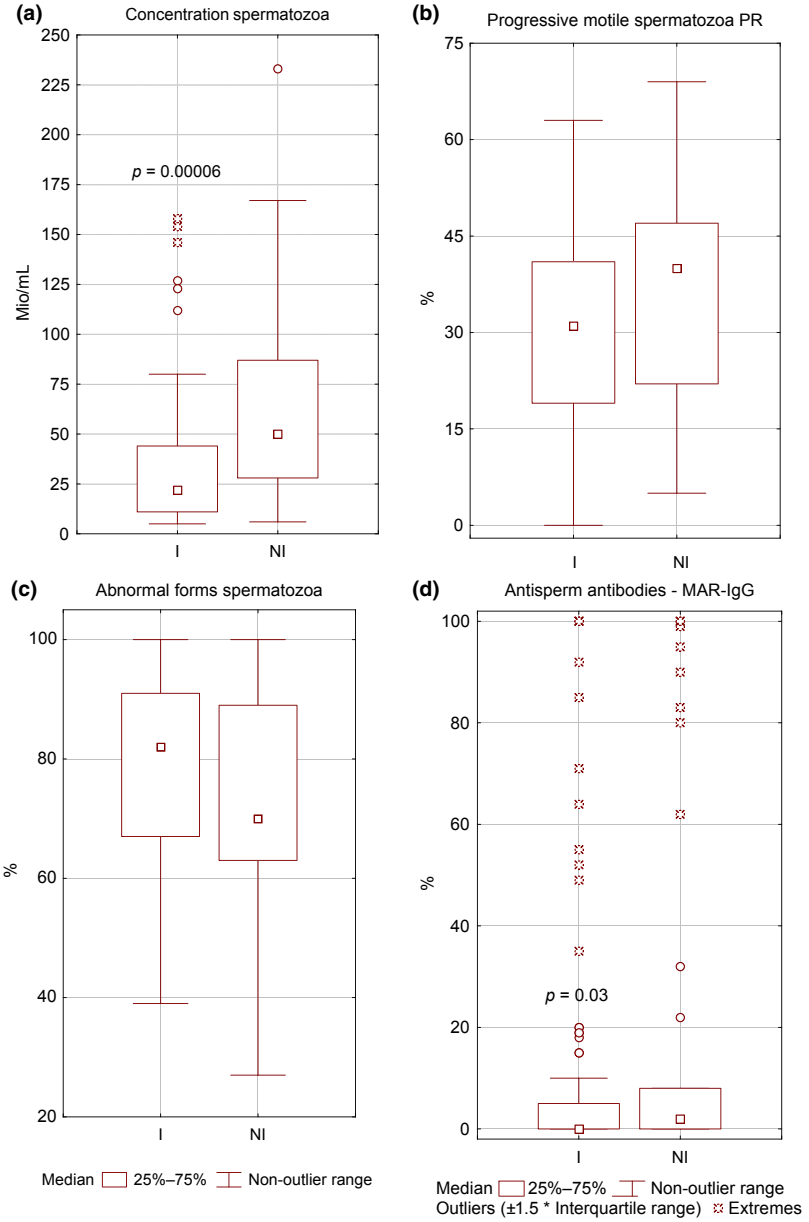
The presented data suggest that ASA is a unfavourable prognostic factor for the sperm improvement after varicocelectomy. It is supported by the next stage of empiric data analysis: the comparison of the surgery results in the selected groups differing in the ASA presence. The subjects' age both in the ASA-positive and the ASA-negative group did not differ significantly: 33 ± 5 and 31 ± 4 years, respectively ($p > 0.05$). The infertility was primary in 68% and 76% of cases, respectively ($p > 0.05$). No differences in left varicocele grade were found: the median grade was 1.5 (25–75% = 1.2) in the both groups ($p > 0.05$). There were no significant differences in the standard spermiogram baseline parameters both in ASA-positive and the ASA-negative group ($p > 0.05$; Tables 1 and 2). In the ASA-positive group, the involuntary infertility was longer: 51.5 ± 39.7 vs. 29.4 ± 27.9 months ($p < 0.01$). No age difference between spouses in both groups were found.

MAR-IgG decrease was found in 16 of 33 subjects (48%; $p > 0.05$), but the average group percentage of IgG ASA-positive spermatozoa in patients with antisperm immune reaction did not decrease during the 3 months period after the surgery (Fig. 2). Immune infertility was diagnosed in 61% of patients prior and 55% after the surgery showing no statistically significant difference ($p > 0.05$). Changes in the volume, concentration and morphology of the spermatozoa were also statistically insignificant (Table 1; $p > 0.05$). Statistically significant increase in the amount of spermatozoa with progressive motility ($p < 0.05$) and in the estimated number of progressively motile and morphologically normal spermatozoa in the ejaculate was demonstrated in 67% of patients ($p < 0.05$). However, the proportion of patients with oligo-, astheno- and teratozoospemia remained unchanged ($p > 0.05$).

The patients from ASA-positive group, who did not show the decrease in ASA level 3 months after the surgery ($n = 17$), were further additionally treated with proteolytic enzymes, antioxidants and carnitines. In the patients who demonstrated decrease in MAR-IgG within 3 months after varicocelectomy ($n = 16$), this parameter continued to improve further on: 11.2% decrease within 6 months (22% relative decrease, $p < 0.05$). However, the spermiogram parameters 6 months after the surgery showed multidirectional insignificant changes ($p > 0.05$).

The ASA-negative varicocele patients ($n = 66$) demonstrated substantially better results after the surgery (Table 2). In 3 months, the group average sperm count rose to 14 Mio/mL (35%; $p < 0.001$), the positive dynamic was found in 73% of patients ($p < 0.01$); as a result, the proportion of oligozoospermic patients decreased from 39% to 15% ($p < 0.01$). Sperm progressive motility increased by 8% (28% relative increase; $p < 0.001$) in 71% of cases ($p < 0.01$); the proportion of

Figure 1 Baseline spermiogram values in patients with (I) and without (NI) sperm quality improvement 3 months after the varicocelectomy: a – concentration of spermatozoa, b – progressive motile spermatozoa, c – abnormal forms of spermatozoa, d – MAR-IgG ASA. The improvement criterion was the increase in the number of progressively motile and morphologically normal spermatozoa in the ejaculate.



asthenospermic patients decreased from 56% to 35% ($p < 0.05$). The number of spermatozoa with morphologic defects decreased by 5% (6% relative decrease; $p < 0.05$). Total number of spermatozoa with normal morphology and progressive motility increased by 16.7 million (+150%; $p < 0.001$), these changes were found in 77% of cases ($p < 0.01$), the proportion of normospermic patients rose up to 53% ($p < 0.01$). In three previously ASA-negative subjects post-surgery examination revealed ASA on the spermatozoa (maximum MAR-IgG = 12%), however, the average group change was insignificant ($p > 0.05$).

Six months after the intervention, there was a two-fold elevation of the sperm concentration and motility that resulted in 2.5 times ($p < 0.001$) increase in number of motile spermatozoa in the ejaculate in 65% of cases ($p < 0.05$), together with 15% decrease in abnormal sperm count ($p < 0.05$).

The pregnancy rate within 1 year after surgery was 39% (25 of 65 followed up cases) in the ASA-negative group compared to 14% (4 of 28 followed up cases) in the ASA-positive group (2.8 times difference, $p < 0.05$).

The initial data of the ASA-positive patients who responded to the surgery (decreased ASA level) ($n = 16$) and those without the autoimmune process reduction ($n = 17$) were also analysed retrospectively (Table 3). The groups with (I) and without (NI) improvement differed in:

- the proportion of patients with more expressed palpable at rest left-side varicocele (grade 2): 56% (9 of 16) in I group compared to 18% (3 of 17) in NI group ($p < 0.05$);
- the occurrence of bilateral varicocele: 50% (8 of 16) in I group compared to 12% (2 of 17) in NI group ($p < 0.05$);

Table 1 Spermiogram values of patients with ASA (MAR-IgG > 10%) prior and 3 months after varicocelectomy, M ± SD, *n* and %

Record forms for semen analyses	Baseline prior surgery	After surgery	Number of patients with improvements, *n* (%)	The degree of parameter change, absolute (%)	Significance level, *p*			
					χ^2	Signs Z	Paired samples *t*-test	Wilcoxon
Volume, mL	3.87 ± 1.58	3.61 ± 1.16	14/33 (42.4)	−0.27 (−6.9)	–	ND	ND	ND
Concentration, Mio/mL	42.3 ± 33.3	51.1 ± 41.3	21/33 (63.6)	8.75 (+20.7)	–	ND	ND	ND
Oligozoospermia[a], *n* (%)	5/33 (15%)	6/33 (18%)	3/5 (60)	7.26 (+74)	ND	ND	–	–
Progressive motile PR, %	34.5 ± 15.9	41.3 ± 15.8	22/33 (66.7)	6.88 (+20.0)	–	ND	0.04	0.04
Asthenozoospermia[b], *n* (%)	14/32 (42)	13/33 (39)	8/14 (57)	19.07 (+50.0)	ND	ND	–	–
Abnormal forms, %	66.5 ± 24.6	69.8 ± 20.2	14/33 (42.4)	3.31 (+5.0)	–	ND	ND	ND
Teratozoospermia[c], *n* (%)	10/33 (30)	9/33 (27)	3/10 (30)	4 (−4.4)	ND	ND	–	–
Normozoospermia[d], *n* (%)	15/33 (45)	15/33 (45)	0/33 (0)	–	ND	ND	–	–
Total number of spermatozoa with normal morphology and progressive motility, Mio/ejaculate	16.7 ± 17.9	23.8 ± 24.3	22/33 (66.7)	7.1 (+42.5)	–	ND	0.038	0.015
MAR-test IgG, %	60.6 ± 32.4	56.7 ± 40.5	16/33 (48.5)	−3.8 (−6.4)	–	ND	ND	ND
Immune infertility (MAR-IgG ≥ 50%), *n* (%)	20/33 (61)	18/33 (55)	4/20 (20)	−5.45 (−6.7)	ND	ND	–	–

The patients with severe oligozoospermia (<5 Mio/mL) and infectious inflammation were excluded from the analysis. ND, the differences are statistically unreliable. [a]Oligozoospermia – concentration of spermatozoa below the lower reference limit (15 Mio/mL). [b]Asthenozoospermia – percentage of progressively motile (PR) spermatozoa below the lower reference limit (32%). [c]Teratozoospermia – percentage of morphologically normal spermatozoa below the lower reference limit (15%). [d]Normozoospermia – concentration of spermatozoa, and percentages of progressively motile (PR) and morphologically normal spermatozoa, equal to or above the lower reference limits.

Table 2 Spermiogram values of patients without ASA (MAR-IgG = 0%) before and 3 months after varicocelectomy, M ± SD, *n* and %

Record forms for semen analyses	Baseline prior surgery	After surgery	Number of patients with improvements, *n* (%)	The degree of parameter change, absolute (%)	Significance level, *p*			
					χ^2	Signs Z	Paired samples *t*-test	Wilcoxon
Volume, mL	3.6 ± 1.6	3.7 ± 1.7	23/66 (35)	0.12 (+3.3)	–	ND	ND	ND
Concentration, Mio/mL	39.7 ± 44.6	53.8 ± 47.1	48/66 (73)	14.0 (+35.3)	–	<0.01	0.004	0.0003
Oligozoospermia[a], *n* (%)	26/66 (39)	10/66 (15)	17/26 (65)	–	<0.01	ND	–	–
Progressive motile PR, %	30.3 ± 15.9	38.8 ± 17.7	47/66 (71)	8.4 (+27.6)	–	<0.01	0.0003	0.00003
Asthenozoospermia[b], *n* (%)	37/66 (56)	23/66 (35)	17/37 (46)	–	<0.05	ND	–	–
Abnormal forms, %	77.2 ± 15.4	72.8 ± 16.7	34/66 (51)	−4.6 (−5.9)	–	ND	0.023	ND
Teratozoospermia[c], *n* (%)	26/66 (39)	18/66 (27)	17/26 (65)	–	ND	ND	–	–
Normozoospermia[d], *n* (%)	18/66 (27	35/66 (53	17/66	–	<0.01	ND	–	–
Total number of spermatozoa with normal morphology and progressive motility, Mio/ejaculate	11.00 ± 20.3	27.4 ± 41.7	51/66 (77.3)	16.7 (+149.8)	–	<0.01	0.0014	0.000003
MAR-test IgG, %	0 ± 0	1.2 ± 2.8	3/19 (16)	1.2	–	ND	ND	ND

The patients with severe oligozoospermia (<5 Mio/mL) and infectious inflammation were excluded from the analysis. ND, the differences are statistically unreliable. [a]Oligozoospermia – concentration of spermatozoa below the lower reference limit (15 Mio/mL). [b]Asthenozoospermia – percentage of progressively motile (PR) spermatozoa below the lower reference limit (32%). [c]Teratozoospermia – percentage of morphologically normal spermatozoa below the lower reference limit (15%). [d]Normozoospermia – concentration of spermatozoa, and percentages of progressively motile (PR) and morphologically normal spermatozoa, equal to or above the lower reference limits.

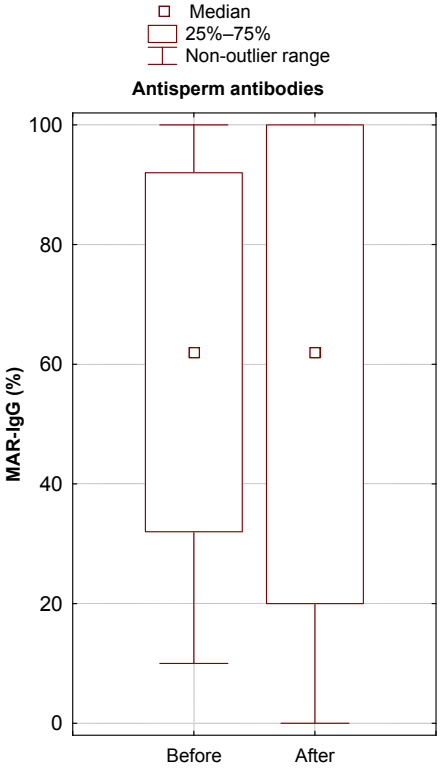
- IgG-ASA level prior to intervention: median value of 48% in I group compared to median value of 92% in NI group ($p < 0.01$).

DISCUSSION

Varicocele is traditionally considered a potentially correctable cause of male infertility (Agarwal *et al.*, 2007; Baazeem *et al.*, 2011; Ficarra *et al.*, 2012; Kroese *et al.*, 2012; Lopushnyan & Walsh, 2012; Miyaoka & Esteves, 2012; Kim *et al.*, 2013). The prevalent opinion is that microsurgical inguinal or subinguinal varicocelectomy is safest and most efficient in the terms of clinical complications and recurrence (Cayan *et al.*, 2009; Diegidio *et al.*, 2011; Ding *et al.*, 2012; Lopushnyan & Walsh, 2012). That is why in this study we used microsurgical sublingual ligation to correct varicocele in the male subjects from infertile couples. However, the surgery often fail to recover fertility and to improve sperm quality even without recurrence: the spermiogram improvement occurs in 60–70% with the pregnancy frequency of 30–40% (Agarwal *et al.*, 2007; Abdel-Meguid *et al.*, 2011; Baazeem *et al.*, 2011; Diegidio *et al.*, 2011; Miyaoka & Esteves, 2012). The results of the surgery are still unpredictable despite the adjustment for the varicocele grade, patient's age, infertility duration, gonadotropin and testosterone levels, etc. (Nieschlag *et al.*, 1998; Redmon *et al.*, 2002; Nieschlag *et al.*, 2010; Miyaoka & Esteves, 2012).

We showed earlier that the antisperm immune response in varicocele patients is associated with the decrease in

Figure 2 The percentage of progressively motile antisperm antibodies (ASA) covered spermatozoa in the patients with autoimmune reactions against spermatozoa prior and 3 months after varicocelectomy.



quantitative parameters of a standard spermogram and the sperm functional deficit. ASA-positive varicocele patients demonstrated a more significant decrease in the semen quality (concentration, total number of progressively motile spermatozoa) which correlated with the grade of spermatic cord veins dilatation (Bozhedomov *et al.*, 2014). The present study of varicocelectomy in 99 patients from infertile couples showed that the autoimmune reactions against spermatozoa is a factor capable to significantly affect the prognosis of fertility recover after the operation. The retrospective analysis of all operated patients data showed that the patients without spermiogram improvement after varicocelectomy had higher ASA levels. The presence of ASA worsened the prognosis of fertility recovery after the varicocelectomy. The ASA-positive sperm count decreased in half of the cases but the average changes in the group were statistically insignificant. The surgery itself did not stop the antisperm immune response. The unfavourable prognosis was associated with higher MAR-positive sperm counts and lower unilateral varicocele. And vice versa, in ASA-negative patients, varicocelectomy resulted in 2.5 times increase in the number of progressively motile and morphologically normal spermatozoa in the ejaculate. With the similar baseline quality parameters, the pregnancies in ASA-positive group were 2.8 times less frequent than in the ASA-negative group.

Comparing our data with the findings of the previous studies, one should pay attention that there are few publications about varicocelectomy on ASA-positive patients. Knudson *et al.* (1994) reported ASA levels in 32 infertile patients with varicocele. In this study, 28% had positive immunobead test results, among which IgG was found to be bound to the surface of the spermatozoa in 100% and IgA in 86% of the cases. The authors were not able to show any significant difference between pre- and post-varicocelectomy ASA concentrations. No correlation between ASA level and the surgical treatment of varicocele was found by Gubin *et al.* (1998). In his doctorate thesis, Sizyakin (1996) ligated dilatated veins in 146 infertile patients, 80% of them had sperm-agglutinating ASA in the concentration of more than 1:32. Sizyakin showed that immune factor of developed infertility makes the varicocelectomy practically inefficient: only 14% of spouses of infertile males became pregnant within 4-year observation period, compared to 13% of pregnant spouses in the similar group which had not underwent the operation. Djaladat *et al.* (2006) using the MAR test found a weak association between varicocele and ASA; moreover, they concluded that even though surgical treatment for varicocele may reduce the ASA level in some patients, it may increase it in other. The semen analysis of these patients showed that sperm morphology and count improved, whereas motility did not. In fact, ASA did not have much debilitating effect before the surgery. However, semen parameters showed improvement after surgery regardless of initial ASA concentration.

In the study of Bonyadi *et al.* (2013), the comparison of patients with ASA-positive serum before (13.6%) and after the intervention (21.7%) showed statistically significant difference. However, this was not true when comparing the patients with ASA-positive semen before (13.7%) and after the surgery (15.7%). Comparing patients who developed ASA after surgery with those who did not, it was shown that among sperm count, motility and morphology, only motility was significantly different. Comparison of patients with positive seminal versus serum ASA showed significant differences in the impaired sperm motility rates. However, the difference was insignificant in terms of sperm

Table 3 The initial varicocele grade and spermiogram parameters in the ASA-positive patients with or without ASA decrease after microsurgical subinguinal varicocelectomy, median (25–75%)

Parameter	Improvement (ASA decrease) (n = 16)	No improvement (no changes or ASA increase) (n = 17)	Mann–Whitney U-test, p
Sperm concentration, Mio/mL	23 (14.5; 68)	38 (33; 80)	ND
Progressive motility category A, %	12 (9; 21)	24 (10; 29)	ND
Progressive motility category B, %	12 (8; 20)	15 (13; 23)	ND
Sperm morphology – abnormal forms, %	69.5 (38.5; 89.5)	73 (54; 83)	ND
MAR-IgG, %	48 (20; 64)	92 (52; 100)	0.007
MAR-IgA, %	19.5 (5.5; 32)	41.5 (21; 71)	ND
Left-side varicocele grade, 1–2	2 (1; 2)	1 (1; 1)	0.043
Right-side varicocele grade, 0–1	0.5 (0; 1)	0 (0; 0)	0.029

ND, the differences are statistically unreliable.

morphology or counts. In other words, varicocelectomy can lead to an improved sperm count and morphology but motility is impaired when it is accompanied by positive ASA. This finding is consistent with other studies (Gilbert *et al.*, 1989; Mehrsai *et al.*, 2005).

Our data may explain the existing contradictions. Djaladat *et al.* (2006) recruited the patients with MAR 10-40%, Knudson *et al.* (1994) included patients with the expressed autoimmune process, Sizyakin (1996) evaluated ASA in the blood serum. Bonyadi *et al.* (2013) defined ASA-positive patients as any patient with ASA level above the threshold (>15%) regardless of absolute values. Therefore, Djaladat *et al.* (2006) concluded that varicocelectomy could reduce ASA concentration. Knudson *et al.* (1994) were not able to show any significant difference between pre- and post-varicocelectomy ASA concentrations. Bonyadi *et al.* (2013) showed that varicocelectomy had no effect on semen ASA. Although serum antibody count was shown to increase after varicocelectomy, sperm motility was not improved. The authors also indicated that varicocelectomy seems to have a beneficial effect on semen parameters in infertile men with varicocele.

As whole, it agrees with our findings. We demonstrated that there were neither significant MAR-IgG decrease nor the increase in concentration and morphological improvements in the group of patients with autoimmune reactions against spermatozoa. The proportion of the subjects with oligo-, astheno- and/or teratozoospermia and immune infertility according to WHO (MAR-IgG > 50%) did not change after the operation. We only found a significant 7% absolute increase in the average proportion of progressively motile spermatozoa in the group (+20% compared to the baseline level). Recent studies suggest that the increased motility is an important predictor of conception possibility (Baker *et al.*, 2013). The estimated average number of progressively motile and morphologically normal spermatozoa in the ejaculate in the group increased by 42% (from 16.7 to 23.8 Mio/ejaculate). Although 45% of ASA-positive patients after surgery demonstrated normozoospermia, only 14% of their spouses became pregnant within a year (4 of 28 remained under observation), that is 2.8 times less than in the ASA-negative group (25 of 65). It is not surprising: 3 months after the surgery, the ASA-negative group demonstrated increased concentration (+35%), progressive motility (+28%), proportion of normal forms (+6%) and estimated number of progressively motile and morphologically normal spermatozoa in the ejaculate (+150%); more than half of the patients (53%) had normozoospermia.

The recent data on the criteria for normal semen parameters suggest that there are no definite cut-off points for semen parameters to distinguish between fertile and infertile men, but fertility should be regarded as a continuum because higher semen parameters reflect a higher chance of pregnancy (Ford, 2010). The couples where the men have a clinical varicocele and mild oligozoospermia or normozoospermia achieve higher spontaneous pregnancy rates after varicocelectomy than couples with moderate-to-severe oligozoospermia (Kamal *et al.*, 2001; Richardson *et al.*, 2008).

Lower pregnancy rate in the couples of ASA-positive patients after the operation is evidently not connected with inadequate number of spermatozoa (45% of patients had normozoospermia in the post-operation period), nor with the sperm functional disorder. Nowadays, the sperm functional disorders, such as inadequate or preterm acrosome reaction, increased DNA fragmentation, etc., are the leading factors of decreased fertility in autoimmune reactions against spermatozoa (Francavilla & Barbonetti, 2009; Krause, 2009; Bozhedomov *et al.*, 2014; and others). This calls for additional research.

Our findings demonstrate that autoimmune reactions against spermatozoa are a significant factor of infertility in varicocele patients. The presence and the number of ASA should be taken into consideration along with other relevant factors when the decision on the necessity and expected efficacy of varicocelectomy in men from infertile couples is being made. Of note, the ASA-positive patients experienced a slight improvement in motility and number of normal sperm cells after surgery suggesting that more data might be required to evaluate its benefit for such patients. However, most urologists ignore the ASA presence when discussing the varicocele efficacy in infertile patients (Cayan *et al.*, 2009; Diegidio *et al.*, 2011; Baazeem *et al.*, 2011; Esteves, 2012; and others). It may result from the decrease in interest to the immune infertility on the part of the researchers. It is caused, on the one hand, by the IVF ICSI technology development which can overcome the infertility in such patients (Zini *et al.*, 2011), and on the other hand, by the fact that the pathogenesis of these conditions remains unclear (Mazumdar & Levine, 1998; Francavilla & Barbonetti, 2009; Walsh & Turek, 2009; Check, 2010) and by the lack of success of contraceptive vaccine which is based on the blocking effect of artificial ASA (Naz, 2011). The ASA-associated disorders, such as lower sperm motility, acrosome reaction impairment, hindered cervical mucus penetration, capacitation and oocyte fertilization failure that are described by many authors (Mazumdar & Levine, 1998; Walsh & Turek, 2009; Francavilla & Barbonetti, 2009; Check, 2010; and others), may not be important from the clinical point of view nowadays? We think that new data on the functional sperm disorders in ASA-positive patients including DNA fragmentation (Bozhedomov *et al.*, 2014) may explain the higher rate of spontaneous abortions in such couples which was found in the beginning of IVF use (Mazumdar & Levine, 1998; Krause, 2009), and may draw the interest of clinicians to this topic.

It must be kept in mind that ASA are frequent in varicocele patients: 25–40% of cases are detected in motile sperm count evaluation (Mazumdar & Levine, 1998; Francavilla & Barbonetti, 2009; Krause, 2009; Walsh & Turek, 2009). It is noteworthy that lack of ASA decrease after varicocelectomy supports the opinion that varicocele is not the immediate cause of the immune infertility as several authors believe (Walsh & Turek, 2009). There are data suggesting that varicocele is a co-factor increasing the risk of autoimmune reaction against spermatozoa if accompanied by additional damaging factors (e.g. testicular trauma) (Bozhedomov *et al.*, 2014).

It should be noted that the small sample size creates certain limitations to the study. The further research and analysis will be performed to evaluate the impact of the oxidative stress and the chromatin structure disorders in the immune infertility pathogenesis.

CONCLUSIONS

Antisperm immune response decreases the varicocelectomy efficacy for reproductive function recovery. The surgery depresses the autoimmune response against the spermatozoa

only in a half of cases. The higher percentage of ASA and lower grade of varicocele are associated with an unfavourable post-surgery prognosis.

CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

V.A.B. was involved in conception and design of the study, acquisition of data, analysis and interpretation of data, critical review of article for important intellectual content, editing the article; N.A.L. was responsible for acquisition of data/laboratory tests; R.A.A. was responsible for acquisition and analysis of data; L.M.A. provided acquisition and analysis of data; M.A.N. provided acquisition of data/laboratory tests; G.T.S. provided critical review of article and final approval of draft.

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