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Male antisperm antibodies: association with a modified sperm stress test and lipid peroxidation

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Summary. We previously reported a modified sperm stress test (MOST), low scores (<0.39) in which were associated with sperm-related abnormal *in vitro* fertilization. Preliminary observations suggested that the presence of male sperm antibodies (ASA) could give low MOST scores. It was therefore decided to undertake a study to verify this possible association and also to ascertain if such a relationship was causal in nature. Six hundred and fifty semen samples from patients consulting for infertility were assessed for basic seminal characteristics, motion parameters (CASA), ASA and MOST. Thirty-nine samples (6%) were ASA-positive. Samples with and without ASA showed similar characteristics, except for percentage of normal forms and MOST scores (0.35 ± 0.03 vs. 0.67 ± 0.01, $P<0.001$, for ASA-positive and -negative, respectively). There was a strong statistical association between presence of ASA and low MOST scores ($P<0.0001$). One-hundred per cent of ASA-positive samples displayed low MOST scores. To verify the nature of this relationship, we incubated ASA-free spermatozoa with ASA-positive and -negative (control) sera. Despite an increase in the percentage of ASA-bearing spermatozoa in those aliquots incubated with ASA-positive serum, their original (pre-incubation) MOST scores remained unchanged. Furthermore, the rate of lipid peroxidation, indirectly reflected in MOST scores, was not different in the aliquots incubated with ASA. In conclusion, there seems to be a strong association between presence of ASA and low MOST values in semen samples of infertile patients; however, the relationship does not appear to be causal.

Introduction

The fact that spermatozoa are immunogenic and can raise specific antibodies has been known since the late 1800s. The prevalence of antisperm antibodies (ASA) in the infertile population varies with the methodology employed to detect them, but generally falls between 5 and 15% (Haas *et al.*, 1980; Witkin, 1988). There is ample evidence that ASA can impair fertility (Mazundar & Levine, 1998), but the mechanisms by which they do so remain unclear.

Sperm membrane lipid peroxidation has also been suggested as a potential cause of male infertility (Aitken & Clarkson, 1987; Aitken *et al.*, 1989). Peroxidative damage of sperm membranes leads to sperm immobility and death (Alvarez & Storey, 1984). Alvarez *et al.* (1996) reported a correlation between a sperm 'stress test', a substitute for lipid peroxidation, motility loss and embryo implantation failure. Modifying this assay, our group described a simple sperm stress test (MOST) with potential to predict sperm-related abnormal *in vitro* fertilization (IVF) (Calamera *et al.*, 1998). A MOST cut-off of 0.39, established through ROC analysis, permits the identification of a subpopulation of infertile men with poor prognosis in standard IVF (i.e. $<50\%$ fertilization rate).

The objectives of the present study were, first, to verify a potential association between the presence of ASA bound to spermatozoa and low MOST scores (<0.39), and, secondly, to ascertain

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whether such a potential association was causal in nature.

Materials and methods

Patients ($n = 650$) consulting for infertility were referred to the Andrology Laboratory for semen analysis. Semen samples were collected by masturbation after 3 days of sexual abstinence and processed within 1 h of collection. All specimens were allowed to liquefy at room temperature and, following liquefaction, basal sperm concentration and motility were measured at 37 °C using a computer-aided sperm analyser (CASA, Cellsoft series 3000; Cryo Resources Ltd, Montgomery, NY), according to a previously established protocol (Calamera *et al.*, 1989).

The studied samples presented no significant sperm agglutination, pyospermia or hyperviscosity. The presence of ASA in the samples was determined by direct MAR (mixed antiglobulin reaction test) (IgG and IgA). In all cases, positive and negative controls were included. Reagents for ASA determination were obtained from Fertility Technologies Inc. (Natick, MA). The direct MAR test was performed by mixing fresh semen with latex particles that had been coated with IgG or IgA. A rabbit antiserum against human IgG or IgA was added to this mixture. Formation of agglutinates between particles and motile spermatozoa indicates the presence of immunoglobulins on the spermatozoa.

Ten microlitres of fresh semen, 10 µl of MAR test particles and 10 µl of antiserum were placed on a slide. The preparation was then mixed 5 times with the edge of a cover-slip. A 20×20 mm cover-slip was placed over the mixture and it was searched, under light microscopy using $40\times$ magnification, for particles attached to motile spermatozoa. One hundred spermatozoa were counted to determine the percentage of reactive spermatozoa. When no attachment of particles to spermatozoa was observed, a new observation 10 min later was performed, maintaining the preparation in a damp chamber. At the same time, after 30 min of liquefaction at room temperature, motile spermatozoa were isolated in Ham's F10 medium with 0.3% BSA, using a swim-up method (Harris *et al.*, 1981). These spermatozoa were used to perform the modified sperm stress test.

Prior to the MOST incubation, a 5-µl aliquot of sperm suspension was placed in a Makler chamber and analysed with CASA to determine per cent motility (initial motility). Polystyrene tubes containing the sperm suspension were incubated at 40 °C in a water bath for exactly 4 h. Following this incubation, another 5-µl aliquot was transferred to a Makler chamber and motility assessed again with CASA (final motility). More than 300 spermatozoa were studied both in the basal and post-incubation aliquots. MOST ratios were calculated by dividing final motility by initial motility.

Settings used during CASA analysis were: number of frames analysed per second = 20; frequency = 30 Hz; threshold velocity = 10 µm s^{-1}; minimum sampling for motility = 1 frame; minimum sampling for velocity = 4 frames, pixel scale = 0.688 µm. The following sperm parameters were obtained: (1) sperm concentration, (2) number of motile and nonmotile spermatozoa, (3) percentage of motile spermatozoa, (4) concentration of motile spermatozoa, (5) speed track (VCL), (6) linear velocity (VSL) and (7) linearity (LIN).

In order to ascertain the impact of exogenous ASA on MOST scores, experiments were performed ($n = 20$) using ASA-free normal spermatozoa. One hundred µl of sperm suspension obtained after swim-up was treated with 50 µl of ASA-positive serum or 50 µl of ASA-negative serum (control). Test tubes were heated at 37 °C for 2 h and the presence of immunoglobulins was detected using a direct MAR test. Samples were centrifuged and the supernatant was discarded and replaced with fresh Ham's F10 (100 µl). MOST was applied as described above.

In a similar experiment, 10 samples were studied to verify the impact of exogenous ASA on the lipid peroxidation rate of ASA-free normal spermatozoa. One hundred µl of sperm suspension obtained after swim-up was treated with 50 µl of ASA-positive or 50 µl of ASA-negative serum (control). Upon incubation, peroxides were assayed by the thiobarbituric acid reaction (TBA) with malondialdehyde (MDA) as the standard (Aitken *et al.*, 1989).

ASA-positive and -negative sera were acquired from Fertility Technologies Inc. We also used a pool of five ASA-positive sera from our own laboratory. No significant differences were observed in the outcome of the experiment, using either the commercial or our ASA-containing sera (data were pooled). The commercial serum produced 80% of spermatozoa positive for IgG and 40% for IgA, while our laboratory standard gave 85% of spermatozoa positive for IgG and 3% for IgA.

Statistical analysis of data was performed using INSTAT software (GraphPad, San Diego, CA).

Results

We studied 650 semen samples of patients referred to our Andrology Laboratory for a basic infertility work-up. Basic semen analysis as well as Kruger's strict morphology, CASA, MAR test, and MOST were performed on all samples. The concentration of seminal leukocytes was also assessed. Six hundred and eleven samples (94%) did not show ASA in a percentage of spermatozoa above the threshold at which they would be considered positive (Table 1). Thirty-nine samples (6%) turned out to be ASA-positive. Seventy-six per cent of spermatozoa from the latter samples were immunoreactive for IgG, while 52% showed membrane-bound IgA.

Samples with and without ASA displayed similar seminal characteristics, except for sperm morphology (Table 1). ASA-positive samples showed poorer morphology than ASA-negative samples (10.7% vs. 15.6% of normal forms; $P<0.05$). The only other parameter in which the two groups differed was the MOST results. ASA-negative samples showed, on average, normal MOST scores (0.67 ± 0.01; Table 1), while the MOST scores of ASA-positive samples were significantly lower (0.35 ± 0.03; $P<0.001$). Interestingly, breaking down ASA-positive and -negative groups according to MOST scores, 83% of ASA-negative samples showed normal MOST scores, while none of the ASA-positive samples were normal by MOST, i.e. 100% of the ASA-positive samples displayed low (<0.39) MOST scores (Table 2). There was a strong statistical association between presence of ASA and low MOST scores ($P<0.0001$, Fisher's exact test).

In order to determine whether there was a cause–effect relationship underlying this statistical association, we incubated ASA-free spermatozoa with commercial ASA-positive and ASA-negative sera and sera of patients with and without ASA. MAR tests and MOSTs were performed before and after incubation. Swim-up spermatozoa originally displaying either normal or low MOST scores were used in this experiment. Despite an increase in the percentage of spermatozoa bearing ASA in the aliquots incubated with ASA-positive serum, their original MOST scores remained unaltered after incubation (Table 3). This was observed regardless of the initial MOST score. In aliquots incubated with ASA-negative serum, there were changes in neither the percentage of ASA-bearing spermatozoa nor the original MOST scores.

To corroborate the lack of change in MOST scores after the exogenous addition of ASA using

Table 2. Distribution of ASA-positive and -negative samples according to normal and low MOST scores

	Number of samples (%)		
	ASA-negative	ASA-positive	Total
MOST <0.39	67 (11%)	39 (6%)	106 (17%)
MOST >0.39	544 (83%)	0 (0%)	544 (83%)
Total	611 (94%)	39 (6%)	650 (100%)

Fisher's exact test: $P<0.0001$. The association between presence of ASA and low MOST scores is highly significant.

Table 1. Selected seminal characteristics of ASA-positive and ASA-negative samples

	Units	ASA-negative samples ($n=611$)	ASA-positive samples ($n=39$)	Statistical significance (P)
Basic analysis				
Concentration	10^6 ml^{-1}	65.9 ± 5.4	68.2 ± 25	<0.95
Motility	%	37.6 ± 2.3	34.5 ± 4.0	<0.60
Morphology	%	15.6 ± 0.7	10.7 ± 1.6	<0.05
VCL	μm s^{-1}	38.9 ± 1.7	36.0 ± 2.0	<0.40
LIN	μm s^{-1}	4.0 ± 0.1	4.6 ± 0.2	<0.90
Specialized tests				
MOST	%	0.67 ± 0.01	0.35 ± 0.03	<0.001
IgG	%	8.1 ± 0.4	76.2 ± 4	<0.001
IgA	%	4.5 ± 0.4	52.0 ± 6.8	<0.001

Patients' semen samples were divided into ASA-positive and ASA-negative samples according to the percentage of spermatozoa bearing bound ASA. Seminal characteristics are presented for both groups and compared statistically with an unpaired t-test.
Data are expressed as mean ± standard error.
VCL = speed track; LIN: linearity; morphology = strict criteria (Kruger's).

Table 3. Effect of incubation of ASA-free spermatozoa with exogenous ASA on the percentage of antibody-bearing spermatozoa and their MOST scores

MOST groups	*n*	ASA-negative serum		ASA-positive serum	
		ASA-positive spermatozoa (%)	MOST scores	ASA-positive spermatozoa (%)	MOST scores
Low (<0.39)	8	5.8±0.8	0.25±0.04	79.8±5	0.25±0.03
Normal (>0.39)	12	4.2±1.0	0.83±0.04	88.0±5	0.85±0.04

Samples containing <10% ASA-positive spermatozoa (ASA-free spermatozoa), displaying normal (>0.39) or low (<0.39) MOST scores, were incubated with ASA-positive serum for 2 h at 37 °C, 5% CO_2, in Ham's F10+0.3% BSA. The percentage of ASA-positive spermatozoa and MOST scores was assessed before and after the incubation. Data represent values after the incubation.

a different endpoint, we evaluated the production of MDA, the end result of lipid peroxidation, under similar experimental conditions, i.e. incubation of normal ASA-free spermatozoa with ASA-positive and -negative sera. No significant differences were seen in the production of MDA by spermatozoa incubated with exogenous ASA or control Ig (Fig. 1). Conversely, the percentage of ASA-bearing spermatozoa increased significantly in the former group.

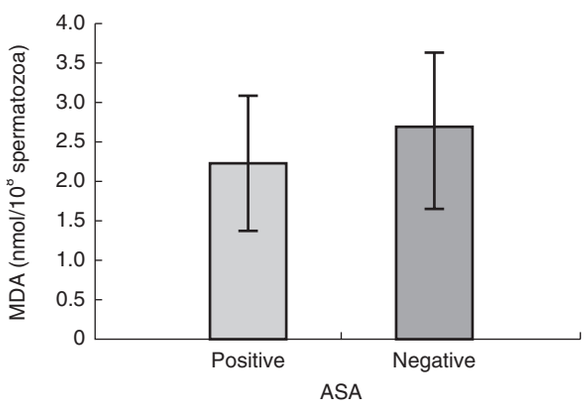

Figure 1. Malondialdehyde (MDA) production after forced lipid peroxidation of ASA-free spermatozoa incubated with ASA-positive and -negative sera for 2 h at 37 °C. Data are expressed as mean (bars)±SE (errors), $n=10$. No statistically significant differences between the two groups were found (paired *t*-test).

Discussion

Antisperm antibodies are prevalent in the infertile male population (Bronson *et al.*, 1984; Clarke *et al.*, 1985). Several hypotheses have been postulated to explain their formation (Mazundar & Levine, 1998): a breach in the blood–testis barrier that results in exposure of immunogenic sperm antigens (Alexander & Anderson, 1979; Mandelbaum *et al.*, 1987), mechanical obstruction of the genital tract due to congenital anomaly, vasectomy or trauma with the consequent extravasation of spermatozoa (Linnet, 1983; Matsuda *et al.*, 1992; Vazquez-Levin *et al.*, 1994), and infection and inflammation of the genital tract and accessory glands (Muñoz & Witkin, 1995; Witkin *et al.*, 1995). All these explanations imply contact between the immune system and sperm antigens, and development of an autoimmune response. Even more intriguing is the mechanism(s) by which ASA mediate infertility. They may adversely affect sperm maturation (Dimitrov *et al.*, 1994), prevent normal sperm penetration of the cervical mucus (Menge & Beitner, 1989; Eggert-Kruse *et al.*, 1991; Kremer & Jager, 1992; Steen *et al.*, 1994), decrease sperm motility (Mathur *et al.*, 1988; Menge & Beitner, 1989), and/or impair sperm–oocyte interaction (Bronson *et al.*, 1981; Mahony & Alexander, 1991; Fann & Lee, 1992). Benoff *et al.* (1993) suggested that ASA binding increased free cholesterol in membranes of capacitating spermatozoa, thereby preventing the membrane fluidity changes needed for putative zona-binding receptor expression.

The time at which human spermatozoa lose motility *in vitro* has been determined, and provides a good estimate of the inverse of the rate of lipid peroxidation in those spermatozoa (Alvarez *et al.*, 1987). Based on this estimate, Alvarez *et al.* (1996) developed a sperm assay that measures motility loss mainly as a result of peroxidative damage. We found a modification of this assay to be a good predictor of sperm-related abnormal IVF (Calamera *et al.*, 1998). The MOST is based on the percentage of motility loss after 4 h of incubation at 40 °C. The rate of lipid peroxidation, provided there is a constant adequate O_2 concentration, is linear over a range of temperature between 34 and 40 °C (Alvarez & Storey, 1985).

In the present study, a significant association between sperm-bound ASA and low MOST scores, which have previously been correlated

with low fertilization potential (Calamera *et al.*, 1998), was observed. All the ASA-positive samples showed low MOST scores (< 0.39), whereas only 11% of ASA-negative samples did so. In view of this finding, we hypothesized that either ASA were the cause of abnormal MOST when present, or low MOST scores and the presence of ASA were two epiphenomena underlain by a common mechanism such as a plasma membrane defect. In order to test these hypotheses, we assessed the MOST and lipid peroxidation of normal, ASA-free spermatozoa incubated with ASA-positive and ASA-negative human sera. Neither the MOST nor the MDA values were significantly different in these two groups, despite a significant increase in the percentage of ASA-bearing sperm in the samples incubated with ASA-positive serum. A small experiment with a prolonged incubation (3 h) produced the same results. Other authors have used the addition of exogenous ASA to prove their action on sperm function under various specific experimental conditions (Bronson *et al.*, 1981; Francavilla *et al.*, 1991; Benoff *et al.*, 1993; Tasdemir *et al.*, 1995).

Our results appear to disprove a causal relationship between ASA and abnormal MOST. However, it is worth noting that, *in vivo*, the antibodies may enter the genital tract at different sites and be in contact with spermatozoa while spermatozoa undergo crucial structural and functional changes such as those occurring during epididymal maturation. This could lead to an alteration of the oxidative/-antioxidative systems in the spermatozoa, which, in turn, would determine the peroxidative potential of those spermatozoa, and consequently their performance in the MOST. Ollero *et al.* (2000) recently reported a decrease in the docasahexanoic acid (DHA) content in human spermatozoa during sperm maturation. They suggested that this event might be part of genomically regulated maturational steps which take place in the epididymis. If these events do not occur, immature spermatozoa in the ejaculate would exhibit cytoplasmic retention and a high rate of lipid peroxidation (Aitken *et al.*, 1994; Huszar & Vigue, 1994). One of the important physiological consequences of the decrease in DHA content during sperm maturation is that removal of DHA from the human sperm membrane during this process decreases the susceptibility of spermatozoa towards oxidative damage (Alvarez & Storey, 1995).

As mentioned before, ASA and abnormal MOST scores may not be causally related but instead be linked by a common phenomenon such as a plasma membrane defect. This would lead to the abnormal release of sperm antigens during their genesis or transport through the genital tract with the consequent generation of ASA, as well as the abnormal maturation of the membrane-related systems involved in lipid peroxidation.

Further investigation of the observed association between ASA, MOST and lipid peroxidation is warranted.

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References

Aitken JR, Clarkson J (1987) Cellular basis of defective sperm function and its association with the genesis of reactive oxygen species by human spermatozoa. J Reprod Fertil 81:459–469.

Aitken JR, Clarkson JS, Fishel S, (1989) Generation of reactive oxygen species, lipid peroxidation and human sperm function. Biol Reprod 40:183–187.

Aitken JR, Krausz C, Buckingham D (1994) Relationships between biochemical markers for residual sperm cytoplasm, reactive oxygen species generation, and the presence of leukocytes and precursor germ cells in human sperm suspensions. Mol Reprod Dev 3:268–279.

Alexander NJ, Anderson DJ (1979) Vasectomy: consequences of autoimmunity to sperm antigens. Fertil Steril 32:253–260.

Alvarez JG, Storey BT (1984) Assessment of cell damage caused by spontaneous lipid peroxidation in rabbit spermatozoa. Biol Reprod 30:323–332.

Alvarez JG, Storey BT (1985) Spontaneous lipid peroxidation in rabbit and mouse epididymal spermatozoa: dependence of rate on temperature and oxygen concentration. Biol Reprod 32:342–351.

Alvarez JG, Storey BT (1995) Differential incorporation of fatty acids into and peroxidative loss of fatty acids from phospholipids of human spermatozoa. Mol Reprod Dev 42:334–346.

Alvarez JG, Touchstone JC, Blasco L, Stone BT (1987) Spontaneous lipid peroxidation and production of hydrogen peroxide and superoxide in human spermatozoa. Superoxide dismutase as major enzyme protectant against oxygen toxicity. J Androl 8:338–348.

Alvarez JG, Minaretzis D, Barrett CB, Mortola JF, Thompson IE (1996) The sperm stress test: a novel test that predicts pregnancy in assisted reproductive technologies. Fertil Steril 2:400–405.

Benoff S, Cooper GW, Hurley I, Mandel FS, Rosenfeld DL (1993) Antisperm antibody binding to human sperm inhibits capacitation induced changes in the levels of plasma membrane sterols. Am J Reprod Immunol 30:113–130.

Bronson RA, Cooper GN, Rosenfeld DL (1981) Ability of antibody-bound human sperm to penetrate zona-free hamster ova *in vitro*. Fertil Steril 36:778–783.

Bronson R, Cooper G, Rosenfeld D (1984) Sperm antibodies: their role in infertility. Fertil Steril 42:171–183.

Calamera JC, Brugo Olmedo S, Quirós MC, Nicholson RF (1989) Computer assisted measurement in normal and pathological human semen, fresh and post swim up technique. Andrologia 21:340–345.

Calamera JC, Doncel GF, Brugo Olmedo S, Kolm P, Acosta AA (1998) Modified sperm stress test: a simple assay that predicts sperm-related abnormal *in vitro* fertilization. Hum Reprod 13:2484–2488.

Clarke GN, Elliot PJ, Smaila C (1985) Detection of sperm antibodies in semen using the immunobead test: a survey of 813 consecutive patients. Am J Reprod Immunol 7:118–123.

Dimitrov DG, Urbanek V, Zverina J, Madar J, Nouza K, Kinsky R (1994) Correlation of asthenozoospermia with increased antisperm cell-mediated immunity in men from infertile couples. J Reprod Immunol 27:3–12.

Eggert-Kruse W, Hofsab A, Haury E, Tilgen W, Gerhard I, Runnebaum B (1991) Relationship between local antisperm–mucus interaction *in vitro* and *in vivo*. Hum Reprod 6:267–276.

Fann CH, Lee CYG (1992) Monoclonal antibodies affecting sperm-zona binding and/or zona-induced acromosome reaction. J Reprod Immunol 21:175–187.

Francavilla F, Romano R, Santucci R (1991) Effect of sperm-antibodies on acrosome reaction of human sperm used for the hamster egg penetration assay. Am J Reprod Immunol 25:77–80.

Haas GG, Cines DB, Schreiber AD (1980) Immunologic infertility: identification of patients with antisperm antibodies. N Engl J Med 303:722–727.

Harris SJ, Milligan MP, Masson GM, Dennis KJ (1981) Improved separation of motile sperm in asthenospermia and its application to artificial insemination homologous (AIH). Fertil Steril 36:219–221.

Huszar G, Vigue L (1994) Correlation between the rate of lipid peroxidation and cellular maturity as measured by creatine kinase activity in human spermatozoa. J Androl 1:71–77.

Kremer J, Jager S (1992) The significance of antisperm antibodies and sperm mucus interaction. Hum Reprod 7:781–784.

Linnet L (1983) Clinical immunology of vasectomy and vasovasectomy. Urology 22:101–114.

Mahony MC, Alexander NJ (1991) Sites of antisperm antibody action. Hum Reprod 6:1426–1430.

Mandelbaum SL, Diamond MP, DeCherney AH (1987) The impact of antisperm antibodies on human infertility. J Urol 138:1–8.

Mathur S, Rosenlund C, Carlton M, Caldwell J, Barber M, Rust PF, Williamson HO (1988) Studies on sperm survival and motility in the presence of cytotoxic sperm antibodies. Am J Reprod Immunol Microbiol 17:41–47.

Matsuda T, Hori Y, Yoshida O (1992) Unilateral obstruction of the vas deferens caused by childhood inguinal herniorrhaphy in male infertility patients. Fertil Steril 58:609–613.

Mazundar S, Levine S (1998) Antisperm antibodies: etiology, pathogenesis, diagnosis and treatment. Fertil Steril 70:799–810.

Menge AC, Beitner O (1989) Interrelationships among semen characteristics, antisperm antibodies, and cervical mucus penetration assays in infertile human couples. Fertil Steril 51:486–492.

Muñoz MG, Witkin SS (1995) Autoimmunity to spermatozoa, asymptomatic *Chlamydia trachomatis* genital tract infection and y8 T lymphocytes in seminal fluid from the male partners of couples with unexplained infertility. Hum Reprod 10:1070–1074.

Ollero M, Powers RD, Alvarez JG (2000) Variation of docosahexaenoic acid content in subsets of human spermatozoa at different stages of maturation: implications for sperm lipoperoxidative damage. Mol Reprod Dev 3:326–334.

Steen Y, Forssman L, Lonnerstedt E, Jonasson K, Wassen AC, Lycke E (1994) Anti-sperm IgA antibodies against the equatorial segment of the human spermatozoa are associated with impaired sperm penetration and subfertility. Int J Fertil 39:52–56.

Tasdemir I, Tasdemir M, Fukuda J, Kodama H, Matsui T, Tanaka T (1995) Effect of sperm-immobilizing antibodies on the spontaneous and calcium-ionophore (A23187)-induced acrosome reaction. Int J Fertil 40:192–195.

Vazquez-Levin MH, Kupchik GS, Torres Y, Chaparro CA, Shtainer A, Bonforte RJ, Nagler HM (1994) Cystic fibrosis and congenital agenesis of the vas deferens, antisperm antibodies and CF-genotype. J Reprod Immunol 27:199–212.

Witkin SS (1988) Sperm-reactive antibodies as measured by enzyme linked immunosorbent assay. In: Perspectives in Immunoreproduction: Conception and Contraception. Mathur S, Fredricks CM (eds) Hemisphere Publishing Co., Washington, pp 93–101.

Witkin SS, Kligman I, Bongiovanni AM (1995) Relationship between asymptomatic male genital tract exposure to *Chlamydia trachomatis* and an autoimmune response to spermatozoa. Hum Reprod 10:2952–2955.