Incidence of Sperm Surface Autoantibodies and Relationship with Routine Semen Parameters and Sperm Kinematics

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Keywords
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Problem
Antisperm antibodies (ASA) are associated with male subfertility. However, results on sperm surface autoantibodies are controversial, the relationship between ASA and semen parameters (WHO, 2010) is unknown, and data on ASA and sperm kinematics are scarce.

Method of study
A retrospective study carried out in men undergoing routine semen analysis (WHO 2010), ASA evaluation (direct SpermMAR™ (IgG) test), and computer-assisted sperm analysis (CASA).

Results
A 2.6% and a 5.9% incidence of ASA-positive cases were found (cut-off 50% and 10%, respectively; $n = 7492$). ASA-positive samples had lower ($P < 0.0001$) sperm concentration, count, motility, and hypo-osmotic swelling (HOS) test score. HOS results did not correlate with sperm vitality in normozoospermic samples with high ASA levels. In unselected samples, ASA-positive samples (cut-off 50%) showed decreased sperm kinematics (VSL, VAP, LIN, ALH, STR, BCF, WOB), but in normozoospermic samples, ASA-positive and ASA-negative subgroups had similar CASA results.

Conclusions
ASA evaluation is highly relevant in full semen assessment.

Introduction

It has been well established that antisperm antibodies (ASA) can interfere with mammalian fertilization, leading to the so-called 'immunological infertility'. The presence of ASA has been linked to impaired conception, having deleterious effects on sperm function, gamete interaction, embryonic development, and implantation.[1–3] Despite its relevance, ASA etiology has not been fully characterized.

Male ASA have been mainly associated with genital tract trauma, inflammation and infection, as well as surgical intervention; antibodies against sperm antigens were detected in the sperm plasma membrane, as well as in serum and seminal plasma.[4–8] The World Health Organization (WHO fifth manual)[9] recommends evaluating ASA by means of either the mixed antiglobulin reaction (MAR) test or the immunobead (IB) test. In particular, a cut-off value of 50% of motile spermatozoa with ASA was

established as clinically relevant.[9] Using these procedures, a 5–39% incidence of sperm surface autoantibodies has been reported in men suspected of infertility.[10–18]

Several researches described the relationship between presence of sperm surface autoantibodies and alterations in routine semen parameters, but most of these publications involved a relatively low number of ASA-positive cases. A recently published systematic review compiled eight independent studies in a total of 238 ASA-positive and 929 ASA-negative samples.[19] Reports included in this analysis came from studies carried out in compliance with the 1999 WHO guidelines.[20] Until present, no similar analysis has been published using current WHO guidelines for semen evaluation.[9]

The presence of ASA has been associated with diminished sperm motility[21–24] and altered sperm membrane integrity.[25,26] With regard to sperm motility, the use of computer-assisted sperm analysis (CASA) has helped to assess sperm movement characteristics, and the results were related to the outcome of intrauterine insemination and *in vitro* fertilization.[27–31] However, information on the impact of sperm surface autoantibodies upon sperm kinematics is still limited and inconclusive.[16,18,32–34]

In a retrospective systematic study based on results obtained within a large group of patients attending an Andrology Laboratory in Argentina, a thorough analysis was carried out aimed at (1) determining the incidence of sperm surface autoantibodies, (2) establishing the association between ASA and semen parameters evaluated in compliance with WHO 2010 guidelines,[9] and (3) assessing the relationship between presence of ASA and sperm movement characteristics determined by CASA.

Materials and methods

Patients

Semen samples were obtained from adult men (>18 y.o.) attending the Laboratorio de Andrología y Reproducción (LAR, Córdoba, Argentina) for full semen assessment (routine semen analysis, ASA and CASA evaluation), as part of a basic clinical andrology evaluation. Data included in this report were retrieved, under patients' written consent, from reports of a total of 9482 samples provided by 7492 men evaluated between July 2010 and September 2015.

Semen Analysis

Semen samples were collected by masturbation after 2–7 days of sexual abstinence. When necessary, samples were transported to the laboratory at ~37°C. In all cases, samples were analyzed within the hour.

After liquefaction, semen analysis was performed following WHO guidelines,[9] unless otherwise indicated. Seminal volume was determined with a graduated conical tube. Sperm concentration and progressive motility (former Grade a + Grade b) were assessed by conventional methods in a Makler counting chamber (Sefi-Medical Instrument, Haifa, Israel). Sperm count was calculated by multiplying sperm concentration and volume of the whole ejaculate. Sperm vitality was determined with the supravital Eosin Y staining. In addition, the hypo-osmotic swelling (HOS) test was performed by incubating spermatozoa in a hypo-osmotic solution, to determine the percentage of membrane-intact (osmotically competent) spermatozoa.[9] Sperm morphology analysis was carried out in samples subjected to Papanicolaou staining and assessed according to Kruger's strict criteria.[35] The concentration of round cells in semen was evaluated using the Makler chamber. Peroxidase-positive cells (PPC; predominantly neutrophils) were identified by means of a colorimetric assay.[36]

All sperm parameters were determined in at least 200 cells in duplicate slides. The WHO lower reference limit (LRL)[9] values were used to define normality (1.5 mL for semen volume; 15 million/mL for sperm concentration; 39 million for sperm count; 32% for sperm progressive motility; 58% for sperm vitality and for HOS test score; 4% for sperm morphology; 1 million/mL for round cells; 1 million/mL for PPC).

In all cases, routine semen parameters and ASA were evaluated by an expert operator, a professional monitored by an in-house laboratory quality assurance protocol (control of monthly means and intra/interoperator variation coefficient) and by an external quality control procedure (accuracy assessment, detection limit, and measuring range) performed at the University of Buenos Aires (Argentina).

Direct SpermMAR™ Test

The presence of sperm surface autoantibodies was assessed using the direct SpermMAR™ test (FertiPro N.V., Beernem, Belgium). Briefly, 10 μL of

homogenized fresh semen was placed on a microscope slide and mixed with 10 μL of the IgG suspension. Next, the 'bridging' antibody (anti IgG) was added for IgG assessment, as indicated by the manufacturer. The drop was covered with a coverslip (22 mm × 22 mm) and, after a 1-min incubation, the preparation was analyzed with a phase-contrast microscope (CX31, Olympus, Tokyo, Japan) at 400 × magnification. The evaluation was repeated after 3 min. Negative and positive controls were included in the assays. At least 200 motile spermatozoa were scored, and the percentage of cells with attached beads was determined.[9] Cut-off values were established at 10% and 50% motile spermatozoa with adhered particles.

Computer-Assisted Sperm Analysis (CASA)

Sperm motility parameters were evaluated using the Integrated Sperm Analysis System ISAS v1 (Proiser R&D, Valencia, Spain). The equipment analyzes 30 frames per second (s).[37] With the aid of a temperature-controlled stage (Proiser R&D), spermatozoa were maintained at constant 37°C during motility assessment. In each sample, at least five microscopic fields were analyzed, and over 300 motile spermatozoa were evaluated. Parameters measured were curvilinear velocity (VCL; μm/s), straight-line velocity (VSL; μm/s), average path velocity (VAP; μm/s), linearity (LIN; arbitrary units, expressed as percentage), amplitude of lateral head displacement (ALH; μm/s), straightness (STR; arbitrary units, expressed as percentage), beat cross frequency (BCF; Hz), mean angular displacement (MAD; degrees), and wobble (WOB; arbitrary units, expressed as percentage).[9]

Statistical Analysis

Data were conveyed as Mean ± Standard Deviation of the Mean (SDM). Comparison of ASA incidence for unselected and normozoospermic (normal sperm concentration, motility, and morphology) sperm populations was made using the chi-square test. Results on routine semen parameters and CASA evaluations in ASA-positive and negative samples were compared by the non-parametric Mann–Whitney test. ROC (receiver-operating characteristic) curves and Spearman correlation analyses were also carried out. Differences between groups were considered statistically significant at a $P < 0.05$. Statistical analyses were carried out using the GraphPad InStat program (GraphPad Software, San Diego, CA, USA).

Results

ASA Incidence

ASA incidence was determined in a total of 7492 men. Semen samples were evaluated by the direct SpermMAR™ test, finding 195 cases with ≥50% motile spermatozoa carrying particles, a 2.6% incidence of surface ASA. Using a 10% cut-off value, a total of 441 cases were classified as ASA-positive (a 5.9% incidence of ASA).

In a subset of semen samples depicting normal sperm concentration, motility, and morphology ($n = 4593$), a 2.0% (cut-off 50%) incidence and a 4.9% (cut-off 10%) incidence of sperm surface autoantibodies were determined. Rates were significantly lower than those found in the unselected population ($P = 0.0237$ and $P = 0.0240$, respectively).

Relationship between ASA and Routine Semen Parameters

A subsequent set of studies analyzed the association between results on surface ASA and routine semen parameters. Findings obtained for all semen parameters are presented in Table I. Considering the established LRL, results can be summarized as follows: 12% of the samples had abnormal semen volume; ~16% of the cases had abnormal sperm concentration and count; over 20% had abnormal sperm motility, and over 33% had abnormal sperm morphology. Overall, a high proportion of spermatozoa were viable in these samples, as can be concluded by the high percentage of sperm vitality and of membrane-intact sperm cells (assessed by HOS test). With regard to round cells and PPC, abnormal values appeared in over 40% and over 9% of the samples, respectively.

Samples were first grouped based on the ASA scores (cut-off 50%), and average values of semen parameters were compared between ASA-positive (86.8 ± 14.1%, 51–99%; mean ± SDM, range) and ASA-negative (2.4 ± 4.2%, 0.1–48.0%) groups. In the ASA-positive subgroup, a lower sperm concentration and count, as well as a reduced percentage of motile and membrane-intact spermatozoa, were observed ($P < 0.0001$) when compared to the

Table I Semen Parameters and MAR Test Results in the Studied Population

Semen parameter[a]	Mean ± SDM	Range	Incidence of abnormal cases (%)
Semen volume (mL semen)	3.1 ± 1.5	0.1–15.2	12.0
Sperm concentration (million spermatozoa/mL)	61.0 ± 53.8	0.02–661.0	15.8
Sperm count (million spermatozoa)	171.4 ± 162.9	0.02–1775.9	16.2
Sperm motility (% progressive spermatozoa)	46.3 ± 18.4	0–89	22.3
Sperm vitality (% live spermatozoa)	83.1 ± 9.3	1–100	0.6
Sperm morphology (% normal forms)	5.4 ± 3.4	0–26	33.2
Round cells (million/mL cells)	1.2 ± 1.6	0.05–71.0	40.5
Peroxidase-positive cells (million/mL cells)	0.4 ± 1.3	0.0–51.1	9.9
HOS test score (% membrane-intact spermatozoa)	78.9 ± 8.7	0.1–98.0	0.4

[a]Total samples evaluated: 9482.

ASA-negative group (Fig. 1). In addition, the ASA-positive subgroup was found to have decreased sperm vitality and round cell concentration, although differences were less significant ($P = 0.0070$ and $P = 0.0293$, respectively). Similar results were obtained when the cut-off value was 10% (ASA-positive = 52.5 ± 35.0%, 10–99%; ASA-negative = 1.8 ± 1.4%, 0.1–9.0%) (Table II), with the exception of round cell concentration, which showed no difference between ASA-positive and ASA-negative samples. Based on these findings, a correlation analysis was performed between ASA and sperm concentration, count, motility, HOS scores, and vitality results. As shown in Fig. 2, a significant association was observed between these parameters. However, no correlation was observed between sperm vitality and ASA values ($P = 0.3588$).

The relationship between ASA and sperm concentration, count, motility, and membrane integrity (HOS test) was confirmed using the ROC curves; this analysis revealed a significant correlation ($P < 0.0001$) between ASA and those semen parameters. In addition, sperm vitality was identified among ASA-related semen parameters, but at a lower significance level ($P < 0.001$) (Table III). Further analysis on ASA-positive (cut-off 50%) samples showing decreased sperm motility ($n = 128$) revealed a reduced sperm concentration and abnormal HOS test scores in 50.8% (65/128) and 80.5% (103/128) of those evaluations, respectively. The three parameters had abnormal values in only one ASA-positive sample.

Studies presented in Fig. 1 revealed significantly lower HOS test scores in the ASA-positive samples than in the ASA-negative ones. Among them, certain samples showed abnormalities in sperm concentration, motility, and morphology that could impact on the sperm ability to respond to an osmotic shock. To overcome this situation, the association between HOS and ASA values was re-evaluated in a subgroup of samples extracted from the total database and showing normal concentration, motility, and morphology ($n = 5442$ cases). HOS test scores were lower in ASA-positive samples (50% cut-off) when compared to ASA-negative samples (78.7 ± 6.5% versus 80.9 ± 6.4%, respectively; $P < 0.0001$). In contrast, sperm vitality was similar in both groups (85.5 ± 0.5% versus 86.2 ± 0.1%, respectively). In line with these results, no correlation was found between sperm vitality and HOS scores in the ASA-positive samples ($R = 0.02988$; $P = 0.052$).

Relationship between ASA and Sperm Kinematic Results

As sperm progressive motility was found significantly decreased in ASA-positive samples, it was of interest to analyze the relationship between ASA and motility characteristics by means of a computerized system. A total of 2838 samples were included in this part of the study, and details on semen parameters in this subpopulation are depicted in Table IV. When

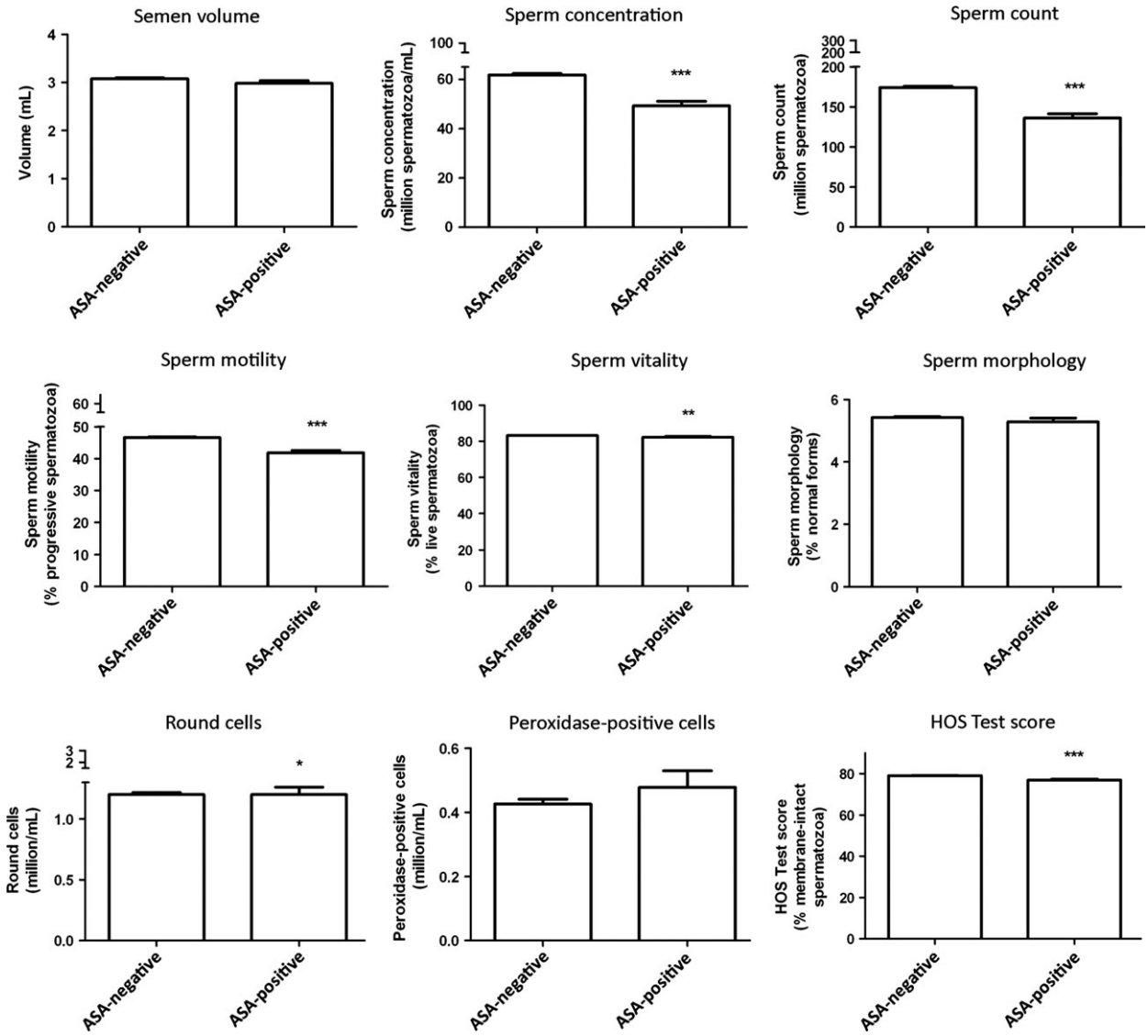

Fig. 1 Semen parameter results in ASA-negative (n = 9196) and ASA-positive (n = 286) samples assessed by the MAR test using the 50% cut-off. Results are expressed as mean ± SDM. ***$P < 0.0001$ for sperm concentration, count, motility, and HOS test score; **$P = 0.0070$ for sperm vitality; *$P = 0.0293$ for round cells.

CASA parameters were analyzed in samples with (cut-off 50%) and without ASA, lower scores ($P < 0.0001$) were found for VSL, LIN, BCF, and WOB in the ASA-positive subgroup. In addition, VAP, ALH, and STR values were lower in the subgroup carrying antibodies, although with lower significance (Fig. 3). When the same analysis was carried out using the 10% cut-off value, only BCF (ASA-positive = 8.9 ± 2.7 Hz; ASA-negative = 8.3 ± 2.8 Hz; $P = 0.03$) and WOB (ASA-positive = 76.8 ± 43.8%; ASA-negative = 78.3 ± 38.9%; $P = 0.01$) were found different. In addition, a significant correlation was found between MAR test results and all kinematic sperm parameters analyzed, except for MAD (Table V).

When movement sperm characteristics were compared in the subset of normozoospermic samples (n = 1587; 56% of the total population analyzed by CASA), no significant differences were found on sperm kinematics between groups (data not shown), despite the high levels of ASA found in ASA-positive samples (83.4 ± 12.1%).

Table II Semen Parameters in the Studied Population Grouped by the MAR Test Results (Cut-Off 10%)

	MAR test (cut-off 10%)			
Semen parameter[a]	<10%	Incidence of abnormal cases (%)	>10%	Incidence of abnormal cases (%)
Semen volume (mL semen)	3.1 ± 1.5	12.2	3.0 ± 1.4	12.9
Sperm concentration (million spermatozoa/mL)	61.8 ± 54.2	16.1	49.3 ± 44.7***	19.0
Sperm count (million spermatozoa)	173.8 ± 164.6	16.5	136.4 ± 130.7***	20.2
Sperm motility (% progressive spermatozoa)	46.6 ± 18.4	22.4	41.8 ± 18.3***	30.8
Sperm vitality (% live spermatozoa)	83.2 ± 9.3	0.7	82.3 ± 9.1**	0.6
Sperm morphology (% normal forms)	5.4 ± 3.5	33.8	5.3 ± 3.1	37.8
Round cells (million/mL cells)	1.2 ± 1.6	40.7	1.2 ± 1.5	37.2
Peroxidase-positive cells (million/mL cells)	0.4 ± 1.3	10.0	0.5 ± 1.3	11.4
HOS test score (million/mL cells)	79.0 ± 8.6	3.1	76.9 ± 9.7***	15.2

[a]Total samples evaluated: 9482.
*$P = 0.0293$.
**$P = 0.0070$.
***$P < 0.0001$.
ASA-positive: 598.
Mann–Whitney test.

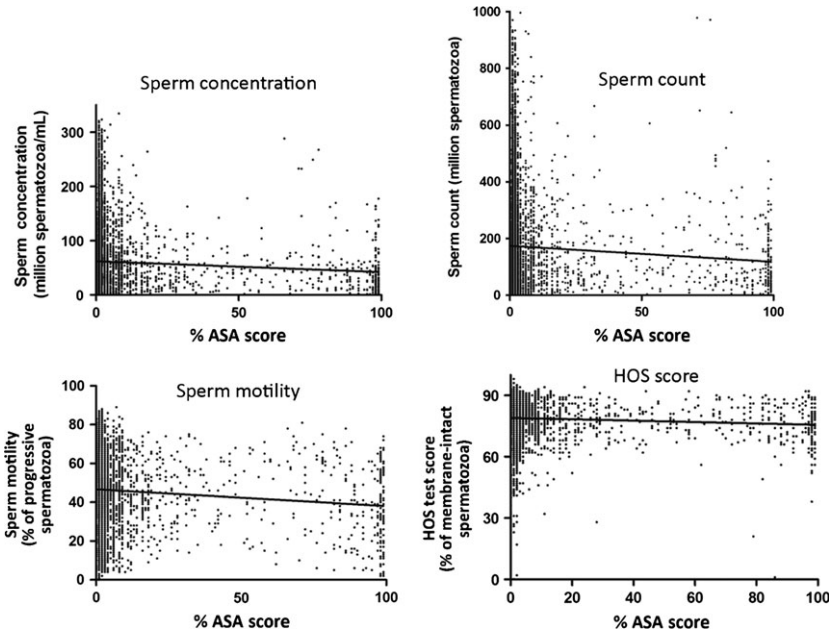

Fig. 2 Correlation analysis between ASA results (MAR test) and sperm concentration, count, motility, and HOS test values. $n = 9482$ samples. Concentration: $R = -0.05560$, $P < 0.0001$; Count: $R = -0.05247$, $P < 0.0001$; Motility: $R = -0.07111$, $P < 0.0001$; HOS test score: $R = -0.05964$, $P < 0.0001$.

Discussion

Several studies have reported the evaluation of surface ASA, with the aim of determining their incidence and relationship with semen parameters. However, the somewhat wide range of results has generated certain confusion among readers. Differences in the reported findings are partly due to the

Table III Semen Parameters and ASA: ROC Curve Analysis

	ROC curve			
	MAR test (cut-off 50%)		MAR test (cut-off 10%)	
Semen parameter[a]	Area	*P* value	Area	*P* value
Semen volume	0.5172	0.3220	0.5161	0.1878
Sperm concentration	0.5593	<0.0001	0.5696	<0.0001
Sperm count	0.5910	<0.0001	0.5709	<0.0001
Sperm motility	0.6001	<0.0001	0.5751	<0.0001
Sperm vitality	0.5468	0.0071	0.5411	<0.001
Sperm morphology	0.5059	0.7480	0.5043	0.7230
Round cells	0.5379	0.0292	0.5199	0.1035
Peroxidase-positive cells	0.5156	0.3685	0.5154	0.2057
HOS test score	0.5869	<0.0001	0.5719	<0.0001

[a]Total samples evaluated: 9482.

Table IV Semen Parameters and MAR Test Results in the Subpopulation Evaluated Using CASA

Semen parameter[a]	Mean ± SDM	Range	Incidence of abnormal cases (%)
Semen volume (mL semen)	2.9 ± 1.5	0.1–15.2	15.1
Sperm concentration (million spermatozoa/mL)	92.9 ± 69.8	0.1–660.9	10.9
Sperm count (million sperm)	250.0 ± 216.2	0.1–1775.9	11.6
Sperm motility (% progressive spermatozoa)	44.6 ± 18.7	1–89	24.5
Sperm vitality (% live spermatozoa)	84.1 ± 8.4	7–96	2.0
Sperm morphology (% normal forms)	5.3 ± 3.2	0–24	33.4
Round cells (million/mL cells)	1.2 ± 2.2	0.1–71.0	37.6
Peroxidase-positive cells (million/mL cells)	0.4 ± 1.7	0.0–51.1	9.3
HOS test (% membrane-intact spermatozoa)	80.5 ± 8.1	0.1–96.0	0.5

[a]Total samples evaluated: 2838.

amount of samples included in each study, the use of diverse methods to evaluate ASA and the threshold values considered for defining ASA-positive samples. Among the available methods for assessing ASA (ELISA: enzyme-linked immunosorbent assay, TAT: tray agglutination test, GAT: gelatin and tray agglutination test, MAR or IB tests, and flow cytometry), the present study reports the results using the direct MAR test, one of the procedures recommended by the WHO 2010 manual[9] to assess surface ASA. Many laboratories selected the MAR test because of its advantages when compared to other methods, that is, analysis requires only a small aliquot of semen and no seminal plasma removal.[38] In particular, in the present study we have used the commercially available SpermMAR™ test, which helps reduce evaluation variability and contributes to assay reproducibility.

Our report is the first to describe ASA evaluation following the guidelines and the cut-off value established by the WHO 2010 manual.[9] Moreover, it involves the largest sample population evaluated by a sole laboratory in which semen analysis is carried out under strict internal and external quality assurance standards. An overall incidence of 2.6 and 5.9% surface ASA (50% and 10% cut-off, respectively) was found. These results are within the range found in studies in which ASA were detected using the 40% cut-off[12,13] (312 cases, 5% incidence;[12] 750 cases; 6.3%[13]) and the 10% cut-off[16] (650 cases; 6%). Contrasting with these findings, 9% surface ASA were reported in other studies[15,26] (111 cases, 9.01%;[15] 1228 cases, 9.4%[26]) using the 20% cut-off. The trend toward lower ASA incidence found in the present study may be associated with the patient population evaluated at the LAR Andrology Laboratory. In this regard, while several cases were suspected of male infertility, others requested the analysis for various reasons, among them as part of the couple infertility workup, after a varicocele repair, after treatment for infection, etc., and may, at least in part, account for the 61.3% normozoospermic samples found in the total population included in the analysis.

Results from our study have also revealed alterations in some semen parameters associated with ASA presence, as shown by the significant decrease in sperm concentration and count, as well as in

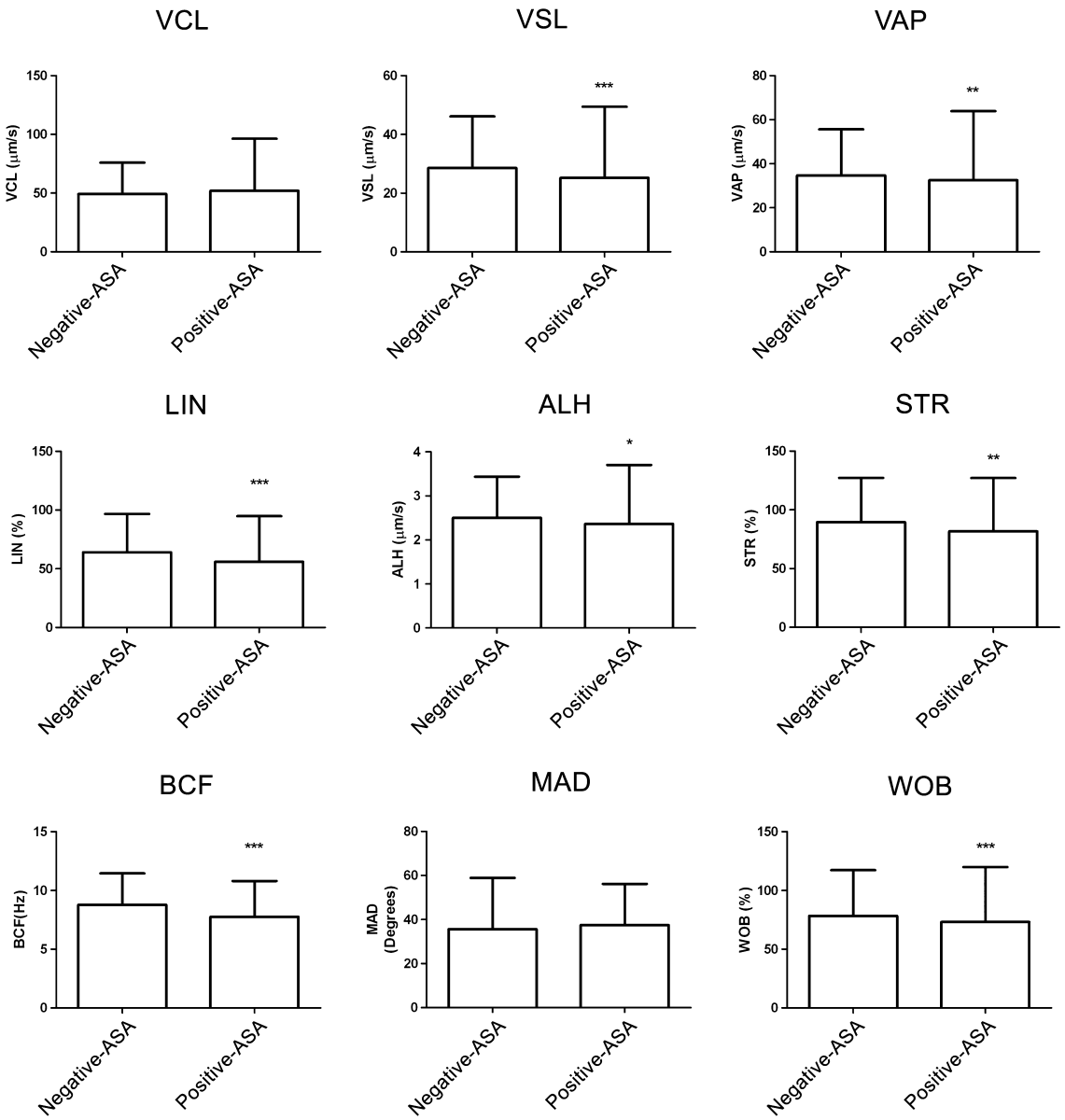

Fig. 3 Sperm kinematics parameters in ASA-negative (n = 2768) and ASA-positive (n = 70) samples using the 50% cut-off. Results are expressed as mean ± SDM. ***P = 0.0007 for VSL and BCF; **P = 0.0015 for VAP; ***P < 0.0001 for LIN and WOB; *P = 0.025 for ALH; **P = 0.0024 for STR.

motility and HOS score, regardless of the cut-off used (50 or 10%). Our findings are in line with the recent report on a systematic literature review and meta-analysis that evaluated the association between ASA and semen parameters in infertile men. In the study, Cui et al. described a reduced sperm concentration and motility in ASA-positive samples from a total of 1167 cases assessed following the WHO 1999 criteria.[19] A decreased sperm concentration and motility in spermatozoa carrying ASA have been linked to alterations in spermatogenesis,[10] sperm agglutination[11,39] and lysis.[40]

Findings from the present study revealed significantly lower HOS test scores in ASA-positive samples when compared to the ASA-negative ones. These findings match those previously reported by Rossato and coworkers,[26] in a study evaluating ~1200 cases using the direct MAR test. Moreover, they are in

Table V Correlation Analysis between CASA Parameters and ASA Results (MAR Test)

Sperm kinematics parameter[a]	Correlation coefficient	*P* value
VCL (μm/s)	−0.04876	0.0094
VSL (μm/s)	−0.05692	0.0024
VAP (μm/s)	−0.06195	0.0010
LIN (%)	−0.06597	0.0004
ALH (μm/s)	−0.05485	0.0035
STR (%)	−0.05253	0.0051
BCF (Hz)	−0.03916	0.0370
MAD (degrees)	0.00999	0.5947
WOB (%)	−0.07928	<0.0001

[a]Total samples evaluated: 2838.

line with a report describing an increase in HOS test results in cases with ASA after corticosteroid treatment that reduced ASA.[41] In our study, lower HOS scores were also observed in ASA-positive samples from a subgroup of cases showing normal sperm concentration, motility, and morphology. In this subgroup, no significant correlation was obtained between the percentage of live spermatozoa and the percentage of membrane-intact sperm cells in samples with high ASA levels (ASA-positives in MAR test ≥50%), suggesting a negative impact of ASA upon membrane functionality rather than on cell vitality. In this regard, the deleterious effect of ASA upon the sperm plasma membrane has been related to the membrane cholesterol content, which prevents membrane fluidity changes needed for the expression of some receptors,[42] as well as to a reduction in the intracellular calcium concentrations rise and the rate of acrosome reaction after hypo-osmotic challenge in spermatozoa with ASA.[26] In line with these findings, a study reported a relationship between ASA presence and abnormal scores for the sperm stress test (MOST) and related these abnormalities to defects in the sperm plasma membrane.[16]

The use of CASA led us to identify changes in sperm kinematics associated with the presence of ASA. The significant decrease observed in the mean value of VSL, VAP, LIN, ALH, STR, BCF, and WOB sperm movement characteristics in samples with high levels of ASA (cut-off 50%) accompanied the diminished progressive motility and the correlation between progressive motility and ASA levels (Figs 1 and 2). In agreement with our findings, Check et al.[32] previously reported a decreased LIN in ASA-positive compared to ASA-negative samples. This difference was no longer observed when using 10% cut-off, as described by Calamera et al.[16]

ASA were also found present in a subgroup extracted from the database of over 5000 cases showing normal sperm concentration, motility, and morphology, as defined by the WHO 2010 manual.[9] In this subgroup, ASA-positive samples from the normozoospermic subgroup also had a high percentage of spermatozoa carrying ASA, as judged by the MAR test results. Moreover, in this target subpopulation, no differences were found in sperm kinematics when comparing results in the ASA-positive and ASA-negative cases. These findings indicate that ASA presence is not always associated with alterations in semen parameters. In this regard, a recent study carried out in 1060 infertile normozoospermic men found an association between higher ASA and acrosome reaction disorders, higher DNA fragmentation, and higher oxidative stress,[18] sperm properties not tested during routine semen analysis.

Altogether, our findings reinforce the relevance of performing ASA analysis in men attending an Andrology Laboratory for semen evaluation, as sperm surface autoantibodies may be present at high levels in spermatozoa with or without abnormalities in other semen and CASA parameters.

Conclusions

- Our study contributed to define the incidence of sperm surface autoantibodies in men attending an Andrology Laboratory.
- Despite the cut-off selected to define ASA-positive samples (50% or 10%), ASA were discovered to be associated with abnormalities in semen parameters (lower sperm concentration, count, motility, and membrane integrity). Moreover, ASA values correlated with results on these sperm parameters.
- HOS test scores did not correlate with the percentage of live spermatozoa in a subset of cases depicting normal sperm concentration, motility and morphology, and high levels of surface ASA, suggesting a deleterious effect of ASA upon sperm membrane integrity.
- A computer-assisted sperm analysis carried out in over 2800 cases extracted from the whole database revealed abnormalities in several kinematic parameters (VSL, VAP, LIN, ALH, STR, BCF, and WOB). Among these cases, normozoospermic samples with high ASA levels

showed no differences in sperm kinematics when compared to ASA-negative samples.

Based on results obtained in the present study, the assessment of sperm surface autoantibodies should be included in every full basic routine semen examination of men attending an Andrology Laboratory.

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