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Review Article
Male reproductive health and infertility

pISSN: 2287-4208 / eISSN: 2287-4690
World J Mens Health 2022 Jul 40(3): 380-398
https://doi.org/10.5534/wjmh.210164

The World Journal of MEN's HEALTH

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Received: Jul 24, 2021 **Revised:** Aug 13, 2021 **Accepted:** Aug 23, 2021 **Published online** Jan 1, 2022
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Antisperm antibodies (ASA), as a cause of male infertility, have been detected in infertile males as early as 1954. Multiple causes of ASA production have been identified, and they are due to an abnormal exposure of mature germ cells to the immune system. ASA testing (with mixed anti-globulin reaction, and immunobead binding test) was described in the WHO manual 5th edition and is most recently listed among the extended semen tests in the WHO manual 6th edition. The relationship between ASA and infertility is somewhat complex. The presence of sperm agglutination, while insufficient to diagnose immunological infertility, may indicate the presence of ASA. However, ASA can also be present in the absence of any sperm agglutination. The andrological management of ASA depends on the etiology and individual practices of clinicians. In this article, we provide a comprehensive review of the causes of ASA production, its role in immunological male infertility, clinical indications of ASA testing, and the available therapeutic options. We also provide the details of laboratory procedures for assessment of ASA together with important measures for quality control. Additionally, laboratory and clinical scenarios are presented to guide the reader in the management of ASA and immunological male infertility. Furthermore, we report the results of a recent worldwide survey, conducted to gather information about clinical practices in the management of immunological male infertility.

Keywords: Antibodies; Infertility, male; Spermatozoa; Sperm agglutination; Survey

INTRODUCTION

Antibodies and auto-antibodies were discovered at the end of the 19th century by three Nobel Prize winners, Elie Metchnikoff, Paul Ehrlich, and Karl Landsteiner. Antisperm antibodies (ASA) were reported in infertile

males as early as 1954 [1]. ASA are immunoglobulins directed against antigens present on the sperm surface [2-4]. Mature spermatozoa are normally located behind the blood-testis barrier. Therefore, spermatozoa are physiologically unexposed to the male immune system. However, when the blood-testis barrier is broken or damaged due to injury or illness, there will be exposure of mature germ cells (antigen carriers) to the immune system leading to the development of ASA [5].

Although not all ASA impair sperm function, ASA may alter the motility, acrosome reaction, capacitation, and fertilizing abilities of the spermatozoon [6,7]. Immunological infertility is only diagnosed when there is evidence of altered sperm functional capacity due to ASA [8,9]. However, the indications for ASA testing are unclear and the clinical significance of detecting ASA in serum is questionable. A large prevalence of ASA has been reported in infertile men (3.9%–15.6%) [10-14], significantly higher than those reported in fertile men (0.9%–2.5%) [10,12,15]. However, in a recent study of more than 10,000 men from infertile couples, the prevalence of ASA was not as high as expected, and was estimated to be between 2% and 4% [16]. This was due to the threshold used to define the positive ASA test.

The relatively low prevalence of ASA has prompted the American Society for the Reproductive Medicine (ASRM), the American Urological Association (AUA) and the European Association of Urology (EAU) to not endorse the ASA test as a first-line test for the evaluation of infertile men [14,17,18]. In this regard, it is noteworthy that only 4.7% (27/572) of clinics and laboratories accredited by the College of American Pathologists (CAP) test ASA across the United States [19].

Direct and indirect methods of ASA detection are described in the World Health Organization (WHO) manual 5th [8] and 6th editions [9]. The prevalence of ASA varies according to the screening method [2,6,7]. A recent study investigated the use of a protein biochip screening for the detection of serum ASA. Using this technique, higher prevalence of ASA (20.9%) was reported in more than 300 infertile patients compared to <2% in fertile men [20]. However, the clinical significance of these "ultra"-detected ASA (at the protein level) remains to be determined. The immunoglobulin A (IgA) and immunoglobulin G (IgG) isotypes which are frequently found in semen could have a negative impact on sperm motility [21], while immunoglobulin M (IgM) antibodies are rarely found [2] due to their larger size and presence primarily in the acute phase of an infection.

Finally, there is no clarity in the treatment of patients with ASA, as the indications for ASA testing are uncertain and variable. In couples undergoing the intracytoplasmic sperm injection (ICSI) procedure, ASA testing is not recommended [18,22,23]. In the present article, we review the causes of ASA formation and immunological infertility, along with the clinical indications for its diagnosis and detailed methodology for its analysis. We also present the results of a short worldwide survey to explore patterns of clinical application and management of ASA testing in male infertility. Finally, we present laboratory and clinical scenarios to help understand the use and importance of ASA testing in a clinical setting.

CAUSES OF ANTISPERM ANTIBODY AND IMMUNOLOGICAL MALE INFERTILITY

The Sertoli cells are responsible for the formation of a blood-testis barrier with their tight inter-cellular junctions [5] that divide the seminiferous epithelium into two compartments, the basal and apical (adluminal) compartments. Germ cells located in the adluminal compartment of the seminiferous epithelium are physiologically protected from exposure to the immune system. Consequently, mature spermatozoa remain hidden from immune system detection. Hence, autoimmune reaction against spermatozoa can occur after testicular, epididymal, or vasal injury that leads to exposure of sperm to the immune system. Surgical trauma to testes or scrotum (therapeutic or iatrogenic), testicular torsion, testicular cancer, undescended testis, urogenital inflammatory conditions, obstruction, or varicocele have been associated with the presence of ASA [24-32]. ASA is strongly associated with obstructive azoospermia, particularly post-vasectomy [32], as ASA is present in 70% to 100% of men after vasectomy [33]. It has also been noted that certain chronic bacterial infections can be accompanied by the presence of ASA. For example, patients with chronic prostatitis are 3 times more likely to develop ASA than control patients without this condition [34,35]. The mechanism is not fully understood but may be related to inflammatory damage to the male genital glands, as well as a local immune dysregulation that can lead to auto-immunity against

spermatozoa [34,36,37]. It has also been suggested that ASA could be produced during cross immune reactions with exogenous antigens (bacteria, virus, fungi, allergens) [36,38-40]. One recent study suggested that human papilloma virus (HPV) infection in men is associated with a greater risk of developing ASA [41]. Finally, in many cases, the cause of these antibodies is unknown or remains idiopathic [42].

SEMINAL ASA TESTING

The indications of ASA testing in male infertility are usually based on the patients' history and routine semen analysis findings. A history suggestive of any of the above-mentioned conditions may warrant an ASA test. Similarly, certain semen parameters may indicate the presence of ASA. Sperm agglutination is an important indicator for conducting an ASA test [6], even though the association between sperm agglutination and ASA is not strong, and sperm agglutination can occur as a result of other factors besides sperm antibodies [8,15,43]. Recently, a study conducted in 195 patients with ASA in their semen investigated the link between sperm agglutination and ASA [44]. Notably, more than one-third of patients with sperm agglutination had ASA with sperm agglutination, compared with less than 3% patients without sperm agglutination [44]. The 2010 WHO laboratory manual for the examination and processing of human semen describes sperm agglutinates as suggestive of the presence of ASA [8].

The presence of ASA is also significantly associated with reduced sperm count, motility, and vitality [43,44] and according to a previous meta-analysis, asthenozoospermia may be an indicator for testing [21]. However, another study involving a large cohort of patients with ASA demonstrated that sperm motility was not correlated with the presence of IgA [16]. Therefore, it is unclear whether ASA should be tested in all patients with asthenozoospermia. Some studies have demonstrated that ASA testing should be considered only in cases of asthenozoospermia with sperm agglutination and normal sperm concentration [17,18,45]. In summary, the presence of ASA may be suspected in the presence

Table 1. Summary of the clinical approach to immunological infertility: testing and treating antisperm antibodies

Indications for testing
• Suggestive history or physical exam: - Trauma to testes or scrotum - Surgery to male reproductive tract–including vasectomy (70%–100% have ASA) - Testicular torsion - Testicular cancer - Urogenital tract inflammation - Varicocele • Sperm agglutination • Asthenozoospermia, especially if agglutination
Methods for testing
• Direct tests (MAR and IB tests): detect antibodies (IgG and IgA) that are directly bound to spermatozoa, results are reported as: - Whether presence of antibodies is positive - The percentage of binding - The area of binding at the spermatozoa • Indirect tests: detect antibodies that are found in free fluids (such as: seminal plasma and cervical mucus)–can be performed in cases of obstructive azoospermia, oligozoospermia, or if semen needs to be stored for later testing.
Management of immunological infertility
• Corticosteroids - Benefit in terms of improving natural pregnancy rates and IVF success rates, but not ICSI - Should consider the many systemic side effects of treatment • Use of ART: - Sperm washing before procedure to dilute antibodies - ICSI can overcome infertility due to ASA and is the recommended ART Note: If the couple is scheduled for ICSI, there is no need to test for ASA, as there will be no effect on outcome.

ASA: antisperm antibodies, MAR: mixed antiglobulin reaction, IB: immunobead, IgG: immunoglobulin G, IgA: immunoglobulin A, IVF: *in vitro* fertilization, ICSI: intracytoplasmic sperm injection, ART: assisted reproductive technology.

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of any suggestive history (as previously described), and sperm agglutination regardless of whether they are associated with asthenozoospermia (Table 1).

METHODS OF SEMINAL ASA TESTING

ASA in semen belong almost exclusively to two immunoglobulin classes, namely IgA and IgG [8]. IgA antibodies may have greater clinical importance than IgG antibodies, but more than 95% of cases with IgA sperm antibodies are also positive for IgG [8,9]. ASA can be measured by direct or indirect tests.

Two direct tests can detect ASA on spermatozoa: the mixed antiglobulin reaction (MAR) test which is performed on a fresh semen sample and the immunobead (IB) test that uses washed spermatozoa [8].

In direct tests, the sample is incubated with latex beads coated with anti-human antibodies (Fig. 1). If ASA are present in the sample, the anti-human antibodies on the beads will bind to the antibodies on the sperm surface: under the microscope, motile spermatozoa will appear coated with the beads. Based on the anti-human antibodies bound to the beads, it is possible to selectively investigate the presence of IgG or IgA with antiserum specific for each antibody. The percentages of motile sperm with the beads attached are counted [8]. These direct tests provide information about the presence of the immunoglobulins, the type of antibodies and their specific localization on the sperm head, midpiece, tail, or all three regions of the spermatozoon [8]. An aliquot of the seminal fluid is incubated with a normal control (previously tested negative for antibody) prior to performing the MAR or IB testing. Direct tests depend on the presence of motile spermatozoa, therefore, in case of inadequate (less than 100) number of motile spermatozoa, indirect tests must be used.

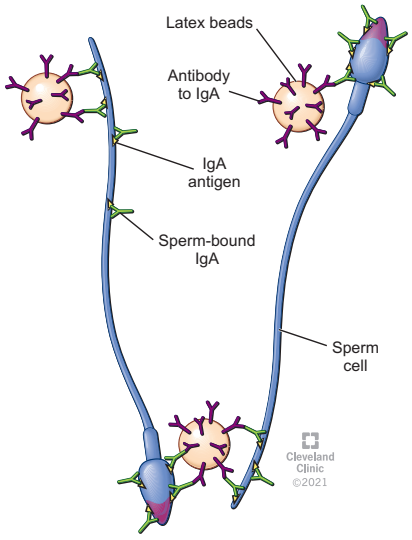

Fig. 1. Graphical representation of sperm agglutinated to the latex beads in the immunobead test.

The indirect test is used to measure sperm-specific immunoglobulins in sperm-free fluids such as seminal plasma, heat-inactivated serum and dissolved cervical mucus. The indirect test uses the suspected fluid incubated with ASA-free donor sperm washed from original seminal fluid. It has to allow time for potential sperm-antibody interaction, although, being dependent of sperm motility, time interferes with these results [9]. When the patient sample is oligozoospermic or asthenozoospermic (alone or in combination), in case of obstructive azoospermia, or if a sample cannot be tested, indirect testing can be performed, and the seminal fluid may be frozen and stored until the time of testing.

DIRECT MAR TEST FOR IGA

After liquefaction, the sperm concentration and the total motile sperm are calculated. SpermMar (FertiPro, Beernem, Belgium) latex particles are a suspension of approximately 2.0 μm in diameter polystyrene latex particles coated with monoclonal antihuman anti-IgA serum (Fig. 2). This is a ready-to-use suspension stored at 2°C to 8°C.

A 10 μL aliquot of fresh sample is placed on a labeled glass slide, and then 10 μL of IgA beads antiserum mixture is placed on the drop of semen. Using a

Fig. 2. Components of the SpermMar test (FertiPro, Beernem, Belgium). Blue top: antiserum bead combination for IgA. White tops: positive and negative controls. Green top: beads for IgG.

Fig. 3. Phase contrast microscope station for evaluating the presence of antisperm antibodies (ASA).

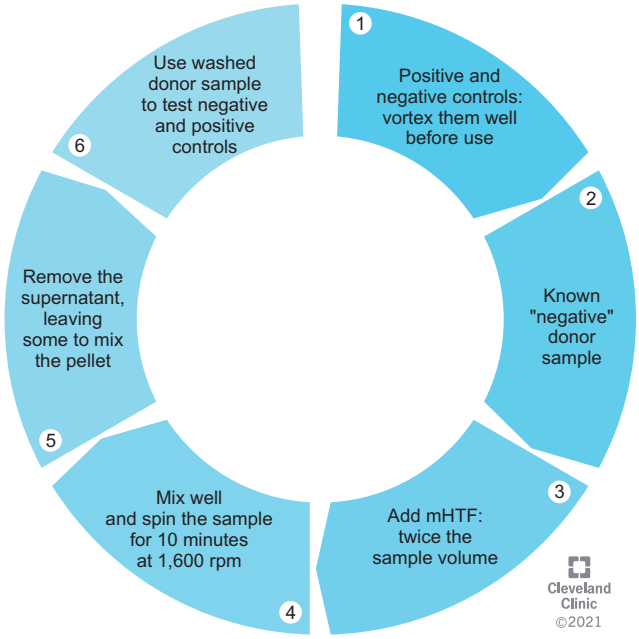

Fig. 4. Workflow chart for SpermMar testing of positive and negative controls. mHTF: modified human tubal fluid.

wooden applicator, the beads are mixed with the semen thoroughly and a 22×50 mm coverslip is placed on top of the mixture. The slide is then placed in a humidified chamber for 3 minutes, and then examined using a phase contrast microscopy using a 40× phase contrast objective with a green filter (Fig. 3). Two hundred motile sperm are counted in replicate and the percentage of motile sperm attached to the beads is reported.

As per WHO manual 5th edition criteria, the reference value for positive ASA test is 50% for both IgG and IgA [8]. The 6th edition of the WHO manual differs from the 5th edition as it does not indicate the exact ASA value for indicating a positive test [9].

Particle binding restricted to the tail tip is not associated with impaired fertility and can be present in fertile men. Furthermore, this edition recommends that, as for all clinical laboratory diagnostics tests, each laboratory should define its normal reference ranges by testing a sufficiently large number of normal fertile men [9]. This will help in establishing the differences between normal and pathological reference ranges. However, as per the manufacturer of the SpermMar kit, an immunological diagnosis of infertility is most likely if 40% or more of the spermatozoa are bound to latex particles. Less than 40% binding is reported as a negative result, but immunological infertility can be suspected when 10% to 39% of motile spermatozoa are bound.

DIRECT MAR TEST FOR IGG

The test with IgG is performed in the same way as the test with IgA, except for different reagents used. For IgG, there are two separate suspensions of SpermMar IgG latex particles and SpermMar antiserum, a monospecific antiserum directed towards the Fc fragment of human IgG.

For in-house testing, the MAR test kit also includes SpermMar IgG positive and negative control samples. These comprise decomplemented patient serum diluted in Ferticult Flushing medium without human serum albumin (2.5 mL). The SpermMar IgG positive and negative controls provided in the kit are in a ready-to-use form. The reagents, and the positive and negative controls must be allowed to warm to room temperature before use. These are stable for 18 months from the date of manufacture and are stored at 2°C to 8°C when not in use (Fig. 4).

To perform the test, a known "negative" control semen sample (previously tested negative for ASA) is used. One volume of sample is mixed with twice the volume of sperm wash medium (mHTF; Vitrolife, Inc., Englewood, Colorado) and centrifuged at 1,600 rpm for 10 minutes. The supernatant is discarded, and the pellet is mixed well in a small amount of the supernatant. A 50 μL aliquot of the positive control is added to 50

μL of the washed semen in a vial. In another vial, 50 μL of the negative control is mixed with 50 μL of the washed semen. The vials are incubated for 60 minutes at 37℃.

For the positive control, 1 drop (10 μL) of IgG–sperm mixture is placed on a clean, dry microscope slide, plus 1 drop (10 μL) of IgG latex beads and 1 drop (10 μL) of IgG antiserum. Similar steps are followed for the negative control on a separate slide. After mixing the latex beads and IgG–sperm mixture with the IgG antiserum using a wooden applicator, a 22×50 mm coverslip is placed on top of the mixture and the slide is placed in a humidified chamber for 3 minutes. After incubation, the slides are examined microscopically using a 40× objective under phase contrast with a green filter. For both IgA and IgG, only motile spermatozoa should be scored, and the percentage of motile spermatozoa that have two or more latex particles attached are scored as bound sperm. Tail-tip binding should be ignored. At least 200 motile spermatozoa should be assessed in replicate, to achieve an acceptably low sampling error.

Initially, motile spermatozoa can be observed with a few beads attached. Then, the bead-binding becomes severe and sperm movement becomes restricted to twitching. Sperm without coating antibodies will be seen swimming freely between the particles. A common problem may occur with non-progressive spermatozoa that are close to, but not attached to the beads. The actual binding of the beads can usually be verified

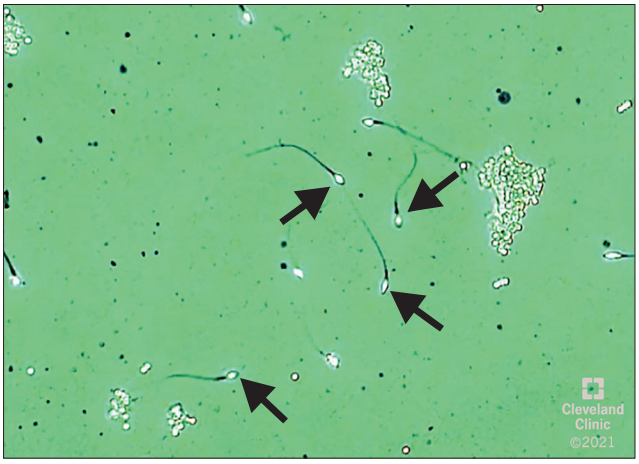

Fig. 5. Negative results. Arrows indicate sperm that are not bound to the beads.

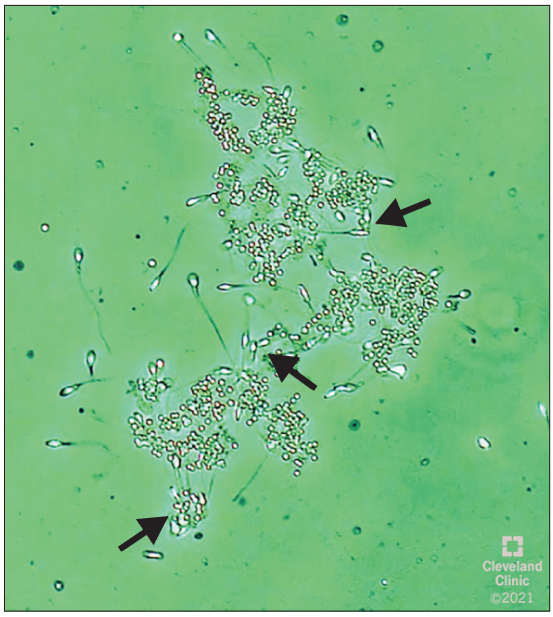

Fig. 6. Positive results. Arrows indicate sperm bound to the beads.

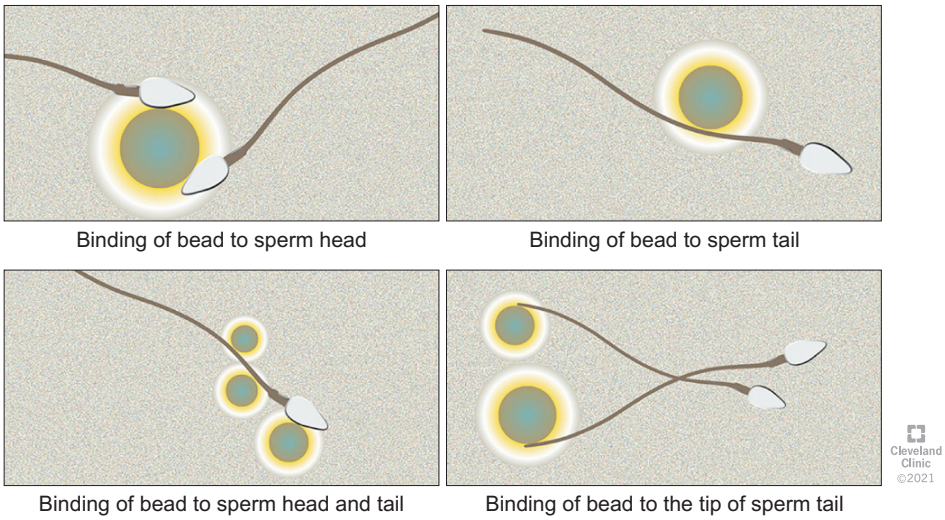

Fig. 7. Different sites of bead attachment. The binding of bead to the tip of the sperm tail may be observed in fertile men, and therefore is not associated with impaired fertility [9].

by lightly tapping the coverslip with a small pipette tip. The movement of beads will be in tandem with the moving sperm.

One of the latest modalities of detection is to detect serum antibodies against ACTL7 protein, which is localized in the acrosome and tail of mature spermatozoa [46]. Serum antibodies against ACTL7 have been reported to reduce fertility to zero in mice. In a recent study, an ELISA test was developed to detect antibodies against ACTL7. The levels of the antibodies were assessed in fertile *vs.* infertile men. The study reported significantly higher levels of this antibody in infertile patients, and also in those who were ASA-positive by tray agglutination test ($p<0.0001$) [46].

Reporting of results:

Negative results: Report as negative. No further explanation is needed (Fig. 5).

Positive results: Report and record as follows:

i. Positive (Fig. 6)
ii. Percent binding
iii. Area of bead attachment (sperm head, tail, or both sperm head and tail involvement) (Fig. 7)

EXTERNAL QUALITY CONTROL

External quality control is a proficiency testing tool to ensure accurate ASA results. The accreditation agency conducts semi-annual surveys for ASA test-related proficiency testing. Two analytes (inactivated serum) are sent each semester. The analytes are tested in routine laboratory flow as patient samples. Positive and negative controls are included in the assay run. The cut-off to report a positive ASA test is when motile sperm binding is >20% as per CAP recommendation [47]. The laboratory results must meet the criteria for the consensus results for the two analytes.

LABORATORY SCENARIOS

Some of the common laboratory scenarios that may be encountered during ASA testing are highlighted here.

1. Case 1

1) Scenario

IgA ASA test performed for a semen sample with a sperm concentration of 15×10^6/mL and sperm motility of 45%. Using a 40× objective, 85 free and 15 bound motile sperm were observed.

2) Solution

The percent binding for IgA is 15% and the result for ASA is negative.

2. Case 2

1) Scenario

Semen sample with 5% motility and 2×10^6/mL sperm concentration with <100 motile sperm on wet preparation are seen. Percent binding is not reported due to low motile sperm count.

2) Solution

In this case, a comment can be made that the ASA results cannot be reported due to the insufficient number of motile sperm.

MANAGEMENT OF IMMUNOLOGICAL INFERTILITY

Immunological infertility is indicated using post-coital test when there is evidence of functional sperm damage due to ASA [8]. However, this test is rarely used today. Likewise, other functional tests to assess the impact of ASA on capacitation, acrosome reaction, or sperm-oocyte interaction are also not available in all laboratories. Possibly for this reason, although the WHO recommends that the diagnosis of immunological infertility should only be made after evaluation of the impact on fertility of ASA, in practice the detection of ASA is considered potentially impacting sperm function and male fertility [9]. We have summarized the clinical approach to immunological infertility in Table 1.

1. Corticosteroids administration

Oral corticosteroids, used to suppress antibody production, have been reported in relatively older literature to have beneficial effects in men with ASA (studies are summarized in Table 2 [48-54]). Taiyeb et al [55] reported in a randomized controlled trial (RCT) that corticosteroid administration in men with ASA could improve conventional *in vitro* fertilization (IVF) outcomes. However, in their study, ICSI outcomes were not improved by corticosteroids, presumably because

Table 2. Summary of studies reporting the effects of corticosteroids treatment on reproductive outcomes

Reference		Observation
Omu et al, 1996 [48]	Study design	Prospective cohort study
	Population	n=40 under treatment n=37 controls
	Dose	5 mg
	Duration	3–6 months
	ASA test	Immunofluorescence in serum Positivity in case of bright 3–4 staining
	Main outcome	Reduction in ASA levels in 50% of patients under treatment
		Significant increase in the motility and viability in therapy group
		Higher PR in therapy group (8 *vs*. 1)
	Study limitation	Low number of patients, no randomization protocol
Hendry et al, 1990 [49]	Study design	RCT
	Population	Prednisolone treatment: 22 Placebo: 21
	Dose	20 mg twice daily, raised to 40 mg if the serum or seminal plasma titers were unchanged in 3 months
	Duration	9 months
	ASA test	TAT in serum and seminal plasma Positivity: ≥32 titers in serum, and/or positive at any titer in seminal plasma
	Main outcome	No significant change in semen parameters
		Lower ASA levels in seminal plasma after prednisolone treatment
		Significantly higher PR in treatment group (9 *vs*. 1)
	Complications	60% of treated patients showed mild side-effects. n=1 withdrew for glaucoma.
	Study limitation	Low number of patients
Hendry et al, 1979 [50]	Study design	Prospective cohort study
	Population	Group 1=15 patients with oligozoospermia Group 2=14 patients with normozoospermia Group 3=18 patients with normozoospermia
	Dose	Group 1=15 mg/day (three times a day) Group 2=15 mg/day (three times a day) Group 3=96 mg/day (received methyl-prednisolone)
	Duration	Group 1=3–12 months Group 2=3–12 months Group 3=7 days
	ASA test	Serum titers of at least 1 in 32 by GAT
	Main outcome	In group 1, sperm-counts became normal in 10 men and 4 of their wives became pregnant.
		In group 2, antibody titers fell slightly and 3 of their wives became pregnant.
		In group 3, antibody titers fell more markedly and 7 of their wives became pregnant
	Study limitation	Low number of patients, no randomization protocol
Lähteenmäki et al, 1995 [51]	Study design	RCT
	Population	Prednisolone treatment: 27 Placebo treatment: 26
	Dose	20 mg/day
	Duration	Day 1–10 of the female partners menstrual cycle, followed by 4 mg on days 11 and 12
	ASA test	MAR to IgG in semen, TAT in serum and FCM
	Main outcome	No significant difference was shown between the groups in terms of fertilization and PRs. Higher PR with IUI (9 pregnancies; p=0.04) than timed intercourse with prednisolone (one pregnancy).
		In patients with normal sperm count (n=14), antibody titers fell slightly and 3 of their wives became pregnant.
		There were no significant associations between antibody levels, sperm count or motility *versus* the incidence of pregnancy.
	Study limitation	Low number of patients

Table 2. Continued

Reference		Observation
Räsänen et al, 1994 [52]	Study design	Prospective cohort study
	Population	n=11 infertile men with positive IgG on MAR screening test
	Dose	20 mg/day for the first 10 days of the partner's menstrual cycle and then 5 mg on days 11 and 12
	Duration	3 cycles (*i.e.*, 3 months).
	ASA test	MAR to IgG and IgA in semen
	Main outcome	A clear reduction of sperm-bound IgG antibody levels was seen in 3/11 (27%) patients, while only IgA was reduced in 2/11 (18%) patients.
		Semen parameters in the before- and after- treatment were not significantly different.
	Study limitation	Low number of patients, no control group
Sharma et al, 1995 [53]	Study design	Prospective cohort study
	Population	n=48 subfertile couples, with males having ≥20% of motile spermatozoa bounded to IgG, IgA, or both
	Dose	40 mg a day, for the first 10 days, then 5 mg on days 11 and 12 of the partner's cycle.
	Duration	9 months
	ASA test	Direct and indirect IBT for IgA and IgG
	Main outcome	Twelve couples became pregnant; a cumulative conception rate of 30.2% was achieved at 9 months Conception rate 30.2% (12 couples)
		In the pregnant group, prednisolone treatment caused a significant increase in grade I motility
		The pregnant group started with significantly higher concentrations of IgG (tail) and grade I motility
		The % of progressive motile spermatozoa was significantly higher following steroid therapy
	Study limitation	Low number of patients
Nagaria et al, 2011 [54]	Study design	Prospective study
	Population	n=9
	Dose	Low-dose prednisolone
	Duration	3 months
	ASA test	ELISA
	Main outcome	Improved sperm motility after treatment PR of 31.6%
	Study limitation	Low number of patients, dosage not reported

ASA: antisperm antibodies, PR: pregnancy rate, RCT: randomized controlled trial, TAT: tray agglutination test, GAT: gelatin agglutination test, MAR: mixed antiglobulin reaction, IgG: immunoglobulin G, FCM: flow cytometry, IUI: intrauterine insemination, IgA: immunoglobulin A, IBT: immunobead test, ELISA: enzyme linked immunosorbent assay.

ICSI bypasses the natural process of zona pellucida binding and fertilization. Notably, the immunosuppressive therapies may have adverse effects outweighing their potential benefits, and thus limiting their routine use. Importantly, the dose and duration of corticosteroid therapy should be carefully considered as steroid overtreatment could result in avascular necrosis of the femur head, hypogonadism, diabetic onset, metabolic decompensation and male infertility [56]. Hence, the clinical utility of prescribing medical immunosuppressive therapy for the sole purpose of suppressing ASA is not clear.

2. Assisted reproductive technology (ART)

When preparing sperm for ART, sperm washing can dilute certain antibodies. Therefore, the sperm-washing techniques can remove unbound antibodies. ASA positive samples collected for ART can be washed even more efficiently, if collected in a container where few milliliters of culture medium are added before collection. As ASA are mainly bound to spermatozoa post-ejaculation, this 'dilution' effect could impede ASA binding to a higher number of motile spermatozoa which can then be separated by the washing technique [57]. The detachment of already bound antibodies appears to be much less obvious [57]. Studies summarizing the impact of ART on reproductive outcomes in ASA-positive patients are listed in Table 3 [29,58-63].

It has been demonstrated that ICSI can overcome immunological infertility due to the presence of ASA [64]. Clinical pregnancy rates were not significantly different in ASA-positive samples (>50% of sperm coated

Table 3. Studies reporting the reproductive outcomes after ART in ASA-positive patients

Intervention	Reference	Observation	
IUI	Barbonetti et al, 2020 [63]	Population	Group 1: 44 men with 100% ASA positive Group 2: 40 men with 50%–99% ASA positive
		ASA testing	IgG-MAR test on semen
		Main outcome	Lower natural LBR in group 1 (p<0.0001) Comparable LBR after IUI
		Study limitations	Retrospective analysis Relatively small sample size
	Ombelet et al, 1997 [58]	Population	Group I: n=14 couples treated with ovarian stimulation/ IUI, followed by IVF if no pregnancy occurred after three IUI cycles. Group II: n=15 patients treated with IVF as a first-choice procedure
		ASA testing	IgG and IgA MAR test in serum and semen (positivity: >50%)
		Main outcome	Take home baby rate of 64.3% (n=9) with 3 IUI cycles Recommend superovulation with IUI as first line of management for immunological infertility
		Study limitations	Small sample size, no randomization protocol
IVF	Lu et al, 2019 [29]	Population	Infertile couples (n=399 cycles): - 39 ASA positive - 360 ASA negative
		ASA testing	ELISA test kit for serum ASA (positivity: ASA >75 IU)
		Main outcome	Lower rates of FR, good embryos, PR, and LBR in ASA positive than ASA negative men (p<0.05)
		Study limitations	Small sample size of ASA positive, selection bias, serum ASA tested
	Clarke, 2006 [59]	Population	Group 1: 51 ASA negative (control) Group 2: 13 ASA positive <80% Group 3: 25 ASA positive ≥80%
		ASA testing	Direct IBT for IgA (positivity: ≥20%)
		Main outcome	Lower FR in ASA positive groups than ASA negative (p<0.05)
		Study limitations	Small sample size
	Vujisić et al, 2005 [60]	Population	Group 1: 38 ASA positive IgG <20% Group 2: 14 ASA positive IgG >20%
		ASA testing	MAR test for IgG, IgA, and IgM on semen
		Main outcome	Comparable FR (73.2% *vs*. 71.5%) and PR (28.9% *vs*. 28.57%)
		Study limitations	Small sample size, lack of control group
ICSI	Lu et al, 2019 [29]	Population	Infertile couples (n=155 cycles): - 19 ASA positive - 136 ASA negative
		ASA testing	ELISA test kit for serum ASA (positivity: ASA >75 IU)
		Main outcome	Comparable PR and LBR between ASA positive and ASA negative
		Study limitations	Small sample size of ASA positive, selection bias
	Esteves et al, 2007 [61]	Population	Group 1: 0%–10% ASA (n=194) Group 2: 11%–20% ASA (n=107) Group 3: 21%–50% ASA (n=33) Group 4: 51%–100% ASA (n=17)
		ASA testing	Direct IBT for IgA, IgG, and IgM
		Main outcome	Comparable results for FR, abnormal FR, cleavage rate and velocity, percentage of good quality embryos, clinical PR, and miscarriage rate
		Study limitations	Retrospective cohort
	Mercan et al, 1998 [62]	Population	207 couples (279 cycles)
		ASA testing	IgG and IgA MAR and IBT in semen (positivity: >30%)
		Main outcome	Comparable clinical PR and delivery rate in ASA positive and ASA negative men
		Study limitations	Number of ASA positive and negative patients not clearly stated, retrospective cohort

ART: assisted reproductive technology, ASA: antisperm antibodies, IUI: intrauterine insemination, IVF: *in vitro* fertilization, ICSI: intracytoplasmic sperm injection, IgG: immunoglobulin G, MAR: mixed antiglobulin reaction, LBR: live birth rate, IgA: immunoglobulin A, ELISA: enzyme linked immunosorbent assay, FR: fertilization rate, PR: pregnancy rate, IBT: immunobead test, IgM: immunoglobulin M.

with ASA) compared with ASA-negative samples (42% *vs.* 52% respectively; odds ratio, 1.45; 95% confidence interval [CI], 0.63–3.30; p>0.05) and ASA in semen were not associated with negative reproductive outcomes (fertilization and clinical pregnancy rate) after ICSI [22,23]. Other studies recommended ICSI as a first choice, as it yields a higher pregnancy rate per cycle compared with intrauterine insemination (IUI) [61,65] or IVF [29]. In a later study by Lu et al [29], ASA negatively correlated with pregnancy rates (odds ratio, 0.630; 95% CI, 0.425–0.932) with IVF. Women coupled with ASA-positive men had half the live birth rates with IVF compared to ICSI. Thus, in the case of ASA, ICSI is the recommended ART technique to be used [18,66].

CLINICAL SCENARIOS

Several clinical scenarios may be encountered when examining patients presenting with sperm agglutination, ASA or cases of failed IUI and IVF. These are described here along with their management.

1. Case 1

1) Scenario

A patient presents with primary infertility and the following semen analysis results: sperm concentration 10×10^6/mL, total motility 25%, moderate to severe agglutination. How do you manage this patient?

2) Solution

With oligoasthenozoospermia and moderate to severe agglutination, ASA testing is required. Evaluate to rule out conditions such as previous testicular/scrotal trauma/surgery, infections, torsion, *etc.* [67].

2. Case 2

1) Scenario #2

A patient presents with history of vasectomy reversal performed 1 year ago and wishes to conceive with a new partner. His semen analysis results are as follows: sperm concentration 4×10^6/mL, total motility 30%, moderate sperm agglutination. How will you manage this patient?

2) Solution

ASA are found in 70% to 100% of men after vasectomy [68]. Sperm agglutination is frequently seen in patients after vasectomy reversal. However, ASA levels following vasectomy reversal are inconsistent in predicting which couples will be successful in conceiving. In this patient, ASA test will be ordered. If the test is positive for ASA, treatment with tapering or low dose corticosteroids may be considered [55]. Alternatively, IUI or ICSI may be recommended.

3. Case 3

1) Scenario

A couple presents with primary infertility. Semen analysis results are as follows: concentration 5×10^6/mL, total motility 31%, moderate to severe agglutination, no female factor infertility. If the couple choose to undergo ICSI, what specific advice would you give them?

2) Solution

Clinical risk factors for ASA should be assessed. However, ASA testing is not recommended in case of ICSI [23].

4. Case 4

1) Scenario

A patient has undergone vasectomy reversal. Semen analysis performed at 6 months after surgery shows the following results: sperm concentration 5×10^6/mL, total motility 25%, moderate to severe agglutination. ASA test results show IgA >50% and IgG >50%, with complete bead attachment to sperm. What is your recommendation to this patient?

2) Solution

The presence of ASA after vasectomy reversal is well-recognized [17,45]. As the ASA test report shows >50% agglutination and complete bead attachment, ICSI should be considered.

GLOBAL ONLINE SURVEY ON ANTISPERM ANTIBODY PRACTICE PATTERNS

1. Methodology

We designed a worldwide online survey to conduct a cross-sectional observational study aiming to investigate the clinical practice of ASA testing in the evalu-

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ation of male infertility (Supplement File 1). A team of experts in the diagnostics of male infertility was recruited from the American Center for Reproductive Medicine (ACRM) (SG, RS, AA, RF) to create a preliminary draft of the survey questions. After an internal revision, these questions were further revised by a pool of international collaborators including andrologists and urologists, to ensure that the questions were appropriate for the target audience (andrologists, urologists, and attending physicians with expertise in male infertility) as well as being easily comprehensible for responders who were non-native speakers of English.

The survey was written in English and included 18 questions organized into 2 different sections: in the first section (7 questions), demographic data of the responders were collected, while in the second section (11 questions), data relating to ASA testing were collected.

Questions were populated online on SelectSurvey (https://www.classapps.com/product_ssv5.aspx), a secured tool approved by the Cleveland Clinic's Information Technology Department. A survey link was sent by e-mail to 106 international experts in male infertility. The link was open from June 19, 2021 to June 28,

Table 4. Demographic data are reported for survey responders (n=66)

Variable	Value
Years of practice	
<2	1 (1.5)
2–5	3 (4.5)
5–10	11 (16.7)
>10	51 (77.3)
Employment	
Uro-andrologist	27 (40.9)
Urologist	18 (27.3)
Andrologist	16 (24.2)
Attending physician	5 (7.6)

Values are presented as number (%).

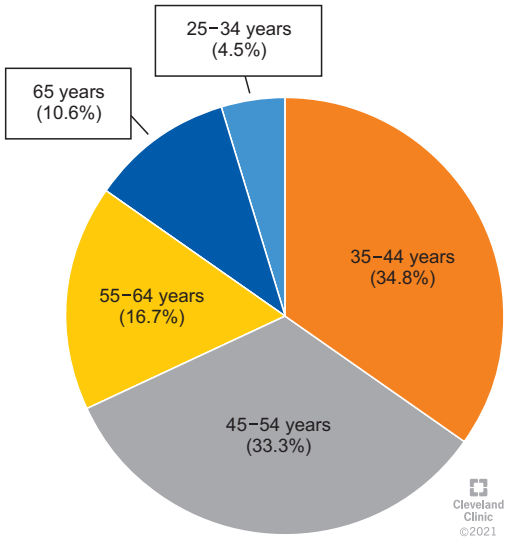

Fig. 9. Distribution of responders as classified based on age.

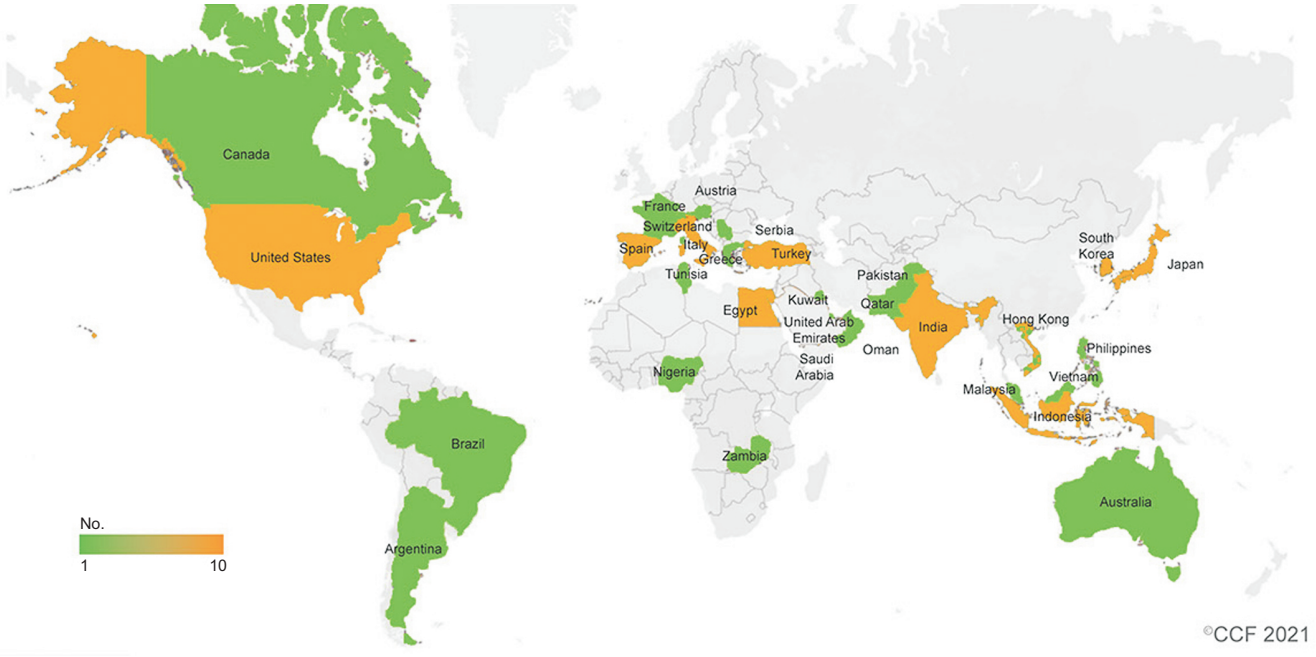

Fig. 8. Geographic distribution of participants in the survey.

2021 to allow the participants to provide their response. Results were downloaded as comma-separated values (CSV) file format and were analyzed by using MedCalc Software (version 19.7.4; MedCalc Software, Ostend, Belgium) after exclusion of incomplete answers. Data are reported as the number of responders and the percentage was calculated on the total number of responders. When responders were allowed to choose more than one option, results in percentage were calculated based on the total number of responders.

A total of 66 out of 106 experts answered the survey, with a response rate of 62.3%. Demographic data are reported in Table 4. Responders originated from 31 different countries (Fig. 8, Supplement File 2), with the majority being between 35 and 54 years old (n=45, 68.2%) (Fig. 9), and with more than 10 years of experience in clinical practice (n=52, 77.6%). Most of the responders identified their primary practice setting as academic hospital/clinic (n=46, 69.7%) or private practice/clinic (n=22, 33.3%) (Fig. 10). Responders were mainly uro-andrologists (n=27, 40.3%), urologists (n=18, 26.9%) and andrologists (n=16, 23.9%).

Almost half of the responders recommended (n=28/66, 42.4%) or did not recommend (n=38/66, 57.6%) ASA testing in clinical practice for patients with male infertility (Table 5). This test was mainly prescribed by responders in cases of sperm agglutination (n=24, 85.7%) and asthenozoospermia (n=15, 53.6%) (Fig. 11), mostly for the evaluation of both IgA and IgG (n=21, 75.0%) by performing the MAR test (n=18, 64.3%).

Within the group of responders who recommend ASA test (n=28), the presence of ASA was considered significant by 71.4% (n=20), particularly when semen

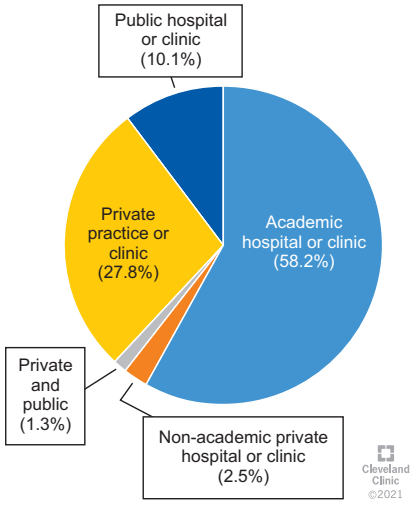

Fig. 10. Distribution of responders as classified based on their primary practice setting.

Table 5. Summary of response from 28 experts recommending ASA testing in male infertility patients

Variable	Value
Indications for ASA testing[a]	
Sperm agglutination	24 (85.7)
Asthenozoospermia	15 (53.6)
Failed IUI	13 (46.4)
Failed IVF	13 (46.4)
Other	4 (14.3)
Antisperm antibody tested	
IgA	3 (10.7)
IgG	4 (14.3)
Both	21 (75.0)
Technique for testing	
MAR test (indirect)	18 (64.3)
Immunobead test (direct)	8 (28.6)
Other	2 (7.1)
Relevance of ASA testing	
Effective	20 (71.4)
Neutral	7 (25.0)
Very effective	1 (3.6)
Biological fluid tested for ASA[a]	
Semen	22 (78.6)
Seminal plasma	8 (28.6)
Serum	4 (14.3)
Cut-off value for abnormal ASA testing (%)	
>20	10 (35.7)
>40	10 (35.7)
>50	8 (28.6)
Frequency of the ASA test ordered	
Monthly	12 (42.9)
Yearly	9 (32.1)
Weekly	7 (25.0)
Number of ASA testing ordered monthly	
1–5	24 (85.7)
6–9	0 (0.0)
10-15	2 (7.1)
>15	2 (7.1)
Initial recommendation in case of positive ASA test	
Steroids	14 (50.0)
ART	8 (28.6)
Sperm washing for IUI	2 (7.1)
Other	4 (14.3)

Values are presented as number (%).
ASA: antisperm antibodies, IUI: intrauterine insemination, IVF: *in vitro* fertilization, IgA: immunoglobulin A, IgG: immunoglobulin G, MAR: mixed antiglobulin reaction, ART: assisted reproductive technology.
[a]Multiple options can be selected.

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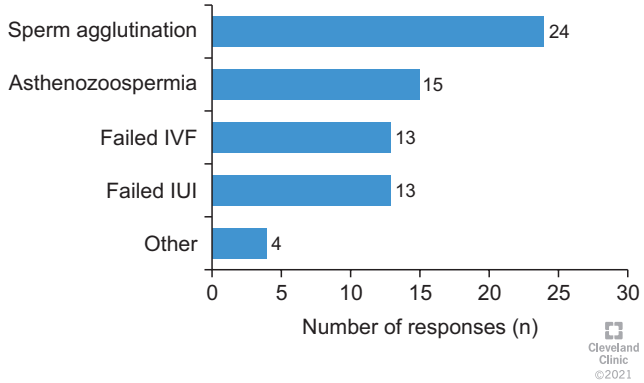

Fig. 11. Reasons for ordering ASA testing. ASA: antisperm antibodies, IVF: *in vitro* fertilization, IUI: intrauterine insemination.

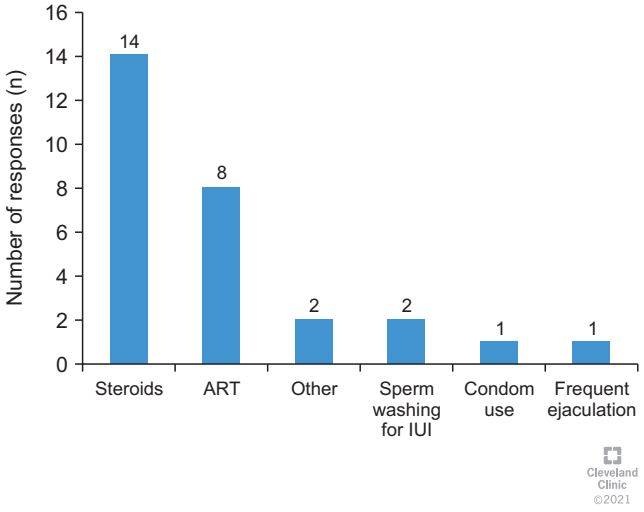

Fig. 12. Recommendations in case of positive ASA at any sperm site (head, mid-piece, tail). ASA: antisperm antibodies, ART: assisted reproductive technology, IUI: intrauterine insemination.

samples are tested (n=22, 78.6%). No consensus was obtained in defining a cut-off value for abnormal ASA testing, as each option (>20%, >40%, or >50%) was indicated by one third of the responders. The results showed that most of the experts (n=12, 42.9%) order the ASA testing at least once per month of practice, with the majority (n=24, 85.7%) ordering between 1 and 5 tests per month of practice. In case of ASA positivity, steroid prescription (n=14, 50.0%) was the most commonly used treatment (Fig. 12), while ICSI was indicated as the best option in case of positive ASA test with majority of sperm head binding (n=19, 67.8%) (Fig. 13).

2. Interpretation of survey results

The survey results showed a response rate of 62.3% from the experts spread across 31 countries. The majority of these experts had more than 10 years of experience practicing (77.3%) in an academic hospital or clinic setting (69.7%), with uro-andrologists (40.3%), andrologists (23.9%) or urologists (26.9%) being the leading groups of practitioners. For these clinicians, the indications for requesting an ASA test included sperm agglutination, asthenozoospermia or failed IUI/IVF cycles. An ASA test was utilized consistently in their practices with 85.7% of those surveyed ordering 1 to 5 ASA tests per month. Most participating specialists requested tests for both IgA and IgG ASA using the MAR direct test while some utilized the IB direct test. Semen was the predominant biological specimen tested for ASA and a cut-off of >20% or >40% as an indication of immunological infertility was utilized by an equal number of clinicians (35.7% *vs.* 37.5%). A cut-off value of >50% (as has been previously recommended in the WHO manual 5th edition) was used by 28.6% of the specialists. Over 70% of the experts indicated that the ASA test is an effective screening test for immunological male infertility.

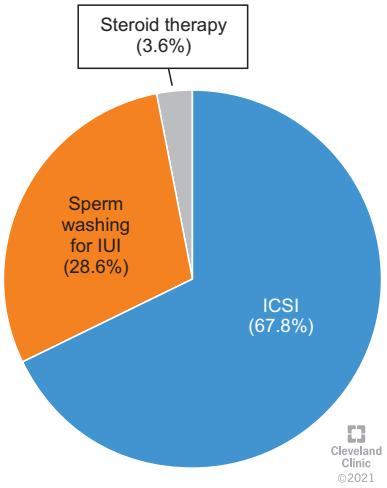

Fig. 13. Recommendations in case of positive ASA testing with majority of sperm head binding. ASA: antisperm antibodies, IUI: intrauterine insemination, ICSI: intracytoplasmic sperm injection.

The survey was also designed to understand the recommendations for practice in patients with positive ASA test. More than 50% of the practitioners recommended low dose steroids as the first-line therapy for patients who had positive ASA testing. More than one third of the experts recommended ART for the management of male infertility associated with immunological causes. ICSI was the preferred recommendation for the majority of cases of positive ASA tests that

bind to the sperm head.

CONCLUSIONS

Despite extensive studies on immunologically-mediated infertility, there is still considerable confusion on the use of ASA testing and the therapy of men with ASA. Many infertility centers and andrology laboratories have abandoned ASA testing and professional societies do not recommend ASA testing in the preliminary phase of male evaluation. The evidence supporting the use of steroids or IUI as a treatment for immunological infertility is sparse, and side-effects from prolonged or high-dose steroid therapy are a valid concern. However, despite these reservations, it appears that ASA testing is widespread amongst clinicians (as shown by our survey) and more than half of them will consider the use of steroids to treat ASA positive cases, and many recommend IUI. This dichotomy between evidence and practice highlights the need for more studies to better understand the practical implications of ASA testing. Meanwhile, ASA testing can be recommended in selected cases, based on suggestive clinical history and semen picture, when the couple is trying for a natural pregnancy, and judicious use of steroid therapy or IUI may be considered for positive cases. However, if a couple is proceeding for ICSI, then there is no need for ASA testing.

ACKNOWLEDGEMENTS

Authors are thankful to the artists Bernastine Buchanan and Ken Kula from the Cleveland Clinic's Center for Medical Art & Photography for their help with the illustrations. The study was supported by the American Center for Reproductive Medicine.

Conflict of Interest

The authors have nothing to disclose.

Author Contribution

Conceptualization: SG, Rakesh S, AA; Data curation: RF; Methodology: AA, Rakesh S, SG, RF; Project administration: AA, Rakesh S, SG, RF; Writing – original draft: AA, SG, FB, RF, Rakesh S, Rupin S; Writing – review & editing: All authors.

Supplementary Materials

Supplementary materials can be found *via* https://doi.org/10.5534/wjmh.210164.

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