The impact of antisperm antibodies on human male reproductive function: an update

Andreia Filipa Silva^{1,2}, João Ramalho-Santos^{1,3} and Sandra Amaral^{1,4}

¹CNC-Center for Neuroscience and Cell Biology, CIBB, University of Coimbra, Coimbra, Portugal, ²PhD Programme in Experimental Biology and Biomedicine (PDBEB), Institute for Interdisciplinary Research (IIIUC), University of Coimbra, Coimbra, Portugal, ³Department of Life Sciences, University of Coimbra, Coimbra, Portugal and ⁴Institute for Interdisciplinary Research (IIIUC), University of Coimbra, Coimbra, Portugal

Correspondence should be addressed to S Amaral; Email: scgamaral@gmail.com

Abstract

Immune infertility occurs due to the presence of antisperm antibodies (ASA). This type of infertility has a relatively low prevalence (2.6–6.6% in infertile men), and its etiology, risk factors, targets, and consequences for male fertility are not completely understood. While it is largely accepted that abnormalities in the blood–testis barrier and/or blood–epididymal barrier are the main factors behind its etiology, and that sperm motility is the most frequently reported altered parameter, few are the well-defined risk factors and ASA targets only now started to be disclosed, with proteins involved in sperm–oocyte interaction rising as the most significant. The development of potential treatments is also limited, being the corticosteroids the more promising. Overall, there are still many knowledge gaps related to immune infertility. With this review, we aimed to gather all the information collected from studies developed in humans in the last decade. Despite the controversial results/inconsistencies, that are not only a result of the complexity of mechanisms/variables involved in ASA infertility but also from the technical approaches to assess ASA and the lack of a consensus regarding the thresholds to be used, this manuscript aims to bring a fresh update on the field. It has become clear that, to obtain more/reliable data, there is a need to assess ASA in all the routine seminal analyses, following WHO recommendations. In this way, it will be possible to obtain consistent and comparable information, that can add to current knowledge. Additionally, multicentric studies with large cohorts are also missing, and future research should take this into consideration.

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Introduction

Infertility is a disease of the reproductive system considered by the World Health Organization (WHO) as a global health issue affecting millions of people at reproductive age. This disease may occur due to both male and female factors, a combination of both, or even due to unexplained reasons (Dohle *et al.* 2010, WHO 2010, Jungwirth *et al.* 2019).

Of the several causes of male infertility, the presence of antisperm antibodies (ASA) leads to so-called immune infertility, an autoimmune disease of the reproductive system with low documented prevalence (Bohring & Krause 2003, Dohle *et al.* 2010, Cui *et al.* 2015, Jungwirth *et al.* 2019). Antisperm antibodies are immunoglobulins that interact with sperm antigens, hindering the function of the male gamete, and thus playing a role in fertility (Azizi *et al.* 2015). In fact, it has been described that the presence of these antibodies can be involved in male fertility impairment (Zini *et al.* 2010, Bozhedomov *et al.*

2014a, 2015, Verón *et al.* 2016, Al-Daghistani 2020). However, the complexity of each individual human body has provided a challenge for research in several areas, and the theme of immunological infertility due to the presence of ASA is not an exception, mainly due to the inherent heterogeneity of human semen samples (Sousa *et al.* 2011).

The aim of this review is thus to provide an overview of the research studies focusing on ASA that were published during the last decade, structured in relevant sections that offer readers an overall state of the art on this issue, in an attempt to clarify what the currently relevant knowledge on this subject actually is, highlighting gaps of information and contradictions, and, finally, shedding some light on what the research on this topic should be in the future.

For this purpose, all the accessible studies performed in humans and written in English found in the PubMed database from 2010 to the end of 2020 were considered, when the search was made using

the keywords ‘antisperm antibodies’. Papers that had several flaws in terms of the concept, experimental design, and/or results expression as well as studies in the female part were excluded. In the same way, whenever justified, papers before 2010 and reviews were included (Fig. 1 and Supplementary Table 1, see section on [supplementary materials](#) given at the end of this article).

The first reported studies on PubMed when the search is made using the keyword ‘antisperm antibodies’ dated from the early 1960s (Fig. 2). However, it is well accepted that the human ASA research journey started in 1954 (Vazquez-Levin *et al.* 2014, Cui *et al.* 2015, Wakimoto *et al.* 2018) with the studies of Rumke (Rumke 1954) and Wilson (Wilson 1954), in which they showed that antibody activity can lead to sperm agglutination and immobilization in the blood (Rumke 1954, Wilson 1954) and seminal plasma (Wilson 1954) in a specific group of men with fertility problems.

Thereafter, the interest in this topic began to increase from the 1970s onwards, reaching a peak in terms of the number of PubMed publications between the late 1980s and early 1990s (Fig. 2). Afterward, the attention in this field starts to decrease, with a constant decline in the published articles in the last 2 decades (Fig. 2). The reasons for such disinterest may be due to the

lack of consistency in the experimental design and methodologies employed, as well as the frequently contradictory results reported.

Despite all the discouraging contradictions that were found during the early years of intense research, systematic reviews and meta-analysis on these issues are scarce. In fact, only a couple of studies started to appear in the last decade. More precisely, six systematic reviews come out (Zini *et al.* 2011a, Cui *et al.* 2015, Foresta *et al.* 2015, Jiang *et al.* 2016, Tiseo *et al.* 2016, Patel *et al.* 2018), in which only half of them includes a consistent meta-analysis (Zini *et al.* 2011a, Cui *et al.* 2015, Jiang *et al.* 2016). Considering the contradictory results obtained through the years, the number of this type of rigorous and compilatory studies, that have the power to identify strengths and weaknesses of the existing studies and eventually improve further research, are frankly insufficient, which once again is reflected in the current disinterest on the topic.

With the upcoming 6th edition of the WHO manual for semen analysis, perhaps more andrology and research centers may become WHO associated laboratories, contributing with their expertise and knowledge, and delivering new and more reliable data, in a global effort to research aspects that are not yet fully understood regarding ASA-related male infertility.

Antisperm antibodies etiology

Despite its potentially high relevance, it is not currently possible to clearly state that the etiology of ASA has been fully characterized. It is generally accepted that developmental abnormalities in the blood–testis barrier (BTB) formation as well as its weakness, damage, and/or disruption can lead to the appearance of ASA (Chamley & Clarke 2007, Azizi *et al.* 2015, Brincat *et al.* 2015, Xu *et al.* 2020) due to the exposure of immunogenic sperm antigens to immunocompetent cells from the immune system (Mazumdar & Levine 1998). In fact, some conditions which are associated, at least to some extent, with the integrity of the BTB, and can also be possible risk factors for ASA development as we will further discuss, have gained attention in the study of ASA-mediated male infertility. Additionally, although not so explored, it is also possible that ASA may be produced at the epididymal level (Lotti *et al.* 2018). In fact, the blood–epididymal barrier (BEB) protects the epididymis and regulates the luminal microenvironment allowing sperm maturation and storage. In the same way, the loss of BEB function/integrity, believed to be less robust than BTB, can more easily expose the sperm cells to the immune system resulting in ASA production (Hedger 2011, Lotti *et al.* 2018). However, in the vast majority of cases, the generation of naturally occurring ASA seems to have an idiopathic origin (Barbonetti *et al.* 2019).

Figure 1 Flow diagram of the studies selection criteria. From the initial 204 studies (initial PubMed search), only studies in humans were included. Furthermore, reviews, non-English and with no access studies were excluded. After full-text screening, and considering support manuscripts, 178 studies were included in this review.

Figure 2 Graphic representation of the distribution of ASA studies throughout the years. This information was assessed at the PubMed database when the search was made using the keywords 'antisperm antibodies'. The publications in this field peaked in the time interval represented between the two arrows. Source: <https://pubmed.ncbi.nlm.nih.gov/> (accessed on 20 January 2021).

Types of antisperm antibodies and their clinical relevance

In men, antisperm antibodies can be found in the blood serum and also in seminal plasma both free and bound to the surface of the sperm cell (Cui *et al.* 2015). They are immunoglobulins against the sperm cell which belong almost exclusively to the class G and A, namely IgG and IgA, respectively (WHO 2010, Vazquez-Levin *et al.* 2014). Both immunoglobulin classes can be detected on the surface of the sperm cells or in biological fluids (WHO 2010). Nevertheless, the principal immunoglobulin found in the blood belongs predominantly to the IgG isotype while those found in external secretions, such as the seminal plasma or even the cervical mucus in women, are mostly from the IgA isotype (Schroeder & Cavacini 2010). The IgA isotype, found at the mucosal surfaces and secretions, has an essential role in the protection from several external agents such as toxins, virus, or bacteria (Schroeder Jr & Cavacini 2010), and this is the reason why this type of antibodies may assume more clinical importance than IgG antibodies (Kremer & Jager 1980, WHO 2010). The targets of these IgG and IgA-ASA are now starting to be disclosed, yet the knowledge is still limited as we will discuss later in this manuscript (see 'Antisperm antibodies targets' section).

Detection of antisperm antibodies

The World Health Organization has recommended the testing for antisperm antibodies as a 'standard procedure' referring to the event of sperm agglutination as a possible indicator of the presence of ASA. Nevertheless, it also notes that agglutination can be caused by factors other than ASA as well as the fact that ASA may be present even without sperm agglutination (WHO 1999, 2010). In our understanding, these recommendations highlight the necessity of routine test for ASA. From a research point of view, the testing of ASA is crucial to gather relevant data regarding ASA-associated fertility problems. However, these tests are not systematically recommended, even in WHO-associated laboratories, unless specific indicative conditions are present, as is the case of sperm agglutination. Nonetheless, Gatimel and co-workers further suggest the history of scrotal trauma or inguinal surgery, as significant factors to

justify the testing for ASA (Gatimel *et al.* 2018). Yet, a consensus about the exact situations in which tests should be performed is lacking (Lu *et al.* 2010, Gatimel *et al.* 2018).

Considering the methodologies to detect ASA, since 1999 that WHO has recommended the use of both direct and indirect tests to detect the presence of IgG and IgA (WHO 1999, 2010). The direct tests, which detect ASA on the surface of motile sperm cells are widely used and comprise the mixed antiglobulin reaction (MAR) test and the immunobead (IB) test. Both of them are well described in the last editions of the WHO manual (WHO 1999, 2010) and despite their similarities, they present important differences that should not be neglected. While the MAR test entails the use of fresh semen samples, the IB test should be performed in washed samples (WHO 1999, 2010). Due to its application in unwashed fresh samples and the possible use of treated erythrocytes as beads, which makes it faster and cheaper (WHO 1999, 2010), the MAR test is the most routinely used in the andrology laboratories (Gatimel *et al.* 2018). Additionally, it is important to remark that the IB test was recently curtailed by the manufacturer, and a progressive replacement of this test by the new immunosphere (IS) test is expected. This new IS test is similar to the IB test considering the concept and execution and also shows a good correlation with the former IB test (Bollendorf & Check 2016).

When necessary or when it is not possible to perform the direct tests, for example, due to a high percentage of immotile sperm cells in the sample, WHO suggests the use of indirect tests. The indirect tests can detect ASA in heat-inactivated sperm-free fluids as is the case of blood serum, testicular fluid, seminal plasma, or even solubilized cervical mucus, using for this purpose antibody-free donor spermatozoa following the direct IB test mentioned above (WHO 1999, 2010).

On the other hand, considering the good specificity and the quantitative power of the enzyme-linked immunosorbent assay (ELISA; Mazumdar & Levine 1998), this serological test is also broadly accepted in the screening of blood serum ASA (Lu *et al.* 2010), probably because it bypasses the complexity and slowness of the indirect tests recommended by the WHO. There are also other indirect tests, such as the tray agglutination test (TAT), gelatin agglutination test (GAT), and sperm immobilization test (SIT; Gandini *et al.* 1995,

Naz & Butler 2013), that were quite used in the past but nowadays are not so common.

Overall, the direct MAR and IB tests along with ELISA are the first line of choice tests for ASA screening, both in semen and serum. However, the results obtained from the different methodologies (Xu *et al.* 2020) and between the different commercially available kits (Lu *et al.* 2019) are not always consistent. In fact, based on studies developed during the 1980s, the WHO reported the fact that the results obtained from MAR and IB tests do not always match (MacMillan & Baker 1987, Scarselli *et al.* 1987, Meinertz & Bronson 1988, Hellstrom *et al.* 1989). Also, a relatively recent report from a survey in China concluded that the methods of semen analysis, as well as results interpretation among laboratories, have marked differences, with several of these laboratories working with other methods than those recommended by the WHO (Lu *et al.* 2010). Therefore, the standardization of ASA detection among laboratories worldwide seems to be urgent.

The recently validated protein biochip, a microarray-based technology, which takes advantage of the high-throughput methodology with minimal input of sample and providing extremely high sensitive and specific results, has shown significant consistency with ELISA in serum ASA detection (Wang *et al.* 2017, Xu *et al.* 2020). In addition, the results obtained with the MAR test were also comparable with the biochip approach (Xu *et al.* 2020). This type of technology was, therefore, suggested as an important tool for the screening of ASA in serum in large cohorts of patients (Wang *et al.* 2017, Xu *et al.* 2020).

Additionally, there are authors that have used flow cytometry as this technique allows the quantification of IgG and/or IgA levels in each live sperm cell (Haas & Cunningham 1984, Räsänen *et al.* 1992), which is an upgrade of the immunofluorescence assays that also use fluorescein-tagged anti-human antibodies to detect ASA but it has to be performed in fixed samples (Helmerhorst *et al.* 1999). Nonetheless, this method has not gained a special relevance.

When it comes to the threshold used to consider ASA levels pathological, WHO recommends 50% motile spermatozoa with adherent particles (WHO 1999, 2010). However, the use of different methodologies to detect ASA, along with differences in thresholds for ASA positivity among the research works, make comparisons between studies hard to accomplish (Supplementary Table 1). Among the WHO recommended tests (MAR test and IB test), the expression of the obtained results is made in percentage (%) of cells while in the case of the ELISA assays, the results are given using the international units (IU) for enzymatic assays, making them difficult to compare. Another example of this difficulty is the case of the already mentioned biochip technology, in which the results are expressed in fluorescent optical density (FOD), highlighting one of the major limitations in the

interpretation of studies focusing on ASA-related male infertility.

In fact, while in the ELISA method, the definition of the thresholds is relatively easy to follow and supported by several studies in which comparisons with the results obtained by WHO recommended tests were done for many years, the most recent technologies, as the case of the biochip approach, do not have this support, and the results are more difficult to understand. These aspects might also justify some of the contradictions found in the literature, stressing the need to homogenize the tests used to assess ASA worldwide.

Antisperm antibodies prevalence

Differences in the occurrence of ASA directly derive from the testing method and obviously from the cut-off definition used (Vazquez-Levin *et al.* 2014, Verón *et al.* 2016, Barbonetti *et al.* 2019). Furthermore, some of the available results were not obtained in a clinical setting, where theoretically, the types of tests used to detect ASA and the cut-off value for ASA positivity are well established by the WHO. This fact leads to significant differences in terms of the percentage of positive ASA samples among distinct research cohorts and a lack of consistency considering the prevalence of this specific group of patients.

Another confounding aspect is the fact that the incidence of ASA is also affected by changes in the WHO criteria for seminal quality throughout the years. Although the threshold value for ASA positivity was maintained unaltered in the last two editions, the same was not verified for the reference levels for seminal quality (WHO 1999, 2010). This detail means that, based on the spermogram results, patients that were considered to have poor parameters according to the 1999 criteria, might be considered normal according to the WHO 2010 edition since in 2010, there was a decrease in the reference levels limit (Jensen *et al.* 2018). This suggests that probably before 2010 more patients were being screened for ASA than now. We hope that the upcoming 6th edition of the WHO manual for semen analysis can definitely help to mitigate these differences. Additionally, several other factors can contribute to the variability of the reported prevalence of these antibodies, such as sample size and the experience of the lab technician that is performing the test, among others.

As expected, the incidence of ASA varies between fertile and infertile men, with the reported percentages associated with fertile men being much lower than the ones reported in the infertile patients (Zini *et al.* 2011a, Cui *et al.* 2015). Yet, the available data in the fertile group are scarce since these individuals are not extensively studied, and here we need to highlight again that the two existing WHO manuals for semen analysis might play a role. During the last decade, two meta-analyses reported similar results regarding the prevalence of ASA

in infertile and fertile men (Zini *et al.* 2011a, Cui *et al.* 2015) with significant levels of ASA detected in the semen of 5–15% of infertile men (Adeghe 1993, Collins *et al.* 1993, Sinisi *et al.* 1993) but only in 1–2% of fertile ones (Sinisi *et al.* 1993, Bates 1997).

Based on the most relevant and recent literature, we noticed a mean ASA prevalence among the infertile male population between 2.6 and 6.6% (Table 1). For this, we considered four studies that used the direct MAR test methodology and the 50% threshold for positivity given by the WHO (Verón *et al.* 2016, Gatimel *et al.* 2018, Lotti *et al.* 2018, Barbonetti *et al.* 2019). Among these research works, there is a recent and wide retrospective study of over 10,000 men that certainly provides a consistent contribution (Barbonetti *et al.* 2019). Also, in one of the studies guided by the same criteria, the ASA incidence among a cohort of fertile men (0.9%; $n=109$) was also reported (Lotti *et al.* 2018), and this percentage is in accordance with the oldest studies mentioned above (Zini *et al.* 2011a, Cui *et al.* 2015).

Interestingly, using the biochip technology and the ELISA methodology, the percentages of ASA prevalence found in serum seem to be quite higher when compared with those obtained on the sperm surface using the direct MAR test (Table 2; Lu *et al.* 2019, Xu *et al.* 2020). In fact, although these types of technologies have the advantage of being more sensitive, comparisons are difficult to make since the direct MAR test measures the sperm cell surface ASA, while the others detect free ASA in the blood serum. Nonetheless, considering biochip technology, there was a study in which the results were comparable with that obtained by ELISA and MAR test methods (Table 2; Xu *et al.* 2020), although in this case, the prevalence determined by the MAR and ELISA tests were higher compared to previous studies (Verón *et al.* 2016, Gatimel *et al.* 2018, Lotti *et al.* 2018, Barbonetti *et al.* 2019, Lu *et al.* 2019).

Antisperm antibodies effects on fertility

Antisperm antibodies may affect male fertility in several ways. The most documented effects are their capacity to reduce sperm motility, cause sperm agglutination, impair sperm ability to penetrate the cervical mucus, or inhibit sperm capacitation, the acrosome reaction, sperm-egg interaction, as well as the initial stages of

Table 2 Prevalence of blood serum ASA among infertile men assessed by protein biochip and ELISA methodologies.

Reference	Samples (n)	Prevalence among infertile men (%)		
		Protein biochip*	ELISA	MAR
Xu <i>et al.</i> (2020)	368	20.92	21.47 [†]	16.03 [‡]
Lu <i>et al.</i> (2019)	554		10.5 [†]	

*ASA positivity: >1547 FOD; [†]ASA positivity: >3 × optical density (OD) of negative controls; [‡]ASA positivity: >75 IU; [§] $n=262$. ASA, antisperm antibodies; FOD, fluorescent optical density; MAR, mixed antiglobulin reaction.

embryo development (Sinisi *et al.* 1993, Nagy *et al.* 1995, Mazumdar & Levine 1998, Lombardo *et al.* 2001, Francavilla & Barbonetti 2009, Krause 2009, Zini *et al.* 2010, Bozhedomov *et al.* 2015, Verón *et al.* 2016, Al-Daghistani 2020). Additionally, although not so unanimous, other parameters such as sperm count, morphology, and viability also seem to be prone to alterations in this specific group of patients (Zini *et al.* 2010, Štula *et al.* 2012, 2014, Cui *et al.* 2015, Verón *et al.* 2016).

The conventional seminal analysis (motility, morphology, concentration) is widely used by clinicians and researchers to categorize the type of male infertility when no other clinical alterations are reported. Motility has gained particular importance in ASA since effects are immediately observable and easily quantified. It is definitely the most accepted altered parameter when it comes to the negative effects of the presence of ASA.

Furthermore, as male infertility is already known to be associated with sperm DNA damage (Evenson *et al.* 1999, Zini & Sigman 2009), it was also possible that a poor DNA integrity may be a consequence of ASA presence and, therefore, an indirect cause of infertility in ASA patients. However, the results regarding this topic are conflicting, and if on one hand there is a study that does not confirm this hypothesis (Zini *et al.* 2010), another one showed an increase in DNA fragmentation in ASA patients along with an increase in sperm reactive oxygen species (ROS) generation (Bozhedomov *et al.* 2015).

Although some doubts and questions still persist, overall, it seems that the presence of ASA may affect male fertility in several ways. Sometimes reducing seminal quality, others impairing steps in the fertilization process, or even a combination of both, but it is also a truth that ASA does not always cause fertility problems, so its relevance to the global male infertility panorama should be re-evaluated. The nature of the ASA targets is now starting to be disclosed. Although the relevance of the proteins identified so far is not always straightforward, it is expected that full and integrated characterization at a molecular level will provide the necessary knowledge to better diagnose ASA-mediated immunoinfertility and eventually to tailor the treatment to these patients.

Table 1 Prevalence of sperm-bound ASA among infertile men assessed by the direct MAR test and using a 50% cut-off for ASA positivity (WHO 2010).

Reference	Samples (n)	Prevalence among infertile men (%)
Barbonetti <i>et al.</i> (2019)	10,025	3.4
Gatimel <i>et al.</i> (2018)	1364	4.4
Lotti <i>et al.</i> (2018)*	181	6.6
Verón <i>et al.</i> (2016)	7492	2.6

*Study also reports 0.9% prevalence among fertile men ($n=109$). ASA, antisperm antibodies; MAR, mixed antiglobulin reaction.

Possible risk factors for antisperm antibodies

Vasectomy

Vasectomy is a surgical procedure for male sterilization in which both vas deferens are cut or sealed. This type of procedure can lead to an immunogenic response thus initiating the production of ASA (Marconi & Weidner 2017). The association between vasectomy and ASA production is well documented (Gubin *et al.* 1998, Marconi *et al.* 2008, Lee *et al.* 2009, Azizi *et al.* 2015). In fact, this is one of the most accepted risk factors associated with ASA generation (Marconi & Weidner 2017). Additionally, it was recently reported that the predisposition for ASA production in vasectomized men seems to be associated with some molecules of the human leukocyte antigen (HLA) system, with men having lower serum ASA levels presenting specific HLA antigens, such as HLA-A2, HLA-B5, HLA-DR53, and HLA-DQ2 (Azizi *et al.* 2015). However, the threshold that used to define ASA positivity was not mentioned (Supplementary Table 2), and the relationship between HLA antigens and ASA production requires additional research since a strong association between other HLA antigens (HLA-A28) with ASA production after vasectomy was also reported (Law *et al.* 1979).

On the other hand, sometimes vasectomized men seek vasectomy reversal surgery, and it is possible that elevated ASA can lead to limited success in fertility recovery. In fact, after vasectomy reversal, an increase in serum ASA levels was observed to be associated with the BMI (overweight and obese) of the studied individuals but, as referred by the authors, the value of this finding is not clear; yet, no association was found between the serum ASA levels and fertility outcomes (Hinz *et al.* 2010). This study has also crucial points that were not mentioned, as is the case of the method to quantify ASA and the threshold used (Supplementary Table 2).

Infection and inflammation

The role of inflammatory processes in the male genital tract in the development of ASA has also been controversial (Krause & Naz 2009, Marconi *et al.* 2009).

For example, regarding the prostate, it is known that the occurrence of an infection and inflammation is associated with prostatitis and may also be implicated in both benign prostatic hyperplasia and prostate cancer scenarios (Naz 1997, Kramer *et al.* 2007, Coussens & Werb 2002). However, the most recent report on this topic has found no association between these prostate conditions and the induction of ASA formation (Hoover & Naz 2012). Additionally, these types of prostate disorders lead to an increase of circulating prostate-specific antigen (PSA), which is a serine protease produced by the prostate gland and secreted into the seminal fluid with the main function of dissolving the seminal coagulum allowing sperm to swim freely (Naz

& Butler 2013). However, there is no evidence of a concomitant induction of immunity to spermatozoa when a PSA rise is observed due to these prostate disorders (Hoover & Naz 2012).

Interestingly, in a group of ASA-positive immunoinfertile men without any prostate disorder, an increase of seminal plasma PSA antibodies was reported (Cuppert *et al.* 2017) and similar observations had already been obtained in a different study, but in the sera of immunoinfertile men, although there the results did not reach statistical significance (Naz & Butler 2013). Considering the main function of PSA in the liquefaction of seminal coagulum, it is expectable that the presence of antibodies against PSA in the seminal plasma may interfere with the fertilization process. However, for these authors, the mechanisms that lead to the production of PSA antibodies in ASA-positive immunoinfertile men are unclear and whether these PSA antibodies are the causative factor of infertility or just associated with it require further investigations (Naz & Butler 2013, Cuppett *et al.* 2017). Nonetheless, these studies are an example of those that use a different threshold for ASA than the one recommended by WHO; in this case, they refer that positivity is 20%, very low considering the WHO criteria of 50%, and it is, therefore, difficult to compare with other works and to hypothesize about the possible role of PSA antibodies in this type of infertility (Supplemental Table 2).

In an orchitis scenario, which is an inflammation of the testis, the most recent data did not find any association between ASA development and previous orchitis (Yasin *et al.* 2016, Lu *et al.* 2019). However, the existing works did not explore this condition properly, and only enrolled a few patients.

Moreover, in a recent study, interesting new aspects were identified in ASA-positive infertile patients as is the case of epididymal abnormalities, history of epididymitis, and high levels of the marker of genital tract inflammation, seminal plasma interleukin 8 (sIL-8), but, surprisingly, not testicular alterations. These results reported by Lotti and collaborators, following the WHO guidelines for ASA positivity, are strongly suggestive of chronic epididymal inflammation in ASA-positive patients (Lotti *et al.* 2018). The authors suggest that the epididymal function should be, therefore, studied in ASA-positive patients. However, in this study, the authors did not observe any correlation of epididymal alterations with seminal parameters, and this aspect should be further explored in further studies.

Viral infections

Seminal infections are widely accepted as a significant contributor to male infertility (Ochsendorf 2008, Ruzs *et al.* 2012). Although these infections may trigger autoimmune reactions accompanied by inherent ASA production (Witkin *et al.* 1995), the association of

viral infections with ASA development has not been extensively studied. As a well-recognized cause of orchitis, mumps was the focus of some studies at the beginning of the 2000s (Kalaydjiev *et al.* 2001, 2002, Jarow 2002), that explored its consequences in terms of ASA production, however, not finding a clear association. During the last years, this question was only addressed in the cases of hepatitis C virus (HCV; Hussein *et al.* 2017), human papilloma virus (HPV; Garolla *et al.* 2013, 2018, Luttmer *et al.* 2016), and adeno-associated virus (AAV; Schlehofer *et al.* 2012).

Despite some differences between studies, the main finding and conclusion considering the context of a viral infection is the existence of a higher prevalence of ASA on the sperm surface, accompanied by a significant decrease in sperm motility (Garolla *et al.* 2013, 2018, Hussein *et al.* 2017). These findings were reported in HCV men (Hussein *et al.* 2017) as well as in HPV infertile men (Garolla *et al.* 2013) along with the decrease of ASA prevalence and motility improvement in the HPV vaccinated ones (Garolla *et al.* 2018). Although several recent findings suggest that viral infections could adversely contribute to the impairment of sperm motility through the development of ASA, contradictory results also exist, in which no association between the presence of both HPV (Luttmer *et al.* 2016) and AAV (Schlehofer *et al.* 2012) with seminal quality and ASA presence were reported. Although these studies present a suitable number of samples, the threshold for ASA positivity was not the same. While in the study of Garolla *et al.* (2013) the threshold for ASA positivity was defined according to the WHO criteria ($\geq 50\%$), in other cases this threshold was $>10\%$ (Hussein *et al.* 2017), $\geq 30\%$ (Schlehofer *et al.* 2012), or not even mentioned (Luttmer *et al.* 2016, Garolla *et al.* 2018). These differences in ASA-positive group definition may lead to variations and, to some extent, explain some of the contradictions reported (Supplemental Table 2).

Bacterial infections

Seminal quality decline has been associated, by several authors, with the process of male genital tract infection and inflammation (Diemer *et al.* 2003) and, among bacterial pathogens, *Chlamydia trachomatis* was reported as the most sexually transmitted bacteria in industrialized countries (Johnson *et al.* 2002, Eggert-Kruse *et al.* 2015). Thus, the majority of the studies on male seminal quality in the context of bacterial infection have been focused on *Chlamydia trachomatis*.

Curiously, a study that used next-generation sequencing technology and bioinformatics analysis to study all seminal bacterial communities and their association with seminal quality did not report the genus *Chlamydia* as one of the most abundant among samples, appearing only in a trace percentage. More than that, they do not mention *Chlamydia* in the genera associated

with normal or abnormal seminal parameters, being *Sphingobium* the only genus significantly abundant in samples with abnormal clinical ASA value (Weng *et al.* 2014).

On the other hand, heat shock proteins (HSP) also known as chaperones seem to have a significant role since these proteins are released in response to stressful conditions, as is the case of infections (Martínez-Prado & Camejo Bermúdez 2010, Naaby-Hansen & Herr 2010). Besides that, the bacterial 60 kd HSP family (bacterial HSP60), which are recognized as the major antigenic determinants during an infection, shares approximately 50% homology with human HSP60 (Shinnick 1991, Morrison *et al.* 1992). This fact can lead to cross-reactivity issues associated with the development of autoimmune and inflammatory diseases (Jones *et al.* 1993, Wick *et al.* 2004), which may result in reproductive failure through ASA generation (Eggert-Kruse *et al.* 2015). However, the most recent studies are not unanimous regarding the association between the presence of chlamydial HSP antibodies and the presence of ASA (Martínez-Prado & Camejo Bermúdez 2010, Eggert-Kruse *et al.* 2015) with some authors reporting an association between them (Martínez-Prado & Camejo Bermúdez 2010) in contrast to others (Eggert-Kruse *et al.* 2002, 2015).

In this context, the existence of molecular mimicry between sperm and bacteria leading to cross-reactivity issues between ASA in humans and bacterial antibodies has been reported. A sperm immobilization factor (SIF) isolated from a bacteria immobilizes both human spermatozoa and motile bacteria (Prabha *et al.* 2009) due to the existence of a common receptor for SIF on spermatozoa and bacteria, that was recently identified (Prabha *et al.* 2011). In this particular study, the authors advanced the possibility of intravaginal therapy using these receptors in an attempt to improve fertility (Prabha *et al.* 2011). Transposing to its relevance in the ASA context, they hypothesize that the existence of this cross-reactivity of sperm antigens and exogenous antigens may be the trigger for ASA development (Prabha *et al.* 2011) which certainly deserves to be more widely studied.

Varicocele

Varicocele is a condition characterized by an abnormal enlargement and a tortuosity of the spermatic cord veins and it is a highly prevalent condition among men, that is often found during routine physical examinations but whose etiology and associated pathophysiological mechanism remain not fully understood (Agarwal *et al.* 2007, Lomboy & Coward 2016). The available literature on the possible influence of varicocele on ASA formation, so far, is contradictory (Marconi & Weidner 2017), ranging from studies reporting an association between both conditions (Golomb *et al.* 1986, Gilbert *et al.* 1989, Knudson *et al.* 1994, Isitmangil *et al.* 1999) to studies reporting that varicocele is not a risk factor for

ASA production at all (Oshinsky *et al.* 1993, Heidenreich *et al.* 1994, Veräjänkorka *et al.* 2003). During the last few years, only two studies contributed to this topic, but no correlation was found between ASA generation and varicocele (Bozhedomov *et al.* 2014a, Lu *et al.* 2019). Nevertheless, there is evidence that ASA in patients who already suffer from varicocele can considerably impair sperm function (Bozhedomov *et al.* 2014a). Indeed, ASA-positive varicocele patients were reported to have lower sperm concentration, progressive motility, and normal morphology, when compared with patients with varicocele, but without ASA. Additionally, the acrosome reaction seems to be impaired in patients with both ASA and varicocele, but in this case, when compared with a fertile control group. Finally, these authors also found a positive correlation between the percentage of sperm immunization and DNA fragmentation rate as well as the production of sperm ROS in ASA-positive varicocele patients, compared with both ASA-negative varicocele ones and a fertile group (Bozhedomov *et al.* 2014a).

The controversy on this topic is necessarily extended to the benefits of the varicocele surgical treatment in the seminal parameters of varicocele patients who already have ASA. Bozhedomov and colleagues have shown an unfavorable prognosis of varicocele surgical repair in cases of an initial high percentage of ASA and also demonstrated that the surgery efficacy in varicocele-ASA patients was lower compared with varicocele patients without ASA, translated by a decrease in the reproductive function reestablishment (Bozhedomov *et al.* 2014b). Despite these results, it was also suggested that the varicocelectomy should not be abandoned and the existence of pre-operative ASA treatment protocols should be considered to maximize surgery benefits in varicocele-ASA patients (Al-Adl *et al.* 2014, Bozhedomov *et al.* 2014b). Considering the possibility of ASA generation after varicocele surgical repair, recent reports did not find any association in semen (Bonyadi *et al.* 2013) and serum ASA levels (Yasin *et al.* 2016, Lu *et al.* 2019). In fact, to our knowledge, only one study reported an increase in serum ASA levels after varicocelectomy (Bonyadi *et al.* 2013).

Cryptorchidism

Cryptorchidism is a common and well-known congenital condition among infant boys. Characterized by the absence of one or both testis from the scrotum, this condition is a well-recognized cause of infertility (Chung & Brock 2011).

Despite the contradictory results from earlier studies (Marconi & Weidner 2017), the most recent efforts did not show any associations between pre-puberty cryptorchidism (Imamoğlu *et al.* 2012, Jiang & Zhu 2013a) or orchidopexy (Jiang & Zhu 2013a) and the presence of ASA. At a genetic level, no differences were found among cryptorchidic patients in terms of

ASA positivity, notably in the natural killer cells surface receptors, which allow them to attack and kill abnormal cells as well as prevent them to attack the normal ones (Niepiekło-Miniewska *et al.* 2015). However, in terms of the human major histocompatibility complex class II HLA molecules, a surprising weak association of these genes and the predisposition to produce ASA among cryptorchidic pre-pubertal boys were described (Kurpisz *et al.* 2011). Nevertheless, the relevance of these observations requires further studies (Kurpisz *et al.* 2011).

Inguinal hernia

Inguinal hernia is a common medical condition that can be rapidly solved through a simple surgical procedure that may, however, potentially impact fertility. The current standardized surgical treatments are laparoscopic or open hernia repair being a mesh used in the majority of the cases (Scott *et al.* 2002, Sršen *et al.* 2008, Perko *et al.* 2011). In fact, it has been postulated that the reason for infertility issues in some of the cases could also be associated with immunological reactions derived from both the surgical procedures as well as the use of a mesh and the subsequent inflammatory process (Štula *et al.* 2012, Gupta *et al.* 2021).

The more recent literature has reported a significant increase in serum ASA levels from the pre- to post-operative situation in patients that underwent open (Štula *et al.* 2012, 2014, Krnić *et al.* 2016, Gupta *et al.* 2021) and urgent incarcerated (Krnić *et al.* 2016) hernia repair, although within the normal range values. Apparently, inguinal hernia repair does not lead to a clinically significant humoral autoimmune response (Marconi & Weidner 2017). However, between the open and laparoscopic methods, the former seems to be the best option with a minimal increase in ASA levels (Štula *et al.* 2012, 2014, Gupta *et al.* 2021).

Autoimmune diseases

An autoimmune disease is a condition in which the immune system mistakenly produces antibodies that attack their own body's cells (NIEHS website: <https://www.niehs.nih.gov/health/topics/conditions/autoimmune/index.cfm> accessed on 16 January 2021). Considering 'immune infertility' is also an autoimmune disease, it would be expectable that ASA could also be produced in association with other autoimmune conditions (Bohring & Krause 2003). However, the last studies in this topic did not find any association between ASA presence (and also seminal quality and hormonal profiles) and autoimmune conditions, as is the case of primary antiphospholipid syndrome (Rabelo-Júnior *et al.* 2012) and ankylosing spondylitis (Nukumizu *et al.* 2012).

On the other hand, an antigen involved in the fertilization process (Busso *et al.* 2005) which was

described as recognized by ASA, the cysteine-rich secretory protein (CRISP-2; Brunner-Agten *et al.* 2013), has shown structural similarities with the major wasp venom allergen (Ves v 5; Müller *et al.* 2008). The authors demonstrated that ASA-positive men had a higher incidence of allergy against Hymenoptera venom, and the results also showed a high amount of autoantibodies that cross-react with both CRISP-2 and Ves V 5 allergen. These autoantibodies lead to sperm immobilization and bind along the sperm surface, including the head, where CRISP-2 is localized (Brunner-Agten *et al.* 2013). Additionally, the fact that these autoantibodies can bind to Ves V 5 led the authors to hypothesize that ASA-positive men may have a higher risk to develop wasp allergy (Brunner-Agten *et al.* 2013). However, ASA positivity in this study was considered above 10% which is very different from the WHO reference value. This, together with the low number of individuals per experimental group, and the lack of discussion on the functional effects of ASA interaction with CRISP-2, that might actually compromise the fertilization process constitute some of the limitations of this study and therefore the value of the obtained results should be further explored and proven.

Finally, an association between programmed cell death 1 (*PDCD1*) gene polymorphism and ASA-related infertility was also suggested (Zamani *et al.* 2015). *PDCD1* has an immunoinhibitory function (Nishimura *et al.* 1999), and polymorphisms in this gene were already associated with several autoimmune diseases including rheumatoid arthritis (Lin *et al.* 2004), type 1 diabetes (Nielsen *et al.* 2003), multiple sclerosis (Kroner *et al.* 2005), ankylosing spondylitis (Lee *et al.* 2006), and allergies (James *et al.* 2005). In this particular study, a significant difference in the genotype and allele frequencies between ASA infertile patients and healthy controls was shown (Zamani *et al.* 2015). This study has, however, several crucial points that were not clear, as is the case of the method to quantify ASA and the threshold used (Supplemental Table 2), so one should be cautious in drawing any conclusions.

Other factors

Besides the supra-mentioned risk factors (often theoretically) associated with the presence of ASA, there are also some research studies that tried to disclose other risk factors associated with this ASA condition.

The production of ASA as a consequence of a vasectomy-induced immune response was suggested as a risk factor for primary progressive aphasia (PPA), language-based frontotemporal dementia, which is a type of primary dementia (Weintraub *et al.* 2006). Although the mechanisms involved in this association were not completely disclosed and are, in fact, difficult to understand, they are mainly based on some antigenic cross-reactivity between sperm and brain (Schachner

et al. 1975, Chaffee & Schachner 1978a,b), and the fact that the sperm is the only cell type, outside of the CNS, that express the cytoskeletal tau protein, whose mutations are linked to familial dementia (Ashman *et al.* 1992). Nonetheless, in Alzheimer's disease, the most common cause of primary dementia, no association was found between the presence of serum ASA and cognitive or language dysfunction in elderly patients with this comorbidity (Han *et al.* 2010). However, the relevance of these studies in the context of male fertility seems to be minor.

Another study focused on a possible association between the testicular microlithiasis and ASA formation. Testicular microlithiasis is an uncommon pathological condition characterized by the deposition of multiple tiny calcifications throughout the testes with possible implications on the structure of the BTB. However, the authors concluded that this condition does not appear to be related with an elevated level of ASA in a cohort of infertile men (Jiang & Zhu 2013b). Another hypothetical risk factor associated with a deleterious impact on the structure of the BTB is testicular sperm extraction (TESE) procedure, a common technique used for testicular sperm retrieval in cases of non-obstructive azoospermia (NOA), and several were the studies that focused on this aspect. Surprisingly, in terms of ASA development, only two studies were published, both concluding that TESE procedure does not lead to a significant increase of serum ASA levels (Komori *et al.* 2004, Ozturk *et al.* 2011). Children testicular rupture, a rare complication of testicular trauma, in which surgery is recommended due to the extrusion of the testicular contents, was also studied in the context of ASA production. However, no alterations were reported in the follow-up regarding the ASA levels after surgical repair (Pogorelić *et al.* 2011).

On the other hand, interesting data came out from another recent study focusing on the bedtime patterns (20:00–22:00 h, after 22:00 h, and after midnight) and duration (short, average, and long) of sleep in healthy men, showing an increase in ASA incidence in the short sleepers among all bedtime patterns (Liu *et al.* 2017). This study included 981 men and the authors attributed to ASA a role in the explanation of how sleep deprivation affects sperm health since they also found that short sleep duration impairs sperm count, viability, and motility (Liu *et al.* 2017). However, they did not suggest a possible mechanism to explain ASA generation in these men.

Overall, despite all the studies that were made over the years, the relevance of certain risk factors for the development of ASA is questionable. In fact, the multifactorial causes and consequences of infertility-related conditions, which can be considered ASA risk factors, along with the not-so-well understood ASA development mechanisms and the distinct research settings, including methodologies to detect ASA and thresholds used, have led to opposing conclusions that, in our opinion, are hindering the knowledge in the

field of immune infertility (Supplemental Table 2). This undoubtedly highlights the need for further objective and systematic research on this subject to allow for more definitive conclusions.

Antisperm antibodies targets

The nature of the exact targets of ASA is the 1 million dollar question in this field, given that with this knowledge it would be possible to both better diagnose and treat ASA-mediated immunoinfertility, as well as to develop novel immunocontraceptive methods.

In fact, many studies have been focused on the identification of ASA targets, and several sperm antigens were already described in immunoinfertile ASA patients through the use of different types of methodologies, such as immunoprecipitation or different types of electrophoresis, nicely summarized by [Vasquez-Levin et al. \(2014\)](#). Some examples of these antigens include proteins involved in sperm-oolemma interaction (P18, P36, SLLP1 or SPRASA), *zona pellucida* binding proteins (NZ-2), among others in which the antigen function is unknown. The main difficulty in these studies is the disclosure of which antigens are effectively blocked by ASA in the entire cell, ultimately leading to the impairment of sperm function (e.g. motility, capacitation, acrosome reaction, and sperm-egg interaction).

Although there has been tremendous technological progress in terms of detection and analysis of biological molecules, only a few studies tried to contribute toward ASA. A recent study using the proteomic analysis of the human sperm proteins resulted in the identification of 32 antigens exclusively detected by the sera of ASA-positive patients, which could be potentially responsible for immunological infertility ([Nowicka-Bauer et al. 2016](#)). Among these 32 antigens 12 (Apg-1 (HSPA4L), 60kDa heat shock protein, angiotensin-converting enzyme (ACE), arylsulfatase A (AS-A), dihydrolipoamide dehydrogenase (DLD), fatty acid amide hydrolase (FAAH), fumarate hydratase, proacrosin binding protein sp32 (sp32), T-complex protein 1 subunit beta isoform 1, triosephosphate isomerase 2 (TP2I), L-lactate dehydrogenase and Zn-alpha-2-glycoprotein (ZAG)) are already known for their involvement in fertilization, mainly in capacitation and sperm-oocyte interaction ([Nowicka-Bauer et al. 2016](#)).

Additionally, another study led to the identification of two human sperm proteins, which are simultaneously recognized by recombinant human *zona pellucida* (rhZP) glycoproteins and by ASA eluted from infertile ASA patients ([Petit et al. 2013](#)). Specifically, the glutathione S-transferase M3 (GSTM) involved in detoxification processes and crucial to sperm as it prevents sperm membrane lipid peroxidation, through ROS elimination via glutathione ([Hemachand & Shaha 2003](#)), and the ionic channel, voltage-dependent anion-selective channel 2 (VDAC2), known to participate in energy

production since it transports adenine nucleotides, Ca^{2+} , and other metabolites at the level of the outer mitochondrial membrane ([Petit et al. 2013](#)), and already associated with male infertility ([Pan et al. 2016](#), [Xu et al. 2016](#)).

Nonetheless, mechanistic confirmation on the actual involvement of these putative targets in mediating a possible effect on male fertility in an ASA context is still lacking.

Antisperm antibodies in assisted reproduction techniques

The screening for ASA as an integral part of semen analysis was reiterated in the last edition of WHO ([WHO 2010](#)), confirming the same clinically relevant threshold of 50% antibody-coated motile spermatozoa ([Barbonetti et al. 2019, 2020](#)). However, the prognostic value and the clinical significance of ASA presence alongside with the choice of the best reproductive treatment strategy, when needed, has been controversial ([Barbonetti et al. 2019, 2020](#)).

In terms of prognostic value, it was recently suggested that only a 100% ASA-positive result (MAR test) can represent the sole determinant of a couple's infertility, having a high negative prognostic value. In fact, only these 100% ASA levels were observed to be highly predictive of a poor post-coital test (PCT) outcome, a surrogate marker of infertility ([Barbonetti et al. 2019, 2020](#)), and a poor live birth rate (LBR), which is a clinical relevant endpoint, with intrauterine insemination (IUI) a relatively effective first-line treatment in these cases ([Barbonetti et al. 2020](#)). A lower degree of sperm autoimmunization (50–99%) does not have such a strong prognostic value considering the aforementioned parameters and it cannot be considered a single determinant of infertility, representing only a contributory factor, according to these authors ([Barbonetti et al. 2020](#)). However, there are authors who advocate a 20% cut-off for ASA positivity in the IB test, based on the results of a PCT ([Koriyama et al. 2013](#)). Indeed, this 20% cut-off value for ASA positivity was already used in the past ([WHO 1992](#)). Nevertheless, the manual also referred that the result given by the IB test only has clinical significance when the threshold for antibody-coated motile spermatozoa was 50% or above. In fact, in the 1999 edition of the WHO manual, the 20% cut-off was abandoned, and the clinical significance threshold of 50% is stressed for all the WHO-recommended tests (MAR and IB; [WHO 1999, 2010](#)).

Considering the natural reproductive process, the presence of ASA always demonstrated some negative effects and the more recent studies also corroborate this association. In fact, the study of Xu and colleagues has shown that serum ASA expression significantly correlates with low natural pregnancy rates ([Xu et al. 2020](#)). Additionally, the PCT and LBR results described in

the studies of Barbonetti and co-workers were reported to be impaired by an elevated level of ASA bound on sperm (Barbonetti *et al.* 2019, 2020).

With the assisted reproduction techniques (ART), the negative effects of ASA on natural conception outcomes can be theoretically bypassed. However, according to the available literature, nicely reviewed by Zini and co-workers, the effects of ASA in the outcomes of *in vitro* fertilization (IVF) have been contradictory (Zini *et al.* 2011a) with several studies showing negative effects of ASA (Junk *et al.* 1986, De Almeida *et al.* 1989, Acosta *et al.* 1994, Lu *et al.* 2019), while others did not report any detrimental effect (Yeh *et al.* 1995, Culligan *et al.* 1998, Zini *et al.* 2011b). When it comes to intracytoplasmic sperm injection (ICSI) outcomes, the results are in general more concordant, with the majority of the studies showing that ASA does not influence the success of this technique (Nagy *et al.* 1995, Check *et al.* 2000, Zini *et al.* 2011b, Yasin *et al.* 2016, Lu *et al.* 2019). In fact, the presence of ASA does not seem to be associated with the success of reproductive outcomes after IVF or ICSI cycles (Zini *et al.* 2011a,b, Yasin *et al.* 2016, Lu *et al.* 2019), suggesting that both techniques are viable options for infertile couples which are facing the problem of ASA presence in semen. This may be not applicable in the context of IUI (Zini *et al.* 2011a), although some studies note that it may be possible to successfully overcome ASA-mediated infertility using IUI techniques (Check & Bollendorf 1992, Lahteenmaki *et al.* 1995, Ombelet *et al.* 1997, Francavilla *et al.* 2009). Importantly, these authors warn that the choice of IUI when ASA are present leads to a reduction of pregnancy probability, especially when ASA levels are high (Check & Bollendorf 1992, Francavilla *et al.* 1992, Check *et al.* 2004, van Weert *et al.* 2005). Also, they even reiterate that in these cases, IUI should not be the first treatment choice (Bates 1997, Check *et al.* 2004, Lombardo *et al.* 2004).

Antisperm antibodies patients' treatments

Several methodologies have been used in an attempt to overcome the negative effects of ASA on male fertility (Shibahara *et al.* 2005). These treatments include methods to remove ASA from the surface of the sperm cell, using repeated sperm washes with modified Hanks balanced salt solution (mHBSS), which has been demonstrated to be relatively inefficient (Haas *et al.* 1988). Another approach is to collect the semen samples directly into a washing buffer, for example, into the culture medium (Bronson 1987) or into a medium containing 50% of heterologous serum or albumin fraction followed by a rapid dilution and washing (Elder *et al.* 1990), based on the assumption that the antibody binding on sperm can occur following ejaculation within seminal fluid, in a time-dependent manner during the liquefaction process (Bronson 1987, Elder *et al.* 1990). This method

seems to be beneficial in some cases, maximizing the sperm recovery and minimizing the amount of ASA-coated sperm (Bronson 1987, Elder *et al.* 1990). Another possible treatment includes the ART options themselves, as already discussed in this review.

Regarding pharmacological approaches, corticosteroids, a class of steroid hormones, have been prescribed to treat men with ASA. Contradictory opinions regarding this topic also exist, however, with some studies confirming the clinical therapeutic relevance of corticosteroids in the treatment of men with ASA (Hendry *et al.* 1990, Keane *et al.* 1995, Omu *et al.* 1996), observing improvements on sperm motility (Keane *et al.* 1995, Omu *et al.* 1996), sperm count (Keane *et al.* 1995), hypoosmotic swelling test (Omu *et al.* 1996), and pregnancy rate (Hendry *et al.* 1990, Keane *et al.* 1995, Omu *et al.* 1996) using the corticosteroid prednisolone. Nevertheless, there were also studies that did not observe any such improvement (De Almeida *et al.* 1985, Haas & Manganiello 1987, Bals-Pratsch *et al.* 1992). In a recent report, the administration of prednisolone was beneficial in men with ASA but without compromised sperm fusogenic capacity, assessed by the sperm penetration assay (SPA). In this case, ASA-positive prednisolone-treated men that undergo an IVF protocol had shown several improvements in IVF outcomes, namely fertilization rate, embryo cleavage rate, average embryo transfer/patient, and pregnancy rate as well as in sperm motility when compared with the non-treated infertile men with ASA (Taiyeb *et al.* 2017). The men who have both ASA and fusogenic problems proceeded to ICSI and the results showed no beneficial effect from the corticosteroid treatment, stressing the usefulness of the SPA test to identify the subgroup of ASA infertile men which may benefit from corticosteroid treatments (Taiyeb *et al.* 2017).

Finally, recently, a new approach has shown the effectiveness of the seminal plasma treatment with *Staphylococcus* protein A in the improvement of sperm penetration ability and in the reduction of ASA titers in immunoinfertile men (Al-Daghistani 2020). The interesting property of the *Staphylococcus* protein A is its high affinity for the Fc portion of IgG and for IgG-containing immune complexes, leading to the selective removal of these immunoglobulins (Silverman *et al.* 2005). This interesting result, certainly warrant further attention, can lead to more tailored interventions in ASA patients.

Conclusions: actual panorama and future perspectives

The main purpose of this manuscript was to show how ASA-related male infertility has evolved during the last 10 years, avoiding irrelevant information, but not disregarding the many contradictory results or limitations in the technical approaches. We have reviewed all the

important aspects, ranging from those that are best known to others that are not so well understood.

It was clear, during our analysis, that many aspects are not straightforward when we talk about ASA-mediated male infertility. Despite the design flaws in some studies, as well as a general lack of consensus regarding the thresholds to be used, it is also clear that the mechanisms by which ASA occur, to their effects on the sperm cells and subsequent fertilization, are still poorly understood. This is, in our opinion, one of the greatest handicaps in the field, and we believe that this is where future research should be firstly focused on producing reliable data on large cohorts.

With a concerted effort, we believe that it will be possible to clarify the real prevalence of this type of infertility, that has been halted by the variety of methodologies and cut-off values used so far, and importantly the exact impact of ASA on sperm and male reproductive function, allowing to define better strategies of intervention, if justified. Hopefully, the upcoming 6th edition of the manual for examination and processing of the human semen, which was already distributed for preview and comments, will have indications in this regard.

Finally, we could not finish this review without saying a word on the actual world pandemic situation due to SARS-CoV-2 infection and of the possible role of COVID-19 disease in male fertility, a subject that has been actively studied in a series of recent papers. It would also be logical to hypothesize the ASA development in patients with COVID-19 and the study on how this will impact fertility. The reasons for this were elegantly summarized in a recent work (Haghpanah *et al.* 2021) and are related with the angiotensin-converting enzyme 2 (ACE2) receptors, which are the virus entrance door in the host cells (Hoffmann *et al.* 2020), and which are present on spermatogonia, Sertoli cells, and Leydig cells (Haghpanah *et al.* 2021). With all these cell types expressing ACE2 receptors, the reproductive system may be a target of infection and taken into account the possible subsequent inflammatory process, certainly, ASA production may be a possibility and should be considered in upcoming studies (Haghpanah *et al.* 2021).

Supplementary materials

This is linked to the online version of the paper at <https://doi.org/10.1530/REP-21-0123>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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Author contribution statement

A F S and S A conceived and designed the study. A F S performed literature search and wrote the draft of the manuscript. A F S, S A, and J R S contributed to the critical discussion of the manuscript. S A and J R S revised the final draft of the manuscript. All authors approved the final version of the manuscript.

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