

# Antisperm antibodies and conception

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**Abstract** Sperm have been known to be antigenic for more than a century. There is a strong body of evidence that in humans and in other species at least some antibodies that bind to sperm antigens can cause infertility. Therefore, these antibodies are of interest today for two practical reasons. Firstly, the association of the antibodies with infertility means that they must be detected and then the couples treated appropriately. Secondly, because these antibodies can induce infertility they have the potential to be developed for contraceptive purposes in humans and also for the control of feral animal populations.

**Keywords** Antisperm antibodies · Fertility · Gamete · Sperm · Conception

## Introduction

Sperm have long been known to be antigenic. Antisperm antibodies (ASA) may be found in both males and females and are reported in up to 9–12.8% of infertile couples [11, 49]. However, these antibodies are present also in approx-

imately 1–2.5% of fertile men [88, 169] and in 1.4% of fertile women [46]. The fact that a significant percentage of fertile couples have detectable ASA clearly shows that these antibodies, at least as they are currently defined, do not all disrupt fertility. The study of ASA and improvements to their detection advanced rapidly during the 1970s, 1980s, and early 1990s but has not advanced as rapidly during the last few years. This is in large part due to the observation that intracytoplasmic sperm injection (ICSI) could be used as an effective treatment for circumventing immunoinfertility [47]. In ICSI, an individual sperm is micro-injected directly into the ooplasm of an oocyte. This technique bypasses most, if not all, of the steps in conception that are adversely affected by antisperm antibodies in human infertility. Thus, much of the clinical impetus for understanding ASA-mediated pathology has been lost. However, there remain several reasons for developing a better understanding of how ASA contribute to infertility. One of these is that ASA appear to act via a wide variety of mechanisms. Improved understanding of these mechanisms along with improved diagnostic testing might allow delineation between those patients with ASA that are causing their infertility and those patients in whom the antibodies are not relevant to their infertility. This distinction could then lead to changes in treatment and a more individualized approach to therapy. Secondly, the first report of systematically tested successful contraceptive vaccination in women is now more than 75 years old [12]. Despite that early success, there has been limited subsequent progress on the highly desirable target of developing a vaccine that can induce reversible but reliable sterility in humans. Enhancing our understanding of naturally occurring ASA may well lead to the identification of antigenic targets for such a vaccine.

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## Structure of the male reproductive tract and sperm

Sperm are produced in the seminiferous tubules of the testis after the onset of puberty in a process called spermatogenesis. The initial stages of spermatogenesis involve several rounds of mitotic division of sperm precursor cells (spermatogonia), which remain connected via syncytial bridges. These pre-sperm cells then move between adjacent Sertoli cells to enter the adluminal and subsequently the luminal compartments of the seminiferous tubules where meiotic divisions reduce the chromosome number to haploid and cytodifferentiation (spermiogenesis) occurs resulting in morphologically mature sperm. The sperm are then transported through the rete testes to the epididymis where functional maturation takes place and extracellular fluid is reabsorbed, resulting in the concentration of the sperm, which are then stored in the cauda epididymis and vas deferens until ejaculated.

Mature sperm consist of a tail, midpiece, and head, which contains the nuclear material with very little cytoplasm. Covering the anterior of the sperm head is the acrosome, which contains many of the proteins required for binding to and penetration of the zona pellucida of the oocyte and receptors for binding to the oolemmal membrane. Antisperm antibodies have been shown to react with each of these major regions of sperm albeit with variable biological effects [17, 51]. Individual men and women may have antibodies that react with several of these sperm regions or only with a single region [17].

## The blood–testis barrier; breaches lead to the production of ASA

The blood–testis barrier is formed by the continuous layer of Sertoli cells contained within the seminiferous tubules that separate the basal and adluminal compartments of the seminiferous tubules. Adjacent Sertoli cells are joined by tight junctions, which form the blood–testis barrier [125]. The blood–testis barrier, among other things, acts to separate haploid cells, sperm, and its precursors from the immune system. This separation is necessary because mature sperm are not present before puberty when much of an individual's central immune tolerance is established; thus, sperm may be seen as antigenic by the adult male immune system. This is clearly seen when sperm are exposed to the male immune system, for example, in the case of trauma to the testes, congenital absence of the vas deferens or vasectomy [51, 145]. After vasectomy, approximately 50% of men will produce ASA [5–7]. Although the blood–testis barrier is important in protecting against the production of ASA, this barrier is obviously not the only protective mechanism that prevents the production of

antibodies against stored sperm in normal men because larger quantities of sperm are present in the epididymis and the vas deferens than in the seminiferous tubules. Like the seminiferous tubules, the lumen of the epididymis is encircled by an epithelial barrier but unlike in the Seminiferous tubules, leukocytes including CD4<sup>+</sup> and CD8<sup>+</sup> lymphocytes and macrophages can be found in the epithelial layer [68, 116, 186]. Whether these lymphocytes normally function to suppress immune responses to sperm antigens remains uncertain but this seems a likely function of at least some of those lymphocytes.

## Antisperm antibodies in women

Why do women produce ASA? A more pertinent question might be: “how is it that despite repeated exposure to relatively large antigen loads during intercourse, most women do not produce ASA?”

During the 1920s and 1930s, Baskin demonstrated that intramuscular immunization of previously fertile women resulted in the production of potent “spermatotoxic” factors (now known to be ASA), which prevented pregnancy for up to 1 year and which were measurable in the serum [12]. However, despite repeated intravaginal insemination, most women and female animals of other species do not produce ASA. Both the vagina and cervix are equipped with active mucosal immune systems and it is well-known that coitus in humans and animals stimulates a rapid influx of leukocytes, particularly neutrophils and macrophages, to the cervix and/or uterus (reviewed in [157]). This inflammatory immune response helps eliminate abnormal or non-fertilizing sperm [174] but it also appears that this immune response to seminal factors is also important in conditioning the female reproductive tract for implantation thereby improving fertility [136, 137, 157, 175]. Despite this apparent inflammatory response, factors present in the seminal plasma, particularly transforming growth factor- $\beta$  (TGF- $\beta$ ) and prostaglandins, prevent the development of paternal-specific immune responses. TGF- $\beta$  is known to inhibit B cell proliferation but it also induces class-switching of human B cells towards IgA [170]. The latter effect might favor the production of ASA in the female reproductive tract. However, the effects of TGF- $\beta$  on antibody production are highly dependent on the dose of TGF- $\beta$  and upon the concentration of other cytokines and signaling molecules that may be present in the local environment [170]. The quantity of active and total TGF- $\beta$  is reported to be highly variable between ejaculates [139]. Thus, the amount of TGF- $\beta$  and other cytokines or immune regulatory molecules present in ejaculates may have a role in whether women develop ASA as has been suggested for men [92]. However, to our knowledge, the question as to whether the

concentration of TGF- $\beta$ , prostaglandins or other immunosuppressing factors in partners' ejaculates correlates with the production of ASA in women has not been examined.

### Which fluids contain ASA?

Antisperm antibodies can be found in both men and women and may be present in the blood or in reproductive tract secretions such as seminal plasma, cervical mucus or follicular fluid [115]. One study determined that women with ASA in their blood also had similar levels of these antibodies in their follicular fluid with the exception of IgM class antibodies [37]. These ASA in the follicular fluid appear to originate as a transudate from the blood and are therefore mainly IgG and IgA. The much higher molecular weight IgM antibodies, whether reactive with sperm or with other antigens, do not appear to enter the follicular fluid in any great quantity [27, 37]. As described later, ASA present in the cervical mucus can have significant impacts on sperm transport and fertility.

The origin of ASA in the male reproductive tract in uninfected men is not clear. However, it has been suggested that sperm-associated and serum-derived ASA recognize different antigens [8, 164] and our own data support that contention (Chiu and Chamley, unpublished). If this is correct, then antibodies present in the male reproductive tract must, to some extent, be produced locally and not simply transduced from the blood. The derivation of ASA in different body fluids has important implications for the selection of the most suitable samples to study both diagnostically and for research purposes. More clarity around this issue would be helpful.

### Do ASA reduce fertility—evidence from clinical studies?

As early as 1899, Landsteiner reported that sperm were antigenic. Similar observations were reported in 1900 by both Metchnikoff and Metalnikov (early references reviewed by Katsh [103]). These reports stimulated considerable research activity with the primary aim of developing immunocontraceptive vaccines. Many groups had reported that fertility was significantly reduced by immunization of various animals with homologous or heterologous sperm preparations. At least one report also appeared describing attempts to induce sterility in women by immunization with semen [12].

The accumulated evidence from animal studies suggested that it was feasible that some cases of human infertility might be associated with immune reactions to sperm. Thus, in 1922, Dr. Samuel R. Meaker [117] reported that serum from several of his infertile female patients

agglutinated and/or immobilized their partner's sperm. In 1954, Rumke [159] published his initial observations on sperm autoimmunity with a report on the detection of sperm antibodies in the serum of two men with oligospermia. Similarly, in 1954, Wilson [183] reported that serum from 2 men with strong sperm agglutination in their semen could cause agglutination of apparently normal sperm from other men. He subsequently reported that the wives of the two men readily conceived using donor insemination. A decade later, Franklin and Dukes [74, 75] published their findings on sperm antibodies in female patients. They found that 20.1% of 214 women undergoing infertility investigations had detectable sperm agglutinating activity in their serum. Within this group, women with unexplained infertility had a much higher incidence (72.1%) of sperm antibodies than women with organic causes for their infertility (8.4%) or fertile women (5.7%). They also reported on a trial of occlusive therapy wherein 13 couples used condoms and/or abstained for 2 to 6 months. They reported that "antibody titers declined markedly in all 13 women and dropped to undetectable levels in 10. These 10 patients were encouraged to resume unrestricted intercourse at the time of expected ovulation with the result that 9 became pregnant." This report stimulated significant interest in the hypothesis that female immunological reactions to sperm could be involved in the etiology of otherwise unexplained infertility and in the concept of an antisperm contraceptive vaccine.

Given that sperm are antigenic and capable of stimulating autoimmunity in men and isoimmunity in women, as demonstrated by the detection of strong sperm antibody responses in some infertile patients, what evidence is there that strong sperm immunity is associated with impaired fertility?

Important studies by Rumke et al. provided convincing evidence that stronger sperm antibody responses in men or women are associated with lowered fertility. In males with positive sperm antibodies, Rumke et al. [160] found that 48% of men with agglutinating titers of less than 1:32 achieved pregnancies, while only 16% of men with titers of 1:32 to 1:512 did so, and none of 11 men with titers of 1:1024 or above. Thus, the higher the level of sperm antibody activity detected in the man's blood, the lower the chance of the couple achieving a pregnancy. An analogous study was conducted with female patients [161]. There were fewer patients included in the latter study, but it did show that women with higher sperm antibody levels had a significantly reduced chance of conceiving within a certain time period.

In another large and rigorous study, Menge et al. [121] tested 698 infertile couples for circulating sperm antibodies and analyzed subsequent pregnancy rates. They found that the pregnancy rate was significantly lower (7.1%) when men had agglutinin titers above 1:16 compared with the

pregnancy rate of 42.7% in the group with negative or low antibody levels. Similarly, in women with higher antibody titers, only 4.0% conceived vs 46.2% in the group with negative or low antibody levels. They also tested many women for the presence of sperm antibodies in cervical mucus. The results indicated that the low conception rates in the presence of sperm antibodies were associated with poor penetration of the cervical mucus [121]. For example, the analysis found a relatively low frequency (10.7%) of good cervical mucus penetration amongst the 28 men with agglutinin titers above 1:128 vs 64.6% with good penetration amongst the 65 men with titers below 1:128 [121]. In another significant study, the proportion of motile sperm coated with antibodies in the patient's semen was analyzed in relation to pregnancy rates in otherwise normal couples [11]. In the group with more than 50% of antibody-coated sperm, only 4/26 (15%) couples conceived during the follow-up period of up to 3 years vs 6/9 (67%) of couples with less than 50% of coated sperm [11].

More recent studies, which have included patients with higher sperm antibody levels have supported the conclusions of the earlier landmark studies. For example, Francavilla et al. [71] treated 19 couples with severe sperm autoimmunity (100% of motile antibody-coated sperm by mixed antiglobulin reaction [MAR] or immunobead test [IBT]) using intrauterine insemination (IUI) for a total of 110 cycles and no pregnancies resulted. In a control group of 86 patients treated by IUI for a total of 411 cycles, they obtained 23 pregnancies. After excluding patients with teratospermia, which also significantly decreased conception rates, they found that the pregnancy rate per cycle in the control group (8.3%) was significantly ( $p < 0.001$ ) higher than that in the group with sperm autoimmunity (0%), wherein none of the 16 patients without teratospermia achieved pregnancies [71]. Similarly, Meinertz et al. [118] found that post vasovasostomy conception rates were strongly influenced by sperm antibody isotype and strength. When the direct MAR was negative, 67% of couples conceived during a follow-up period of up to 3 years, whereas no pregnancies occurred when 100% of motile sperm were coated with IgA class antibodies and the serum sperm agglutinin titer was at least 1:256 [118].

It is reasonable to expect that higher levels of sperm immunity would lead to compromised fertility because there have been numerous reports describing the deleterious effects of sperm antibodies on sperm function in vitro. High levels of ASA have been shown to block penetration of cervical mucus by highly motile sperm and also to significantly reduce fertilization of human oocytes. Results of several studies of patients with ASA who underwent fertility treatment and the relationship of their ASA to treatment outcome are summarized in Table 1.

In summary, thorough evaluation of the literature in this area has led us to a firm conclusion that stronger sperm antibody responses are associated with lowered fecundity. However, it is important for clinicians to appreciate that variables such as antibody specificity, immunoglobulin class, and dose come into the equation when evaluating patient's results. Thus, a positive sperm antibody screening result alone, without further assessment, should not be accepted by the clinician as necessarily indicative of immunoinfertility.

### Clinical detection of ASA

The initial investigation of the male partner of an infertile couple should include a direct IBT or MAR screen for sperm-bound antibodies [182]. A positive result (>50% of motile sperm being antibody-coated) should be followed-up with a repeat test and mucus penetration testing to make an assessment of the functional significance of the antibodies [45]. It is also recommended that the female partner should be tested for circulating sperm antibodies. High levels of circulating antibodies may severely reduce the chances of successful treatment by in vitro fertilization (IVF) [43] or donor insemination. Assessment of in vitro sperm–mucus interaction by means of the capillary (Kremer) test and/or the semen/cervical mucus contact test (SCMCT) may suggest the likely presence of sperm antibodies in cervical mucus, even though circulating antibodies may have been weak or undetectable. The presence of antibodies in cervical mucus should be confirmed by testing liquefied cervical mucus using the indirect IBT. The presence of high cervical mucus antibody levels and associated negative or low titer circulating antibodies suggests a good prognosis for the treatment of the couple by intrauterine artificial insemination. In contrast, the presence of high antibody concentrations or titers both locally and systemically suggests a poor prognosis. Couples with apparently intractable immunoinfertility can be effectively treated using ICSI [47].

The preceding discussion describes the current best practice for the detection of ASA in the routine fertility laboratory. However, the tests we have suggested all suffer from the problem that these investigations will be limited to showing whether there are ASA in the relevant samples but will not identify the antigens with which those ASA react. This is a major limitation of such tests. Many of the antibodies that are detected, especially low titer antibodies, may have no relevance to infertility as is demonstrated by their presence in some fertile couples [88, 141, 169]. The field of ASA diagnostics still awaits the development of antigen-specific tests.

**Table 1** Several studies of patients with ASA who underwent fertility treatment and the relationship of their ASA to treatment outcome

ASA and method of detection (source of ASA)	Method of fertilization	Endpoints/outcomes	Reference
i-IBT (semen)	IVF	FR decreased	[100]
i-IBT (FF, serum)	IVF	FR decreased	[114]
i-IBT (serum sperm)	IVF	FR decreased, FR unaffected	[185]
IBT	IVF	FR decreased	[56]
MAR	IVF GIFT	FR decreased, PR decreased, CR unaffected, EQ decreased	[143]
IBT (sperm, female serum)	IVF	FR decreased, PR decreased	[31]
IBT–TAT	IVF	FR decreased	[98]
i-IBT (female serum as culture media)	IVF	FR decreased, PR decreased	[178]
MAR–IBT (IgG or IgA) TAT	IVF	FR decreased	[156]
MAR–TAT	IVF	FR decreased, FR unaffected	[108]
IBT–i-IBT (female serum)	IVF	FR, PR unaffected	[142]
IBT	IVF–GIFT	FR decreased, PR decreased	[2]
IBT	IVF	FR, CR, PR unaffected	[171]
IBT	IVF	FR decreased	[188]
MAR–FCM	ICSI	FR unaffected	[109]
MAR–IBT	ICSI	FR, PR unaffected	[127]
i-IBT (seminal plasma)	IVF	FR decreased	[69]
MAR	IVF	FR decreased, PR decreased, IR decreased	[179]
IBT	ICSI	FR, PR unaffected	[47]
i-MAR(serum and follicular fluid) and MAR (sperm)	IVF	FR, PR unaffected	[180]

*GIFT*: gamete intrafallopian transfer, *IBT*: direct immunobead test, *i-IBT*: indirect immunobead test, *TAT*: tray agglutination test, *ICSI*: intracytoplasmic sperm injection, *IVF*: in vitro fertilization, *IVF-ET*: in vitro fertilization-embryo transfer, *MAR*: mixed antiglobulin reaction, *FR*: fertilization rate, *CR*: embryo cleavage rate, *EQ*: embryo quality, *PR*: pregnancy rate, *IR*: embryo implantation rate.

### Potential mechanisms of ASA

It is possible to envisage a number of processes that ASA could disrupt leading to reduced fertility. The following sections describe some of these functions with examples of studies investigating the effects of ASA. These examples are by no means a comprehensive list of all studies but rather are intended to be a reflection of the types of studies and results obtained.

#### Affects of ASA on sperm transport

In humans, semen is deposited into the vagina (in other animals semen may be deposited directly into the uterus, for example, the pig) and must pass through the cervix before entering the uterus and fallopian tubes. In the periovulatory period, abundant, watery, mucus lines the cervical canal. In humans, sperm have been shown to align themselves with the longitudinal axis of mucin fibres in the cervical mucus and their swimming is more vectorial than in culture medium [104]. Thus, the cervical mucus can act as a guide for normal sperm to enter the uterus but it may also act as a filter to prevent the passage of some very abnormal sperm into the upper reaches of the female reproductive tract.

The ability of ASA to disrupt normal interactions of sperm with the cervical mucus and inhibit sperm penetration into cervical mucus has been described in many studies [23, 32, 36, 38, 144, 150, 151]. Morgan et al. have shown that high titer antibodies on sperm inhibit the passage of sperm through the cervical mucus even when the sperm appeared to be normal in the semen analysis [124]. In several studies, the proportion of motile sperm or the level of ASA detected by MAR or tray agglutination test (TAT) correlated with the inhibition of sperm penetration in the cervical mucus [23, 28, 95, 121]. Hence, the cervical mucus may aid in the selection of the most fertile sperm of an ejaculate by acting as an immunological filter preventing the passage of sperm coated with ASA.

ASA may also be detected in the cervical mucus of up to 29.6% of women with unexplained infertility [121, 123] and at a lower incidence in unselected infertile women [36, 38, 64]. Comparison of the isotype distribution of IgA ASA in the sera or cervical mucus suggested that at least some of the cervical mucus antibodies were produced locally [107]. These cervical mucus-derived ASA immobilized sperm and prevented passage through the cervical mucus [121, 123]. It has been suggested that sperm surface attached IgA are more important than IgG ASA in inhibiting penetration

through the cervical mucus and further suggested that ASA reacting with the sperm head or tail main piece but not those reacting with the tail tip prevented mucus penetration [42, 85, 180].

The mechanisms by which ASA interfere with the movement of sperm in the cervical mucus have been extensively studied. It has been suggested that IgA ASA are particularly important in inhibiting penetration of the cervical mucus by sperm [63]. The secretory component of sperm IgA ASA binding to the glycoproteins of the cervical mucus has been suggested to cause the shaking of sperm in the cervical mucus [94, 106]. Jager et al. reported that IgG can also induce a shaking phenomenon but suggested that fragment crystallizable (Fc) region of the IgG molecule may be impairing the motility of antibody-bound sperm in the cervical mucus [96, 97]. Clarke then observed that IgA class antisperm antibodies from serum could induce a strong shaking reaction and that the reaction was not affected by absorption of the serum with rabbit antiserum, thus confirming that the Fc region of IgA can interact with the cervical mucus [39]. The ability of IgA1 protease, which cleaves the Fc region from the IgA molecule, to improve the ability of IgA-coated sperm to penetrate the cervical mucus further supports the involvement of the Fc fragment in ASA/cervical mucus interactions [24]. It has been suggested that ASA on sperm bind to Fc receptors, such as a 15 kDa protein with amino-terminus identical to that of secretory leukocyte protease inhibitor in human cervical mucus, and this inhibits sperm transport [89].

In an interesting study examining the transport of sperm through the higher reaches of the female reproductive tract, Shibahara et al. have shown that ASA can retard or inhibit the passage of sperm from the uterine cavity to the fallopian tubes in women by examining the presence of sperm in peritoneal fluid 30 min after artificial insemination [166].

From this discussion, it should be clear that at least a proportion of ASA, whether produced by the male or the female partner, can inhibit the transport of sperm through the female reproductive tract.

#### Effects of ASA on capacitation and the acrosome reaction

After ejaculation, sperm undergo several changes in the female reproductive tract, which together are termed capacitation. Although the full spectrum of changes associated with capacitation are unclear, this process involves protein phosphorylation and lipid redistribution resulting in destabilization of the membrane (reviewed in [57, 67]). Capacitation of sperm is essential to facilitate the acrosome reaction, an exocytotic event during which the sperm plasma membrane fuses with the outer acrosomal membrane allowing the exposure of the contents of the acrosome, such as acrosin and hyaluronidase that are

important to allow penetration of the oocyte vestments by sperm. The acrosome reaction also results in the exposure of the inner acrosomal membrane proteins, such as the complement control protein CD46 [29], to the exterior of the sperm. The ability of ASA to bind to sperm can be affected by capacitation while the binding of ASA to sperm can also conversely affect the processes of capacitation and acrosome reaction.

Fusi et al. suggested that the change in the sperm membrane induced by capacitation and acrosome reaction can affect the binding of ASA [76]. These workers incubated ASA-containing sera with capacitated, acrosome-reacted sperm and found that 48% of ASA-positive and 20% of ASA-negative sera demonstrated a different pattern of indirect IBT binding with capacitated sperm [76]. Evidence has also emerged that ASA might prevent membrane fluidity changes needed for capacitation before fertilization [13]. In addition, sperm incubated with serum containing immobilizing ASA were found to have lower rates of spontaneous and induced acrosome reactions than sperm that were not incubated with the serum [126, 172]. Furthermore, ASA can inhibit the ability of sperm to undergo spontaneous capacitation as an antibody raised against a human sperm protein, BS-17, prevented capacitation of human sperm [181].

The effects of ASA on acrosome reaction have been contradictory. For instance, Romano et al. found that the proportion of acrosome-reacted sperm was higher in ASA-coated sperm [158], while other authors found no effect of ASA on acrosome reaction [70]. Lansford et al. found that IgG ASA from different individuals could inhibit the acrosome reaction, while other ASA initiated or had no effect on the acrosome reaction [110]. Likewise, Marin-Briggiler et al. found variable effects of ASA on the acrosome reaction [115]. While Francavilla et al. found 7 out of 12 ASA-containing sera inhibited the zona pellucida-induced acrosome reaction [73]. In addition, Saragueta et al. found heavy and light chains of human IgG in follicular fluid that were capable of inducing the acrosome reaction [163]. Taken together, these examples suggest that ASA have a variable effect on the acrosome reaction and capacitation; while some ASA can adversely alter the ability of sperm to undergo capacitation or acrosome reaction, other ASA do not.

#### Complement as a mediator of ASA effects

Complement is a cascade of proteins of the innate immune system, which, among other functions, can bind to antibody/antigen complexes on a cell surface and cause lysis of the cell via deposition of a membrane attack complex. IgG isotype antibodies are efficient at stimulating complement while IgA is a relatively poor activator of comple-

ment. One study demonstrated that complement-fixing but not non-complement-fixing ASA reduced the ability of sperm to penetrate zona-free hamster oocytes [22], while D’Cruz et al. have shown that incubating complement-activating ASA with normal sperm caused a significant reduction (87–43%) in mobility and also observed alterations in sperm morphology with subsequent sperm lysis *in vitro* [52]. D’Cruz et al. [53] have also demonstrated that sperm-bound ASA obtained from the ejaculates of men with ASA are capable of activating complement. However, whether complement exists in the female reproductive tract at physiologically relevant concentrations is questionable. Price et al., using a haemolysis assay, have shown that complement is present in the cervical mucus, but at approximately one tenth of the levels found in the blood [152]. This level of complement appeared to induce immobilization of 70% of ASA-coated sperm after 3 h. In contrast to the low levels of complement in the cervical mucus, follicular fluid has been shown to contain levels of complement approximately one half that found in plasma [50]. While other authors suggest that at least some of the major components of complement are present in follicular fluid at similar levels to plasma [37, 147, 148]. However, while follicular fluid may contain adequate levels of complement to cause sperm damage *in vitro*, the dilution of follicular fluid following ovulation may reduce its potency *in vivo*. This situation is further complicated by the suggestion that complement and complement regulatory proteins, such as CD46 (MCP), which is localized to the inner acrosomal membrane of sperm, may be involved in sperm–oocyte binding [156, 173].

#### Effects of ASA on fertilization

In order for sperm to fertilize an oocyte, several molecular interactions must take place. Firstly, the sperm must penetrate the tightly packed cumulus cells that surround the oocyte. This process is facilitated by the sperm acrosomal enzyme hyaluronidase, also known as PH-20, which breaks down the cumulus cell matrix, which contains high levels of hyaluronic acid [55, 162]. Interactions between PH-20 and the cumulus matrix also result in increased calcium levels in the sperm making the sperm more responsive to the subsequent zona pellucida-induced acrosome reaction [33]. An acrosome intact sperm then attaches to the zona pellucida in a process referred to as primary binding. The acrosome reaction is triggered as part of this primary binding event leading to secondary and more permanent binding of the sperm to the zona [19]. Finally, having penetrated the zona pellucida, sperm bind to the outer membrane of the oocyte, the oolemma, so that the sperm nucleus can be released into the oocyte. Both primary and secondary binding to the zona pellucida and

binding to the oolemma are thought to be mediated by specific protein or protein–carbohydrate interactions, which ASA could potentially disrupt. Unfortunately, despite there being many candidate proteins on sperm, the exact nature of the sperm ligands for the zona pellucida and oolemma remain indeterminate. However, several lines of evidence indicate that ASA might interfere with recognition of sperm binding sites on the zona pellucida. Bronson et al. reported binding of head-directed IgG or IgA ASA to sperm reduced sperm binding to human zona pellucidae [21]. Another study showed that donor sperm when incubated with ASA-containing serum was unable to fertilize human oocytes or bind to the human zona pellucida. When the serum ASA were pre-absorbed by normal sperm, the serum no longer showed this negative effect on sperm–zona binding [176]. Additional evidence that ASA can affect sperm binding to zona pellucida was provided by Mahony et al. [112] who, using the hemizona assay, demonstrated that seven of the ten ASA-containing sera tested reduced zona binding. Other authors reported that ASA could affect fertilization but not clinical pregnancy rates once fertilization had occurred [41, 155]. Liu et al. observed that antibody-coated sperm from men with sperm autoimmunity had an impaired ability to bind to the human zona pellucida, but that oolemma binding was unaffected. In contrast, other authors found that ASA did not affect sperm–oocyte binding [111]. Using zona-free hamster oocytes, other authors found that ASA were capable of both stimulating and suppressing sperm–oocyte fusion [4, 26]. It is possible that not all ASA affect sperm–oocyte binding/fusion and it is likely that the antigenic specificity of ASA is important in their effects on fertilization.

Clarke et al. showed that under controlled experimental conditions, sperm antibodies from female sera significantly inhibited *in vitro* fertilization of fresh human oocytes [44]. The fertilization rate was related to the IgG class sperm antibody titer. The inhibitory activity was removed by absorption of the highest titer serum with protein A sepharose to remove most of the IgG class sperm antibodies. The antibodies subsequently eluted from the protein A sepharose retained their ability to inhibit fertilization [44]. However, because spare human oocytes are rarely available, it is difficult to study how ASA might affect sperm–oocyte interactions using human sperm and oocytes. Consequently, this has often been investigated by observing the ability of ASA-coated sperm to adhere and penetrate zona-free hamster oocytes. This assay utilizes hamster oocytes stripped of the cumulus oophorus and zona pellucida to test the penetrating ability of acrosome-reacted human sperm [187]. Numerous investigators have shown that human ASA can inhibit the penetration of hamster oocytes by human sperm [1, 72, 83, 102, 167]. In addition, antibodies experimentally raised against specific sperm

proteins are also capable of inhibiting sperm penetration of hamster oocytes [9, 153, 167]. For example, rabbit antibodies raised against sperm proteins of 36 and 18 kDa reduced the binding and penetration of hamster zona-free egg by human sperm [9] and monoclonal antibodies reactive with sperm proteins inhibited sperm fusion with zona-free rodent eggs [153, 167]. In contrast, some serum ASA have been found to promote the penetration and adhesion of human sperm to hamster oocytes [25, 26]. Mixed results were also reported by Aitken et al. [4] who found that ASA could promote, inhibit or be neutral in their influence on sperm penetration of oocytes. These lines of conflicting data outline the importance of knowing the specificities of the ASA involved; some of the ASA do not affect fertilization and some do.

Several sperm proteins (or their isoforms) have been postulated to have more than one function in sperm–oocyte interactions. Examples of such proteins are triosephosphate isomerase (TPI), and acrosin/proacrosin. ASA reacting with each of these proteins have been demonstrated in samples from infertile individuals [10, 33, 91]. There is evidence that antibodies reactive with TPI can inhibit both the secondary binding of sperm to the zona pellucida and also binding of sperm to the oolemmal membrane [9, 10]. Thus, the confusion regarding the functionality of ASA is exacerbated by the potential for an antibody reacting with a single protein to disrupt more molecular mechanism involved in the sperm–oocyte interactions that are required for fertilization. It might also be possible that an ASA could react with a functional domain within a protein, which inhibits one function of that protein but not another function of the protein.

#### Post fertilization effects of ASA on fertility

Definitive studies in various animal models have shown an association between sperm antibodies and pre- or post-implantation embryonic degeneration [119]. In one study on rabbits, reproductive tract secretions containing ASA were found to cross-react with rabbit morulae and blastocysts, resulting in embryotoxic effects during in vitro culture [120]. In a number of well-controlled experiments, this group demonstrated that only secretory IgA (sIgA) from the uterine fluid of semen-immunized does was embryotoxic during in vitro culture. In contrast, blood sera with high titers of sperm antibodies were not embryotoxic nor were IgG fractions isolated from the immune uterine fluid (IUF) [120]. Absorption of IUF with either sperm or anti-sIgA removed the embryotoxicity, thereby providing evidence of specificity. Other experiments indicated that the sperm antigen stimulating the sIgA embryotoxic antibody in IUF was distinct from the antigen stimulating IgG and IgA class antisperm antibodies with the ability to inhibit

fertilization. In unpublished observations, absorption of the IUF with paternal lymphocytes did not abrogate the embryotoxicity, therefore implying that transplantation antigens were unlikely to be involved. Additional investigations suggested that the antigen responsible for the sIgA-associated embryotoxicity was a subsurface component. Thus, immunization of does with isolated sperm membrane fractions resulted in reduced fertilization, whereas immunization with submembrane fractions caused only the post fertilization effects on embryos [120].

Why should sperm antibodies react with embryos? Firstly, the sperm membrane is integrated as a mosaic into the zygote membrane during the process of fertilization so that sperm antigens are incorporated, albeit at relatively low densities, into the developing embryo [138]. Secondly, embryonic gene expression commencing from the four to eight cell stage results in the synthesis of various developmental antigens, which can cross-react with sperm antigens (for review, see Menge and Naz [122]). Consequently, during embryo development and perhaps particularly around the time of blastocyst hatching, there is an opportunity for the ASA to bind to cross-reacting embryonic antigens and potentially cause embryo degeneration or possibly block implantation.

There is also some evidence for post fertilization effects associated with sperm immunity in humans. With respect to deleterious effects, Warren Jones [99] first reported that around 50% of pregnancies conceived in women with ASA subsequently ended in first trimester spontaneous miscarriages. Similar observations have been reported by other groups [121, 184]. In the study by Witkin and David, it was found that 7/16 (44%) of women who miscarried were positive for sperm antibodies in their serum compared with only 2/17 (12%) of women who had successful ongoing pregnancies [184]. Examination of the immunoglobulin classes of the antibodies revealed that IgA was significantly ( $p < 0.01$ ) more common in women who miscarried. The IgA class antibodies in serum may be indicative of local secretory IgA in the female reproductive tract. However, despite the strong evidence in rabbits, it is not known whether sIgA class antisperm antibodies in humans are embryotoxic. In another clinical study [84], it was found that of 173 women referred for a history of 3 or more consecutive spontaneous miscarriages, there was a significantly higher incidence of sperm immobilizing antibodies when compared with the infertile group. It is interesting to note that they also observed a higher incidence of sperm antibodies in the group of women identified as having an immunological basis for their recurrent miscarriages (for example, couples sharing at least three human leukocyte antigen (HLA) determinants or couples with the female showing a relatively low response to her partner's lymphocytes in mixed lymphocyte culture). Other groups have

reported a significant association between ASA and some autoantibodies such as antiphospholipid antibodies and it may be that it is these coincidental autoantibodies, which have deleterious effects on the fetus rather than the ASA themselves [65, 66]. In contrast to the studies cited above, which have reported an association between sperm antibodies and recurrent miscarriage, other authors have not found a statistically significant association [46, 93]. Further investigations in this area are warranted, particularly focusing on the possible involvement of subsurface sperm antigens, which react with IgA class antisperm antibodies.

With respect to the positive effects of sperm immunity, there is preliminary evidence from analysis of IVF data suggesting that sperm antibodies are associated with increased implantation rates [48, 54]. If confirmed, this could potentially add an interesting new dimension to our analysis and understanding of sperm immunity.

### **Current research on defining the antigenic specificity of ASA and distinguishing those ASA that induce infertility from those that do not**

From the preceding sections, it should be clear that ASA are highly variable both in terms of their antigenic specificity and their biological effects. Most techniques used in clinical practice to detect ASA rely on detecting gross binding of antibodies to whole sperm or extracts of whole sperm, either directly or indirectly. Although clinical laboratories have developed considerable expertise in using these assays and judging the value of their results, even in the best of settings these existing tests fall short of being able to distinguish reliably between ASA that contribute to infertility and those that do not. A significant step forward in this field would be the identification of specific antigens that when bound by ASA would specifically affect fertility. In an attempt to distinguish between the antigenic specificities of infertility-inducing ASA and the specificities of ASA that do not reduce fertility, we used differential display Western blotting to compare the reactivity of ASA from patients in whom the antibodies were the only identified cause of infertility and normal fertile individuals [34]. While this type of analysis was able to identify protein bands, which reacted with both types of ASA, and protein bands, which reacted only with infertile ASA, it was not possible to identify the individual proteins involved. However, the rapid advances in proteomics have allowed this approach to be refined using two-dimensional gels and Western blots. Two-dimensional electrophoresis allows much greater separation of proteins than traditional one-dimensional gels and the separated protein “spots” identified by Western blotting can then be characterized by peptide mass fingerprinting and/or peptide sequence anal-

ysis. Using this approach, we recently identified a novel sperm protein (which we termed SPRASA) as the antigen for ASA from infertile but not fertile men [35]. With the aim of characterizing the sperm proteome, a study by Mandal et al. [113] also identified the same protein (SLLP-1) concurrently and demonstrated that antibodies reactive with SLLP-1 prevent the fertilization of zona-free hamster oocytes by human sperm.

Other researchers have recently employed the power of proteomic techniques combined with Western blotting to identify the antigens of ASA [14]. One group identified 38 proteins and isomeric forms of those proteins, which reacted with ASA. Of those 38, 14 were reactive with ASA from both fertile and infertile patients. One of the proteins, which reacted with ASA from infertile patients only, was identified as a novel protein (FLJ32704). An immunodominant peptide derived from the sequence of FLJ32704 reacted with ASA from 36 of 40 infertile patients but none of 40 fertile persons [14]. Others have used a similar approach and again identified a range of proteins that were reactive with ASA [15, 16]. However, the use of this technology has at least one major limitation. Under ideal conditions, samples loaded onto the first dimension of two-dimensional gels are treated with harsh reagents including reducing reagents with the aim of maximally reducing molecular interactions. However, by modifying or removing the conformation of three-dimensional proteins, this treatment renders many potential antigens of ASA non-antigenic. Alternative approaches will need to be employed to characterize many of the as yet unidentified antigens of ASA.

### **Antisperm antibodies as contraceptives**

There has been considerable interest for more than a century in developing a contraceptive vaccine for use in humans. Today, such a vaccine would need to be both highly efficacious in its contraceptive effect, such that it would at least be as effective as existing contraceptives, and be reversible. There is also considerable interest in developing contraceptive vaccines for animal species, particularly feral animal pest species. Vaccines for feral animal control have rather different considerations to those for human contraception with a permanent contraceptive effect being the target and delivery systems having to be more sophisticated with the likelihood that they would be delivered via recombinant DNA vaccines, possibly using a viral vector.

The first systematic study of ASA as a contraceptive in humans was published by Baskin [12] who intramuscularly immunized a series of 20 fertile women with approximately 3 ml of fresh semen (3 immunizations, 7 days apart) in the absence of adjuvant. All of these women, except the one who received the lowest immunizing dose of semen,

produced strong spermatotoxins and were infertile for up to 1 year. This infertility was apparently reversible with at least 1 woman becoming pregnant 1 year later [12]. This immunization protocol apparently produced no systemic side effects and only moderate pain at the injection site for 24 h. Thus, research into contraceptive vaccines had a promising start decades before the availability of steroid-based contraceptives. More recent attempts to produce contraceptive vaccines reactive with sperm have focused on particular candidate proteins. There are now a large number of candidate proteins, which have been studied and we will illustrate the types of study with a few examples. The topic has been comprehensively reviewed recently by several authors [58, 59, 132].

PH-20, the bifunctional hyaluronidase, was used in a number of animal studies as an immunocontraceptive target. An early report of a study in guinea pigs suggested that PH-20 would be an excellent contraceptive vaccine target with vaccination producing a good contraceptive effect, which was reversible [154]. However, subsequent studies have failed to reproduce these promising results in other species [87].

SAGA-1 was identified as a potential target for a contraceptive vaccine as part of the Sperm Antigen Program of the World Health Organization Task Force on Antifertility Vaccines [60, 90]. SAGA-1 has recently been shown to be CD52 [61], a protein expression of which is not restricted to sperm. However, it is now apparent that variations in the glycosylation of CD52 occur between tissues and that antibodies reactive with the sperm isoform have limited reactivity with other isoforms of CD52. The S19 monoclonal antibody, which reacts with the sperm isoforms of CD52 does not react with either lymphocytes or female reproductive tract epithelium, among other tissues, making the S19 epitope a potential target for a contraceptive vaccine [135].

Using the phage peptide display technique, Naz et al. identified a 12 amino acid peptide (YLP(12)) derived from an approximately 50 kDa sperm protein [131]. A monoclonal antibody reactive with this peptide blocked the acrosome reaction [131] while naturally occurring ASA from some infertile, but not fertile, men reacted with the peptide [129]. Immunization of mice with the YLP(12) peptide reduced their fertility [130]. It has also been shown that the YLP peptide can be delivered as a recombinant DNA-based vaccine, which reduced fertility in mice over a prolonged period (1.3 years) [133].

FA-1 has also been reported to be a potential target for contraceptive vaccines. Antibodies reactive with this sperm-specific protein block binding of sperm to the zona pellucida [101] and can inhibit fertilization in animals including primates [128, 134]. Further, a vaccine using a

combination of YLP(12) with the FA-1 antigen caused a greater reduction in fertility in mice than either the YLP(12) peptide or FA-1 antigens alone, suggesting complex antigens involving more than one sperm protein may produce an attractive vaccine [134]. Others have also recently demonstrated the utility of using vaccines with combinations of peptides [86].

The sperm-specific isoform of lactate dehydrogenase C4 (LDH-C4) has also been shown to be an effective contraceptive in several species including primates [30, 79, 80, 165] with 73% of matings in female baboons immunized with LDH-C4 being infertile compared to 23% in controls [79]. It has also recently been shown that LDH-C4 can be delivered effectively as a DNA-based vaccine, which might be important in the delivery of a vaccine to feral animal populations [30].

There are many potential targets for contraceptive vaccines that we have not discussed, some of which may not yet have been discovered and much work remain to be done before any sperm-reactive contraceptive vaccine is likely to be useful. It must also be remembered that sperm antigens are not the only potential target for a contraceptive vaccine and the utility of other antigens involved in reproduction, such as hormones [171] and zona pellucida components [62, 82, 185] must also be considered.

### **Treatment of infertility where ASA are a causative factor**

As the detection of ASA became easier with the introduction of tests, such as the IBT [20, 40], attempts were made to treat these antibodies by a variety of mechanisms including removal of antibodies from sperm by washing or proteolysis [146, 168], immunosuppressive therapy in men or women [81, 168, 177], and other approaches based on the frequency of ejaculation. Nearly a decade ago, a survey of all fertility clinics in the United Kingdom concluded that there was much confusion over how to manage couples with antibody-related infertility [105]. However, particularly for women with ASA in the cervical mucus, IUI in which washed sperm are deposited directly into the uterus thereby avoiding adverse interactions between sperm and the cervical mucus has been shown to be effective [3, 32, 77]. Despite previous confusion regarding the optimal treatment, the advent of ICSI, which bypasses almost all potential effects of ASA, has rendered more or less redundant all of these earlier therapies with the exception of IUI. It has been shown that the presence of ASA does not reduce the effectiveness of ICSI as a treatment [47]. However, ICSI should not be regarded as a panacea for the treatment of ASA, as several studies

including a large international collaborative study have shown that children conceived by ICSI/IVF have more defects or do not do as well in some aspects of physical health in childhood as naturally conceived children [18, 140]. Although other studies suggest there is no difference between ICSI/IVF and naturally conceived children [149]. The genetic and epigenetic risks arising from ICSI have been reviewed recently by Georgiou et al. [78] and there remains confusion as to whether ICSI has detrimental effects on the offspring. Despite that confusion, it is much clearer that to undergo ICSI in most centers, the female partner undergoes an intensive regime of hormone therapy to induce ovarian stimulation and multiple follicle development, followed by the highly invasive oocyte recovery and embryo replacement steps of this procedure. Particularly, when it is the male partner who has the ASA that are suspected of causing the couples infertility, the burden of the treatment falls primarily on the partner who does not have the causative pathology raising potential issues around the use of ICSI under these circumstances.

## Conclusions

Some ASA cause infertility or contribute significantly to infertility in humans, but current diagnostic techniques fail to distinguish between those antibodies that cause and those that do not cause infertility. This is at least in part because current diagnostic tests do not examine the antigenic specificity of ASA but rather look at the gross binding of the antibodies to sperm or regions within sperm.

The development of ICSI has removed much of the clinical imperative to develop better diagnostic tests because this technique circumvents almost all of the mechanisms of action of ASA. However, there remain questions about the safety of ICSI and there are potential issues surrounding the use of this technique to treat patients who may not have the antibodies themselves (where they occur in the male partner). For some ASA, lower cost and less invasive techniques, particularly IUI, may be a highly desirable alternative therapy to ICSI. There is further drive to understand the specificity of ASA to help in the design of contraceptive vaccines for use both in the human population and in pest control. For these reasons it is important that work in this field continues.

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