

Antisperm antibodies and human reproduction

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Summary

Purpose: To present strategies in diagnosing and treating infertility related to antisperm antibodies. **Methods:** Antisperm antibodies (ASA) were detected on sperm using the direct immunobead (IDB) test. Treatments included intrauterine insemination (IUI) with pretreatment with chymotrypsin/galactose vs in vitro fertilization (IVF) with intracytoplasmic sperm injection (ICSI). **Results:** Intrauterine insemination with protein digestive enzyme treatment was much more effective than IUI without enzymatic therapy. However IVF with ICSI provided three times the pregnancy rate for males with sperm coated with ASA than IUI with chymotrypsin treated sperm. **Conclusions:** It is advisable to include measurement for ASA on the initial semen analysis. However, another option is to perform it initially only with an abnormal post-coital test. The decision for IUI with chymotrypsin pretreatment of the sperm vs IVF with ICSI may depend on insurance and financial issues.

Key words: Antisperm antibodies; Post-coital tests; Intracytoplasmic sperm injection; Protein digestive enzyme; Intrauterine insemination.

Variable effect of antisperm antibodies on fertility

There is no question that in some instances the presence of antisperm antibodies (ASA) coating the sperm can cause infertility such that intercourse, intrauterine insemination (IUI) with or without treatments of sperm aimed at neutralizing or eluting the ASA or even conventional in vitro fertilization (IVF) (where approximately 50,000 sperm are incubated with each egg) are associated with a very low chance of fertilization [1-8]. Sometimes the only way that fertilization can occur is through IVF with intracytoplasmic sperm injection (ICSI) [9-12].

However it is also known that ASA is present in 1-2.5% of fertile males [13, 14] and possibly in 4% of fertile females [15]. There are many reasons why in some people the presence of ASA requires IVF with ICSI to allow fertilization of eggs whereas in some patients there is no adverse effect and pregnancies occur after natural intercourse. To better understand why some antibodies, but not all, inhibit the achievement of pregnancy it is first important to understand how ASA impairs fertilization.

ASA may inhibit sperm from progressing through the cervical mucus, thus preventing them from reaching the oocyte [16-20]. We evaluated post-coital tests in women whose male partners had > 50% of their sperm coated by ASA [18]. Only 31% (4/13) of males with $\geq 50\%$ ASA by the direct immunobead test demonstrated sperm with progressive linear motion following post-coital testing. All four of these males' wives achieved a pregnancy within six months with just intercourse.

The reason why some males can have > 50% ASA coating their sperm and yet have normal post-coital tests could be related to various factors.

1) Though at least 50% of the sperm may have some antibody attached the immunobead does not detect the concentration of ASA per sperm. Thus possibly the antibody load is not sufficient to cause immobilization of the sperm when coming in contact with the complement in the cervical mucus (ASA typically does not immobilize the sperm when testing the semen analysis because of the absence of complement in the normal ejaculate) [20].

2) The antibodies are not those that lead to immobilization in the mucus. One should be careful though of making the assumption that if ASA does not lead to poor post-coital tests that they do not cause infertility. There are various sperm antigens to which antibodies can be made [21]. There are data showing that ASA may contribute to infertility by disrupting sperm-oocyte recognition and fusion [22, 23], inhibiting sperm from undergoing capacitation [24], inhibiting the acrosome reaction [24, 25], or inhibiting the binding of sperm to the zona pellucida [26-28], and possibly ASA may be directed to specific sperm cell membrane antigens that are essential for oocyte division and thus can inhibit the ability of a fertilized oocyte to cleave to an embryo [29].

3) There is a possibility that the antibodies can be directed against antigens that are not involved in sperm motility or in the fertilization process but are merely inert. This could explain how the four women with normal post-coital tests achieved pregnancies with intercourse [18].

In the aforementioned study, 44 of 59 women who had intercourse 8-16 hours previously demonstrated some sperm with progressive forward motion in cervical mucus obtained at the time of a mature follicle and before the luteinizing hormone (LH) surge [18]. Only 9% (4/44) of women with adequate post-coital tests had male partners with sperm with $\geq 50\%$ ASA. In contrast in 15 women not demonstrating sperm with progressive forward motion nine (60%) had male partners with ASA $> 50\%$ [18].

Over six months of therapy, IUI achieved a pregnancy in five of nine (56%) positive for ASA vs five of six (83%) of those negative for ASA [18]. These findings could suggest that when ASA are present and cause immobilization of sperm in the mucus there may be ASA also present that are directed against antigens that are needed for the process of fertilization. Merely bypassing the mucus by doing an IUI is therefore not sufficient to allow pregnancy to ensue.

Testing for antisperm antibodies

Understanding the type of limitation of immune testing and the source of material helps in determining strategies in diagnosing the possible association of ASA and infertility. Though many types of assays have been developed the two most common assays used are the immunobead (IBD) assay and the mixed antiglobulin reaction (MAR) test [30-34].

The IBD assay is composed of polyacrilimide beads that are coated with a specific anti-immunoglobulin. The coated beads are then mixed with fresh, viable washed or unwashed sperm samples and ultimately bind to sperm-bound ASA. Using the light microscope one can detect the percentage of sperm that have beads attached so that the percentage of sperm coated with ASA can be determined. Furthermore by using antibodies specific for head, tail, and tail tip the location of the antibodies can be detected. Also by using IgA, IgG and IgM antibodies the immunoglobulin class that is involved can be detected.

The MAR assay is similar to IBD testing. Blood group O – Rh-positive erythrocytes are coated with human IgG or IgA and subsequently mixed with washed or unwashed viable sperm. Antibodies specific to the immunoglobulin used to coat the erythrocytes is added and sperm agglutination occurs in the presence of ASA.

Both tests detect what percentage of sperm are coated with ASA but neither detect the concentration of ASA per sperm. There is probably a correlation such that the higher the percentage of sperm coated with ASA the higher the concentration of ASA per sperm. However, there may be some instances when ASA is directed to a key sperm antigen needed for mobilization or fertilization and a high percentage of sperm are positive and yet the concentration of the ASA per sperm is not sufficient to affect fertility. Some of the variability in conclusions as to the significance of ASA as a cause of infertility may be related to what percentage of sperm coated with ASA is considered a positive test. Some studies have considered a positive IBT test as $> 20\%$, some $> 50\%$ since some normal fertile sperm donors have $\leq 50\%$ ASA, and some consider $\geq 80\%$. Some studies have evaluated 100% of sperm showing ASA.

The effect of ASA isotype and location of the binding of ASA in achieving pregnancies

Some studies have suggested that sperm coated with IgG reduces fertilization rates more than sperm coated with IgA or IgM [34]. Another study found that only IgG in the sperm reduced fertilization rates with IVF whereas only IgM in female sera reduced fertilization rates [35]. IgA in the sera was associated with lower pregnancy rates possibly by impairing progression of sperm through the cervical mucus [35]. Some studies have concluded that the combination of IgG and IgA ASA have a synergistic negative effect on oocyte fertilization [36, 37]. It should be noted that not all studies agree that ASA of any particular isotype reduces fertilization rates [38, 39].

There are several studies dealing with the location of the antibody isotype [40-42]. One study found a significant reduction in fertilization when IgA was present on the sperm head [40]. Another study using sperm complement mediated immobilization tests found that a high degree of immobilization was found only when IgG ASA was bound to the distal 2/5 of the principal piece of the tail [42].

Nevertheless there are other studies that fail to show any connection between the location of IgG or IgA ASA [43-45]. Thus it is hard to make "head or tails" of the significance of ASA isotype and location and these extra measurements markedly increase the cost of performing the IBD or MAR assay. Since there are no clear cut data as to modifying treatment protocols based on these parameters, I have eliminated these extra parameters and I am content to merely measure IgG and IgA without location and I do not measure IgM. Perhaps measuring IgG alone may be sufficient.

Philosophy of evaluation and treatment

Though a positive ASA test cannot with certainty diagnose an infertile male, I believe the information provided even with some limitations can be very helpful in management of the infertility and can save the couple time and money in the long run. Thus I believe the slightly extra cost is justified.

Though the presence of ASA is not always an etiologic factor in infertility, a higher percentage of sperm bound with ASA is more likely than not to be a contributing factor either by impeding sperm to progress through the cervical mucus or by preventing fertilization of the oocyte.

One theoretical way to avoid the immobilization of sperm coated with ASA is to avoid the cervical mucus by performing an IUI. Since the sperm coated with ASA is immobilized by the complement in cervical mucus, by washing the sperm and placing it directly into the uterine cavity this deficit could theoretically be overcome. However though the ASA that immobilizes the sperm in mucus (as determined by a properly timed post-coital test) may be obviated by this procedure, pregnancy rates are not very high - especially when there is a high percentage of sperm coated with antibody [46, 47].

A study was performed involving 16 couples where all infertility factors were corrected except a poor properly timed post-coital test (no sperm with progressive linear motion) despite what appeared to be appropriate quality cervical mucus [46]. Furthermore the male partner was found by IBD testing to have > 50% of sperm with ASA. Intrauterine insemination was performed with all the males ejaculating into 5 ml of equal parts of modified human tubal fluid buffered with HEPES solution and 7.5% bovine serum albumin in an attempt to dilute the sperm to theoretically negate the attachment of antibodies at the time of ejaculation. Another group ejaculated into media with the protein digestive enzyme chymotrypsin with galactose in order to cleave part of the immunoglobulin molecule to neutralize function prior to sperm washing [46-51]. The 16 couples were randomly assigned one of the two sperm preparations and if no pregnancy was achieved, the other preparation was used for the second cycle of treatment [46]. With each failure they were switched to the other protocol for the next treatment [46]. There were 65 treatment cycles - 32 with chymotrypsin/galactose and 33 with albumin. Pregnancies were achieved in eight of 32 (25%) cycles following IUI with chymotrypsin galactose vs only one of 33 (3%) performed with sperm ejaculated into albumin fortified media [46]. When 100% of sperm was found to be coated by ASA Francavilla *et al.* found no live pregnancies following 119 IUI cycles [47]. In contrast, a pregnancy rate of 25% per cycle was found following IUI with chymotrypsin-treated sperm including couples whose male partner had 100% of the sperm coated with ASA [46].

These data suggest that when immobilizing antisperm antibodies are present merely bypassing the source of complement, i.e., the cervical mucus, is not very effective in correcting the infertility [46, 47]. The data also suggest that diluting the effects of sperm attaching at the time of ejaculation is not very effective either [48, 49]. The fact that merely bypassing the complement-laden cervical mucus by an IUI did not overcome the problem of ASA coating the sperm strongly suggests that when sperm immobilizing auto antibodies are present they probably co-exist with ASA that also inhibit fertilization. Thus improved efficacy would be gained by either neutralizing the ASA by enzymatic cleavage [46, 50] or possibly eluting the ASA from the sperm, e.g., with fertilization antigen-1 [51]. Possibly this treatment renders less toxic the ASA that are inhibiting oocyte fertilization.

Since the presence of ASA on sperm does not generally have an adverse effect on motility when performing the semen analysis [52] and since a normal post-coital test does not preclude the presence of ASA that can inhibit oocyte fertilization, I recommend that ASA be measured by one of these two simple inexpensive sperm tests (direct IBT or MARS) rather than allowing expensive inappropriate treatments to be rendered.

In 1990 a meta-analysis was published stating that performing a post-coital test was not cost-effective [53]. Though the suggestion to abandon the test was criticized by several clinicians including this author, since that meta-analysis was published many infertility specialists do not perform this simple inexpensive test [54]. As previously mentioned, it is our policy to perform screening for ASA on the male partner's first semen analysis. To cut costs we merely eliminate IgM and eliminate localization of ASA. I could understand an argument that only a small percentage of males will have ASA that merely effect fertilization of the oocyte but do not inhibit mobilization in cervical mucus. So why not perform only ASA in males with apparently normal semen parameters but who fail to demonstrate sperm with progressive movement in cervical mucus of apparent good quality collected at the proper time. Thus measurement of ASA could be reserved for those failing to conceive despite what seems to be an adequate number of treatment cycles for that woman's age. However, a poor post-coital test should immediately prompt the measurement of ASA by a test, e.g., the direct IBT on the sperm and if negative, then the cervical mucus should be evaluated for ASA by the indirect IBT.

Some fertility specialists argue that they perform IUI every cycle to improve the odds of conception. Thus if a poor post-coital test did exist, the IUI would "correct" the problem. It is true that there are some circumstances, e.g., poor quality cervical mucus (especially following clomiphene citrate therapy) where IUI would correct the problem [55-57]. However, Griffith and Grimes argue that \$50,000,000 is wasted yearly on a one-time post-coital test. Imagine the amount of money wasted on far more expensive IUI procedures performed monthly for many cycles! There are no clear-cut data to suggest that IUI improves pregnancy rates in women with normal post-coital tests to justify the immense extra expense and time lost from work [58]. What would be even more inexcusable would be to undergo the expenditure of far less effective "plain" IUI if ASA were present when some type of sperm treatment, e.g., chymotrypsin galactose, should precede the IUI. Thus if a couple-physician still choose to empirically try clomiphene citrate therapy/IUI despite normal ovulation or use clomiphene because of not attaining a mature follicle, if the post-coital is poor it certainly could be a side-effect of the drug. However, in this circumstance the sperm should still be checked for ASA to be sure a less efficacious IUI procedure are not performed.

One question that arises is when should a woman be checked for ASA and should the specimen that is tested be mucus or serum? Antisperm antibodies could be present in the serum but not secreted into the mucus. Except for lowering fertilization rates when used as a protein source in IVF media (which is not done much any more by IVF facil-

ities), antibodies exclusively in serum only should not negatively affect pregnancy rates. Bypassing the cervical mucus by performing an IUI (in this case no special sperm treatment is needed) should obviate the problem if ASA in cervical mucus is impeding sperm progression. Based on these assumptions there is little need other than curiosity to determine if ASA in mucus is the cause of an unexplained poor post-coital test. One study showed that only ASA in the cervical mucus as determined by the indirect IBD test (which is more expensive than the direct IBD because donor sperm is needed) occurred in only 7% of female partners [57]. Thus testing for ASA in cervical mucus is not nearly as important as testing sperm for ASA.

In vitro fertilization with intracytoplasmic sperm injection vs IUI for sperm coated with ASA

The first publication claiming an adverse effect of ASA bound to sperm on fertilization rates was published in 1986 [59]. However, subsequently there were some small studies finding no reduction in fertilization rates with IVF-ET using conventional oocyte insemination [60, 61]. Nevertheless, the majority of studies did find that ASA bound to sperm does reduce the fertilization rate [1-7].

In contrast to conventional oocyte insemination with sperm coated by ASA, most data show that ICSI allows normal fertilization rates [9-12].

At the Cooper Center for IVF when sperm coated with ASA seem to be at least partially responsible for the infertility of a given couple, the couples are given the choice of IUI with chymotrypsin/galactose or IVF with ICSI. A priori, the latter would probably be more successful since IVF is generally more successful per cycle than IUI but at a much greater cost.

Considering insurance coverage and personal income over the last ten years there have been slightly more IUI cycles (60.7%) than IVF with ICSI (in women \leq age 42 whose male partners had $> 80\%$ ASA). The clinical pregnancy rate per cycle of IUI with chymotrypsin galactose was 13.1% (34/258) with a miscarriage rate of 15%. In contrast the clinical pregnancy rate per embryo transfer with IVF and ICSI was 40.7% (68/167) with a miscarriage rate of 19% (unpublished data).

Obviously IVF with ICSI will give a couple a three-fold increased chance of achieving a pregnancy compared to IUI. However, three IUI cycles are still a lot cheaper than one IVF-ET cycle. Obviously insurance coverage and finances will help guide a couples' decision. The decision for IUI vs IVF with ICSI would be made easier if it was known whether the ASA was only of the immobilization type, as evidenced by a poor post-coital test, and not one that would inhibit fertilization. Similarly if upon routine testing of a semen analysis ASA is detected despite a normal post-coital test, it would be very helpful to know if these ASA are those that can adversely effect oocyte fertilization or not. Research is presently ongoing with proteomics to try to identify specific immunogenic antigens that are important in the fertilization process [62].

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