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OPINION

The significance of antisperm antibodies for sperm–cervical mucus interaction*

J.Kremer[1] and S.Jager

Section of Reproductive Medicine, Department of Obstetrics and Gynaecology, University Hospital, Groningen, The Netherlands

[1]To whom correspondence should be addressed at: Parklaan 16, NL-9724 Groningen, The Netherlands

An overview is presented of the effects of antisperm antibodies on the sperm–cervical mucus interaction. Antisperm IgA on spermatozoa or in cervical mucus can severely inhibit sperm penetration of cervical mucus and migration through it. Disturbance of the sperm–cervical mucus interaction is the only firmly established effect of these antisperm antibodies and leads to reduced fertility, as shown by a poor or negative result of the post-coital test. The presence of antisperm IgA in the male or female partner can be investigated more specifically with the sperm–cervical mucus contact test and can be confirmed by the sperm agglutination test on bromelin-liquefied cervical mucus, by the mixed antiglobulin reaction test or by the immunobead test for IgA.

Key words: antisperm IgA/cervical mucus/immunobead test/mixed antiglobulin reaction test/spermatozoa

Introduction

Antisperm antibodies in men and women belong to the IgG, IgA or IgM class (Hjort, 1983). The systemically produced circulating antisperm IgG is a monomeric immunoglobulin which leaks into the secretions of the male and female genital tract. These relatively large molecules do not easily traverse the mucosa and the concentration of antisperm IgG in semen and cervical mucus is, therefore, < 1% of the serum concentration (Rümke, 1974). This concentration is usually too low to cause sperm agglutination in the ejaculate or to interfere with the interaction of spermatozoa and pre-ovulatory cervical mucus. The pentameric IgM, like IgG, is a circulating antibody but the molecules which are much larger than IgG do not transude at all. We have never found antisperm IgM in semen or cervical mucus.

The sperm agglutinating antibodies in seminal plasma and cervical mucus are IgA and are at least partly of the dimeric secretory type IgA (Friberg, 1974; Ingerslev *et al.*, 1982), most probably produced in the mucosal stroma of the cervix (Schumacher, 1988) and epididymis (Linnet and Fogh-Andersen, 1979). Antisperm IgA, but not antisperm IgG, present on spermatozoa or in cervical mucus (Figure 1), disturb the sperm–cervical mucus interaction (Kremer *et al.*, 1978; Jager *et al.*, 1980; Bronson *et al.*, 1984; Clarke, 1988; Eggert-Kruse *et al.*, 1991), presumably by cross-linking the spermatozoa to the high viscosity component of cervical mucus (Jager *et al.*, 1981). Many couples with anti-IgA in semen or cervical mucus are therefore subfertile. The achievement of pregnancy, however, depends not only on the concentration of antisperm IgA in cervical mucus or the degree of antisperm IgA coating on spermatozoa but also on the presence or absence of other factors influencing fertility.

Misunderstanding about antisperm antibodies

Antisperm antibodies have often been stated to be a frequently occurring cause of reduced fertility in men and women. However, in patients visiting our fertility unit, we found an incidence of not more than 5% in men and only 0.2% in women (Jager *et al.*, 1984). A second misunderstanding is that serum testing is needed to detect antisperm antibodies. Testing serum for sperm agglutination titres and sperm immobilization activity can only provide additional information to support the diagnosis based on test results of semen and cervical mucus. A third incorrect allegation is that antisperm antibodies are found in many couples with unexplained (including normal sperm–cervical mucus interaction) infertility, particularly in the female partners. We have screened hundreds of couples with unexplained infertility during the last 15 years and have never found antisperm antibodies in either male or female partners. We did find women in this group with sperm agglutinating activity in serum but this was not due to antisperm immunoglobulins. Some women with unexplained infertility were positive in the sperm immobilization test when spermatozoa from ony one donor was used but the test was negative with spermatozoa from another donor. We conclude that unexplained infertility is not due to the presence of antisperm antibodies.

A fourth incorrect statement is that a poor result of the post-coital test is often caused by immobilizing antisperm antibodies in cervical mucus. Several investigators reported a rather high percentage of women with antisperm antibodies in their cervical mucus when the result of the post-coital test was negative or poor. We found a positive result of the sperm immobilization test with cervical mucus in only one of 52 couples with a poor result of the post-coital test. One reason for this discrepancy is that we considered the result as positive only if obtained with spermatozoa from at least two donors. Another reason for the discrepancy is that the liquefied cervical mucus was diluted with human serum without antisperm antibodies (Kremer and Jager, 1983; Jager *et al.*, 1984), while other investigators used a salt solution for dilution or extraction of the

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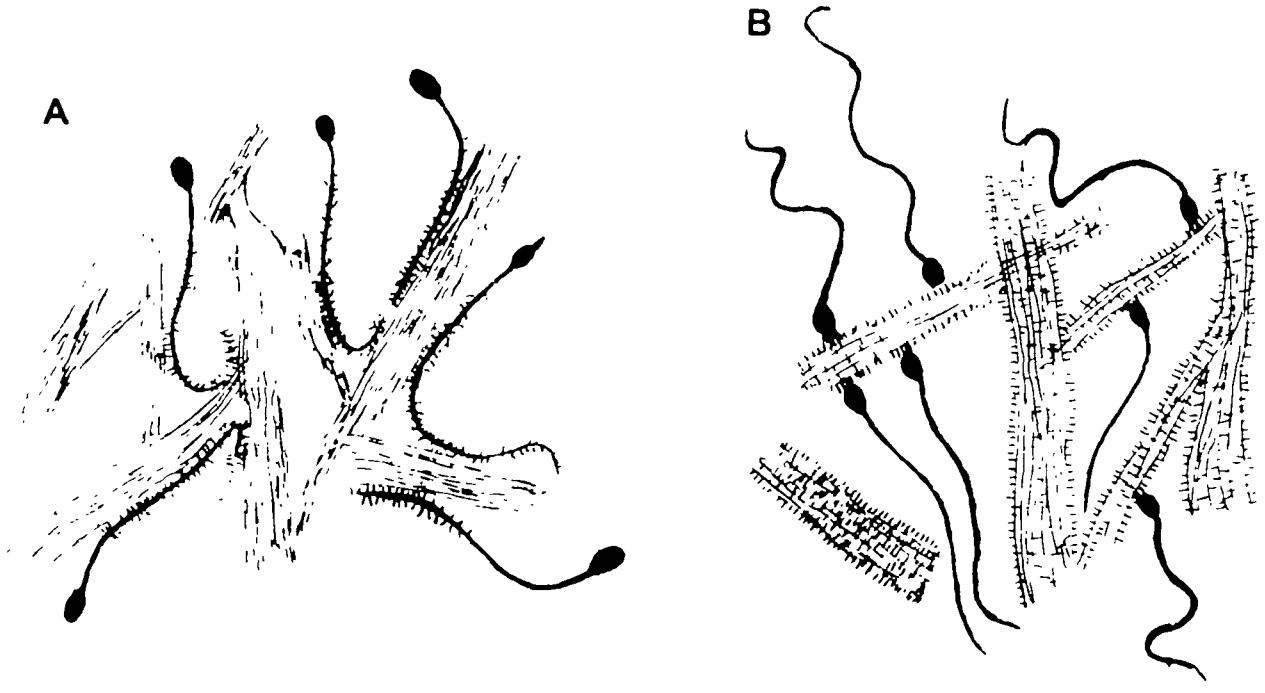

Fig. 1. Disturbed interaction between spermatozoa and cervical mucus due to antisperm antibodies attaching the spermatozoa to the glycoprotein micelles of the cervical mucus. (**A**) Secretory antisperm IgA in semen attaches the spermatozoa usually via the tail. (**B**) Secretory antisperm IgA in cervical mucus attaches the spermatozoa via the head.

mucus. It is known that rabbit and guinea-pig sera used as the source complement contain a factor, probably IgG, which can be toxic to spermatozoa in combination with complement and thus may produce a false positive outcome of the test. The addition of human serum reduces this spermotoxicity considerably (Hellema and Rümke, 1978; Hancock, 1979). Even if complement-fixing antisperm antibodies are present in cervical mucus, complement-dependent sperm immobilization is unlikely to cause poor results of the post-coital test since complement concentrations in cervical mucus are borderline at most (Schumacher, 1988).

Conditions where the presence of antisperm antibodies can be suspected

Table I summarizes the conditions in which the presence of antisperm antibodies can be expected. Sperm auto-agglutination in the ejaculate is not necessarily pathognomonic for the presence of antisperm antibodies, since it can occur in their absence, particularly in ejaculates which have been standing for a long time. On the other hand, absence of sperm auto-agglutination does not exclude the presence of secretory IgA on the spermatozoa. The presence of antisperm IgA on spermatozoa or, in rare cases, in cervical mucus should always be considered if the result of the post-coital test is unexpectedly negative or poor, i.e. when semen, pre-ovulatory cervical mucus and coital techniques are normal (Kremer *et al.*, 1978).

Tracing of antisperm antibodies in gynaecological practice

In a gynaecological practice, tracing of antisperm antibodies in couples with fertility problems should be carried out step by step. The first step is the performance of a post-coital test during the correctly timed pre-ovulatory phase of a menstrual cycle, between 8 and 12 h after intercourse. The presence of antisperm IgA, which disturbs fertility, in semen and in cervical mucus can be excluded if many forward-moving spermatozoa are observed. Antisperm IgG may be present but this has no consequence for cervical transport and survival of spermatozoa or their fertilizing capacity. If immotile or only locally motile spermatozoa, or even no spermatozoa are seen in normal pre-ovulatory cervical mucus, despite normal semen characteristics, then the presence of IgA on the spermatozoa or in the cervical mucus is probable.

The woman is subsequently requested to return during the pre-ovulatory phase of her next menstrual cycle and to bring a sample of her husband's semen within 2 h after ejaculation. A drop of the semen and a small amount of the cervical mucus are thoroughly mixed on a microscope slide, covered with a coverslip and studied with the microscope. If in this test, the sperm cervical mucus contact (SCMC) test (Kremer and Jager, 1976), >75% of the forward-moving spermatozoa change their motility into rapid movements on the spot upon contact with the cervical mucus

Table I. Groups of patients with an increased probability of having antisperm antibodies

(1)	Men operated on for inguinal hernia during early childhood
(2)	Men with unilateral or bilateral regression (complete or partial) of the Wolffian duct(s)
(3)	Vasectomized men
(4)	Men with a history of genital tract infection
(5)	Men with sperm agglutinates in the ejaculate
(6)	Men or women if the result of the post-coital test is unexpectedly negative or poor

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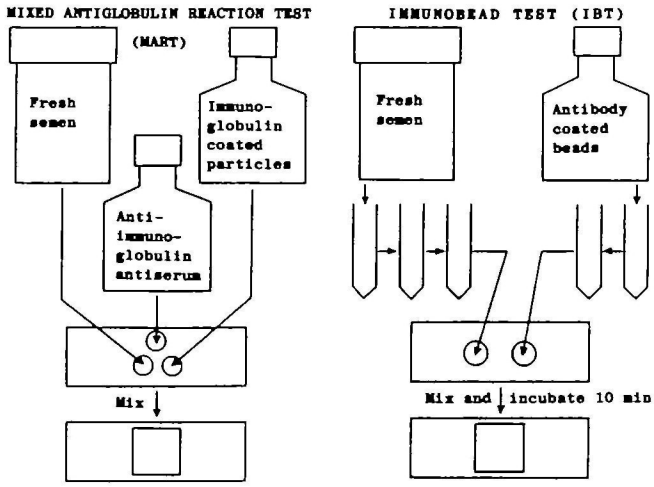

Fig. 2. Detection of sperm surface antibodies with the mixed antiglobulin reaction test and the immunobead test. The mixed antiglobulin reaction test is performed by mixing on a microscope slide a drop of fresh, untreated semen with a drop of immunoglobulin-coated particles and a drop of a strong monospecific antiserum reactive with human immunoglobulins. The test is read by estimating the percentage of motile spermatozoa attached to the particles (Jager *et al.*, 1978, 1980). The immunobead test is performed on spermatozoa washed free from seminal plasma components by centrifugation and resuspension. The washed spermatozoa are mixed with particles coated with monospecific antibodies against human immunoglobulins.

Table II. Results of mixed antiglobulin reaction (MAR) test and immunobead test (IBT) on a consecutive series of ejaculates from 140 men visiting the fertility unit

Results	IgG		IgA	
	MAR	IBT	MAR	IBT
No result[a]	33	44	38	46
Negative	90	81	90	83
Positive	17	15	12	11

[a]Due to poor sperm motility or due to loss of spermatozoa in the centrifugation steps.

Table III. Time needed for a single mixed antiglobulin reaction (MAR) test and immunobead test (IBT) (IgG + IgA)

Step	Test type	
	MAR	IBT
Washing (3×)	–	24 min
Mixing, incubating and reading	8 min	11 min
Whole procedure	8 min	35 min
Each additional test	4 min	7 min

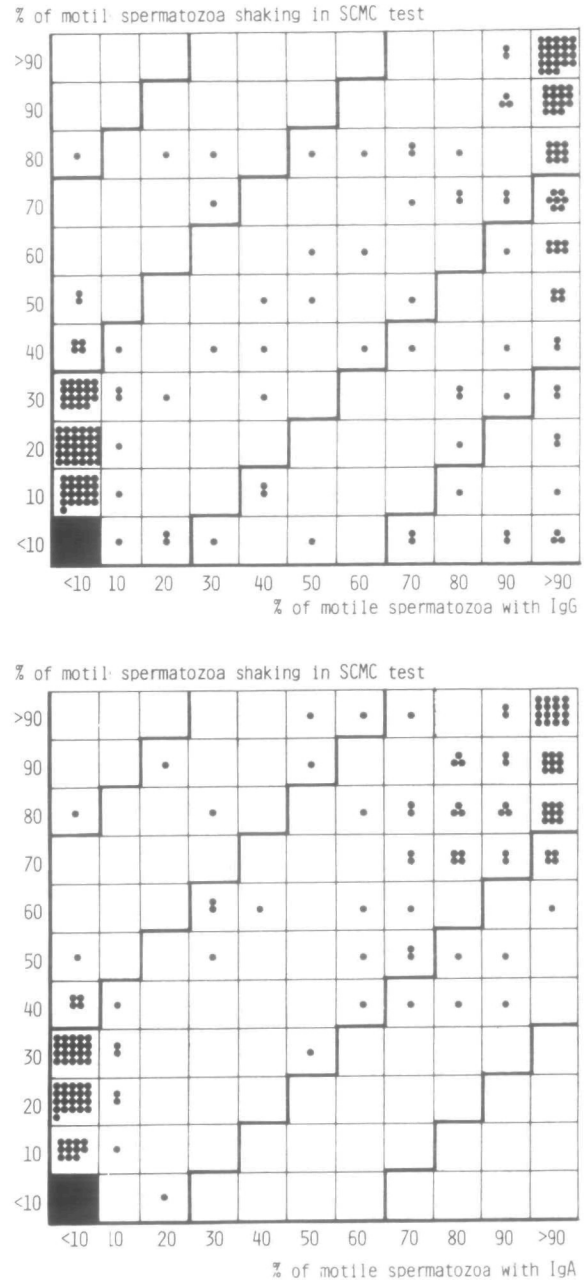

Fig. 3. Comparison between the results of the sperm–cervical mucus contact (SCMC) test and the IgG mixed antiglobulin reaction (MAR) test (upper half) and the IgA MAR test (lower half). The results of both the SCMC test and the MAR test are expressed in multiples of 10 (Jager *et al.*, 1978). Taking into account the uncertainty of the results (20% at most; Jager *et al.*, 1980), agreement is considered to be reasonable in the central area. Results in the extreme outer areas are considered to disagree and those in the areas in between represent doubtful agreement. The areas are delineated by bold lines.

(the so-called shaking phenomenon), then IgA is likely to be present on a large majority of the spermatozoa. This shaking phenomenon is probably caused by cross-linking of spermatozoa with glycoprotein micelles in the cervical mucus. By cross-testing with donor spermatozoa and donor mucus, the antisperm antibodies can be located either in the husband's semen or in the wife's cervical mucus.

If the crossed SCMC test indicates presence of antisperm antibodies in the cervical mucus, this can be confirmed by observing the sperm agglutinating activity of the mucus. For this purpose, cervical mucus is liquefied with the same volume of a bromelin solution (Jager *et al.*, 1977) and incubated with donor

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spermatozoa. If antisperm IgA is present, head-to-head sperm agglutinates are observed. Further confirmation can be obtained by determining sperm agglutinating and sperm immobilizing activity in serum, since local antisperm IgA is almost always accompanied by circulating antisperm IgG. If the crossed SCMC test indicates the presence of antisperm antibodies in the semen, confirmation of the diagnosis can be obtained by determining the percentage of motile spermatozoa with IgA with either the mixed antiglobulin reaction (MAR) test (Jager *et al.*, 1978, 1980) or the immunobead test (Clarke *et al.*, 1985) (Figure 2).

A comparison of the MAR test with the immunobead test shows that more semen samples can be tested by the former method (Table II). Due to the centrifugation steps in the immunobead test, some semen samples lost their motility and some mucus samples were lost since no sediment was obtained. Moreover, the immunobead test is more time-consuming (Table III). We perform the IgG MAR test as part of routine semen analysis. The corresponding IgA MAR test is done only if the result of the first IgG MAR test is positive. In 90% of these cases, the IgA mixed antiglobulin reaction test is also positive, albeit generally with a lower percentage value than in the IgG test. In contrast to the IgG MAR test, the corresponding IgA MAR test cannot be performed with commercially available coated particles. These particles must therefore be coated in the laboratory with human colostral IgA (also commercially available). Instead of an IgA MAR test, an immunobead IgA test can be performed when the IgG MAR test gives a positive result.

The agreement between the results of the sperm cervical mucus contact test and the mixed antiglobulin reactions test is demonstrated in Figure 3. The percentage of spermatozoa with IgA (IgA/MAR%) is roughy equivalent to the percentage of spermatozoa shaking in the sperm cervical mucus contact test. This agreement is expected since the shaking phenomenon in the SCMC test is almost always caused by antisperm IgA.

Conclusion

Sperm penetration into cervical mucus and subsequent migration can be severely inhibited by antisperm IgA on the spermatozoa or, in rare cases, in cervical mucus. This condition is a cause of negative or poor results of the post-coital test in ~5% of infertile couples. The tentative diagnosis, infertility due to presence of antisperm antibodies, can be supported with the sperm cervical mucus contact test. If the antisperm antibodies are present in the husband, it can thereafter be confirmed with the IgA mixed antiglobulin reaction test or the IgA immunobead test. The presence of antisperm IgA in cervical mucus can be demonstrated by adding donor spermatozoa to cervical mucus liquefied with bromelin. A sperm agglutination test and a sperm immobilization test with serum have additional value only for the diagnosis.

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