Survey of the diagnosis and management of antisperm antibodies

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A questionnaire was sent to all Human Fertilization and Embryology Authority-registered reproductive medicine centres throughout the UK to survey their policy for the diagnosis and management of antisperm antibodies. Forty-eight responses were received from the 74 units that use husbands’ spermatozoa for treatments (65%). Most centres use at least one test to detect antibodies, although a minority perform no tests on the basis that their clinical practice would be unaltered if antibodies were present. Positive tests are classed as clinically significant at levels varying from ≥10% to ≥50% for direct sperm binding tests (mixed antiglobulin reaction, immunobead test), and ranging from any positive reaction to ≥1:32 for the microtitre tests (gelatin and tray agglutination tests, microimmobilization test). Strategies for managing affected patients include no intervention, artificial insemination and intrauterine insemination (IUI) using spermatozoa prepared by various techniques, in-vitro fertilization (IVF) with or without increased insemination concentration, and intracytoplasmic sperm injection. Criteria for the latter are diverse, some centres managing all antibody-positive patients this way, while others resort to it only in severe cases or after other treatments have failed. Half of the respondents occasionally or regularly employ steroids, either alone or in conjunction with IUI or IVF. Overall, it appears that much confusion exists as to how best to manage couples presenting with antibody-related infertility.

Key words: antibody detection/antisperm antibody/assisted conception/ICSI/male infertility

Introduction

A diagnosis of antisperm antibodies during the investigation of a sub-fertile couple is generally considered to be of some clinical importance (Rümke et al., 1974; Bronson et al., 1984; Matson et al., 1988). Antibodies found in serum and cervical mucus are usually directed to sperm tails, and are associated with an inhibitory effect on sperm transport through the female reproductive tract. However, the presence of antibodies in serum is not always indicative of sperm-head antibodies (Adeghe et al., 1986), which are thought to be of greater clinical significance. Antisperm antibodies directed against the sperm head, whilst not affecting semen parameters such as count and motility (Adeghe et al., 1989; De Almeida et al., 1991), are known to interfere with sperm binding to the oocyte, resulting in reduced fertilization both in vivo and in vitro (Bronson et al., 1982; Clarke et al., 1985; De Almeida et al., 1989).

The detection of antisperm antibodies can be performed by a variety of tests using spermatozoa, seminal plasma, serum or cervical mucus, and is a common component of the semen evaluation carried out by specialist reproductive medicine centres. The most widely employed tests are those for immunoglobulin classes G and A (IgG and IgA). These may be direct measures of the percentage of affected spermatozoa, such as the immunobead test (IBT), or may be qualitative microtitre assays, for example the gelatin and tray agglutination tests (GAT and TAT) using serum or seminal plasma. The lymphocyte proliferation assay has also been used to measure antisperm cell-mediated immunity (Focacci et al., 1997). However, the results relate poorly to those from other methods for detecting antisperm antibodies, and this technique is not considered to be useful for the diagnosis of immunological infertility. The results of all these methods must be interpreted with regard to clinical significance, as low to moderate antibody levels may not have a profound effect on fertility (Rümke et al., 1974; Barratt et al., 1992). Treatments used with varying degrees of success have included antibiotics (Fällbrant and Nilsson, 1977), immunosuppression (Hendry et al., 1986), artificial insemination (Yovich and Matson, 1988), methods for preventing binding to or removal of antibodies from spermatozoa (Elder et al., 1990; Grundy et al., 1991), in-vitro fertilization (IVF) (Palermo et al., 1989), and, more recently, intracytoplasmic sperm injection (ICSI), with the direct introduction of a single spermatozoon into the ooplasm (Nagy et al., 1995). Recent reports on the efficacy of ICSI in the treatment of antibody-related infertility suggest that it may be more effective in achieving pregnancy than other methods (Clarke et al., 1997). However, there appears to be some confusion as to whether it is really necessary to resort to this more invasive treatment as the first line of management, as well as to the level of antibodies that is deemed to be critical for a reduced chance of success with other treatments.

In order to determine the current practice regarding the diagnosis and management of antisperm antibodies, a questionnaire was circulated to all Human Fertilization and Embryology Authority (HFEA)-registered reproductive medicine centres throughout the UK. The objectives were to discover which methods were employed to detect antisperm antibodies, to learn what levels of antibodies were considered to be of
clinical significance, and to survey the strategies for managing couples presenting with infertility linked to antisperm antibodies.

Materials and methods
The questionnaire was sent to the clinical directors of each of the 117 centres on the HFEA List of Licensed Centres (April 1997 edition), of which 74 are licensed for treatment using husbands' sperm. A simple format was used to request information on each of the three main areas of interest as described above. The questions asked were:

- Which test/tests do you use to detect antisperm antibodies in: male serum, semen, female serum, cervical mucus?
- What would you consider to be a significant level of antisperm antibodies?
- How do you manage patients with antisperm antibodies: expectant, artificial insemination (AIH), intratubal insemination (IUI), intracytoplasmic sperm injection (ICSI), steroids, other?

Centres were asked to give as much information as necessary to provide details of methods of sperm protection when antibodies were present, and to note whether they altered the number of spermatozoa used for insemination in IVF. They were also requested to state their criteria for ICSI if this was considered to be an appropriate treatment for couples with antibodies. In the section concerning the use of steroids, centres were asked to specify which (if any) regimen was employed, and whether this was used alone or with any of the other treatments described.

Results
Forty-eight replies were received from the 74 centres who carry out IVF and related techniques (the remaining 43 are licensed only for sperm storage and donor insemination), giving a response rate of 65%.

Testing for antisperm antibodies
Three centres use no tests for antisperm antibodies in semen or serum. Table I shows the distribution of the various methods used for testing semen, seminal plasma, male and female serum and cervical mucus. The majority of responding centres (44/48) test for antibodies in semen using one or more of several methods, with the mixed antiglobulin reaction (MAR) and direct IBT tests being most commonly used by 28 and 19 laboratories respectively. Other methods include different microtitre assays of antibodies to sperm in seminal plasma, specifically the tray agglutination, microimmobilization, and gelatin agglutination tests [TAT, microimmobilization (MIT) and GAT; seven, three, and two centres respectively].

Of 48 centres, 17 test serum from male partners using one or more assays (TAT, n = 10; MIT, n = 5; indirect IBT, n = 4; GAT, n = 2; MAR, n = 2), and 15/48 centres test female serum, with the TAT most often utilized (n = 8), followed by the indirect IBT (n = 5) and MIT (n = 2). The MAR and gelatin agglutination tests are used less often, by only one and two laboratories respectively.

Only seven of the 48 centres that replied stated that they test cervical mucus for antisperm antibodies. The methods used are the sperm-mucus interaction (SMI) and sperm-cervical mucus contact (SCMC) tests, used in four and three centres respectively.

Levels of clinical significance of antisperm antibodies
The responses given to this question varied widely. The MAR test is considered significant by six laboratories if >10% of spermatozoa are bound to particles, if binding is ≥15% (n = 12 centres), ≥40% (n = 1) and ≥50% (n = 4). The World Health Organisation (WHO) Laboratory Manual for the Examination of Human Semen and Sperm–Cervical Mucus Interaction (1992) states that this test is regarded as positive if >10% of spermatozoa are bound to particles, that it is of suspected clinical significance if there is 10–50% binding, and of probable significance if there is 50% or more sperm binding.

Similarly, the IBT is interpreted as significantly positive by three centres when >10% of spermatozoa are bound to immunobeads, by seven centres if binding is 20–40%, and by five centres if >50% of spermatozoa are attached to the beads. The WHO manual sets out 20% as the minimum binding to be regarded as a positive test, with >50% of sperm binding constituting a clinically significant test.

For centres using the gelatin agglutination and microimmobilization assays, positive reactions in titres ranging from ≥1:4 to ≥1:32 were reported as significant. For the tray agglutination test, any positive reaction in female serum, and positive tests at a dilution of ≥1:32 in male serum were stated as being significant, which is in agreement with the guidelines set out by Boettcher et al., (1977).

Management of antisperm antibody-positive patients
The responses regarding the management of antibody-positive patients are presented in Table II. Couples with low levels of antisperm antibodies are managed expectantly by two centres and with artificial intracervical insemination with washed or unwashed husband’s semen by four centres. Of the 48 centres, 35 perform IUI with spermatozoa washed and prepared by a variety of techniques. Six centres specify a cut-off level of 20–50% antibodies for treatment with IUI, two others require a minimum number of 5–10×10^6 motile spermatozoa/ml available in the final preparation, while a further four centres use IUI only with spermatozoa of proven fertilizing ability. Around half of the laboratories (16/35) use samples produced into medium supplemented with a high concentration of protein, and most use a density gradient to separate spermatozoa (31/35), although eight centres perform a ‘swim-up’ to prepare
IVF is used by the majority of units (34/48) when antisperm antibodies are present. Most of the respondents did not specify a threshold for treatment by this method, although two centres stated that not more than 50% of the spermatozoa may be antibody-bound, and another three use a limit of ≈80%. Two centres report using IVF after 3–6 months unsuccessful treatment by IUI. The methods of sperm preparation vary considerably and follow a similar distribution to those used for IUI. Half of the centres stated that they use an altered insemination concentration for IVF, most increasing it to more than $10^5$ motile spermatozoa/ml, although one unit reported inseminating with a lower concentration than usual for cases where there are high levels of antibodies. The rationale for this is not stated.

Not all of the centres replying to the questionnaire perform ICSI, but of the 35 that either provide this service or refer patients elsewhere, four recommend ICSI when there are >20% of spermatozoa affected by antibodies, another four when antibodies exceed 50%, and a further four resort to ICSI only when there are >70% antibodies. Another centre remarked that ICSI is the method of choice when motility is significantly affected due to agglutination of spermatozoa, and 13 of the 35 units choose to perform ICSI after an IVF treatment cycle which has resulted in poor (<25–30%) or complete failure of fertilization.

Almost half of the replies (22/48) stated that steroids are used regularly or occasionally, either alone or in conjunction with IUI or IVF. The protocols for steroid administration vary widely, the most common regimen being 20 mg daily of prednisolone for days 1–10 of the female partner’s follicular phase or follicle-stimulating hormone stimulation, then 5mg daily for days 11 and 12. Several replies stated that the use of steroids has been obviated with the advent of ICSI for the treatment of antibody-related infertility.

**Discussion**

Finding antisperm antibodies in either partner of a couple presenting with infertility provides the clinician with information which may be useful in their subsequent management. A variety of tests are in regular use for the detection of antisperm antibodies. Most of the centres responding to the questionnaire employ direct assays for antibody attachment to spermatozoa, as recommended in the WHO laboratory manual for semen evaluation (1992), although other techniques are also in use. A wide range in the level of antibodies that is considered to be of clinical significance was reported.

In a pilot study for the establishment of an external quality assessment scheme for seminology laboratories, Matson (1995) found a high degree of variation in the results obtained from the several methods used by 20 laboratories detecting antibodies in standardized samples, with positive test values using the IBT ranging from 21–82%. As individual laboratories showed reasonable reproducibility of results in this study, the greater between-laboratory variation was suggested to be due to the presence of consistent errors in the performance of the tests. Results from the now-established UK National External Quality Assessment Scheme show similar variation; in a recent distribution 13/26 tests on a known positive sample were reported to be antibody-negative, and a known negative sample was reported as positive by 5/26 participants (A.Atkinson, scheme manager, personal communication). Many clinics establish their own ‘normal’ ranges and cut-off points for clinical significance, based on correlations between the tested parameters and subsequent outcomes of treatment. Given the reported variation in test results, the differing levels of significance of the various antibody assays described here may reflect individual clinics’ own experience in the treatment of couples with antisperm antibodies.

Several replies to the questionnaire noted that the actual quantification of antibodies was of less importance than other parameters such as sperm concentration and motility, which affect the number of non-antibody-coated spermatozoa available for use in treatment. Some centres claimed that testing for antisperm antibodies was ‘of academic interest only’, as the results often relate poorly to subsequent fertilization rates following attempts at IVF. Furthermore, a minority of clinics do not perform any tests for antisperm antibodies, justifying this by stating that they would not alter their clinical management if antibodies were detected.

Strategies for the management of couples with significant numbers of antisperm antibodies show the same degree of variation. In their response to the survey, several centres claim that, despite ‘significant’ numbers of antibodies, IUI using spermatozoa prepared by a variety of techniques is used as the first line of treatment for three to six cycles. Some centres specify a minimum number of freely motile spermatozoa in the final preparation (typically $>5–10\times10^6$/ml) or previously proven fertilization as criteria for suitability for this method. Others elect to use IUI only with lower antibody levels ($<20–50\%$), moving straight to IVF when levels are higher than this. A recent study (Ombelet et al., 1997) compared IUI using spermatozoa prepared by conventional swim-up (if normal semen parameters) or Percoll (if subnormal) after production of the sample into protein-supplemented medium, with IVF at an insemination concentration of $10^5$ spermatozoa per oocyte in two groups of patients with antibody-related infertility. High

### Table II. Management of antisperm antibodies and methods for preparing spermatozoa, where given

<table>
<thead>
<tr>
<th>Management</th>
<th>Expectant</th>
<th>AIH</th>
<th>IUI</th>
<th>IVF</th>
<th>ICSI</th>
<th>Steroids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of centres using method</td>
<td>2</td>
<td>4</td>
<td>35</td>
<td>34</td>
<td>35</td>
<td>22</td>
</tr>
<tr>
<td>Sperm preparation methods</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Protein-supplemented medium</td>
<td>–</td>
<td>–</td>
<td>16</td>
<td>16</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Split ejaculate</td>
<td>–</td>
<td>–</td>
<td>6</td>
<td>10</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Chymotrypsin/galactose</td>
<td>–</td>
<td>–</td>
<td>3</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Pentoxifylline</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Swim-up</td>
<td>–</td>
<td>–</td>
<td>8</td>
<td>4</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Density-gradient centrifugation</td>
<td>–</td>
<td>–</td>
<td>31</td>
<td>31</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>IVF: altered insemination concentration</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>17</td>
<td>–</td>
</tr>
</tbody>
</table>

AIH = artificial insemination; IUI = intrauterine insemination; IVF = in-vitro fertilization; ICSI = intracytoplasmic sperm injection.

samples. Other methods include split ejaculates ($n = 6$) and pentoxifylline ($n = 1$) to stimulate motility, while three report making use of chymotrypsin/galactose in an effort to strip antibodies from the spermatozoa.
pregnancy rates ensued in both groups (64.3% after three cycles of IUI and 93.3% after three IVF treatments), and the report concluded that IUI was a worthwhile first line of treatment for couples with antisperm antibodies.

A common approach is to employ a method to try to reduce antibody binding to spermatozoa, such as production of the ejaculate into medium supplemented with a high concentration of protein (Elder et al., 1990), followed by prompt processing of the ejaculate. Attempts to strip antibodies from spermatozoa using enzymatic digestion with chymotrypsin/galactose (Tucker et al., 1990; Bollendorf et al., 1994) and to separate antibody-bound from antibody-free spermatozoa using immunobead adsorption (Grundy et al., 1991; Verheyen et al., 1994) prior to IUI or IVF have met with varying degrees of success, and were not widely reported to be in use by the respondents to the questionnaire.

Other centres state that IVF is the preferred treatment for patients with moderate to high antibody levels and, while some clinics make no alteration to either sperm separation techniques or insemination concentrations, it is more usual to prepare spermatozoa from a sample produced into protein-supplemented medium, and to increase the number of spermatozoa incubated with each oocyte by up to 10 times the standard concentration. Both IgA and IgG antibodies are associated with impaired fertilization in IVF (Clarke et al., 1988; Lahteenmaki, 1993), depending on the proportion of affected spermatozoa and the localization of antibody binding on the spermatozoon. In an effort to counteract a suspected zona-binding problem due to antisperm antibodies, many laboratories choose to inseminate oocytes with a much higher number of spermatozoa than usual. This strategy of high insemination concentration (generally ≥1×10^6 motile spermatozoa/ml) has been demonstrated to be a useful technique for achieving fertilization in some male factor cases (Hall et al., 1993; Cowan et al., 1996), but does not always result in good fertilization rates. Moreover, inseminating oocytes with a very high number of spermatozoa which have been exposed to oxidative stress under conditions of leukocytospermia, often associated with antibodies following vasectomy or genital tract infection, may cause cell membrane damage, resulting in poor quality embryos with a reduced potential for implantation and further development (Dumoulin et al., 1992). Following the successful introduction of ICSI for the treatment of couples with other forms of male factor infertility, it is an attractive alternative for the management of couples affected by antisperm antibodies.

Some of the groups surveyed reported using ICSI for any patient with positive antibody tests ranging from ≥20% to levels >80%. In a retrospective analysis of the results of a series of 55 ICSI treatment cycles in 37 couples with high levels of seminal antisperm antibodies (≥80% on MAR testing), Nagy et al. (1995) found an average fertilization rate of almost 76%, in comparison with only 13% following standard IVF for nine of these patients. It was observed that a significantly higher proportion of embryos was of poor quality than in antibody-negative ICSI patients, a finding also noted by this group in antibody-positive patients undergoing routine IVF (Palermo et al., 1989). Furthermore, Lahteenmaki et al. (1995) suggested that ICSI patients with high levels of antibodies may suffer an increased rate of early pregnancy loss. However, a subsequent study (Clarke et al., 1997) based on a similar group of patients did not confirm either of these reports, and concluded that ICSI was an effective treatment for couples with severe antisperm antibody-related infertility.

The occasional or regular use of steroids was reported by almost half of the respondents to the questionnaire, either alone or in combination with IUI or IVF. Continuous treatment with steroids over a period of at least 6 months may improve semen parameters and pregnancy rates (Hendry et al., 1986) but, in view of the possible serious side-effects of corticosteroid administration, many clinicians prefer other options. As ICSI provides an alternative treatment for the management of these patients, many centres have chosen to abandon the use of steroids. ICSI generally results in good fertilization rates, although it too may carry its own, as yet undiscovered, long-term side-effects.

In conclusion, the results obtained in the survey highlight the wide range of tests used for antibody detection, as well as the discrepancies in the interpretation of these tests and strategies for managing affected patients. ICSI has so far proved to be the most effective means of managing other categories of male factor infertility, and this may also be the case where antisperm antibodies are thought to be responsible for a couple’s inability to conceive. However, ICSI is still a new technique, and the long-term safety aspects are as yet unknown; hence a degree of caution should be exercised in its application. Several of the centres that responded to the survey do not appear to adhere strictly to the recommendations set out by the WHO, and there is a need for laboratories to employ more standardized tests, using control samples, in order to achieve comparable results. It may then be possible to establish critical levels of antibodies by relating test results to subsequent treatment outcomes for IUI and IVF, as well as improved evaluation of the various methods for preparing spermatozoa for use in these treatments. The current confusion that exists regarding the management of antisperm antibodies requires further attention in order for these patients to be treated effectively, efficiently, and safely.

References
Diagnosis and management of antisperm antibodies


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