Validity and cost-effectiveness of antisperm antibody testing before in vitro fertilization

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Objective: To determine the usefulness of and cost-effectiveness of antisperm antibody testing in the prediction of poor fertilization rates in couples undergoing IVF.

Design: Retrospective cohort study.

Setting: A hospital-based reproductive endocrinology and infertility practice.


Main Outcome Measure(s): Fertilization rates in couples undergoing conventional IVF.

Result(s): One hundred nineteen couples were evaluated for antisperm antibodies; fertilization rates were similar in those couples whose husbands were and were not tested (64% versus 68%). Antisperm antibodies were detected in 16 men. Four (25%) of the 16 couples whose husbands had antisperm antibodies fertilized 50% of oocytes, compared with 31 (30%) of the 103 couples whose husbands did not have these antibodies. Overall, 21 couples (8.4%) experienced complete fertilization failure. In a program that included antisperm antibody testing for selected couples and intracytoplasmic sperm injection (ICSI) for those who tested positive, it would cost $11,735 to prevent a fertilization failure (assuming ICSI were 100% effective), whereas it would cost $9,250 to perform ICSI in a second IVF cycle for those who initially failed.

Conclusion(s): In this practice setting, antisperm antibody testing has low sensitivity in predicting low or no fertilization and does not appear to be cost-effective when selectively ordered as part of an IVF workup. (Fertil Steril 1998;69:894 – 8.

Key Words: Antisperm antibodies, in vitro fertilization, fertilization, human, infertility, hospital charges

The fact that animals are capable of developing autoimmunity to spermatozoa has been known for more than a century, but it was not until 1954 that such antibodies were described as a causative factor for human infertility (1, 2). Although spermatozoa can be very antigenic, they are normally protected from entering the seminiferous tubules by the blood-testis barrier. If this barrier sustains an insult, such as infection, trauma, or surgery, sperm proteins may come into contact with the immune system, resulting in antisperm antibodies.

Estimates suggest that a male factor is at least partially responsible for 30%—50% of infertility (3). Because a significant percentage of male infertility is believed to be immunologic, the specific role that antisperm antibodies play in infertility may become crucial in certain patient populations. Antibodies have been shown to interfere with sperm motility and with the sperm’s penetration of the cervical mucus and the zona pellucida. In vitro fertilization has been used as a means of overcoming these problems (4, 5); however, antisperm antibodies can interfere with sperm-oocyte interaction in IVF as well (6, 7). Because of these potential interactions, many assisted reproductive technology programs screen all couples entering IVF programs for sperm antibodies.

The purpose of this article is to examine the clinical utility and cost-effectiveness of antisperm antibody testing in our IVF population.

MATERIALS AND METHODS

Patient Selection

The study group consisted of 251 consecutive couples who had undergone IVF in our institution between 1992 and 1997. Men were
tested for antisperm antibodies if the couple had no history of pregnancy together, if the screening semen analysis showed significant agglutination, or if postcoital testing was suggestive of antisperm antibodies. In all, 119 of the 251 couples met these criteria and underwent antisperm antibody testing by immunobead assay (2 couples were subsequently retested) before undergoing IVF. Patient information for this study remained confidential and within the institution.

**Immunobead-Binding Assays**

Semen specimens were collected before the start of the IVF cycle. A commercially available direct immunobead test was used (BioRad, Richmond, CA). After liquefaction of the semen, the sample was diluted 1:5 with Biggers, Whitten, and Whittingham (BWW) medium (Irvine Scientific, Irvine, CA). The samples were centrifuged at 500 \( \times \) g for 3 minutes, and the supernatant was discarded. The pellets were resuspended in BWW with 5% serum albumin and centrifuged in microcentrifuged tubes. The pellets were washed three times. The final pellet was then suspended with BWW with 5% serum albumin and adjusted to a concentration of 100 \( \times \) 10^6 motile sperm/mL.

Ten microliters of each washed immunobead suspension was incubated for 10 minutes (room temperature) on a slide with 10-\( \mu \)L of sperm suspension. The immunobeads used were polyacrylamide beads covalently bound to antibodies containing rabbit antihuman IgG, IgA, or IgM antibody raised against human immunoglobulin classes IgA, IgM, and IgG. The immunobead method is used to detect antibodies on sperm in patients in IVF cycles. The method localizes the beads on the different regions of the sperm and scores them on progressively motile sperm because of the nonspecific adhesion.

Through the use of a phase-contrast microscope, a minimum of 100 motile sperm were analyzed for the binding of immunobeads. The percent of motile sperm with beads bound to the head, neck, or tail was determined, and a total value was calculated. Specimens were considered positive when \( \geq 15\% \) of motile sperm had immunobeads associated with them (7, 8). Assays using known positive and negative controls were run on each day of testing. Full details of direct immunobead testing have been reported (8). Testing of the samples was performed by the same investigator throughout the study period. Because donor sera were used in our IVF laboratory, the female partners of the couples undergoing IVF were not tested for antisperm antibodies.

**Semen Preparation and Analysis**

Semen samples tested were those used to inseminate oocytes for IVF-ET and were collected as per the IVF protocol (9, 10). All specimens were collected after a mandatory omission of sexual activity for at least 48 hours. Semen specimens were placed into a 37°C incubator for up to 30 minutes to allow liquefaction to take place. After gentle vortexing, the samples were evaluated for volume and pH. Each specimen also was evaluated for sperm agglutulation, motility, concentration, morphology (10), and concentration of white blood cells. All dilutions were analyzed manually two times on a hemacytometer (Baxter Healthcare Corporation, McGraw Park, IL) and/or a 20 MicroCell Chamber (Conception Technologies, Inc., La Jolla, CA) for sperm concentration. The MicroCell also was analyzed on the computer-assisted semen analyzer (Hamilton-Thorne Research, Beverly, MA), using first the standard and then the high-density parameter settings (11). Specimens were prepared for in vitro insemination with the use of a swim-up technique as per the IVF protocol (9–13).

**In Vitro Fertilization-Embryo Transfer**

Full details of the IVF protocol have been reported elsewhere (9). Briefly, all female partners underwent ovulation induction using gonadotropins, usually using a midluteal leuprolide acetate suppression protocol. Response was monitored through transvaginal ultrasound and serum estradiol levels. When two or more follicles were \( \geq 16 \) mm in size, hCG, 10,000 IU, was administered intramuscularly. Follicles were aspirated 34–35 hours later, and recovered oocytes were evaluated for maturity. They were exposed to sperm after reaching the metaphase II stage of development characterized by the presence of the first polar body. No attempt was made, based on immunobead-binding results, to alter the sperm concentration (100,000–400,000 motile sperm/mL). Gametes were left undisturbed for approximately 18 hours after insemination.

Fertilization was confirmed when two pronuclei were observed within the cytoplasm (9, 13). Reinsemination was performed on oocytes that failed to fertilize initially (14, 15) and was followed by reexamination the following day for delayed fertilization. After confirmation of fertilization, the zygotes were placed in fresh culture medium and allowed to remain undisturbed in the incubator until ready for ET (9).

**Statistical Methods**

Two- and three-group comparisons were made with exact tests (SPSS, Version 7.5, Chicago, IL). Differences in means were assessed by the Mann-Whitney U test (two-group comparisons) or the Kruskall-Wallis (three-group) tests and proportions by \( \chi^2 \). All P values are two-sided.

**RESULTS**

In the 119 couples who were tested for antisperm antibodies versus the 132 couples who were not tested, we compared several variables that, in our practice, predict successful IVF. Test couples had lower mean percent motile sperm (48.7% versus 53.7%, \( P = 0.04 \)) and lower mean percent normal morphology (29.5% versus 37.2%, \( P = 0.0005 \)); there were no statistically significant differences (all \( P > 0.05 \)) with respect to age of the female partner, number of oocytes retrieved and transferred, sperm straight-line velocity, or percent fertilization (63.9% versus 68.1%).

**FERTILITY & STERILITY**
Sperm concentration, although lower in males who were tested than in those who were not (76.5 versus 87.7 $10^6$/mL, $P = 0.13$) was not significant.

Sixteen men in the IVF program were found to have autoimmunity to sperm; a threshold level of $15\%$ total binding of either IgG, IgA, or IgM was used as the determination point (Table 1). Six men were positive for IgG only, three were positive for IgA only, and none were positive for IgM alone. Two men tested positive for both IgA and IgG, and one was positive for both IgG and IgM. The remaining four were positive for all three immunoglobulins (Table 1). Only two patients (nos. 148 and 291) were positive for head, neck, and tail with at least one of the three gamma globulins. Three patients (nos. 143, 190, and 308) were positive for the head alone in at least one of the three Ig classes. Likewise, only two patients (nos. 156 and 181) were positive for tail alone in at least one of three classes. The combination of sites is given in Table 1.

The overall fertilization percentage for 103 patients without antisperm antibodies was 62.8% compared with 66.6% fertilization rate for the 16 antisperm antibody–positive patients. The difference was not significant ($P = 0.68$; data not shown). In addition, the cleavage rate for the negative antisperm-antibody group (82.0%) was not significantly different ($P = 0.08$) when compared with the positive antisperm-antibody group (93.5%).

Only 25% (4 of 16) of the couples whose male partners were positive for antisperm antibodies had <50% fertilization of oocytes. Of these four couples, one was positive for IgG, one for IgA, one for both IgG and IgA, and one for all three. In the group that was negative for antisperm antibodies, 30.1% (31 of 103, $P = 0.78$) had <50% fertilization (Table 2). The sensitivity for antisperm antibody testing in predicting ≥50% fertilization was 11.4% (specificity 85.7%; positive predictive value 25%).

Overall, 8.4% (21 of 251) of the couples undergoing IVF had total fertilization failure on their first attempt at IVF. Of this group, 14 had undergone antisperm antibody testing, and three were positive (21.4%).

All 251 couples were subdivided into 0, 1%–50%, and >50% fertilization rate groups (Table 3). There was a clear increase in means across those three groups for sperm concentration, motility, normal morphology, and straight-line velocity (all $P<0.01$). By contrast, the proportion of abnormal antisperm antibody tests in the three fertilization groups was 21.4% (3 of 14 tested), 4.8% (1 of 21 tested), and 14.3% (12 of 84 tested), respectively ($P = 0.394$). There were no

### Table 1

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<th>Patient no.*</th>
<th>IgA titer</th>
<th>IgG titer</th>
<th>IgM titer</th>
<th>Age of female (y)</th>
<th>No. of oocytes</th>
<th>Oocytes fertilized (%)</th>
<th>No. of zygotes</th>
<th>Zygotes fertilized (%)</th>
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<td>5</td>
<td>20</td>
<td>1</td>
<td>100</td>
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Note: N = neck; T = tail; H = head region of attachment for immunobeads in ≥15% of motile sperm.
* A list of all patients tested before IVF, regardless of outcome.

### Table 2

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<th>Fertilization</th>
<th>≤50%</th>
<th>&gt;50%</th>
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<tr>
<td>No. (%) who were positive for ASA</td>
<td>4 (25)</td>
<td>12</td>
</tr>
<tr>
<td>No. (%) who were negative for ASA</td>
<td>31 (30)</td>
<td>72</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>84</td>
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</table>

Note: The proportions of couples who had ≤50% fertilization were similar in ASA-positive and in ASA-negative groups ($P = 0.78$). ASA = antisperm antibodies.
Statistically significant differences in these groups with respect to the number of oocytes retrieved (Table 3). The only statistically significant difference (P<0.05) between the non- and the low-fertilization groups was for sperm concentration (Table 3).

### DISCUSSION

We did not find antisperm antibody testing to be helpful in predicting low or complete fertilization failure in our study population; indeed, the sensitivity was 11%, and the positive predictive value was 25%. Overall, 21 of the 251 couples experienced complete failure, and although sperm concentration, motility, morphology, and straight-line velocity were all lower in the no-fertilization males, only concentration reached statistical significance.

In the past, various studies have looked specifically at the relationship between antisperm antibodies and IVF fertilization rates. Both prospective and retrospective studies have shown clearly that antisperm antibodies from female sera can inhibit human IVF when maternal serum was used in culture (16, 17). Chang et al. (18) found that antisperm antibodies in female sera had no adverse effect on the outcome of IVF-ET when maternal serum was not used in the culture.

These findings have prompted most assisted reproductive technology programs to screen maternal serum for antisperm antibodies. Mathur and co-workers (19) found that IVF results were not affected when antisperm antibodies were present in the male partners exclusively. However, other investigators have suggested that fertilization inhibition may be caused by a synergistic effect of IgG and IgA class antibodies in seminal plasma (7, 16). In our study population, however, there were six men who had either IgG and IgA or all three classes of immunoglobulins, yet only two of these patients had <50% fertilization.

In our study population, 13.4% (16 of 119) of men tested for antisperm antibodies were found to be positive. These findings are similar to those of Hendry and associates (20), who found a positive antisperm antibody rate of 8% in men who attended an infertility clinic and who had no prior history of surgery. Two men in our group had identifiable risk factors for antisperm antibodies. One of these men had undergone reversal of a vasectomy, and the other gave a history of testicular torsion. It should be noted, however, that testicular torsion has not been definitively linked to autoimmunization (21).

Several studies have evaluated patients with total failure of fertilization. In our study population, 8.4% of couples (21 of 251) experienced fertilization failure. In 1990, Barlow et al. (22) reported a fertilization failure rate of 16% (95 of 587). They attributed most of these cases to sperm defects. Another retrospective analysis by Bedford and Kim (23) reported a 6.2% fertilization failure rate (25 of 402). On the basis of patterns of sperm attachment to and penetration of the zona, the researchers concluded that many cases of fertilization failure may be attributed to an egg defect.

Specifically, these investigators hypothesized that a resistance to the passage of spermatozoa past the inner portion of the zona pellucida may exist in some patients. In our population, 14 of the 21 couples with fertilization failure were tested, based on the criteria described in the previous section, for antisperm antibodies. If the remaining patients who failed fertilization had been tested, a different trend in predictive value of the test may have been found.

In this institution, the goal of antisperm antibody testing was to help predict poor fertilization. If an IVF program tests selected couples for antisperm antibodies and then uses intracytoplasmic sperm injection (ICSI) for those who test positive, our results would indicate that total charges (based on charges above the basic [8,500] fee in our practice) for preventing the three fertilization failures would be $35,205 (119 couples tested for antisperm antibodies at $195 each + 16 couples who were antisperm antibody positive who received ICSI at $750 each), and that assumes ICSI is 100% effective. Thus, it would cost $11,735 ($35,205 divided by three fertilization failures) per fertilization failure salvaged in cycle one versus only $9,250 ($8,500 + $750) to use the

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### TABLE 3

<table>
<thead>
<tr>
<th>Fertilization group</th>
<th>Sperm concentration (10⁶/mL)</th>
<th>Sperm motility (%)</th>
<th>Sperm morphology (% normal)</th>
<th>No. with ≥15% ASA</th>
<th>VSL</th>
<th>No. of oocytes retrieved</th>
</tr>
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<tbody>
<tr>
<td>0 fertilization (n = 21)</td>
<td>34.7</td>
<td>38.9</td>
<td>21.9</td>
<td>3 (21%)*</td>
<td>34.8</td>
<td>9.6</td>
</tr>
<tr>
<td>1%–50% fertilization (n = 43)</td>
<td>66.9</td>
<td>46.0</td>
<td>24.6</td>
<td>1 (5%)*</td>
<td>37.4</td>
<td>12.9</td>
</tr>
<tr>
<td>&gt;50% fertilization (n = 187)</td>
<td>91.3</td>
<td>54.0</td>
<td>36.8</td>
<td>12 (14%)*</td>
<td>46.0</td>
<td>13.4</td>
</tr>
</tbody>
</table>

P value

All groups | <0.0001 | 0.0008 | <0.0001 | 0.394 | <0.0001 | 0.10 | 0.10 | 0.35 | 0.07 |

0 versus 1%–50% | 0.003 | 0.17 | 0.15 | 0.10 | 0.35 | 0.07 |

Note: ASA = antisperm antibodies; VSL = straight-line velocity. Semen analysis data refers to prewashed specimen results.

* Antisperm antibody testing was done in 14, 21, and 84 patients in the 0, 1%–50%, and ≥50% groups, respectively.
ICSI strategy on all oocytes during a second IVF cycle for each couple who failed initially.

Perhaps a better strategy would be to eliminate the anti-sperm antibody testing and to perform second-day ICSI on any set of oocytes that do not fertilize. However, the success of second-day ICSI in general and specifically for this indication is under investigation.

In conclusion, we were able to identify 16 patients with antisperm antibodies (13.4%), and four of them had fertilization rates of <50%. Overall, of the 21 couples who experienced fertilization failure, 14 had been tested for antisperm antibodies, and 3 were positive. In this practice setting, antisperm antibody testing has low sensitivity for predicting successful fertilization and continued development and does not appear to be cost-effective when ordered as part of a standard IVF workup.

This finding should not be confused with the ability of the test to predict fertilization failure because the number of antisperm antibody–positive men in our study was small and because not all of the men in the study population were tested. Perhaps further investigation with larger numbers of patients with antisperm antibodies will reveal a subset of patients for whom this testing proves beneficial.

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References