FERTILITY AND STERILITY®
VOL. 69, NO. 5, MAY 1998

Published by Elsevier Science Inc.
Printed on acid-free paper in U.S.A.

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

Patrick J. Culligan, M.D.,† Martin M. Crane, Ph.D.,‡ William R. Boone, Ph.D.,* Thomas C. Allen, Ph.D.,§ Thomas M. Price, M.D.,* and Keith L. Blauer, M.D., M.B.A.*‖*

Greenville Hospital System, Greenville and North Greenville College, Tigerville, South Carolina

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.

Patient(s): Male partners of 251 couples undergoing IVF between 1992 and 1997.

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. ©1998 by American Society for Reproductive Medicine.)

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

Received August 6, 1997; revised and accepted November 24, 1997.

Presented at the 3rd Annual C. M. Easley Symposium, Greenville, South Carolina, May 16–17, 1997.

Reprint requests: William R. Boone, Ph.D., Reproductive Endocrinology and Infertility, 890 West Faris Road, Suite 470 Greenville, South Carolina 29605 (FAX: 864-455-8492).

* Department of Obstetrics and Gynecology, Greenville Hospital System.

† Present address: Division of Urogynecology, Evanston Continence Center, Northwestern University, Evanston, Illinois.

‡ Department of Research, Greenville Hospital System.

§ Department of Biology, North Greenville College, Tigerville, South Carolina.

‖ Present address: Genetics and IVF Institute, Fairfax, Virginia.

0015-0282/98/$19.00
PII S0015-0282(98)00034-X

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 100×10^6 motile sperm/mL.

Ten microliters of each washed immunobead suspension was incubated for 10 minutes (room temperature) on a slide with 10-μL 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 agglutination, 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 χ^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%).

TABLE 1

Fertilization and cleavage rates for 16 couples with positive antisperm antibody titers.

Patient no.*	IgA titer	IgG titer	IgM titer	Age of female (y)	No. of oocytes	Oocytes fertilized (%)	No. of zygotes	Zygotes fertilized (%)
1	4	15	3	38	11	91	3	30
52	1	15	3	29	17	0	0	0
61	1	22	2	39	2	100	2	100
91	13	$35^{N,T}$	15	37	9	78	6	86
130	13	15	13	35	4	100	4	100
143	$31^{H,T}$	20^{H}	9	39	0	0	0	0
148	12	$100^{H,N,T}$	2	34	14	79	10	91
156	28^{T}	$50^{H,T}$	16^{T}	33	12	92	5	45
181	40^{T}	$43^{N,T}$	6	29	7	86	6	100
190	32^{H}	13	3	36	3	0	0	0
210	15	12	3	36	3	100	3	100
222	12	17	10	26	12	83	9	90
230	21	10	11	31	14	79	9	82
231	20	32^{H}	15	36	6	83	5	100
291	$86^{H,N,T}$	$94^{H,N,T}$	16	28	4	50	2	100
308	17	18^{H}	4	42	5	20	1	100

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.

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 2

Fertilization rates for couples with and without positive antisperm antibodies.

	Fertilization	
Group	≤50%	>50%
No. (%) who were positive for ASA	4 (25)	12
No. (%) who were negative for ASA	31 (30)	72
Total	35	84

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.

TABLE 3

Semen parameters and fertilization rates for couples undergoing IVF.

Fertilization group	Sperm concentration (10^6/mL)	Sperm motility (%)	Sperm morphology (% normal)	No. with ≥15% ASA	VSL	No. of oocytes retrieved
0 fertilization (n = 21)	34.7	38.9	21.9	3 (21%)*	34.8	9.6
1%–50% fertilization (n = 43)	66.9	46.0	24.6	1 (5%)*	37.4	12.9
>50% fertilization (n = 187)	91.3	54.0	36.8	12 (14%)*	46.0	13.4
P value						
All groups	<0.0001	0.0008	<0.0001	0.394	<0.0001	0.10
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.

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 no- 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

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 antisperm 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.

Acknowledgment: The authors thank Nancy D. Taylor, Ph.D., for editorial assistance.

References

1. Jequier AM, Crich JP. Tests for anti-sperm antibodies. In: Jequier AM, Crich JP, editors. Semen analysis: a practical guide. London: Blackwell Scientific Publications, 1986:98–121.
2. Rümke PH, Hellinga G. Autoantibodies against spermatozoa in sterile men. Am J Clin Pathol 1959;32:357–63.
3. ACOG Technical Bulletin 142. Male infertility. Washington DC: ACOG, June 1990:1–80.
4. McLaren A. Immunological control of fertility in female mice. Nature 1964;201:582–5.
5. Edwards RG, Ferguson LC, Coombs RRA. Blood group antigens on human spermatozoa. J Reprod Fertil 1964;7:153–61.
6. De Almeida M, Gazagne I, Jeulin C, Herry M, Belaisch-Allart J, Frydman R, et al. In-vitro processing of sperm with autoantibodies and in-vitro fertilization results. Hum Reprod 1989;4:49–53.
7. Junk SM, Matson PL, Yovich JM, Bootsma B. The fertilization of human oocytes by spermatozoa from men with antispermatozoal antibodies in semen. J In Vitro Fert Embryo Transfer 1986;3:350–2.
8. Bronson RA, Cooper GW, Rosenfeld DL. Sperm-specific isoantibodies and autoantibodies inhibit the binding of human sperm to the human zona pellucida. Fertil Steril 1982;38:724–9.
9. Boone WR, Johnson JE, Price TM, Bowers BK, Morris MD, Crane MM, et al. In vitro fertilization and embryo transfer (IVF-ET) comes to the Upstate. J SC Med Assn 1994;90:360–6.
10. World Health Organization. Laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 3rd ed. Cambridge: Cambridge University Press, 1992.
11. Johnson JE, Boone WR, Blackhurst DW. Manual versus computer-automated semen analyses. Part I. Comparison of counting chambers. Fertil Steril 1996;65:150–5.
12. Jones HW Jr, Jones GS, Hodgen GD, Rosenwaks Z, editors. In vitro fertilization: Norfolk. Baltimore: Williams & Wilkins, 1986.
13. Veeck LL. Atlas of the human oocyte and early conceptus. Vol. 2. Baltimore: Williams & Wilkins, 1991.
14. Talansky BE. Fertilization and early embryonic development in the human. In: Cohen J, Malter HE, Talansky BE, Grifo J, editors. Micromanipulation of human gametes and embryos. New York: Raven Press, 1992:84–112.
15. Catt J, O'Neill C. Manipulation of sperm before intracytoplasmic sperm injection improves fertilization rates. Fertil Steril 1995;64:1210–2.
16. Clarke GN, Hyne RV, du Plessis Y, Johnston WIH. Sperm antibodies and human in vitro fertilization. Fertil Steril 1988;49:1018–25.
17. Mandelbaum SL, Diamond MP, DeCherney AH. Relationship of antisperm antibodies to oocyte fertilization in in vitro fertilization-embryo transfer. Fertil Steril 1987;47:644–51.
18. Chang T-H, Jih MH, Wu T-CJ. Relationship of sperm antibodies in women and men to human in vitro fertilization, cleavage, and pregnancy rate. Am J Reprod Immunol 1993;30:108–12.
19. Mathur S, Mathur RS, Holtz GL, Tsai CC, Rust PF, Williamson HO. Cytotoxic sperm antibodies and in vitro fertilization of mature oocytes: a preliminary report. J In Vitro Fert Embryo Transfer 1987;4:177–80.
20. Hendry WF, Morgan H, Stedronska J. The clinical significance of antisperm antibodies in male subfertility. Br J Urol 1977;49:757–62.
21. Fraser I, Slater N, Tate C, Smart JG. Testicular torsion does not cause autoimmunization in man. Br J Surg 1985;72:237–8.
22. Barlow P, Englert Y, Puissant F, Lejeune B, Delvigne A, Van Ryssel-berge M, et al. Fertilization failure in IVF: why and what next? Hum Reprod 1990;5:451–6.
23. Bedfore JM, Kim HH. Sperm/egg binding patterns and oocyte cytology in retrospective analysis of fertilization failure in vitro. Hum Reprod 1993;8:453–63.