

## Male immunologic infertility: sperm performance on in vitro fertilization

Mónica H. Vazquez-Levin, Ph.D.\*†

Judith A. Notrica, M.Sc.‡

Ester Polak de Fried, M.D.‡

*Centro de Salud Reproductiva CER, and the Instituto de Biología y Medicina Experimental, Buenos Aires, Argentina*

**Objective:** To analyze sperm performance in a group of patients with male immunologic infertility treated with IVF-ET.

**Design:** Retrospective clinical study.

**Setting:** Patients attending a private IVF clinic.

**Patient(s):** The study group comprised seven men with significant levels of surface-bound antisperm antibodies treated in nine IVF cycles. The control group comprised nine couples with female tubal infertility and no indication of male factor infertility treated on the same cycle.

**Intervention(s):** None.

**Main Outcome Measure(s):** Fertilization rate, early embryonic development, implantation, and clinical pregnancy rate (PR).

**Result(s):** Forty-six (44.2%) of 104 inseminated oocytes were fertilized in the study group compared with 65 (84.4%) of 77 in the control group, which was a significant difference. Surface-bound antisperm antibodies significantly inhibited early embryonic cleavage in the study group (13 [28.3%] of 46 embryos with at least 3 blastomeres) compared with the control group (41 [63.1%] of 65 embryos, with at least 3 blastomeres). The percentage of good-quality embryos (grades 1 and 2) was similar in the study and control groups (71.7% and 78.5%, respectively). The percentage of poor-quality embryos (grade 4 and two pronuclei) was higher in the study group compared with the control group (13.9% versus 9.2%, respectively); however, the difference was not significant. The implantation rate and clinical PR were lower in the study group (3% and 11%, respectively) compared with the control group (9.5% and 44%, respectively), but the difference was not statistically significant.

**Conclusion(s):** The fertilization rate and early embryonic cleavage of human embryos was found to be reduced significantly in patients with high levels of surface-bound antisperm antibodies. Moreover, embryonic quality and the PR may be compromised by the presence of significant levels of surface-bound antisperm antibodies. (Fertil Steril® 1997;68:675–81. © 1997 by American Society for Reproductive Medicine.)

**Key Words:** Immunologic infertility, antisperm antibodies, in vitro fertilization, early embryonic development, human embryos, pregnancy rate

Antisperm antibodies are detected in men suspected of infertility in 8% to 21% of the cases (1, 2). In vitro fertilization-ET has been recommended as

an effective procedure for achieving fertilization in couples with male autoimmune infertility (3–5). Although this procedure has been found to bypass the inhibitory effect of high levels of antisperm antibodies on sperm motility, fertilization rates are reduced in approximately 40% of the attempts (3, 5).

It has been postulated that surface-bound antisperm antibodies may interfere by impairing forward progression motility and by inhibiting fertiliza-

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Received January 8, 1997; revised and accepted May 22, 1997.  
Supported by the Consejo Nacional de Investigaciones Científicas y Técnicas from Argentina.

Presented at the 51st Annual Meeting of the American Society for Reproductive Medicine, Seattle, Washington, October 7 to 12, 1995.

\* Reprint requests: Mónica H. Vazquez-Levin, Ph.D., Instituto de Biología y Medicina Experimental, Vuelta de Obligado 2490, 1428, Buenos Aires, Argentina (FAX: 54-1-375-4822; e-mail: VAZQUEZ@IBYME.EDU.AR).

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† Instituto de Biología y Medicina Experimental.

‡ Centro de Salud Reproductiva CER.

tion either by reducing sperm binding to and penetration of the oocyte zona pellucida (ZP) or by inhibiting the acrosome reaction and/or sperm fusion to the egg (6–9). Moreover, surface-bound antisperm antibodies also may interfere with early embryonic development, as evidenced by a report showing abnormal embryo cleavage in a patient with sperm-specific antisperm autoantibodies localized on the acrosomal region of the sperm head and on the sperm tail (10).

The present study was performed to analyze retrospectively sperm performance in a group of patients with male immunologic infertility undergoing IVF, by means of fertilization rate, early embryonic development, and pregnancy outcome.

## MATERIALS AND METHODS

### Patients

Of 111 couples treated with IVF, 10 of the male partners (9.01%) were found to have significant levels of surface-bound antisperm antibodies (i.e., at least 20% of the sperm were swimming with adhered particles between the clumps of erythrocytes; see later). Seven of these men were treated in nine IVF cycles (the study group, patients 1 to 9). The remaining 3 men were not included in the study.

In all cases, the female partners had tubal infertility and no other apparent reproductive dysfunction. In vitro fertilization-ET was carried out using a standard procedure. The results were compared with those of a control group of 9 patients (controls 1 to 9) with tubal infertility in the female partners and no indication of male factor infertility who were treated on the same cycle (using the same batch of medium and supplements and similar gamete culture conditions).

### Semen Analysis and Evaluation of Surface-bound Antisperm Antibodies

Semen analysis was performed according to the World Health Organization (WHO) guidelines (11). Routine analysis included evaluation of the physical characteristics of the semen; the volume of the semen; and the concentration, motility, and morphology of the sperm. The percentage of normal forms in the semen samples was determined after sample staining using the Papanicolaou stain modified for sperm and was evaluated using the strict criteria previously described by Kruger et al. (12). In our laboratory, the cutoff value predictive of success in IVF has been determined to be 10% instead of 14% (data not shown).

The presence of surface-bound antisperm antibodies was evaluated on all fresh semen specimens us-

ing the mixed antiglobulin reaction (MAR) test, according to WHO guidelines. Briefly, 10  $\mu\text{L}$  of fresh semen and 10  $\mu\text{L}$  of immunoglobulin G (IgG)-coated erythrocytes were mixed with 10  $\mu\text{L}$  of anti-IgG antibody (Coatgen; Organon Corp., Durham, NC). The reaction observed under the microscope was considered to be positive if at least 20% of the sperm were swimming with adhered particles between the clumps of erythrocytes.

### In Vitro Fertilization-ET Procedure

Controlled superovulation was initiated with the LH-releasing hormone analogue leuprolide acetate (LA, Lupron; Abbott Laboratories, Buenos Aires, Argentina) starting in the middle of the luteal phase of the previous cycle (1 mg/d SC) and continuing until the day of hCG (Profasi; Serono Laboratories, Buenos Aires, Argentina) administration. Simultaneously, patients received FSH (Metrodin; Serono Laboratories) at a dosage of 150 IU/d, from day 2 to day 5 of the cycle, and hMG at a dosage of 150 IU/d from day 2 of the cycle until the day of hCG administration.

On the day of oocyte retrieval, semen samples were collected by masturbation into sterile plastic jars. After liquefaction, semen volume, sperm concentration, and sperm motility were evaluated. In all cases, a fraction enriched in motile sperm was obtained by performing a three-step Percoll (Pharmacia, Uppsala, Sweden) gradient using a standard procedure. Briefly, the semen was layered over a gradient of 50%, 70%, and 95% Percoll and, after 30 minutes of centrifugation at  $300 \times g$ , the 95% layer containing the highly motile fraction was removed and washed twice by centrifugation for 10 minutes at  $600 \times g$  with culture medium. The final pellet was resuspended in culture medium, adjusted to a final concentration of  $5 \times 10^6$  motile sperm and used for oocyte insemination. Sperm quality after overnight incubation in the IVF medium was evaluated in all samples, by scoring sperm viability and motility.

Follicular transvaginal aspiration was performed 36 hours after hCG administration. After retrieval, the oocytes were cultured and, 4 hours later, they were inseminated with 150 to  $180 \times 10^3$  motile sperm from the enriched motile fraction. Oocytes and sperm were cultured at  $37^\circ\text{C}$  (5%  $\text{CO}_2$  in air) in human tubal fluid (HTF) media (Irvine Scientific, Santa Ana, CA) supplemented with 0.5% human serum albumin.

After 16 to 20 hours of incubation, the remaining corona cells were removed from each oocyte, and the presence of polar bodies and pronuclei (PN) was evaluated to determine oocyte maturity and fertilization. Oocytes with 2PN were considered to be fer-

tilized. Unfertilized oocytes lacking polar bodies were considered to be immature and were excluded from the study. The early embryos were examined 22 hours later for evaluation of early embryonic cleavage and embryo quality.

The fertilization rate was calculated as follows in each group: (number of fertilized oocytes/number of inseminated mature oocytes)  $\times$  100. Early embryonic development was evaluated on day 2 after retrieval, by scoring embryonic cleavage (determining the number of blastomeres in each embryo and calculating the number of embryos with at least 3 blastomeres divided by the total number of embryos found in each group) and embryonic quality (the percentage of high-quality grades 1 and 2 embryos, with respect to the total number of embryos in each group).

In addition, poor embryo quality was defined as the percentage of grade 4 embryos and undivided fertilized eggs (2PN) with respect to the total number of embryos in each group. Embryo grading was carried out according to the criteria previously described by Veeck (13).

Clinical pregnancies were defined by detecting increasing serum hCG levels in at least two determinations 14 days after ET and were confirmed by ultrasound screening of the gestational sac 7 days after the last hCG determination. The pregnancy rate (PR) was calculated as the percentage of clinical pregnancies with respect to the total number of cases in which at least one embryo was transferred. In addition, the implantation rate was determined.

### Statistical Analysis

Differences in semen parameters between groups were evaluated using the Mann-Whitney nonparametric test. Comparative evaluation of fertilization rates, early embryonic development, implantation, and PRs between groups was done using  $\chi^2$  analysis.

## RESULTS

Routine evaluation of semen samples was performed in all cases by determining semen volume, sperm concentration, sperm motility, and sperm morphology (Table 1). All patients but one (patient 8) from the study group were found to have a normal sperm concentration. Patient 8 corresponds to the second cycle done on patient 5, who had a normal to low sperm concentration ( $20 \times 10^6/\text{mL}$ ) in the first cycle. The sperm concentrations of the two groups were not significantly different (Mann-Whitney nonparametric test). The percentage of motile sperm was slightly lower in the study group compared with the control group ( $P < 0.1$ ; Mann-Whitney nonparametric test). Sperm morphology was not significantly

different between the two groups (Mann-Whitney nonparametric test) and ranged from 12% to 39% normal forms for patients with surface-bound anti-sperm antibodies, and from 12% to 38% normal forms for control patients.

In all cases, good sperm recovery and  $>90\%$  sperm motility was obtained after subjecting semen samples to the Percoll gradient procedure. Moreover, sperm motility was good in all cases but one (not determined) after overnight culture under IVF conditions (Table 1).

The percentage of sperm with adhered particles ranged from 65% to 100%, except in patient 7, in whom it was 31% (Table 1). The presence of surface-bound antisperm antibodies was associated with a significant decrease in the ability of the sperm to fertilize the oocytes under standard IVF conditions. As shown in Figure 1, of a total of 104 inseminated mature oocytes, only 46 were fertilized in the study group, for a fertilization rate of 44.2% (range, 14%–92%). These results were significantly lower compared with those of the control group, in which 65 of 77 oocytes were fertilized, for a fertilization rate of 84.4% (range, 60% to 100%;  $P < 0.0001$ ). The fertilization rates were not associated with either the levels or the localization of antisperm antibodies (data not shown).

In addition to their inhibitory effect on fertilization, surface-bound antisperm antibodies appeared to have a detrimental effect on early embryonic development. Embryonic cleavage was reduced significantly in patients with surface-bound antisperm antibodies (Fig. 2). From a total of 46 fertilized oocytes, only 13 embryos (28.3%) were found to have at least three blastomeres at the time of transfer. In contrast, 41 (63.1%) of 65 embryos in the control group had at least three blastomeres ( $P < 0.0001$ ).

Embryonic quality was evaluated by analyzing the proportion of good- and poor-quality embryos. With regard to good-quality embryos, the study group showed a slight decrease in the proportion of grades 1 and 2 embryos (33 of 46, 71.7%) compared with the control group (51 of 65, 78.5%); however, the difference was not statistically significant ( $P = 0.556$ ) (Fig. 3, top panel). With regard to the presence of poor-quality embryos, significant levels of surface-bound antisperm antibodies were associated with an increase in the proportion of grade 4 and 2PN embryos (15.2%) compared with the control group (9.2%) (Fig. 3, bottom panel). However, the difference did not reach statistical significance ( $P = 0.505$ ).

Finally, the effect of surface-bound antisperm antibodies on the ability to develop a clinical pregnancy was evaluated. An average of 3.6 embryos per patient were transferred in the study group, compared

**Table 1** Semen Parameters, MAR Test Results, Sperm Survival, and Fertilization Rate in Patients From the Study and Control Groups

	Semen volume (mL)	Sperm concentration ( $\times 10^6/\text{mL}$ )	Sperm motility (%)	Sperm morphology <sup>†</sup> (%)	MAR test <sup>‡</sup>	Sperm survival <sup>§</sup>	Fertilization rate <sup>  </sup> (%)
<b>Study group</b>							
Patient 1	1.3	150.0	60	39	100	+++	67
Patient 2	2.8	33.6	60	32	90	++	57
Patient 3	1.5	90.0	70	32	80	+++	43
Patient 4	4.0	52.0	55	14	95	+++	14
Patient 5	5.0	20.0	65	12	100	++	23
Patient 6	1.3	95.0	70	25	65	+++	63
Patient 7	4.0	55.0	70	28	31	+++	20
Patient 8¶	3.0	9.0	55	16	100	++	41
Patient 9**	1.5	80.0	65	32	90	+++	93
<b>Control group</b>							
Control 1	4.3	87.0	65	33	Negative	+++	60
Control 2	1.8	56.0	70	16	Negative	+++	100
Control 3	4.4	40.0	75	38	Negative	+++	64
Control 4	3.0	4.5	75	29	Negative	+++	100
Control 5	4.0	102.0	60	12	Negative	+++	100
Control 6	4.2	96.0	65	24	Negative	+++	100
Control 7	4.2	70.0	60	33	Negative	+++	100
Control 8	1.7	120.0	75	26	Negative	ND	90
Control 9	2.4	75.0	70	32	Negative	+++	67

Note: Semen volume, sperm concentration, and sperm motility were evaluated according to the WHO guidelines.

<sup>†</sup> The percentage of normal forms in the semen samples was determined as outlined in the text.

<sup>‡</sup> The MAR test was conducted as described in the text. The result was considered to be positive when  $\geq 20\%$  of the sperm were swimming with adhered particles between the clumps of erythrocytes and negative when  $< 20\%$  of the sperm were swimming with adhered particles.

<sup>§</sup> Sperm survival was determined as outlined in the text. +++, very good ( $> 80\%$  motile sperm); ++, good ( $> 60\%$  motile sperm); +, poor (30% to 60% motile sperm); ND, not determined.

<sup>||</sup> The fertilization rate for each patient was calculated as follows: (no. of fertilized oocytes/no. of total inseminated mature oocytes)  $\times 100$ .

¶ Patient 8 corresponds to the second cycle done on patient 5.

\*\* Patient 9 corresponds to the second cycle done on patient 2.

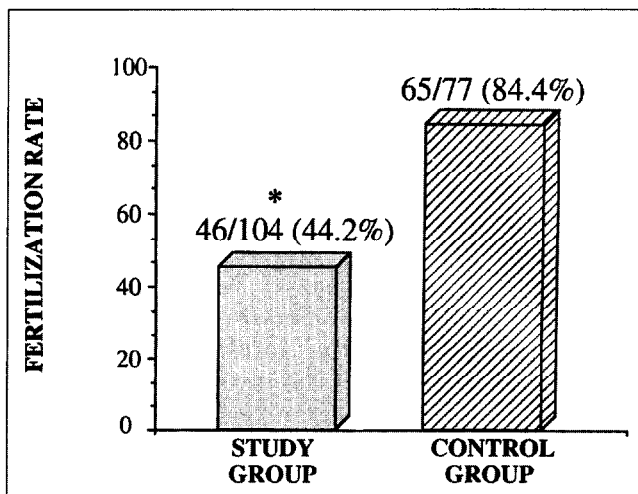
with 4.5 in the control group. An overall decrease in the PR (1 of 9, 11%) as well as the implantation rate (3%) was found in the study group compared with the control group (4 of 9, 44%; and 9.5%, respec-

tively). However, these differences were not statistically significant ( $P = 0.294$  and  $P = 0.388$ , respectively).

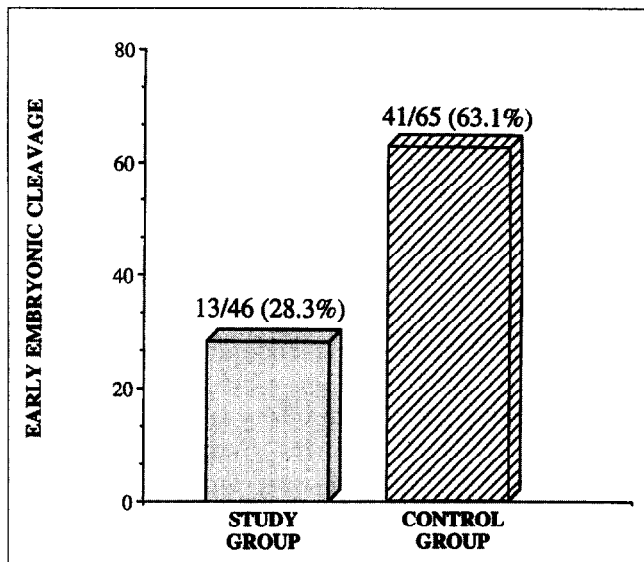
## DISCUSSION

To our knowledge, there are few studies analyzing the effect on the fertilization rate in IVF of different levels of surface-bound antisperm antibodies as measured by the MAR test. Lahteenmaki (14) reported fertilization rates of 42%, 35%, and 17% in patients with weakly positive ( $\geq 10\%$  and  $< 40\%$ ), positive ( $\geq 40\%$  and  $< 90\%$ ), and strongly positive ( $> 90\%$ ) sperm MAR test results, respectively. He emphasized that the group with strongly positive MAR test results was significantly different from the group with positive MAR test results (14). However, the three groups showed a trend toward a decrease in the fertilization rate, when compared with the 62.5% rate reported for the control group.

In a study by Rajah et al. (15), patients with low levels of antisperm antibodies as determined by the MAR test (0% to 20% surface-bound antisperm antibodies) had fertilization rates ranging from 43% to 60%; those with intermediate levels (21% to 80%) had fertilization rates ranging from 0% to 100%, and



**Figure 1** Effect of surface-bound antisperm antibodies on fertilization rate in human IVF. Fertilization rate was calculated in the study and control groups as the no. of fertilized ova/total no. of inseminated ova (%). Differences between groups were evaluated using the  $\chi^2$  analysis. \* $P < 0.0001$ .



**Figure 2** Effect of surface-bound antisperm antibodies on early embryonic cleavage. Early embryonic cleavage was determined in the study and control groups. The values on top of the bars represent the no. of embryos with at least 3 blastomeres/total no. of embryos (%). Differences between groups were evaluated using the  $\chi^2$  analysis ( $P < 0.0001$ ).

those with high levels (>80%) had fertilization rates ranging from 0% to 83%. For most of these patients, the results of the direct immunobead binding test (IBT) revealed no association with the fertilization rate, suggesting that the effect of surface-bound antisperm antibodies may be associated with differences in the localization of the IgGs and their ability to block epitopes involved in the process of sperm-egg interaction.

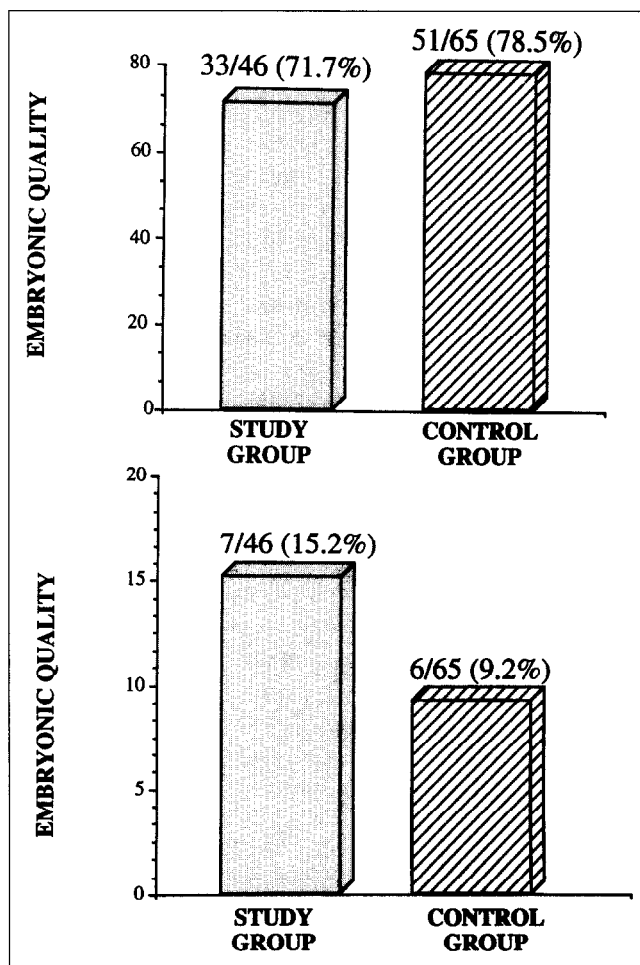
In consequence, we decided to include in the study group all patients with a MAR test result showing >20% sperm with particles adhered to the erythrocytes and to designate as the control group patients with <20% sperm with particles adhered to the erythrocytes.

The results of our study show that high levels of antisperm antibodies on the sperm surface are associated with an overall significant decrease in the ability of the sperm to fertilize human oocytes under standard IVF conditions. This is in agreement with previous reports showing a deleterious effect of surface-bound antisperm antibodies on fertilization rates in standard IVF procedures (14–16). However, the presence of surface-bound antisperm antibodies is not necessarily associated with complete failure of fertilization; the fertilization rate in the study group ranged from 14% to 93%, and was >50% in four cycles.

This wide range in the fertilization rate may be explained by differences in the combination of Igs in the semen sample. Evaluation of surface-bound antisperm antibodies was carried out using the MAR

test, rather than the IBT, which can identify IgG, IgA, and IgM. The MAR test is easy and cost-efficient to perform, but it only measures the contribution of IgG to the surface-bound antisperm antibodies in semen. This represents a limitation of the study, because the possible influence of IgA (and IgM) on the study outcome is unknown.

Several studies have shown high titers of one of the two isotypes to be sufficient to inhibit sperm binding to the ZP (7, 17, 18); however, the presence of both IgA and IgG has been suggested to have a synergistic inhibitory effect on sperm-olemma binding and fertilization in IVF (4, 7). Variability in the fertilization rate also may be associated with Ig localization and blockage of epitopes involved in the interaction, as mentioned previously, rather than the amount of Ig present. Finally, sperm preparation for IVF may have reduced the amount of antibody bound to sperm, as discussed previously (14). Altogether, this may have allowed fertilization to occur



**Figure 3** Effect of surface-bound antisperm antibodies on early embryonic quality. Good (top panel) and poor (bottom panel) early embryonic quality in both the study and control groups. Differences between groups were evaluated using the  $\chi^2$  analysis.

even in those cases with a strongly positive MAR test in semen. This phenomenon has been reported previously by Rajah et al. (15), who described fertilization rates ranging from 0% to 83% in patients with MAR test results showing >80% sperm adhered to the erythrocytes.

In addition to their inhibitory effect on fertilization, surface-bound antisperm antibodies appeared to have a negative effect on the early development of human embryos. Patients in the study group showed an overall significant decrease in the percentage of advanced embryos. These results are in agreement with a report by Naz (10) describing the deleterious effect of surface-bound antisperm antibodies on early embryonic cleavage in a patient with significant levels of these antibodies and a normal fertilization rate.

High levels of surface-bound antisperm antibodies also have been associated with abnormal cleavage of mouse embryos (19). Moreover, Mandelbaum et al. (20) and Vazquez-Levin et al. (21) previously reported the detrimental effect of female sera antisperm antibodies on early embryonic cleavage of human embryos from IVF. Sperm-specific plasma membrane isoantigens have been detected on the egg plasma membrane after fertilization (22); surface-bound antisperm antibodies recognizing these epitopes could impair early embryonic development, cleavage, and possibly implantation.

A slight decrease in the percentage of good-quality embryos and a trend toward an increase in the percentage of poor-quality embryos was found in patients in the study group. A detrimental effect of antisperm antibodies present in female sera upon embryonic quality was previously reported by Vazquez-Levin et al. (21). In addition, Nagy et al. (23) reported a decrease in the proportion of excellent and good-quality embryos, as well as a significantly increased proportion of poor-quality embryos in a group of 37 patients with severe male immunologic infertility who were treated with intracytoplasmic sperm injection (ICSI). Their study suggested that the presence of antisperm antibodies may impair embryonic quality. However, they did not rule out an indirect effect mediated by the altered sperm.

Evaluation of PRs in both groups again showed a trend toward a decrease in implantation and clinical PRs in patients with significant levels of surface-bound antisperm antibodies. These results are in contrast to those of previous studies suggesting that once fertilization has been obtained in patients with male immunologic infertility, PRs do not differ from those of controls (14, 15). However, because of the low number of pregnancies in each group, the data are not conclusive.

Abnormal fertilization and cleavage rates were

not caused by abnormalities in the embryo culture conditions used in the IVF procedure; as indicated previously, the control group was comprised of patients who were treated with the same batch of medium and supplements, and under similar culture conditions. Patients in the control group had a significantly higher fertilization rate, as well as a higher proportion of advanced and good-quality early embryos.

The significant decrease in the fertilization rate and in early embryonic cleavage did not appear to be associated, at least in some cases, with abnormalities in oocyte quality. Four of the seven couples (patients 2, 5, 6, and 7) from the study group participated in the ovum donation program and donated in six of the nine cycles part of their cohort of ova to a total of 10 recipients. The fertilization rate in the recipients ranged from 75% to 100%, with the exception of one patient with male factor infertility caused by abnormalities in sperm morphology, in whom the fertilization rate was 30%. The fertilization rates in the oocyte donors were 20%, 23%, 57%, 63%, and 93%.

The development of early embryos in the oocyte recipient couples was found to be normal; 72% of the embryos had at least three blastomeres at the time of ET, and 87.5% were good-quality embryos (grades 1 and 2). Of the 10 oocyte recipient couples, 5 developed a clinical pregnancy from this oocyte cohort, including the case with a low fertilization rate.

Although the identity of the antigens recognized by the surface-bound antisperm antibodies during fertilization and early embryonic development has not been determined, several studies have been done in an attempt to elucidate the nature of these entities (24). Cleavage signal CS-1 is a protein that appears to be involved in early embryo cleavage; antibodies to CS-1 present in serum from a patient with immunologic infertility inhibited the first cleavage of the human pronuclear stage zygotes (13).

In addition to CS-1, the fertilization antigen FA-1 has been found to have a role in sperm binding to human ZP, and antibodies directed against FA-1 were found to inhibit fertilization and early embryonic development (2); FA-1 is recognized in 78% of sera from patients with immunologic infertility. Finally, a recent report by Browning and Strome (25) first described in *Caenorhabditis elegans* a sperm factor required for embryogenesis. If it were present in humans and had a similar role, this protein could be recognized by specific antibodies and then inhibit the development of early embryos.

A report from Nagy et al. (23) described the use of ICSI in the treatment of male immunologic infertility. Their results suggest that the presence of antisperm antibodies does not interfere with the fertilization process when sperm are microinjected into

the cytoplasm of the egg. In our patient population, patient 7 from the study group, as well as a group of patients with male immunologic infertility who previously had not undergone standard IVF, were treated successfully using IVF in combination with ICSI. These results suggest the benefit of ICSI in patients with male immunologic infertility.

In conclusion, the results of our study show that the presence of antisperm antibodies reduces the ability of the sperm to fertilize the oocyte and in some cases, affects early embryonic development and implantation after treatment with standard IVF.

*Acknowledgments.* The authors thank Liliana Blanco, M.D., from the clinical staff, and Alejandra Piazza, M.S., and Mercedes Gomez, M.S., from the laboratory staff, for performing the laboratory IVF procedures.

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