

# The Effect of Sera Antisperm Antibodies in the Female Partner on In Vivo and In Vitro Pregnancy and Spontaneous Abortion Rates

JEROME H. CHECK, DIANE KATSOFF, ANIELA BOLLENDORF, AND CARRIE CALLAN

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**PROBLEM:** To determine the incidence of antisperm antibodies (ASA) in female sera from infertile couples or those suffering from recurrent abortions. Also to determine if the pregnancy and/or abortion rates are any higher in those positive versus those negative for ASA. **METHOD:** All registered patients had sera drawn and ASA measured by indirect immunobead test on initial study. Pregnancy and abortion rates were determined for patients undergoing in vivo or in vitro therapy.

**RESULTS:** There was a low incidence of ASA in patients having in vivo or in vitro treatment. There was no decrease in pregnancy rates (PRs) or increase in spontaneous abortions (SAB) in those positive for ASA.

**CONCLUSION:** Antisperm antibodies in female sera do not seem to be etiologic in causing infertility or SAB. Future studies might consider changing the antigen source from donor sperm to husband's sperm.

## Key words:

Sera immunoglobulins, immunobead test, fecundity, miscarriage

JEROME H. CHECK  
DIANE KATSOFF  
ANIELA BOLLENDORF  
CARRIE CALLAN

The University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School at Camden, Cooper Hospital/ University Medical Center, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility, Camden, New Jersey.

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Address reprint requests to Jerome H. Check, M.D., 7447 Old York Road, Melrose Park, PA 19027.

## INTRODUCTION

Sera antisperm antibodies (ASA) have been associated with infertility.<sup>1</sup> Furthermore, several reports have suggested a relationship of ASA and fetal wastage. Some early studies reported that approximately 50% of women with a history of early recurrent spontaneous abortion (SAB) were positive for circulating sperm agglutinating or sperm-immobilizing antibodies.<sup>2</sup> Another study found that 35.9% of women with "immunologic causes" of recurrent abortions and 24.8% of women without such causes were found to have ASA in one of four tests used.<sup>3</sup> However, many of the positive results were from an ELISA assay which can detect antibodies to internal sperm antigens which may not be clinically important.

Another study found an association between SABs and circulating IgG antibodies in female sera to the male partners' (not donor) sperm.<sup>4</sup> These latter authors hypothesized that the ASA may be a marker for defective immunosuppression in women with recurrent SAB or, alternatively, exposure

of the sperm-sensitized pregnant women may activate the maternal immune system to respond to paternal antigens present on the embryo.<sup>4</sup>

The study presented herein evaluated the importance of sera ASA in the female partner in a large series of consecutive patients seeking help for infertility rather than for habitual abortion. Both pregnancy rates (PRs) and SAB rates were then prospectively compared in women whose sera tested positive for ASA (immunobead test [IBT]  $\geq 50\%$ ) with the PRs and SAB rates in those who were negative.

## MATERIALS AND METHODS

The study evaluated 2,363 consecutive patients presenting to the private practice clinic of the Division of Reproductive Endocrinology and Infertility of the Robert Wood Johnson Medical School at Camden, Cooper Hospital/University Medical Center in New Jersey. Those patients who upon initial assessment required in vitro fertilization-embryo transfer (IVF-ET) were assigned to the Cooper Institute for In Vitro Fertilization. A total of 878 were assigned to IVF-ET so the final totals for the study were 1,485 patients attempting conception through in vivo techniques and 878 by IVF. If a patient initially was treated through in vivo techniques and failed to conceive and was now recommended IVF-ET, only their in vivo studies were evaluated in the study. The number of pregnancies and the number of cycles needed to achieve the pregnancies were compared in those patients with and without ASA. The maximum number of cycles evaluated was six, but many had fewer.

The in vivo patients were treated for a variety of conditions, e.g., anovulation, luteal phase defects (LPD), male factor, cervical factor, and endometriosis by a variety of methodologies, but those positive for ASA were not treated any differently from those negative for antibodies.

Each serum sample obtained during their initial visit was evaluated for ASA using the indirect IBT.<sup>5,6</sup> The serum was heat-inactivated at 56°C for 20 min, then mixed with donor sperm negative for ASAs and then incubated for 30 min at 37°C. The donor sperm was washed three times with 0.5% BSA/BWW. The washed sperm was then mixed with IgG beads on one slide and IgA beads on the other slide. Sperm with  $\geq 50\%$  bead binding were considered positive, 20–49% binding weakly positive, and  $<20\%$  negative.

Chi-square analysis was used to compare the PRs by presence of sera antibodies within the in vivo and

in vitro groups. A t-test for independent group was used to compare the mean fertilization rates in the in vitro group by presence of sera antibodies. All tests were done using a *P* value of .05 as indicating a significant difference.

## RESULTS

The frequency of positive ASA in the in vivo group was 112 of 1485 (7.5%) and was 53 of 878 (6.0%) in the in vitro group.

The clinical PR for in vivo patients (counting a patient as pregnant only if there was ultrasound evidence of pregnancy) was 31 of 112 (27.7%) of women with positive antibodies in 3.2 cycles versus 380 of 1,373 (27.6%) in 4.4 cycles in those negative for ASA. Spontaneous abortions occurred in 5 of 31 (16.1%) with positive ASA versus 56 of 380 (14.7%) in those patients who were negative for ASA. If chemical pregnancies are included (beta human chorionic gonadotropin [hCG] level  $> 100$  mIU/mL but no gestational sac seen by sonography), then there were 40 pregnancies (35.7%) in those positive versus 463 pregnancies (35.0%) in those negative for antibodies, and the SAB data found 14 (35.0%) and 139 (30.0%) in those groups, respectively.

There were 13 pregnancies in the 53 (24.7%) IVF patients with positive ASA. Since they were treated for 71 cycles, the clinical PR per cycle was 18.3%; there were two SAB (15.4%) and the viable PR was (15.5%) (per induction cycle). There were 162 pregnancies in the 825 (18.6%) patients negative for antibodies in 1,131 cycles (14.2% PR per induction cycle); this group had 31 SAB (19.2%) and the viable PR was 11.6% per stimulation cycle.

The implantation rate for those with positive antibodies was 4.6% (8 of 175) versus 4.5% (120 of 2,643) in the women with negative antibodies who went through IVF-ET (chi square, *P* = .960). The mean number of embryos transferred was  $3.1 \pm 1.8$  for those with positive antibodies vs  $2.8 \pm 1.8$  for patients without antibodies (t-test, *P* > .05).

There were no differences found in the PRs by presence of sera antibodies as a result of the chi-square analysis. The mean fertilization rate was  $59.1\% \pm 31.7\%$  for the group with positive ASA as compared to  $48.3\% \pm 31.3\%$  for the group with negative ASA (*P* < .05, t-test).

There were no statistical differences in any parameters comparing antibody negative versus positive females.

## DISCUSSION

These data failed to demonstrate any adverse effect of sera ASA in female partners on subsequent pregnancy or SAB rate either in patients attempting to conceive through in vivo treatment or those undergoing IVF. Though there were some women who also had ASA in the cervical mucus, this problem was obviated by timed intrauterine insemination (IUI).

Antisperm antibodies did not adversely affect IVF PRs either. The Cooper Institute for IVF does not use maternal serum (but rather uses bovine serum albumin) as the protein source in the culture media; other centers using maternal sera positive for ASA may suffer decreased FRS and PRs and should thus be screened for ASA and the culture media adjusted with another protein source if positive.<sup>7</sup>

There have been other studies failing to demonstrate an adverse effect of ASA on subsequent pregnancy or SAB rates. One study failed to find any women with ASA in a group of 34 women with recurrent pregnancy losses including 20 with unexplained losses.<sup>8</sup> Another study using agglutination assays failed to demonstrate an increased abortion rate in ASA positive women.<sup>9</sup> Clarke and Baker measured ASA in 1,203 women presenting for infertility or recurrent abortion; they found a lower incidence of ASA also using the indirect IBT ( $\geq 50\%$  considered positive) in the recurrent aborters (1.4%) versus the infertility patients (7.4%);<sup>10</sup> this prevalence matches very well with our findings.

The data presented herein suggest that sera ASA in the female partner by themselves are not etiologic in causing infertility, as long as techniques to overcome cervical factor are used, e.g., IUI or IVF. When ASAs have been found in sera there is also a likelihood of being present in other body fluids. However, even if so, PRs do not seem to be adversely affected. Perhaps in a subgroup of habitual aborters it may be a marker for other problems that could lead to SAB, e.g., antiphospholipid antibodies.<sup>8</sup>

These data evaluated association of SAB and ASA in a different manner than other studies; rather than determine merely the frequency in recurrent aborters versus non-aborters, which may only provide information that the antibodies could be markers for but not etiologic for SABs, our study compared SAB rates in patients positive for ASA with rates of those who are negative and found no differences. Thus, at least with the use of the IBT, there appears to be little reason to measure sera ASA in infertile women or patients with

recurrent abortions. One exception may be IVF centers using maternal sera for the oocyte culture medium.

The data presented herein do not necessarily refute the results of Witkin and Chaudhry, in which an association of sera ASA in women and SAB was found.<sup>4</sup> They used as the source of sperm the husband's specimen rather than donor sperm typically used in the indirect IBT; furthermore, the former uses sperm prepared by swim-up and the latter uses semen; possibly prepared sperm may facilitate the detection of sperm-coating antigens, whereas seminal plasma might tend to inhibit binding to absorbed sperm coating antigens on the sperm surface. Thus future studies might evaluate female ASA by using only husband's and not donor sperm prepared by swim-up. Mathur et al. also suggested that antibody titers are higher against husband's than donor sperm.<sup>11</sup>

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