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IX

Sera antisperm antibodies (ASA) in female partners does not reduce IVF pregnancy rates (PRs) (1)

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SUMMARY

The study presented herein determined the incidence of antisperm antibodies (ASA) in female sera from infertile couples or those suffering from recurrent abortions. ASA was measured by indirect immunobead test. Pregnancy and spontaneous abortion (SAB) rates were determined for patients undergoing in vivo or in vitro therapy. There was a low incidence of ASA in patients having in vivo or in vitro treatment. There was no decrease in pregnancy rates or increase in spontaneous abortions in those positive for ASA. Thus, ASA 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.

INTRODUCTION

Sera antisperm antibodies (ASA) have been associated with infertility (2). 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 SAB were positive for circulating sperm agglutinating or sperm-immobilizing antibodies (3).

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 (4). 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 (5). 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 (5).

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 prospectively compared in women whose sera tested positive for ASA (immunobead test (IBT) $\geq 50\%$) vs 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. Those patients who, upon initial assessment required in vitro fertilization-embryo transfer (IVF-ET) were assigned to the Cooper Institute for In Vitro Fertilization. 878 patients were assigned to IVF-ET; therefore total numbers evaluated were 1485 patients attempting conception through in vivo techniques and 878 by IVF. If a patient was initially 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 a lot less.

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 than those negative for antibodies.

Each serum sample obtained during their initial visit was evaluated for ASA using the indirect IBT (6,7). 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 groups was used to compare the mean fertilization rates (FRs) 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 AND CONCLUSIONS

The frequency of positive ASA in the in vivo group was 112/1485 (7.5%) and was 53/878 (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 31/112 (27.7%) of women with positive antibodies in 3.2 cycles vs 380/1373 (27.6%) in 4.4 cycles in those negative for ASA. Spontaneous abortions occurred in 5/31 (16.1%) with positive ASA vs 56/380 (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) there were then 40 pregnancies (35.7%) in those positive vs 463 pregnancies (35.0%) in those negative for antibodies and the SAB data found 14 (35.0%) and 139 (30.0%) in those respective groups.

There were 13 pregnancies in the 53 (24.7%) IVF patients with positive ASA. Since they were treated for 71 cycles the clinical PR/cycle was 18.3%; there were two SABs (15.4%), the viable PR was (15.5%) (per induction cycle). There were 162 pregnancies in the 825 (18.6%) patients negative for antibodies in 1131 cycles (14.2% PR/induction cycle); this group had 31 SAB (19.2%), the viable PR was 11.6%/stimulation cycle.

The implantation rate for those with positive antibodies was 4.6% (8/175) vs 4.5% (120/2643) in the women with negative antibodies who went through IVF-ET (chi square, $p=.960$). The mean number of embryos transferred was 3.1 ± 1.8 for those with positive antibodies vs 2.8 ± 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 FR 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.

These data failed to demonstrate any adverse effect of sera ASA in female partners on subsequent pregnancy or SAB rate in patients attempting to conceive through in vivo treatment nor 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 effect 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 FR and PRs and should thus be screened for ASA and the culture media adjusted with another protein source if positive (8).

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 (9). Another study using agglutination assays failed to demonstrate an increased abortion rate in ASA positive women (10). Clarke and Baker measured ASA in 1203 women presenting for infertility or recurrent abortion; they found a lower incidence of ASA using also the indirect IBT ($\geq 50\%$ considered positive) in the recurrent aborters (1.4%) vs the infertility patients (7.4%); (11) 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 (9).

These data evaluated association of SAB and ASA in a different manner than other studies; rather than determine merely the frequency in recurrent aborters vs 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 vs 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 where an association of sera ASA in women and SAB was found (5). 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 whole sperm; possibly prepared sperm may facilitate the detection of sperm-coating antigens, whereas, seminal plasma might tend to inhibit binding to adsorbed 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 (12).

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