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# Determination of the Clinical Significance of Antisperm Antibodies Using an in vivo Insemination Technique

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The immunobead assay has the advantage of being able to directly determine the presence of antisperm antibodies (ASA) on the sperm surface [1, 2]. However, it is a qualitative rather than quantitative test; the test determines the percentage of the sperm coated by ASA but the assay does not provide any idea of how many antibodies are attached to each sperm.

The intrinsic quality of the sperm, motile density, and concentration of the antibody on the sperm surface may all effect the clinical significance of the presence of antibodies as measured by the immunobead assay. In fact, the present cut-off level of 50% that distinguishes normal from subnormal was arbitrarily chosen based on the fact that many normal donors have levels up to that point [3].

A highly effective therapy for cervical factor has been previously described using a dose of ethinyl estradiol that would suppress ovulation but maximally stimulate cervical mucus; follicular maturation would be simultaneously stimulated with human menopausal gonadotropins [4, 5]. It was reasoned that by using the high dose estrogen to maximally stimulate mucus and block ovulation one could determine the likelihood of an immunological problem by the comparison of first male partner's sperm and then donor sperm following therapeutic donor insemination (TDI). Theoretically, demonstration of sperm with progressive forward motion (PFM) after insemination of male partner's sperm (AIH) would suggest

absence of ASA in semen or mucus; failure to show PFM following AIH but showing PFM following TDI would suggest ASA in the male partner; failure to demonstrate PFM following AIH and TDI would suggest ASA in cervical mucus.

A study was thus performed to see the correlation of ASA as detected by direct immunobead test (IBT) on the sperm and by indirect IBT on the mucus with the PFM of sperm following AIH and TDI. Furthermore, in patients where ASA was suspected as being etiologic the couple would try intracervical inseminations for 6 months without hyperstimulation. Those failing to conceive would be offered corticosteroids and the pregnancy rates for the next 6 months were then determined.

Finally, the presence of positive ASA may render the sperm incapable of fertilization without necessarily causing a poor postcoital test. For example, antibodies could prevent sperm attachment to the zona pellucida, therefore to evaluate the pathologic effect of ASA even when the postcoital test was adequate consecutive couples composed of positive and negative patients with good and poor postcoital tests would have their pregnancy rates compared over a 6-month time period.

## *Materials and Methods*

*Study 1: Unexplained Poor Postcoital Tests*

A total of 30 couples were selected where suspicion of a possible immunological etiology for a poor postcoital test was based on demonstration of normal quality mucus with a Moghissi score above 10 [6]. The semen analysis was required to have a minimum volume of 1.5 ml, $20 \times 10^6$ sperm/ml with a minimum of 60% with PFM. Each patient was required to demonstrate at least five sperm/high-powered field but with none demonstrating PFM. Furthermore, no other infertility factors were apparent except for the poor post-coital test.

A direct IBT was performed on all semen specimens using the technique described by Bronson et al. [2]. The percent of IgA and IgG were measured. An indirect immunobead assay was performed on cervical mucus which was heat inactivated to remove complement. Antibody-free donor sperm were employed; collection was obtained by a 'swim-up' technique [7]. An aliquot of antibody-free sperm was then incubated with the cervical mucus to allow for the antibody attachment to the sperm. The direct IBT was performed on these donor sperm specimens that had been exposed to cervical mucus.

High-dose estrogen (ethinyl estradiol, 50 μg daily) is given from day 3 to day 14 of cycle. On day 14 a serum estradiol ($E_2$) is obtained to ensure breakthrough ovulation did not occur (ethinyl estradiol does not cross-react with the 17 Beta $E_2$ assay). The patient is then ready for AIH and assessment of sperm movement in the mucus which is performed at 2 and 8 h. Poor PFM leads to repeat testing the next day after TDI.

*Study 2: Evaluation of Consecutive Patients with a Female Infertility Factor Identified (Other than Cervical Factor) and Corrected – Influence of Antisperm Antibodies in the Male on Postcoital Testing and Pregnancy Rates following Timed Intrauterine Insemination*

Another group, consisting of 60 consecutive patients were evaluated to compare the correlation of postcoital testing with ASA. A good-to-fair postcoital test was considered if at least 3–5 sperm/high-powered field with PFM were seen at least 8 h after intercourse. Pregnancy rates after 6 months were then determined. Those couples with poor postcoital tests were treated with timed intrauterine insemination (IUI) (approximately 36 h from initiation of serum luteinizing hormone (LH) surge or 12 h from peak LH surge; daily LH levels were obtained beginning 10 days prior to expected menses until ovulation was determined).

## *Results*

Three groups were separated based on the response of the female partners to AIH and TDI. Group 1 consisted of 8 couples who demonstrated sperm with PFM following AIH. This group would not be expected to have either ASA in the semen or cervical mucus. Group 2 was composed of 17 couples where no sperm with PFM were seen following AIH but PFM was seen following TDI; this group would be predicted to have the largest percentage of male partners with positive ASA levels. The final 5 patients comprised group 3 where neither AIH nor TDI resulted in sperm with PFM. Cervical mucus ASA would be predicted.

The incidence of positive ASA ($\geq$ 50%) as determined by IBT according to different groups is seen in table 1. Slightly positive was considered between 20 and 49%. The response to high-dose estrogen did in fact predict the groups with the significant ASA levels. Not one of the Group 1 patients demonstrated positive ASA whereas 47% of group 2 males were positive and if slightly positive is counted, the level was 71%. In contrast, not one of the females were positive for ASA. Group 3 was in fact the only one demonstrating ASA in cervical mucus (40%).

The differential response of AIH and TDI to the estrogen stimulated mucus and the presence or absence of ASA determined the type of therapy. Group 1 women were treated with the high dose estrogen-hMG technique using ethinyl estradiol [5] and 4/8 (50%) conceived in 6 months; 2/4 (50%) of the remainders conceived during the next 6 months with the same technique and timed IUI. A total of 3/17 (17.6%) of group 2 females became pregnant following intracervical insemination. These pregnancies were

*Table 1.* Incidence of positive ASA in groups 1–3

| | Total | +IgG | +IgA | +IgG or IgA | Percent +ASA | PCT + ASA IgG or IgA | +ASA and PCT+ |
|---|---|---|---|---|---|---|---|
| *Group 1* | | | | | | | |
| Male | 8 | 0 | 0 | 0 | 0 | 0 | 0 |
| Female | 8 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Group 2* | | | | | | | |
| Male | 17 | 8 | 4 | 8 | 47 | 4 | 12 (70.6%) |
| Female | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Group 3* | | | | | | | |
| Male | 5 | 1 | 0 | 0 | 20 | 1 | 2 (40%) |
| Female | 5 | 2 | 2 | 2 | 40 | 0 | 2 (40%) |

achieved in the female partners of the males without ASA (3/9, 33%). None of the 8 females with partners with positive ASA became pregnant during the first 6 months following intracervical insemination but 6/8 (75%) conceived following male treatment with methylprednisolone 96 mg × 1 week each month during the next 6 months. One of 2 couples with slightly positive ASA in the males treated with corticosteroids also achieved pregnancies during the second 6 months. All couples conceiving following methylprednisolone therapy first demonstrated sperm with PFM in the mucus. One of 5 group 3 females conceived following intracervical insemination during the first 6 months; 1 of 2 having positive ASA in the mucus was treated with corticosteroids during the second 6 months and she conceived.

The correlation of postcoital tests and presence of antisperm antibodies and pregnancy rates (6 months) following timed intrauterine insemination is seen in table 2. One of the original 60 patients dropped out of the study. Only 9% of couples (4/44) with normal postcoital tests were positive for ASA compared to 60% of couples with poor postcoital tests (9/15). Statistical analysis was performed using the Fisher's exact test; $p < 0.001$. There was a higher pregnancy rate over 6 months in the group with normal postcoital tests (84%) compared to those with poor results treated by timed IUI (67%). However the Fisher's exact test did not show statistical differ-

*Table 2.* Correlation of postcoital tests and presence of ASA and pregnancy rates (6 months)

| | Total | Pregnant | | +ASA | | Pregnancy +ASA | | −ASA | Pregnancy −ASA | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | n | % | n | % | n | % | | n | % |
| Good postcoital tests | 44 | 37 | 84 | 4 | 9 | 4 | 100 | 40 | 33 | 83 |
| Poor postcoital tests | 15 | 10 | 67 | 9 | 10 | 5 | 56 | 6 | 5 | 83 |

+ASA refers to a level over 50% employing immunobead test on the spermatozoa.

ence, $p = 0.14$. Considering all patients with positive ASA only 4/13 (30%) had normal postcoital tests compared to 40/46 patients (87%) with negative ASA. Fisher's exact test was significant, $p < 0.001$. The pregnancy rate in the couples with positive ASA in the male partner was 69% (9/13) compared to 83% (38/46) with negative ASA in the male partner but the results were not statistically different (Fisher's exact test $p = 0.24$).

The cumulative probability of pregnancy in couples with positive ASA is seen in table 3a, negative ASA in table 3b, good postcoital tests in table 3c and poor postcoital tests in table 3d.

## *Discussion*

The data from the first study demonstrated that not all patients with unexplained poor postcoital tests have an immunological etiology. The differential response to AIH or TDI in estrogen-stimulated mucus was able to predict fairly well whether ASA is present or not and in which partner. Though only 47% of group 2 men were found positive for ASA the slightly positive levels $\geq 20\%$ but $< 50\%$ may still have contributed so that if positive is considered $\geq 20\%$ then 71% would be positive. Thus, where reliable immunobead assays are not available, a reasonable idea of whether ASA is present may be obtained by this insemination technique. Furthermore, the data showed that the majority of couples with unexplained poor postcoital tests do not necessarily have an immunological basis (only 33%). Although the Moghissi score was over 10 in all cases, it is of interest that in 27% (8/30) of the cases improving the mucus even further with estrogen

*Table 3.* Cumulative probability of pregnancy in couples under different conditions

| Number of cycles | Number of patients | Pregnancies achieved | Lost to follow-up | Patient months of treatment | Pregnancy rate per month | Cumulative probability of pregnancy |
|---|---|---|---|---|---|---|
| *a* *Positive ASA* | | | | | | |
| 1 | 13 | 6 | 0 | 10.00 | 0.60 | 0.60 |
| 2 | 7 | 1 | 0 | 6.50 | 0.15 | 0.66 |
| 3 | 6 | 0 | 0 | 6.00 | 0.00 | 0.66 |
| 4 | 6 | 0 | 0 | 6.00 | 0.00 | 0.66 |
| 5 | 6 | 0 | 0 | 6.00 | 0.00 | 0.66 |
| 6 | 6 | 2 | 0 | 5.00 | 0.40 | 0.80 |
| | | 9 | | | | |
| *b* *Negative ASA* | | | | | | |
| 1 | 46 | 17 | 0 | 37.50 | 0.45 | 0.45 |
| 2 | 29 | 4 | 0 | 27.00 | 0.15 | 0.53 |
| 3 | 25 | 3 | 0 | 23.50 | 0.13 | 0.59 |
| 4 | 22 | 8 | 0 | 18.00 | 0.44 | 0.77 |
| 5 | 14 | 2 | 0 | 13.00 | 0.15 | 0.81 |
| 6 | 12 | 4 | 0 | 10.00 | 0.40 | 0.89 |
| | | 38 | | | | |
| *c* *Good postcoital* | | | | | | |
| 1 | 44 | 17 | 0 | 35.50 | 0.48 | 0.48 |
| 2 | 27 | 3 | 0 | 25.50 | 0.12 | 0.54 |
| 3 | 24 | 2 | 0 | 23.00 | 0.09 | 0.58 |
| 4 | 22 | 7 | 0 | 18.50 | 0.38 | 0.74 |
| 5 | 15 | 2 | 0 | 14.00 | 0.14 | 0.78 |
| 6 | 13 | 6 | 0 | 10.00 | 0.60 | 0.91 |
| | | 37 | | | | |
| *d* *Poor postcoital* | | | | | | |
| 1 | 15 | 6 | 0 | 12.00 | 0.50 | 0.50 |
| 2 | 9 | 2 | 0 | 8.00 | 0.25 | 0.63 |
| 3 | 7 | 1 | 0 | 6.50 | 0.15 | 0.68 |
| 4 | 6 | 1 | 0 | 5.50 | 0.18 | 0.74 |
| 5 | 5 | 0 | 0 | 5.00 | 0.00 | 0.74 |
| 6 | 5 | 0 | 0 | 5.00 | 0.00 | 0.74 |
| | | 10 | | | | |

can result in improved postcoital tests. Thus, if the estrogen alone allows PFM with AIH one can use the ethinyl estradiol-hMG technique to try for pregnancy.

The second study did not require good quality cervical mucus and yet the positive ASA in the male was still present in 60% (9/15) of the cases. The fact that all 4 of the couples with positive ASA in the males with good to fair postcoital tests conceived, does not support the concept that even if sperm progression in the mucus is not impaired, probably some other aspect of the fertilization process is adversely effected. Thus, one should never treat a patient merely on the presence of ASA but must first demonstrate a way that the immunological factor is causing infertility. It is clear, however, that only a minority of patients with good postcoital tests (9%) are positive for ASA whereas a majority (60%) are negative for ASA.

Achievement of good postcoital tests resulted in a better pregnancy rate (84%) than timed IUI by LH surge (67%) but no statistical difference was seen. However, the fact that a reasonable pregnancy rate of 67% over 6 months was accomplished by timed IUI, the latter seems to be a more appropriate therapy than corticosteroid therapy (which resulted in a 75% pregnancy (6/8) rate in the partner of the treated males), since one of the side effects of therapy could be aseptic necrosis of the femoral heads [8, 9].

Many clinicians when treating with IUI also hyperstimulate the patient with hMG, which increases the cost and exposes the patient to a greater risk of multiple gestation and ovarian hyperstimulation syndrome. Perhaps by carefully observing the serum LH levels a good 6-month pregnancy rate can be achieved without the use of hMG.

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