

EFFECT OF ANTISPERM ANTIBODIES ON POSTCOITAL RESULTS AND EFFECT OF INTRAUTERINE INSEMINATION ON PREGNANCY OUTCOME

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The effect of antisperm antibodies (ASA) in males was determined in 59 men using the direct immunobead test (IBT). Postcoital tests were evaluated in couples for whom all female factors appeared to be corrected. Pregnancy rates in 6 months were compared in couples with good postcoital tests vs. those with poor results; the latter group was treated with timed intrauterine insemination (IUI). Thirty-one percent of males with positive ASA ($\geq 50\%$) had normal postcoital tests and all four achieved pregnancies. Fifty-six percent of men with positive ASA and poor postcoital scores achieved pregnancies following IUI therapy of their wives in 6 months; 83% of couples with normal postcoital tests achieved pregnancies as did couples treated with IUI when the postcoital was poor but the male ASA negative.

Key Words: Antisperm antibodies; Intrauterine insemination; Computer-assisted semen analysis.

INTRODUCTION

The study presented herein was aimed to evaluate the efficacy of intrauterine insemination in couples where the postcoital test was poor and antisperm antibodies (ASA) were present on the sperm. Furthermore, the presence of ASA on the sperm was evaluated for their effect on postcoital testing. Comparisons of pregnancy rates were made with similar couples with the apparent exclusive cause of infertility relating to a cervical factor, but where the male partner was ASA-negative. Finally, the pregnancy rates were compared to patients who initially had poor postcoital tests but where some sperm with progressive forward motion was demonstrated after therapy.

The presence of ASA in the male ejaculate is associated with decreased fertility potential [2]. Certainly its effect on inhibiting sperm progression in the cervical mucus, shown by poor postcoital tests, can be expected to result in subfertility [11]. Furthermore, the possibility exists that the ASA might impair fertility in other ways, as, for example, by impairing binding

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to the zona pellucida [6]. Thus, simply bypassing the cervical mucus by performing intrauterine insemination may not be sufficient to allow pregnancy to occur, even when the semen analysis has been interpreted as normal.

Alternatively, the immobilization of ASA-coated spermatozoa may be delayed after sperm contact with the reproductive tract because the antisperm antibody-complement process is not immediately completed and, in fact, the delay may amount to several hours [5]. If the only adverse effect that results from the ASA is sperm immobilization in mucus, then theoretically intrauterine insemination (IUI) performed as close to the time of ovulation as is possible might prove an effective treatment of male infertility related to ASA on the sperm surface [10]. If the oocyte is already in the tube, fertilization may take place because the immobilization of the sperm may be delayed a few hours.

IUI cannot be considered an effective therapy for sperm that have severe motility impairment immediately after ejaculation [17]. Poor motility may be related to an intrinsic sperm defect with the coincidental presence of ASA. However, in other circumstances, the ASA might be etiological because although normally the male's ejaculate should be devoid of complement, injury to the male ejaculatory system may have allowed leakage of complement in from the outside [4, 14].

The present study was conducted to evaluate the efficacy of IUI for couples with poor postcoital tests when ASA was present in the ejaculate of the male partner. Further evaluation was performed to see whether the presence of ASA in the male ejaculate impairs fertility even when the postcoital test demonstrated sperm with progressive forward motion (PFM).

MATERIALS AND METHODS

General Design. The study consisted of new couples registering for infertility evaluation during a specific 10-week period. Each male partner was to have two baseline semen samples, including morphology and immunobead assay for ASA. Patients who failed to comply with obtaining both semen samples, agreement to IUI, or did not comply to the recommended correction of other infertility factors were not included in the study. Each couple was to have as the exclusive cause of infertility an ovulation disorder and/or cervical factor problem.

Antisperm Antibody Assay. A direct immunobead test (IBT) was performed on all semen specimens similar to that described by Bronson et al. [3]. The percentage of sperm with ASA was noted. At least three beads had to be attached to be considered positive [1]. A level $\geq 50\%$ was considered positive and $\geq 20\%$ to 49% weakly positive based on previous data from Jennings et al. [19]. The location (head, tail, or tail tip) was also determined as well as immunoglobulin class (IgG or IgA). The minimum requirement for each of the two baseline specimens was a volume of 1.5 ml with a sperm concentration of at least $20 \times 10^6/\text{ml}$ and greater than 50% normal forms.

Timing of the IUI. Daily serum luteinizing hormone (LH) (Amersham Corp., Arlington Heights, IL) and serum estradiol (E_2) (Diagnostic Products, Los Angeles, CA) levels were obtained beginning 16 days before expected menses. The radioimmunoassays were performed by double-antibody and solid phase radioimmunoassay, respectively. Timing of IUI was aimed for at least 36 h and no more than 48 h from the initiation of the LH surge, or 12-24 h from the peak. The onset of the LH surge was defined as a doubling of the level from the preceding day as long as the rise continued the next day and the peak LH surge generally attained at least a fivefold rise over baseline.

Patient Population. Only couples for whom female infertility factors were considered corrected (except for cervical factor) were included in the evaluation of pregnancy rates. Ovulatory disorders (anovulation or luteal phase deficiencies) were included as long as full correction of ovulatory cycles was evident. Each of these females was to demonstrate, with or without corrective therapy, a mature follicle as evidenced by a sonographic size of an 18- to 24-mm average diameter associated with a serum E_2 of at least 200 pg/ml. Furthermore, release of the ovum as demonstrated by a 5-mm collapse 2-3 days after maturity was required. Two late luteal phase endometrial biopsies were to be in phase. Finally, a laparoscopic procedure was conducted to demonstrate bilateral tubal patency and no endometriosis or adhesions. In fact, no patient was included who had previous tuboplasties or fulguration or vaporization of endometriotic implants even if the laparoscopy was now perfectly normal. Should follicle-maturing drugs, i.e., clomiphene citrate, or human menopausal gonadotropins (hMG) be employed, the objective was never to try to stimulate multiple folliculogenesis, but merely to try as best as possible to stimulate just one mature follicle.

Bromocriptine was employed for hyperprolactinemia associated with immature follicles [13]. Subsequent to these procedures, the pregnancy rate for all couples in 6 months time was determined and comparisons were made between couples in whom the male was antibody-positive (> 50% ASA level of sperm) or negative and between couples with postcoital tests demonstrating sperm with PFM vs. those receiving IUI for postcoital tests without any progressive sperm. Semen specimens weakly positive were considered as negative for this study.

IUI vs. Intercourse. Postcoital tests were performed 8-12 h following intercourse. If sperm with PFM were demonstrated, the couple was allowed to continue with intercourse for the next 6 months and IUI was not offered. Females who demonstrated no sperm with PFM were subsequently treated with IUI; the first IUI cycle initiated the six cycles for evaluation of pregnancy rates. Sexual relations in the group was advised at the time of follicular maturity and for three consecutive nights thereafter. If mucus quality was found to be subpar, but there were still sperm with PFM, attempts to improve the mucus were made with therapy, i.e., guaifenesin [7] or a short course of estrogen [8]. Postcoital tests were rechecked in each cycle and IUI performed only if the postcoital test failed to show any sperm with PFM. Repeat testing was done for male immunoglobulins monthly. In each patient achieving a pregnancy, the male partner was required to repeat the semen analysis and be rechecked for antisperm antibodies.

Statistical Analysis. Differences between pairs of groups were analyzed using either Fisher's exact test or χ^2 analysis when comparing the groups. A p value < 0.05 was required to claim statistical significance.

RESULTS

Fifty-nine couples fulfilled the criteria for evaluation of 6-month pregnancy rates. Table 1 evaluates postcoital results and antibody testing in the 59 couples used for fertility investigation. Postcoital tests showing sperm with PFM were found in 44/59 couples (75%). Only 4 of these 44 couples (9%) showing sperm with PFM in mucus had males with positive ASA ($\geq 50\%$) and 40 (19%) were negative. In contrast, 9/15 couples (60%) without sperm with PFM in postcoital tests had males with positive ASA. The majority of the males were negative for ASA (46/59-78%) and sperm with PFM were found in 87% (40/46). However, only 31% (4/13) of males with positive ASA had postcoital tests demonstrating sperm with PFM (χ^2 analysis showed statistical significance with $p < 0.001$). It should be noted that all 13 males with > 50% ASA present in the semen had positive tests in the sera also.

TABLE 1 Correlation of Postcoital Tests and Presence of Antisperm Antibodies^a

	Total	+ ASA	- ASA
Postcoital tests with PFM sperm	44	4 (9%)	40 (91%)
Postcoital tests without PFM sperm	15	9 (60%)	6 (40%)

^aASA was considered positive if the direct immunobead test had $\geq 50\%$ of sperm with at least three beads attached.

The correlation of postcoital tests and the presence or absence of ASA in the male partner and subsequent pregnancy rates are seen in Table 2. The 6-month pregnancy rate for couples with postcoital tests showing sperm with PFM was 84% (37/44) compared to those with poor (no sperm with PFM on testing) postcoital treated with IUI whose pregnancy rate was 67% (no statistical difference; $p = 0.14$). The pregnancy rate in couples with positive ASA in the male partner was 69% (9/13) compared to 83% (38/46) in couples where the male was ASA negative (no statistical difference; $p = 0.24$). All four couples with positive ASA in the male but "normal" postcoital test, demonstrating progressively motile sperm achieved pregnancies; 56% (5/9) of couples with zero progressing sperm on postcoital tests and positive ASA in the male achieved pregnancies. In all cases where pregnancies were achieved by males positive for ASA, repeat testing of the semen continued to demonstrate the presence of immunoglobulins. Repeat postcoital testing never demonstrated sperm with PFM when repeated in those previously not demonstrating PFM.

Table 3 provides the actual percentage of sperm with at least three immunobeads attached and indicates the location of antibody as to whether attached to head, tail, or tail tip. Furthermore, the results are subdivided into IgA and IgG antibodies and whether the patient achieved a pregnancy or not. Only 2 of 9 in the pregnant group had 50% IgG attached to sperm head compared to 2 of 4 in the nonpregnant group, and the same findings were seen regarding localization to tail. Concerning IgA attachment 4 of 9 males in the pregnant group had $> 50\%$ IgA to head compared to 2 of 4 nonpregnant, and 3 of 9 with positive IgA to tail in pregnant group compared to 2 of 3 nonpregnant.

DISCUSSION

There have been several manuscripts suggesting that corticosteroids may be the most effective therapy for an immunological male factor [18, 22]. Others have failed to demonstrate

TABLE 2 Correlation of Postcoital Tests and Presence of Antisperm Antibodies in Patients Achieving Pregnancy

	Total Pregnant	+ ASA ^a	- ASA
Postcoital with PFM sperm	37/44 (84%)	4/4 (100%)	33/40 (83%)
Postcoital without PFM sperm	10/15 (67%)	5/9 (56%)	5/6 (83%)

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

Note: The pregnancies are within a 6-month interval once all female factors were corrected.

TABLE 3 Antisperm Antibody Levels in Accordance with the Location on the Sperm and Immunoglobulin Isotype

IgG%	IgG Head	IgG Tail	IgG Tail Tip	IgA%	IgA Head	IgA Tail	IgA Tail Tip
+ Pregnant Group							
91	11	3	85	70		20	68
100	99	99	80	100	99	95	42
73			75	88	26	2	68
80			80	95	1		94
89		7	87	100	11	52	78
90	17	3	90	100	98	11	76
11	11		2	96	95	23	12
100	80	2	75	99	72	1	64
100	3	50	99	93	10	50	90
- Pregnant Group							
96	79	41	96	98	88	53	99
71	18	2	68	98	17	23	98
100	97	65	100	100	84	59	100
91	3		99	78	2		78

improved pregnancy rates in the female partners of corticosteroid-treated males compared to controls [16, 23]. Whether this therapy is effective or not, all agree that the treatment has frequent side effects and also carries the risk of causing aseptic necrosis of the femoral head [24].

Although IUI of washed sperm would obviate the problem of poor sperm penetration of the mucus, intrauterine loss of sperm viability might still occur. However, timing close to ovulation could prove to be quite advantageous since fertilization might then occur before sperm immobilization. In fact, the ASA could be present in the uterine cavity, so that IUI may just be a mechanism of placing the sperm in the uterine cavity and immobilization could still occur there prior to fertilization if there is sufficient delay to allow the complement reaction. Thus, if timing could be such that an oocyte is ready for the sperm rather than 48 h later, fertility results might significantly improve. Margalioth et al. reported a 13% pregnancy rate in 4.5 cycles in couples with the male having positive ASA employing IUI in natural cycles [21]. In contrast, 30% and 39% conceived within the same time frame when hyperstimulation with clomiphene citrate or hMG was employed [21].

The study presented herein demonstrates a 56% pregnancy rate in six cycles (five pregnancies in 32 cycles; 15.6%/cycle) using IUI without hyperstimulation. Perhaps some of the infertility cases in Margalioth's study ascribed to ASA might have been related to more subtle and therefore unevaluated follicular maturation defects in the female partner.

Even when the reason for IUI is a cervical factor without an immunological basis, some have reported poor success rates when hyperstimulation was not employed [15, 20]. However, we have demonstrated a 21.2% pregnancy rate per cycle with IUI for cervical factor when carefully timed and performed 36-48 h from the initiation of the serum LH rise or 12-24 h from the peak [10]. We believe that our relatively good success with IUI for male immunologi-

cal factors may similarly be partly related to proper timing with LH surge and meticulous correction of all female factors. We think that some of the previously reported poor results may have been secondary to not doing the IUI at the proper time, not adequately correcting other female factors, and perhaps making a wrong diagnosis of cervical factor by not performing the postcoital tests in accordance with follicle maturation studies (test possibly performed at the wrong time, so false diagnosis of cervical factor, or the failure to identify poor mucus related to follicle immaturity [12] or premature luteinization [9]). Spontaneous remission of the immunological problem was ruled out by finding not one male who was negative for ASA at the time of conception in their partner.

In conclusion, the data reported here suggest that carefully timed IUI might be the most cost-effective method with the least risk/benefit ratio for male factor immunoglobulin problems. Therefore, IUI should be attempted prior to corticosteroid therapy, or IUI with hyperstimulation, or the use of in vitro fertilization/embryo transfer. A larger series is needed to determine in couples with poor postcoital tests treated with IUI if males with positive ASA are less likely to achieve pregnancies in their female partners than males who are ASA-negative.

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