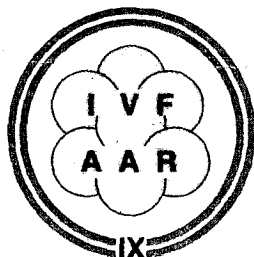


REPRINTED FROM:



WORLD CONGRESS ON IN VITRO FERTILIZATION AND ASSISTED REPRODUCTION

Vienna (Austria), April 3 - 7, 1995

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Mild enzymatic treatment of sperm bound with antisperm antibody prior to oocyte insemination improves IVF pregnancy rates (1)

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SUMMARY

The study presented herein was designed to determine if chymotrypsin-galactose (CG) treatment of sperm bound with antisperm antibodies (ASA) improves pregnancy rates (PRs) following in vitro fertilization (IVF). Patients with >50% ASA who failed to conceive despite six intrauterine insemination (IUI) cycles were included. There was a significantly lower fertilization rate in those patients inseminated with sperm incubated in culture medium vs CG (27% vs 47%, $p < .05$ t-test). Similarly, a higher percentage of patients receiving culture medium treatment of sperm had failed fertilization (45%) compared to CG (11%). Though the clinical PRs were higher with CG (21%) than medium (9.5%), there was no statistical difference. CG did not reduce ASA levels; possibly CG neutralizes ASA action.

INTRODUCTION

Various tests to measure ASA have been described; one of the most popular is the immunobead test (IBT) (2). This assay allows

identification of the type of antibody (i.e., IgG or IgA) and the location on the sperm surface of the antigen binding site.

Some couples can achieve a pregnancy following intercourse, despite the presence of ASA on the sperm surface or in seminal plasma. Others may require timed intrauterine insemination (IUI), possibly enhanced by ejaculating into culture medium where the disruption of coagulum may prevent attachment of those antibodies contributed only at the time of ejaculation (3). Recently, a technique was described demonstrating an improved PR in couples when the male partner had ASA, if their specimens were pre-treated with CG (4). The initial hope was that the enzyme would elute or neutralize the adverse effect of the ASA.

Unfortunately, in some couples, despite many cycles of timed IUI, even with CG-treated sperm, pregnancies may fail to occur. The next step in these couples might be in vitro fertilization (IVF). The fertilization rates (FRs) of human oocytes following IVF had been found in some studies not to be impaired even where ASA levels have been >70% (5,6). Another study did show reduced IVF FRs with ASA, but only when at least 80% of the motile sperm were found with both IgA and IgG; IgG alone did not impair fertilization (7).

The study presented herein evaluated the efficacy of IVF-ET in couples with CG treated sperm who also failed to conceive after six cycles of IUI; the majority of these IUI cycles used CG for sperm treatment. Furthermore, the study compared both PRs and FRs following sperm collected in culture medium vs media with CG. Finally, the effects of antibody location on the PRs and FRs were evaluated.

MATERIALS AND METHODS

The study group consisted of 36 patients whose sperm were bound with >50% ASA and who failed to conceive despite six cycles of timed IUI with sperm processed with either CG or culture medium (0.5% BSA/HTF). A total of 59 IVF-ET cycles were evaluated from 6/88 to 10/91.

All patients were screened for ASA with their initial semen analysis and re-tested prior to IVF. Patients were determined to have >50% IgA; IgG, or both. The direct IBT, as previously described, was used (2). Initially, 21 patients were randomized into CG vs culture medium. Since there were no pregnancies in the first 11 patients selected for culture medium treatment and 4/10 pregnant in the first cycles treated with CG, the next 15 patients were all given CG. The 11 patients previously randomized to culture medium maintained the same therapy in succeeding cycles. Thus, of the 36 patients in the study, 25 had the CG preparation and 11 had the culture medium with albumin preparations.

Collection and processing with albumin supplemented media

Patients ejaculated into 2.5 mL/IVF culture medium. The specimen was then processed using Percoll density gradient.

The percent fertilization, viable PR/cycle, and PR/patient for all cycles during the same time period where there were no ASA present were also calculated. Patients were divided into male factor and tubal factor categories. Male factor was defined as a motile density <8x10⁶/mL

or <20% progressive motility. Male factor was not defined according to strict morphology, though this measurement was used in calculating the number of sperm used for insemination.

RESULTS AND CONCLUSIONS

Though the clinical PR/cycle was higher with CG (21.1% or 8/38) than culture medium (9.5% or 2/21) the numbers were insufficient to show significance. Interestingly, if comparisons were made between techniques of sperm preparation where there were only tail or tail-tip directed antibodies, a significantly higher PR with CG (4/11; 36.4% per cycle) vs culture medium (1/16; 6.2% per cycle) was found. Two of the pregnancies in the CG group and one in the culture medium group failed to progress to demonstrate ultrasound evidence of pregnancy; therefore, the final viable PR/cycle was 16.7% vs 5.0% for CG vs culture medium, respectively.

All patients in the study had at least 68% mature oocytes to inseminate with the majority of the patients having >80% mature oocytes. The mean number of sperm used to inseminate each oocyte in the ASA group treated with CG was 8,654 vs 9,140 in the ASA control group vs 7,255 in the male factor control group vs 9,985 in the tubal factor control group. The mean FR for the CG group was 46.7 ± 29.4 vs $27.3 \pm 36.6\%$ for culture medium ($p < .05$, t-test). The majority of media treated patients (12/20 or 60%) had poor fertilization, as defined as <30%, compared to CG-treated specimens (13/36 or 36.1%). There were 13 cases of zero fertilization; 7 (53.8%) occurred in patients where ASA were directed to tail-tip only.

We have previously demonstrated an adequate PR for infertile couples with poor post-coital tests (PCTs) related to the males having ASA following IUI with sperm collected in media (8). Because the numbers were small in this previous study we could not demonstrate any significant differences between those with PCTs treated by IUI where no ASA was present (83% PR by six months) vs those with antibodies present (5/9 pregnant or 55.5%) (8). However, the possibility certainly exists that with a larger sample, a significant difference might be found, thus suggesting that the adverse effect of ASA may go beyond merely inhibiting the sperm from reaching the fallopian tubes.

In fact, a larger study comparing PRs following IUI of sperm bound with ASA treated with media and albumin vs CG found the latter to result in significantly higher PRs (4).

All patients having IVF-ET for male factor related to ASA were required to have failed at least six timed IUI cycles. Possible reasons for failure with IUI may include chance alone, poor timing, or the antibodies are interfering with fertilization, (beyond mere immobilization of sperm in mucus). In-vitro fertilization may, theoretically, be more effective after failed IUI. A very small percentage of sperm may not be affected with ASA so that with IVF, but not IUI, sufficient sperm free of antibodies reach the oocyte.

These data were collected from June 1988 to October 1991 at the Cooper Institute for IVF-ET, during which time 1374 IVF-ET cycles were performed. However, only 35 patients having 56 cycles were found during this time who attained the minimum selection criteria of >50% ASA by

direct IBT, and failure to achieve a pregnancy despite a minimum of six failed IUI cycles (and of course the finances and desire to undergo IVF). Thus, a multi-center study may be needed to corroborate or refute these data demonstrating the beneficial effects of CG treatment of semen specimens positive for ASA. Since many men positive for ASA are fertile and others may achieve pregnancy by timed IUI when PCTs are poor, it is essential if we want to adequately assess the importance of IVF-ET for an ASA problem to properly select the study group (as in the cases described herein). For example, the PR with CG treatment and IUI was 12/80 or 15%/cycle. It seems logical that these patients would have achieved pregnancies also by IVF and thus considerably increase the cost and patient risk. Obviously, if some centers go right to IVF instead of IUI, then a better PR should be attained.

The quality of semen parameters in males with ASA are not severely impaired (9); however, one mechanism by which males with ASA may be subfertile is immobilization of the sperm in the cervical mucus requiring the presence of complement in cervical mucus, thus preventing access to the endometrial cavity and then subsequently the fallopian tubes. Though there may be complement also present in uterine and fallopian tube fluids, possibly the concentration is less and further impairment of sperm progression only occurs to those sperm with the highest concentration of antibodies bound to the membrane which is not measured by the IBT, since it is not a quantitative assay. One hypothesis as to why CG treatment improved IUI PRs without reducing the percent of sperm having bound IBT was that it may reduce the amount of the antagonistic action of ASA on sperm through fragmentation of the antibodies. Pattinson et al., hypothesized that chymotrypsin reduces the effects of the antibody on sperm function by disruption of the immunoglobulin molecule however, still allowing recognition of the anti-immunoglobulin on the immunobead (10).

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IX World Congress on
IN VITRO
FERTILIZATION and
ALTERNATED
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Vienna, Austria
3-7 April 1995

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