

Chymotrypsin-Galactose Treatment of Sperm With Antisperm Antibodies Results in Improved Pregnancy Rates Following *In Vitro* Fertilization

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PROBLEM: To determine if chymotrypsin-galactose (CG) treatment of sperm bound with antisperm antibodies (ASA) improves pregnancy rates (PRs) following *in vitro* fertilization (IVF).

METHOD: Patients with >50% ASA who failed to conceive despite six intrauterine insemination (IUI) cycles were included. Initially the sperm treatments were randomized with CG vs culture medium; subsequently only CG treatment was used.

RESULTS: 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.

CONCLUSIONS: Though the percentage of sperm bound with antibodies are not reduced, we hypothesize that the CG treatment improves fertility by possibly mitigating the antagonistic action of these antibodies.

Key words:

Antibody location, fragmentation, IgA, IgG

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INTRODUCTION

The incidence of significant antisperm antibody (ASA) in the semen of men undergoing treatment for infertility has been estimated to be 8.0%.¹ 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 pregnancy rate (PR) in couples when the male partner had ASA, if their specimens were pre-treated with chymotrypsin-galactose (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.⁸

MATERIALS AND METHODS

Selection of Patients

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). Timing of the IUI was based on follicular maturation studies as previously described.⁹ A total of 59 IVF-ET cycles were evaluated from 6/88 to 10/91.

Randomization

All patients were screened for ASA with their initial semen analysis and retested 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 versus culture medium. Since there were no pregnancies in the first 11 patients selected for culture medium treatment and four of ten 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.

Method of Collection and Processing With Chymotrypsin-galactose

The males in the CG group ejaculated into 5 ml of Earle's Balanced Salt Solution (EBSS) with 0.1M D(+)-galactose (Sigma #G5388) added to a 5 mg vial of chymotrypsin (Sigma #CHY-5S); 5-10 min after production, the specimen was transferred to a 15 ml conical centrifuge tube and diluted with 5 ml of EBSS and galactose and centrifuged for 5 min at 300g. The supernatant was removed and the pellet was resuspended in IVF culture medium (Irvine Scientific Human Tubal Fluid (HTF) with 5 mg/ml, Irvine Scientific BSA #1092). The pellet was then layered onto a Percoll density gradient.

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.

Percoll Density Gradient

The suspension was then layered onto a 1 ml 90% Isotonic Percoll column and centrifuged for 20 min at 300g. The supernatant was discarded and the pellet was washed 2x with 1 ml of IVF culture medium. The motile sperm pellet was resuspended in 200-500 μ l of IVF culture medium and used for IVF insemination.

Oocyte Insemination

For both groups $\leq 10,000$ morphologically normal motile sperm were added to each oocyte. Sixteen hours after insemination, corona cells were removed by gentle suction through a fine bore pasteur pipet and checked for the presence of pronuclei (PN) and polar bodies. Oocytes containing 2PN were considered fertilized and those with a visible polar body were considered mature.

Data on Patients Without ASA During Same Time Period

The percent fertilization, viable pregnancy rate (PR)/cycle, and PR/patient for all cycles during the same time period where there were no ASA present was also calculated. The patients were divided into male factor and tubal factor categories. Male factor was defined as a motile density $< 8 \times 10^6$ /ml or $< 20\%$ pro-

gressive 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

Though the clinical PR/cycle was higher with CG (21.1%) than with culture medium (9.5%), the numbers were insufficient to show significance (Table I). 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 of 11; 36.4% per cycle) compared to culture medium (1 of 16; 6.2% per cycle) (see Table II) 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/patient was 25% for CG and 9.1% for culture medium and per cycle 16.7% vs 5.0%, respectively. Table III, which includes any patients with a head-directed antibody (only three had exclusively head directed ASA) did not show any advantage of CG treatment. Table IV demonstrates FRs and PRs for male factor and tubal factor cases without ASA undergoing IVF-ET during the same time period.

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 versus 9,140 in the ASA control group versus 7,255 in the male factor control group versus 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 distribution of cycles by fertilization is presented in Table V. 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; seven (53.8%) occurred in patients where ASA were directed to tail-tip only.

DISCUSSION

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.¹⁰ 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 6 months) and those with antibodies present (5/9 pregnant or 55.5%).¹⁰ 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 that compared PRs following IUI of sperm bound with ASA treated with media and albumin to 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 merely 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.

There is still no uniform opinion as to the most important location of ASA on the sperm surface associated with poor fertility prognosis. Clarke et al.⁶

TABLE I. Comparison of In Vitro Fertilization Pregnancy Rates Following CG Vs Medium Treatment of Sperm Coated by Antisperm Antibodies

	No. patients	No. cycles	No. pregnant ^a	PR/patient	PR/cycle
CG ^b	25	38	8	32.0%	21.1%
Medium	11	21	2	18.2%	9.5%
<i>P</i> value				.34	.23

^aDefined as ultrasound evidence of gestational sac.

^bCG: Chymotrypsin-galactose.

TABLE II. Comparison of Pregnancy Rates for CG Vs. Medium Treatment for In Vitro Fertilization No Head Antibodies

	No. patients	No. cycles	No. pregnant ^a	PR/patient	PR/cycle
CG ^b	9	11	4	44.4%	36.4%
Medium	8	16	1	12.5%	6.25%
<i>P</i> value				.14	.04

^aDefined as ultrasound evidence of gestational sac.

^bChymotrypsin-galactose.

reported that only >80% of IgA or a combination of IgA and IgG will have an adverse effect on fertilization, regardless of location. Mandelbaum et al.⁸ stated that only head-directed antibodies, regardless of percentage, are important. However, our data showed failed fertilization even when the antibodies were exclusively directed to tail tip and thus refute this concept. In contrast, no extra impairment of fertility seems to be caused by head directed antibodies using IVF.

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 had failed to achieve a pregnancy despite a minimum of six failed IUI cycles (and of course had the finances and desire to undergo IVF). Thus, a multicenter 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% per cycle, and it seems logical that these patients would have achieved pregnan-

cies 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;¹¹ 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 occurs only 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 is 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.^{12,13}

Reduced FRs despite IVF may still be related to an oocyte factor rather than to the male factor;¹⁴ but

TABLE III. Comparison of IVF Pregnancy Rates Following CG to Medium Treatment of Sperm With Head Directed Antisperm Antibodies

	No. patients	No. cycles	No. pregnant	PR/patient	PR/cycle
CG ^a	16	27	4	25.0%	14.8%
Medium	3	4	1	33.3%	25.0%
<i>P</i> value				.50	.26

^aChymotrypsin-galactose.

TABLE IV. Pregnancy Rates in Male Factor and Tubal Factor Patients Without Antisperm Antibodies^a

	No. Patients	No. cycles	Avg. age	Avg % fert.	# Viable preg.	% Preg/pts	% Preg/cycle
Male factor	69	121	33.5	36	8	11.6	6.1
Tubal factor	219	405	33.3	56	44	20.1	10.9

^aFert. = fertilized; preg. = pregnancy.

even if the CG is able to fragment the antibody, there are some data that have shown that antigen-binding fragments (Fab) which have lost their sperm agglutinating and immobilizing abilities can still inhibit fertilization.^{15,16} Perhaps chymotrypsin negates the antifertilization qualities of ASA with or without affecting sperm agglutinating or immobilizing properties. To determine if CG would have a positive effect on all sperm, we performed a small randomized study (unpublished), on patients who were negative for ASA and have had IUI performed. Pregnancy rates/cycle were 9/76 (11.8%) with no CG and 4/72 (5.6%) when CG was used. The study was stopped once we saw that CG may have a possible negative effect on PRs in non-ASA patients.

The data only extended to 6/91 because at that time the randomized study stopped and all male patients with ASA were treated with CG. The PRs of the group not treated with CG were comparable to the patients with ASA not treated with CG. These data were collected during the incipient years of our IVF program. The PRs in tubal factor and male factor have significantly improved,^{17,18} but also the PR for ASA has increased to 28.7% per cycle.

There have been previous studies suggesting improved PRs with ASA using treatment with 50% serum/culture medium.¹⁹ It would be interesting to randomly compare the efficacy of CG to 50% serum/culture medium following IVF for male factor related to ASA.

TABLE V. Distribution of Cycles by Fertilization Percentage—Chymotrypsin—Galactose (CG) Versus Albumin Pretreatment

Fertilization	CG	Albumin
0%	4 (11.1%)	9 (45.0%)
1–30%	9 (25.0%)	3 (15.0%)
31–70%	13 (36.1%)	6 (30.0%)
71–100%	10 (27.8%)	2 (10.0%)

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