

Two methods of achieving pregnancies despite subnormal hypo-osmotic swelling test scores

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Objective: To determine whether either the use of intracytoplasmic sperm injection (ICSI) or chymotrypsin-galactose pretreatment of sperm before IUI can improve pregnancy results in female partners of men with sperm with subnormal hypo-osmotic swelling test scores.

Design: Randomized controlled study.

Setting: University-based private practice of an infertility center and IVF center.

Patient(s): Couples with infertility who presented during a specific time interval and in whom the male partner had a hypo-osmotic swelling score of <50% in two consecutive cycles.

Intervention(s): Controls were treated by conventional IUI. Members of the treatment group were given an option of treatment of sperm with chymotrypsin-galactose before undergoing IUI or of undergoing IVF-ET with ICSI.

Main Outcome Measure(s): Pregnancy rate (PR) per patient and per cycle.

Result(s): None of the 14 control patients conceived despite 38 IUI cycles, whereas conception occurred for 4 (50%) of 8 couples in whom sperm was pretreated with chymotrypsin-galactose and for 2 (50%) of 4 couples who underwent ICSI. Patients who had enzymatic sperm treatment underwent 12 IUI cycles and 4 ICSI cycles; the PR per cycle was 33.3% in the former and 50% in the latter.

Conclusion(s): These data suggest that treating sperm with chymotrypsin-galactose before IUI or injecting only one sperm into the oocyte overcomes to some degree the block to successful pregnancy seen in women whose male partners have subnormal hypo-osmotic swelling tests. (Fertil Steril® 1997;68:549-51. © 1997 by American Society for Reproductive Medicine.)

Key Words: Implantation, intracytoplasmic sperm injection, chymotrypsin-galactose, membrane integrity

Couples in whom the male partners have sperm specimens demonstrating a score of <50% on the hypo-osmotic swelling test have extremely poor in vivo pregnancy rates (PRs), even when the men had normal motile densities (1). Enthusiasm for the subnormal hypo-osmotic swelling test as a predictor of subfertile semen specimens waned when it was found to be a poor predictor of subnormal fertilization rates after IVF-ET (2). However, the study by Barratt et al. (2) and other supporting studies (3-5) showing normal fertilization rates despite low hypo-osmotic swelling scores never evaluated PRs.

A dissimilarity between fertilization rates and PRs after IVF-ET using sperm specimens with hypo-osmotic swelling scores of <50% has been demonstrated (6). This might be explained by the hypothesis that the sperm may secrete some toxic substance that not only damages the sperm membrane but damages the oocyte membrane, which, although not interfering with fertilization or early cleavage, somehow is interfering with subsequent implantation.

The objective of this study was to determine whether clinical pregnancies could be achieved by IVF-ET despite subnormal hypo-osmotic swelling scores of the sperm by limiting exposure of the oocyte to only one sperm by performing intracytoplasmic sperm injection (ICSI). Furthermore, because enzymatic treatment of sperm coated with antisperm antibodies has been shown to improve PRs (7), this

Received January 24, 1997; revised and accepted May 12, 1997.

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study also evaluated whether chymotrypsin-galactose treatment of sperm could improve hypo-osmotic swelling scores and/or subsequent PRs.

MATERIALS AND METHODS

Patient Selection

The study group included all couples presenting with infertility in whom the male partners had hypo-osmotic swelling scores of <50% in two consecutive semen analyses during the period from October 1, 1993, to August 1, 1994. Men with antibodies anti-sperm were excluded from the study. Couples were randomized into a group who received normal IUI and a treatment group who were given the option to choose either chymotrypsin-galactose treatment or ICSI. Those offered treatment were advised of the theoretical benefit of the procedures and were also advised that we had tried chymotrypsin-galactose treatment previously in four men, with one couple achieving a successful pregnancy (6).

The sperm was processed on a three-layer discontinuous Percoll gradient. One microliter of sperm suspension with a concentration of 1×10^6 to 2×10^6 /mL was added to 1 μ L of 10% polyvinylpyrrolidone (PVP) (Scandinavian IVF Science AB, Goteborg, Sweden) under oil. Motile normal-appearing sperm were then selected for ICSI (one could not tell if the sperm selected had normal or subnormal functional integrity of the sperm membrane). One immobilized sperm was injected into each mature oocyte. Subsequent fertilization rates and PRs then were determined.

Chymotrypsin-Galactose Treatment of Sperm

Patients who were undergoing IUI were randomized into a group who would have standard Percoll three-layer discontinuous density gradient separation of sperm and a group who had chymotrypsin-galactose treatment before separation.

For chymotrypsin-galactose treatment, 0.1 M galactose was dissolved in 5 mL of Earle's balanced salt solution and added to 5 mg of chymotrypsin. The patient ejaculated directly into this chymotrypsin-galactose mixture. The semen immediately was mixed to break up the coagulum. Bovine serum albumin (30 mg/mL) was added to stop the enzymatic reaction. The hypo-osmotic swelling test was performed as described previously (1).

This study was approved by the research review board of the Cooper Institute for Reproductive and Hormonal Disorders, which is composed of 4 physicians, 3 embryologists, 3 andrologists, 1 ultrasonographer, and 1 biostatistician.

Chi-square analysis and Fisher's exact test were

Table 1 Comparison of Pregnancy Rates in Female Partners of Males with Subnormal HOS Test According to Treatment

Treatment	No. of patients	No. of cycles	Pregnancies	PR per patient (%)	PR per cycle (%)
ICSI	4	4	2	50	50
CG	8	12	4	50	33.3
Control	14	38	0	0	0

used to compare the PRs by treatment. A *P* value of <0.05 was used to define statistical significance.

RESULTS

Twelve patients were randomized to the treatment group, and 14 patients were randomized to the control group. Four of the 12 treatment patients chose to have ICSI; their choice was influenced by the presence of evidence of tubal disease, e.g., adhesions or unilateral tubal occlusion. The remaining 8 couples had chymotrypsin-galactose treatment of semen before IUI.

The control group had 38 cycles of IUI, whereas the treatment group had 16 cycles. Of the 16 cycles, ICSI was performed in 4, and in 12 cycles, chymotrypsin-galactose treatment was rendered followed by IUI. Two of the 4 couples undergoing IVF-ET with ICSI conceived, as did 4 of the 8 couples undergoing IUI with chymotrypsin-galactose-treated sperm, compared with no pregnancies in the 14 control couples undergoing IUI without enzyme treatment (Table 1) (*P* < 0.05). The PR per cycle was 37.5% for the treatment group versus 0 for the control group, as seen in Table 1 (*P* < 0.05).

All pregnancies demonstrated viability by ultrasound at 12 weeks. Of the 8 patients in the chymotrypsin-galactose-treated group, 6 showed improvement of their hypo-osmotic swelling scores to >50%; the two pregnancies were in this group.

DISCUSSION

The dissimilarity between what appear to be normal fertilization and cleavage rates and poor PRs may be explained by sperm with subnormal hypo-osmotic swelling scores somehow impairing subsequent implantation. The exposure of the egg to only one sperm in the ICSI cases is consistent with the possibility of avoiding exposure to a hypothetical toxic factor that is released by the larger numbers of sperm to which the zona pellucida (ZP) comes in contact under normal fertilization conditions; this factor theoretically results in an implantation defect of the embryo. This could be related to the sperm with low hypo-osmotic swelling scores that remain

attached to the oocyte or even the 2 pronuclei embryo.

The data also suggest that this factor could be proteinaceous in nature, given that a protein digestive enzyme such as chymotrypsin was able to improve the subnormal hypo-osmotic swelling scores in some cases and allow pregnancies to ensue.

The frequency of low hypo-osmotic swelling scores is low in the general population (1). The possibility exists that some men with apparently normal semen parameters, including hypo-osmotic swelling scores, also may be responsible for infertility in light of the disparity found between fertilization rates and implantation rates. Evaluation of possible adverse effects of the supernumerary sperm attached to the ZP deserves further investigation. If some abnormal cytokine ratio or unusual protein is found in men with subnormal hypo-osmotic swelling scores, then possibly this marker can be sought in the semen of men with unexplained infertility in cases where pregnancy does not occur despite transferring of embryos.

An interesting concept is that a possible toxic factor may be present in higher concentrations in men with subnormal hypo-osmotic swelling scores but may be present to a lesser degree in some semen specimens that would be considered to be "fertile" under normal in vivo conditions. This factor may create an implantation defect when larger numbers of sperm come into contact with the oocyte under

IVF conditions. Such a hypothesis may lead to the investigation of the use of lower insemination concentrations or shorter incubation periods in all IVF cases until this possible implantation factor(s) can be identified.

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