



ICSI AS AN EFFECTIVE THERAPY FOR MALE FACTOR WITH ANTISPERM ANTIBODIES

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This study was conducted to evaluate if in vitro fertilization (IVF) with intracytoplasmic sperm injection (ICSI) is an effective treatment for infertility complicated by the presence in the male partner of sperm autoantibodies. Over a 1-year study period comparisons of fertilization, pregnancy, and implantation rates were made in couples where the male partner was negative or weakly positive for sperm autoantibodies (<50%) (gr 1); autoantibodies were strongly positive (>80%) (gr 2); or autoantibodies were moderately positive (50–80%) (gr 3). Only patients having oocytes fertilized by ICSI were included. The fertilization, clinical pregnancy, implantation, and miscarriage rate for group 1 ($n = 67$) was 56, 43, 21, and 14%. Comparable values for group 2 ($n = 20$) were 55, 40, 23, and 25%, and for group 3 ($n = 6$) were 63, 33, 23, and 0%. IVF with ICSI demonstrates comparable fertilization, pregnancy, implantation, and miscarriage rates in female partners of males with and without sperm autoantibodies.

Keywords antibodies, ICSI, male factor, sperm autoantibodies

High-level sperm autoantibodies have been associated with decreased fecundity [6, 13]. One way that antisperm antibodies inhibit fertility is by impairing the sperm's ability to penetrate the cervical mucus [8, 17, 18, 22]. However, with the advent of conventional in vitro fertilization (IVF) it became clear that sperm autoantibodies reduce fertilization rates [1, 2, 5, 7, 11, 14, 23]. Some improvement in fertilization rates with IVF and sperm autoantibodies was reported following enzymatic treatment of the sperm with chymotrypsin/galactose [15]. In fact, improved pregnancy rates (PRs) have been reported following intrauterine insemination (IUI) with chymotrypsin pretreated sperm [4]. However, good PRs, despite the presence of sperm autoantibodies following IUI without chymotrypsin treatment, have been reported [20]. The

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technique that theoretically should give best success would be IVF with intracytoplasmic sperm injection (ICSI), since the main adverse effect of sperm autoantibodies (ASA) seems to be sperm/zona pellucida binding [6].

Nagy et al. [19] was the first to apply ICSI for ASA and found that the fertilization rate was significantly higher than sperm autoantibody-negative patients undergoing ICSI. However, there was a higher proportion of poor quality embryos resulting in a clinical PR of only 26%, a rate lower than usual in couples undergoing IVF with ICSI [19]. In a similar report, Lahteenmaki et al. [16] found comparable fertilization and cleavage rates in couples with male factor problems as compared to those with and without sperm autoantibodies. Embryo quality, however, was lower when using ICSI [16]. Though they had a good clinical PR (46%), 35% had spontaneous abortions (SAB), with a lower delivery rate of 27% [16].

The studies by Nagy et al. [19] and Lahteenmaki et al. [16] detected autoantibodies using the mixed antiglobulin reaction (MAR) test. The Immunobead Test (IBT), another method to detect antisperm antibodies, was used by Clarke et al. [10], who used ICSI to treat sperm autoimmunity. In contrast there was no evidence of an overall decrease in embryo quality nor an increase in SAB rates in couples where the male partner had sperm autoimmunity [10]. However, for that series, at least, the clinical PRs with IVF and ICSI were not particularly high for either the group with antibodies (19%) or the group with severe male factor negative for sperm antibodies (12%) [10].

These studies prompted us to examine our own data to evaluate the efficacy of ICSI when sperm autoantibodies are present as measured by the IBT, compared to ICSI cycles using patients with subfertile specimens that are negative for sperm autoantibodies.

MATERIALS AND METHODS

Patient Selection

The records of all couples having IVF and ICSI in 1997 were reviewed. Patients were stratified by the results of their sperm direct IBT test. There were 3 groups: (1) autoantibody negative, (2) autoantibody strongly positive (IgG and/or IgA >80%), and (3) autoantibody moderately positive (IgG and/or IgA 50–80%).

Couples with very severe oligoasthenozoospermia did not have sperm autoantibodies measured and were excluded from the study. Only the first oocyte retrieval and embryo transfer (ET) for each patient was used to assure independent observations. If the ET was deferred on the retrieval cycle, either for risk of ovarian hyperstimulation syndrome or inadequate sonographic endometrial parameters, then the first frozen ET was evaluated. At our Center there is usually an equal chance of conception with fresh or frozen ET. All couples with sperm autoantibodies were required to have had previously at least 3 cycles of IUI following treatment of the sperm with chymotrypsin/galactose [4]. These couples also would have performed a postcoital testing at least 8 h after intercourse showing no sperm with progressive motion. There were 2 exceptions where the female partner also had tubal disease. There were no age restrictions, and, in fact, over 25% of the female partners in groups 1 and 2 were >40 years old.

Immunobead Test

Semen were evaluated for autoantibodies using the direct IBT [7]: 5–10 × 10 spermatozoa were washed 3 times using 0.5% bovine serum albumin (BSA) in Biggers–Whitten–Whittingham

(BWW) medium. After centrifugation the final pellet was resuspended in 5% BSA/BWW. The sperm were mixed with IgG and IgA beads and read microscopically for the percentage of sperm bead binding. In cases where there were insufficient motile sperm to perform direct IBT, an indirect IBT test was done. Donor antibody-negative sperm were then washed free of seminal plasma and processed in the same manner as the direct assay.

In Vitro Fertilization

Female partners either were stimulated by using leuprolide acetate in the midluteal phase for 10 days prior to the initiation of gonadotropins (first making sure the estradiol was <50 pg/mL and the progesterone was <2 ng/mL), or were given leuprolide acetate in the early follicular phase with gonadotropins started 1–3 days after the leuprolide was initiated. The daily dosage of leuprolide acetate varied according to the stimulation protocol with as little as 0.05 mg up to 1 mg. The type of gonadotropin also varied according to protocol with either all FSH and/or highly purified FSH or in combination with hMG. The daily dose ranged from 75 to 600 IU. Oocytes were retrieved 36 h after IM injection of 10,000 hCG. ET was performed 3 days after retrieval. Most fresh or frozen-thawed embryos had assisted embryo hatching using acidic Tyrodes solution [9].

Intracytoplasmic Sperm Injection

This procedure was performed as previously described [21]. For each oocyte, a motile sperm, immobilized with an injection pipette in a drop of polyvinylpyrrolidone (Scandinavian IVF Science AB, Goteberg, Sweden), was injected into the ooplasm. The injected oocytes were placed in human tubal fluid (HTF; Irvine Scientific, Irvine, CA) and 10% synthetic serum substitute (Irvine Scientific) and incubated for ≥ 16 h before evaluation for signs of fertilization (two pronuclei).

Embryo Preselection

Most women had 3 embryos transferred; sometimes 4 were transferred, especially in the older group. Preselection was performed by allowing twice as many embryos, as to be transferred, to develop to the 3-day-old stage. The ones with the best morphology (most symmetry of blastomeres and least fragmentation) and greater cell numbers were transferred 72 h after fertilization and the rest were frozen. If there were more fertilized oocytes than the 6 or 8 needed for preselection, the remainder were cryopreserved at the 2 pronuclear stage. Cryopreservation used a simplified freezing method and one-step thawing protocol [3].

Statistical Analysis

Analysis of variance and chi-square analysis were applied to evaluate outcome differences in group, fertilization rates, clinical PRs (gestational sac on ultrasound), implantation rates, and delivery rates. A p value of .05 was used.

RESULTS/DISCUSSION

The average fertilization rates and fertilization failure were similar in all groups (Table 1). The clinical PR per retrieval, the implantation rates, and SAB rates were similar in all groups. The delivery rates for the 3 groups were 37% for group 1 (sperm autoantibody negative); 30% for group 2 (sperm autoantibody strongly positive), and 33% for group 3 (autoantibody mod-

Table 1. Comparison of IVF outcome by sperm antibody testing

	ASA			
	Negative	Strongly positive (>80%)	Moderately positive (50–80%)	Not tested
Number of patients	67	20	6	57
Fertilization rates				
Average*	56 ± 27	55 ± 21	63 ± 25	50 ± 25
% with failed fertilization	5 (8%)	1 (5%)	0	5 (9%)
Clinical pregnancies*	29 (43%)	8 (40%)	2 (33%)	23 (40%)
Implantation rate*	21%	23%	23%	21%
Miscarriages*	4 (14%)	2 (25%)	0 (0%)	2 (9%)
Sperm parameters				
Count (10 ⁶ /mL)	(0.1–127) 2	(1.65–87) 21	(9.4–129) 20	(0–111) 2
Motility (%)	(0–80) 17	(8–81) 46	(23–66) 56	(0–100) 27
Hypoosmotic swelling test percentage	(26–89) 59	(2–84) 46	(36–81) 63	(8–71) 54
Normal morphology strict criteria	(0–60) 6	(2–16) 8	(5–16) 14	(0–12) 3

erately positive). The use of IVF with ICSI is a very effective treatment for infertility related to the presence of sperm autoantibodies in the male partner. IVF with ICSI was not any less efficient than when it is used for male factor but without sperm autoantibodies. The clinical pregnancy, implantation, and delivery rates are also comparable to IVF without ICSI when male factor is not present.

This is the fourth report confirming that IVF with ICSI can improve PRs when it is at least partially related to ASA in the male partner. However, in contrast to the report of Nagy et al., the PRs when ASA are present are not lower than those for the usual patient having IVF [19]. Lahteenmaki et al. found a higher rate of SAB when ASA was present, but our study did not support this conclusion [16]. Thus, these data support the conclusion of Clarke et al. that sperm autoantibodies when treated by IVF and ICSI do not lead to a decrease in PRs or an increase in miscarriage rates [10]. Our data are more convincing than Clarke's because the PR following IVF with ICSI for ASA was low even though comparable to groups without ASA, whereas the PRs in this study were much higher [10].

Unfortunately, the methodology used in our IVF program would preclude evaluating embryo morphology as previously described [10, 19]. We use an embryo preselection process where we allow only twice as many embryos as intended to transfer to reach the multicell stage, transfer the best half, and freeze the remaining multicell embryos; all other embryos would have been frozen at the 2 pronuclear stage where embryo morphology could not be assessed. Furthermore, there are data suggesting that embryo morphology does not correlate well with pregnancy outcome [12].

Sometimes patients achieve pregnancies despite the presence of sperm autoantibodies in the male partner, especially if the postcoital tests are normal or if the autoantibodies are only moderately positive [8]. We tried to ensure a study population requiring IVF with ICSI by

including only couples who failed to achieve pregnancies despite at least 3 treatment cycles with IUI of sperm treated by a protein digestive enzyme that has been previously shown to be an effective therapy for some cases of infertility related to sperm autoantibodies [4, 15].

IVF with ICSI is a very effective method to achieve pregnancies when sperm autoantibodies are contributing to a couple's infertility. There does not seem to be any increase in SABs related to this problem.

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