

Repeat intracytoplasmic sperm injection

Clinical perspective

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ABSTRACT

Objectives: To report the results of repeat intracytoplasmic sperm injection (ICSI) after complete failure of fertilization with initial ICSI.

Methods: The medical records of the couples undergoing repeat ICSI at the Human Reproductive Biology Unit, Soliman Fakeeh Hospital, Jeddah, Kingdom of Saudi Arabia, after complete failure of fertilization by initial ICSI between December 1994 and December 2000 were retrospectively examined.

Results: Seven hundred and eighty two oocytes from 146 women failed to fertilize by initial ICSI. The main indications for the procedure were severe oligoasthenoteratozoospermia or azoospermia. Fresh sperms were used in 136 cases, of which 98 (72%) were ejaculated, 33 (24.3%) were obtained by testicular sperm extraction, and 5 (3.7%) by testicular sperm aspiration. Of

the remaining 10: 3 were from cryopreserved semen samples and 7 were from cryopreserved testicular biopsies. The age of the women (mean \pm standard deviation) was 31 ± 16.2 years. The duration of infertility was 10.5 ± 9.4 years. A total of 151 (19.3%) oocytes were fertilized after repeat ICSI. The number of cleaved embryos was 125 (15.9%); of which 2 (1.6%) were grade 5, 47 (37.6%) were grade 4, 65 (52%) were grade 3, and 11 (8.8%) were grade 2. A total of 122 embryos were transferred to 71 women. This resulted in one pregnancy and the birth of a healthy full term baby.

Conclusion: In cases of complete failure of fertilization with initial ICSI, fertilization and pregnancy can follow repeat ICSI. Further clinical and cytogenetic studies in this area are necessary.

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Complete fertilization failure may occur after an initial intracytoplasmic sperm injection (ICSI) procedure due to a large variety of factors including some fundamental inadequacies of the sperm or the oocyte. Such an event can be psychologically devastating and expensive. An attempt at a "rescue" operation is a natural response that has to be weighed against the risk of transferring chromosomal abnormalities. The concept of trying a repeat fertilization modality is not new. In 1994 subzonal sperm injection was used in the treatment of oocytes failing to fertilize after an initial microinjection.¹ In

this report, we present data on our experience with repeat ICSI in couples with complete fertilization failure after initial ICSI.

Methods. Between December 1994 and December 2000, there were 146 cycles of complete failure of fertilization with initial ICSI at the Human Reproductive Biology Unit, Soliman Fakeeh Hospital, Jeddah, Kingdom of Saudi Arabia. These ICSI cycles were carried out with the purpose of alleviating infertility mainly due to severe

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oligoasthenoteratozoospermia or azoospermia. Controlled ovarian hyperstimulation was performed using standard regimens. The regimen varied according to the couples' day of contacting the hospital asking for a treatment cycle to be commenced and pattern of past response. Pituitary down regulation was achieved by one of 4 regimens: 1) Decapeptyl (Ferring, Germany) 0.1 mg on a daily basis by a subcutaneous route; 2) Superfact (Hoechst, United Kingdom) nasal spray in divided 6-12 sniffs per day starting day 2 of the menstrual cycle; 3) Decapeptyl (Ferring, Germany) 3.75 mg in a single intramuscular injection; 4) Zoladex (Astra-Zeneca, Switzerland) 3.6 mg in a single subcutaneous injection, administered in the mid luteal phase of the preceding cycle. The gonadotrophin used was either human menopausal gonadotrophin (hMG) (Pergonal; Serono, Switzerland) or purified follicle-stimulating hormone (FSH) (Metrodin; Serono, Switzerland). Standard stimulation was daily dosing of 225-400 international units (IU) of hMG for women 30-40 years of age. Women >40 years or with a history of low gonadotrophin response were given a daily maximum of 600 IU of hMG, administered in 2 doses. Women <30 years, or with a history of high gonadotrophin response, were given a single daily dose injection of 150-225 IU of hMG. Follicle growth monitoring was achieved with the use of ultrasonography and measurement of serum estradiol. This was begun on stimulation day 6 and was then performed every 1-2 days, as indicated. A dose of 10,000 IU of human chorionic gonadotrophin (hCG) (Profasi; Serono, Italy) was administered intramuscularly when 3 follicles reached a minimum mean diameter >18 mm in the average patient or when only 2 follicles reached a minimum mean diameter >18 mm in the poor responders with an estradiol level >500 pg/ml. Transvaginal oocyte retrieval was performed 36 hours after hCG administration. Sperm parameters were assessed according to the World Health Organization guidelines.² Different methods of sperm preparation were employed, namely, swim-up and Percoll gradient centrifugation. The choice of the method for sperm preparation depended upon the sperm parameters found at the initial assessment. Briefly, for the swim-up method, 1-2 ml of semen was mixed with 3-4 ml of growth medium and centrifuged at 200 gm for 5 minutes. The supernatant fluid was decanted and the sperm pellet was gently dislodged. With the use of a sterile Pasteur pipette the dislodged sperm pellet was dispensed to the bottom of another test tube containing one ml of growth medium and incubated at 37°C. After one hour the top layer containing the washed motile sperms was aspirated and analyzed for count and progression. For Percoll gradient centrifugation, an aliquot of one ml suspended spermatozoa was placed on a discontinuous Percoll gradient (usually 45/90%) and

centrifuged at 600 gm for 15 minutes. The pellet was resuspended in growth medium and the Percoll was removed by centrifugation at 200 gm for 5 minutes. The sperm suspension was transferred to another test tube with one ml of growth medium. Oocytes were treated with 0.5% hyaluronidase (Sigma Co.) to induce lysis of the cumulus oophorus cells. Cells of the corona radiata were removed mechanically with a pasteur pipette under stereomicroscopic guidance at a magnification of X50. Subsequently the maturity of the oocytes was determined. Only oocytes in the metaphase were used for the ICSI procedure. Immediately prior to the ICSI procedure, 5 µl of 10% polyvinylpyrrolidone solution was added to the sperm-containing droplet to reduce sperm motility if this was felt necessary,³ or the sperms tail was crushed against the bottom of the holding dish. The basic culture system employed a medium formula prepared at own assisted reproduction laboratory. Briefly, the stock solution was prepared by dissolving 60 mg of Penicillin, 50 mg of streptomycin and 11 mg of Sodium Pyruvate in 200 ml of ultra high purity (UHP) water to which one gram of sodium hydrogen carbonate and 100 ml of earle's balanced salt solution (EBSS) X10 concentrate is added. The solution was made up to 1000 ml by adding UHP water. The growth medium was prepared by adding 250 ml of the stock solution to a 250 ml volumetric flask containing 275 mg of sodium hydrogen carbonate. The osmolarity was measured and adjusted to 283-287 mOsm/kg. Finally the solution was sterilized by filtration through 22 µm millipore filters. This medium was used to support embryo growth from days 1-3 (day one being the day of the fertilization check). The remaining 750 ml stock solution was used as a flushing medium by adding 15 ml N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES) buffer and 100 IU of Heparin/ml. The pH was adjusted to 7.3-7.4. The resulting solution was sterilized by filtration through 0.22 µm millipore filters. Injected oocytes were examined at 16-18 hours after injection to determine whether or not they were fertilized. Embryos were categorized according to the following grading system: Grade 5 - Equal-sized symmetrical blastomeres with clear cytoplasm and visible nuclei. Grade 4 - Equal-sized symmetrical blastomeres with cytoplasmic granularity but demonstrable nuclei. Grade 3 - Unevenly sized blastomeres with or without granularity and <25% fragmentation. Grade 2 - At least 2 or more normal blastomeres with 50% fragmentation. Grade 1 - > 50% fragmentation with at least one normal blastomeres. Grade 0 - Evidence of cleavage but the gross fragmentation and no normal looking blastomeres. Embryo transfer was performed 2 days after fertilization (2-3 days after retrieval). All patients received luteal phase support with Cyclogest (Cyclogest 400; Hoechst Pharmaceutical) progestogen vaginal pessaries 400

mg/day from the day of embryo replacement. Clinical pregnancy was defined as the presence of a gestational sac as well as fetal heart beat on ultrasonographic screening. No routine biochemical pregnancy testing was implemented.

Results. Between December 1994 and December 2000, 782 oocytes from 146 women failed to fertilize after initial ICSI for oligoasthenoteratozoospermia or azoospermia. Women were between 19 and 47 years of age with a mean age of 31.1 ± 16.2 years. The duration of infertility ranged between one and 25 years with a mean of 10.5 years. Fresh sperms were used in 136 cases, of those, 98 were ejaculated, 33 were obtained by testicular sperm extraction, and 5 by testicular sperm aspiration. Of the remaining 10: 3 were cryopreserved semen samples and 7 were from cryopreserved testicular biopsies. The documented percentage of abnormality on 125 samples showed a mean of 46.2%. Of 782 oocytes with primary fertilization failure, 151 (19.3%) oocytes fertilized after repeat ICSI. The number of cleaved embryos was 125 (15.9%); of which 2 (1.6%) embryos were grade 5, 47 (37.6%) were grade 4, 65 (52%) were grade 3, 11 (8.8%) were grade 2. A total of 122 embryos were transferred to 71 women. Seven embryo transfers were difficult and 64 were easy. One pregnancy was achieved and a healthy baby was delivered at term.

Discussion. To our knowledge, this study is the first of its kind to report on the clinical use of repeat ICSI when the initial procedure fails. The idea of "rescue" procedures is relatively old. Reinsemination in conventional in vitro fertilization (IVF) and by intracytoplasmic sperm injection in cycles of complete fertilization failure had started soon after the first utilization of those technologies.⁴⁻⁷ The percentage of fertilization over the 6-years of study is 19.3%. As expected those results would not compare favorably with the fertilization rate of first attempt IVF,⁸ first attempt ICSI⁹ and "rescue" ICSI post IVF failure¹⁰ but they do compare favorably with rates obtained in studies on reinsemination of aged, failed-fertilized oocytes by standard IVF⁴ and by partial zona dissection.¹¹ In this study, the oocytes inseminated with a fresh semen sample on the initial ICSI were inseminated with sperms from the same sample on the repeat attempt. Using a fresh sample in the subgroup were ejaculated samples were used may offer a small added advantage.¹² From the logistical point of view, because the oocytes are already denuded, repeat ICSI is less time consuming than the primary procedure. It has, however, to be remembered that this procedure cannot be anticipated, but its possibility has to be born in mind and planned for.

In conclusion, the present study demonstrates that it is possible to achieve a satisfactory fertilization rate and even a term pregnancy and delivery of a healthy child, utilizing the technique of repeat ICSI. It has to be born in mind that embryos derived from older failed-fertilized oocytes have a lower developmental potential due to a higher proportion of chromosomal anomalies than those in fresh fertilized oocytes.¹³ Further cytogenetic studies are also warranted for the added reason of excluding late fertilization, rather than the repeat ICSI, as the mode of operation in this particular method of fertilization.

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