Intracytoplasmic sperm injection as a treatment for unexplained total fertilization failure or low fertilization after conventional in vitro fertilization

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Objective: To determine whether IVF or intracytoplasmic sperm injection (ICSI) should be the choice of treatment in case of a previous IVF attempt with unexplained total fertilization failure or low fertilization (<25%).
Design: Prospective study.
Setting: Leiden University Medical Center.
Patient(s): Thirty-eight couples undergoing IVF and ICSI on sibling oocytes after a first IVF attempt with total fertilization failure or with low fertilization (<25%).
Intervention(s): Performing IVF and ICSI on sibling oocytes.
Main Outcome Measure(s): Fertilization and (ongoing) pregnancy rate.
Result(s): A total of 271 oocytes were collected in 24 oocyte retrievals in the total fertilization failure group. Hundred nine oocytes were randomly allocated to IVF and 12 were fertilized (11%); 162 sibling oocytes were allocated to ICSI and 78 were fertilized (48%). In 8 of the 24 patients fertilization occurred after IVF. The pregnancy rate after transfer of 1 IVF and 1 ICSI embryo (n = 3) was 67% and after the transfer of 2 ICSI embryos (n = 21) this was 52%. In the low fertilization group 169 oocytes were collected in 14 oocyte retrievals. Seventy-two oocytes were randomly allocated to IVF and 16 were fertilized (22%). Ninety-seven sibling oocytes were allocated to ICSI and 58 were fertilized (60%). In 7 of 14 patients fertilization occurred after IVF. The pregnancy rate after the transfer of 1 IVF and 1 ICSI embryo (n = 5) was 80% and after the transfer of 2 ICSI embryos (n = 9) this was 33%.
Conclusion(s): Performing ICSI on some oocytes of a cohort may avoid total fertilization failures both in patients with a history of total fertilization failure and in patients with a history of low fertilization, as the percentage of fertilization is higher after ICSI compared to IVF and the recurrence of total fertilization failure and low fertilization is high after IVF treatment. (Fertil Steril® 2005;83:612–7. ©2005 by American Society for Reproductive Medicine.)

Key Words: Total fertilization failure, low fertilization, ICSI, IVF, fertilization rate, pregnancy rate

Intracytoplasmic sperm injection (ICSI) rather than conventional IVF in case of severe male factor infertility has been accepted as a highly successful method in terms of fertilization and pregnancies resulting in live births. The results in couples with mild male factor infertility were discordant (1, 2) as were those in case of non-male factor infertility. Using sibling oocytes Khamsi et al. (3) reported a higher fertilization and pregnancy rate after ICSI relative to IVF, whereas Staessen et al. (4) found no difference.

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In case of normospermia total fertilization failure and low fertilization (defined as <25% fertilization) occurs in 5%–15%, and 20%, respectively, of the couples undergoing IVF, with a recurrence rate of about 30%–50% (5–7). This failure of oocytes of the female patient to be fertilized by the spermatozoa of the male partner undergoing infertility treatment may be explained by lack of penetration of the zona pellucida, an oocyte activation failure, or a defect in the oocyte. Intracytoplasmic sperm injection circumvents those obstacles and might therefore be effective. Only one study on the efficacy of IVF and ICSI after a previous attempt with total fertilization failure has been published, reporting in favor of ICSI, despite the use of a high insemination concentration in the IVF procedure (8). However, most of the couples included in that study suffered from oligoasthenoteratozoospermia.

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The aim of this study is to compare the efficacy of IVF and ICSI in case of a previous IVF attempt with either total fertilization failure or low fertilization in couples with normospermia. It is a prospective study using sibling oocytes that have been randomly assigned to either an IVF or an ICSI procedure with fertilization and pregnancy rate as primary outcome.

MATERIALS AND METHODS

Patients

The couples that were included in this study showed total fertilization failure or low fertilization (<25%) in a first attempt (between September 1995 and September 2002) in which all retrieved oocytes were treated with (conventional) IVF. In the second attempt (between November 1995 and February 2003) part of the retrieved sibling oocytes of those patients were treated with IVF and the other part with ICSI.

Other inclusion criteria were the retrieval of at least five oocytes (sufficient number for an IVF as well as an ICSI procedure) and semen characteristics of >20% motility after sperm preparation (discussed later) and $>1 \times 10^6$ total motile sperm count after sperm preparation. The criteria were fulfilled both on the day of the first IVF attempt as well as on the day of the second attempt. A total number of 24 different couples fulfilled the inclusion criteria for the total fertilization failure group, as did 14 different couples for the low fertilization group.

The mean age of the women was 32.2 ± 3.9 years (range 23–41 years). The results reported all refer to the second treatment.

Each couple included in this study was informed that due to the possibility of recurrence of total fertilization failure or low fertilization, ICSI and IVF would be performed on sibling oocytes. Because ICSI is a proven method for the treatment of infertility there was no need for Institutional Review Board approval. However, written informed consent of each included couple to perform ICSI on at least part of the retrieved oocytes was obtained.

Ovarian Stimulation

Ovarian stimulation was performed by a combination of GnRH agonist: Decapeptyl (Ferring, Hoofddorp, The Netherlands); Synarel (Pharmacia, Woerden, The Netherlands), FSH: Gonal F; Pergonal; Metrodin HP (Serono Benelux, Den Haag, The Netherlands), and hCG: Profasi (Serono Benelux, The Hague, The Netherlands); Pregnyl (Organon, Oss, The Netherlands). Luteal phase supplementation was given by intravaginally administered P, Progestan (Organon) and a single hCG injection, Pregnyl (Organon).

Semen Preparation

Freshly ejaculated semen from the male partner was allowed to liquefy. After measuring the volume of the sample, concentration and percentage of motile spermatozoa was assessed in a Makler counting chamber (Sefi-Medical Instruments, Haifa, Israel). Earle's medium buffered with HEPES and supplemented with 0.5% human serum albumin (Cealb, CLB, Amsterdam, The Netherlands) was added to the semen sample and mixed by pipetting. Depending on the total number of motile spermatozoa, the mixed sample was pipetted on top of either a layer of 70% or 80% Percoll (from 1995 to 2000)(Pharmacia, Woerden, The Netherlands) or PureSperm (from 2000 to 2003) (Nidacon, Goteborg, Sweden) layer and centrifuged ($800 \times g$, 10 minutes).

The supernatant was removed and the sperm pellet was resuspended in HEPES-buffered Earle's medium. Depending on the total number of motile spermatozoa, this suspension was either pipetted again on top of an 80% Percoll or PureSperm layer and then washed twice, first in HEPES-buffered medium and the second time in Universal IVF medium (Medicult; Lucron, Milsbeek, The Netherlands), or washed twice with these media after the first Percoll or PureSperm treatment.

Volume, concentration, motility, and the total motile sperm count were redetermined after processing. The spermatozoa were kept at 37°C and 5% CO_2 in air in an incubator until the insemination or injection procedure. The same semen sample was used for both the insemination and the injection.

Oocyte Retrieval and Preparation

The retrieved oocyte–cumulus complexes (OCCs) were pooled and washed in HEPES-buffered Earle's medium and then randomly transferred in groups of two to six OCCs (depending on the total number of OCCs retrieved) to droplets of 25-μL culture medium (Universal IVF medium [Medicult]) under mineral oil (Sigma, Brunswig Chemie, Amsterdam, The Netherlands) and then stored into an incubator (37°C, 5% CO_2).

Before injection or insemination the OCCs were taken out of the incubator and the OCCs in the first droplets were assigned to ICSI and the OCCs in the last droplets were assigned to IVF in a ratio of 3:2. A higher number of oocytes were assigned to ICSI to secure occurrence of fertilization, as not every oocyte can be injected (about 10%–20% due to their maturational stage) or will survive after injection (about 10%).

The OCCs that were assigned to ICSI were denuded of their surrounding cumulus cells both enzymatically and mechanically between 0 and 4 hours after retrieval (9). The maturation stage was checked and the oocytes that had extruded a polar body were selected for injection. The ICSI was performed as described in detail elsewhere (9). After injection the oocytes were transferred to 25-μL droplets of Universal IVF medium, where they were cultured individually.