Conventional in vitro fertilization versus intracytoplasmic sperm injection in patients with borderline semen: a randomized study using sibling oocytes

Lucette van der Westerlaken, M.Sc., Nico Naaktgeboren, Ph.D., Harjo Verburg, M.D., Sandra Dieben, M.D., and Frans M. Helmerhorst, Ph.D.

Department of Reproductive Medicine, Leiden University Medical Center, Leiden, The Netherlands

Objective: To determine whether patients with borderline semen should be treated with conventional IVF or intracytoplasmic sperm injection (ICSI).
Design: Randomized study.
Setting: A university medical center in The Netherlands.
Patient(s): One hundred six couples with borderline semen who were undergoing IVF and ICSI on sibling oocytes.
Intervention(s): Performing IVF and ICSI on sibling oocytes.
Main Outcome Measure(s): Fertilization and pregnancy rates.
Result(s): One thousand five hundred eighteen oocytes were collected in 106 oocyte retrievals: 849 oocytes were randomly allocated to ICSI, of which 761 were microinjected, and 669 oocytes were randomly assigned to IVF. In 26 of the 106 patients, there was fertilization only after ICSI and not after IVF (IVF− group). The fertilization rate was 51% (92/182 oocytes). In 78 patients, there was fertilization after both IVF and ICSI (IVF+ group); the fertilization rate was 51% for both the IVF- and ICSI-treated oocytes (271/528 oocytes and 334/658 oocytes, respectively). In 2 patients, there was no fertilization after either IVF (0/6 oocytes) or ICSI (0/9 oocytes). Patients of the IVF+ group had a higher total motile sperm count after preparation than did those of the IVF− group. More high-quality embryos were obtained after ICSI in patients of the IVF+ group. In 101 patients, embryo transfer was performed: 26 in the IVF− group and 75 in the IVF+ group. No significant differences were found with regard to pregnancy rates between those two groups: pregnancy rates were 54% in the IVF− group and 48% in the IVF+ group.
Conclusion(s): Performing ICSI on at least some of the oocytes will avoid unnecessary fertilization failure in patients with borderline semen: in this study, 26 of 104 cycles (25%) were rescued by ICSI. (Fertil Steril® 2006; 85:395–400. ©2006 by American Society for Reproductive Medicine.)

Key Words: ICSI, IVF, borderline semen, fertilization rate, embryo quality, pregnancy rate

Threshold values of sperm parameters for assisted procreation are based mainly on the World Health Organization standard (1) and widely are used to discriminate between male fertility and subfertility (2–4). However, the prognostic value of those parameters is questionable (5–7). Mahutte and Arici (8) conducted a review of different screening tests. Their conclusion was that more sophisticated methods such as sperm–zona binding ratios and zona pellucida–induced acrosome reaction tests may improve the ability to predict fertilization capacity, but unfortunately, no test can exclude the possibility of fertilization failure. In patients with borderline semen, the decision to choose either IVF or intracytoplasmic sperm injection (ICSI) is critical because the chance of total fertilization failure after a conventional IVF or of performing an unnecessary ICSI procedure is hard to predict.

Received February 28, 2005; revised and accepted May 23, 2005.
Reprint requests: Lucette van der Westerlaken, M.Sc., Department of Reproductive Medicine, IVF Center, PO Box 9600, 2300 RC Leiden, The Netherlands (FAX: 31-71-5266701; E-mail: l.a.j.van_der_westerlaken@lumc.nl).

The majority of failed fertilized oocytes do not contain sperm nuclei after conventional IVF (9, 10), indicating that most cases of fertilization failure relate to an inability of the sperm cell to penetrate the oocyte. Oocyte-related factors that might account for fertilization failure in some cases could be defects in the pronuclear formation or an oocyte activation failure (8).

The aim of this study was to determine the optimal treatment (IVF or ICSI) for patients with borderline semen while minimizing the risk of total fertilization failure with fertilization rate as primary outcome and pregnancy as secondary outcome. We therefore performed IVF and ICSI on sibling oocytes in a first cycle in a cohort of patients with borderline semen.

MATERIALS AND METHODS

Patients

Between September 1995 and September 2003, 106 couples suffering from mild male-factor infertility were treated in a first cycle with IVF and ICSI on sibling oocytes. Mild male-

factor subfertility was defined by the presence of at least one abnormal semen parameter, that is, concentration $<20 \times 10^6$ per milliliter and/or <40% motility according to the World Health Organization (1) criteria.

Patients were included in this study on the basis of previous diagnostic semen analyses and when, on the day of oocyte retrieval, their semen fulfilled the above criteria again. The other inclusion criterion was the retrieval of at least five oocytes. The mean age of the women was 31.3 (± SD) ± 4.4 years.

Institutional review board approval was obtained to perform ICSI, and all patients were informed that because of the low semen quality, total fertilization failure after conventional IVF was possible and therefore ICSI and IVF would be performed on sibling oocytes. Written informed consent was obtained from each included couple to perform ICSI on at least some of the retrieved oocytes.

Ovarian Stimulation

The patients were stimulated according to a standard short-stimulation protocol, starting with down-regulation on the 1st day of menstruation with GnRH agonist, given as a nasal spray (Synarel; Pharmacia, Woerden, the Netherlands) or SC (Decapeptyl; Ferring, Hoofddorp, the Netherlands). This was followed by ovarian stimulation with purified FSH (Metrodin; Serono Benelux, Den Haag, the Netherlands) or recombinant FSH (Gonal F, Serono Benelux), starting either on day 4 with 150 IU when the women were 37 years of age or younger or on day 2 with 225 or 300 IU if the women were 38–39 or 40–41 years of age, respectively. The patients were monitored by vaginal ultrasound scans and serum E_2 measurement. If necessary, the dose was adjusted during stimulation. When the desired follicle growth was achieved, hCG was given SC (10,000 IU; Profasi, Serono or Pregnyl, Organon, Oss, the Netherlands), followed by oocyte pickup 36 hours later. Luteal-phase supplementation was given by intravaginally administered P (Progestan; Organon) and by a single hCG injection (Pregnyl, Organon).

Semen Preparation

Freshly ejaculated semen was allowed to liquefy. Volume was determined, concentration and percentage of motile spermatozoa were assessed in a Makler counting chamber, and the total number of motile spermatozoa was calculated. The semen samples were diluted 1:1 with *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES)–buffered Earle's medium supplemented with 0.5% human serum albumin. The diluted sample was pipetted on top of a 1-mL layer of 70% Percoll (during 1995–2000; Pharmacia, Woerden, the Netherlands) or a 1-mL layer of 70% PureSperm (during 2000–2003; Nidacon, Goteborg, Sweden) in a 12-mL tube and was processed by centrifuge ($800 \times g$, 10 minutes). The supernatant was removed, and the sperm pellet (0.1–0.5 mL) was resuspended in 5 mL of HEPES-buffered Earle's medium. This suspension was then washed twice, first in HEPES-buffered medium and a second time in universal IVF medium (Medicult; Lucron, Milsbeek, the Netherlands). Volume, concentration, motility, and the total motile sperm count were redetermined after processing. The spermatozoa were kept at 37°C in a CO_2 incubator until IVF or ICSI took place.

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 of culture medium (universal IVF medium; Medicult) under mineral oil (Sigma, Brunswig Chemie, Amsterdam, the Netherlands) and then put into an incubator (37°C, 5% CO_2).

Before injection or insemination, the OCCs were taken out of the incubator. 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, respectively. A higher number of oocytes were assigned to ICSI to secure the occurrence of fertilization: not all oocytes can be injected because of their maturational stage (about 10%–20%), and not all oocytes will survive the injection (about 10%).

The OCCs that were assigned to ICSI were denuded of their surrounding cumulus cells both enzymatically and mechanically at between 0 and 4 hours after retrieval (11). 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 (11). After injection, the oocytes were transferred to 25-μL droplets of universal IVF medium, in which they were cultured individually.

The OCCs that were assigned to IVF kept their surrounding cumulus cells, and they were cultured individually in 25-μL droplets of universal IVF medium. Each oocyte was inseminated with 75,000–150,000 motile spermatozoa (standard number is 75,000), 2–6 hours after oocyte retrieval, in a total volume of 25–30 μL.

The same semen sample was used for both the insemination and the injection.

Assessment of Fertilization

Fertilization was scored 16–18 hours after injection and insemination. For the IVF oocytes, the surrounding cumulus cells were removed mechanically by repeated pipetting of the OCCs in and out of a hand-drawn Pasteur pipette, and the oocytes were transferred to new 25-μL droplets of universal IVF medium.

Both the IVF and the ICSI oocytes were checked for normal fertilization (the presence of 2 pronuclei) or no and abnormal fertilization (0 and 1, >2 pronuclei, respectively) or whether the oocyte had degenerated.