
Transport–in-vitro fertilization/intracellular sperm injection: a prospective randomized study

T.Coetsier1,3, A.Verhoeff2, P.De Sutter1, J.Roest2 and M.Dhont1

1Department of Obstetrics and Gynaecology, University Hospital, De Pintelaan 185, 9000 Gent, Belgium and 2Department of Obstetrics and Gynaecology, Zuiderziekenhuis, Groene Hilledijk 315, 3075 EA Rotterdam, The Netherlands

3To whom correspondence should be addressed

We performed a prospective randomized clinical trial to investigate whether long distance oocyte transport prior to an intracytoplasmic sperm injection (ICSI) procedure influences fertilization rates, embryo quality and/or embryo implantation rates. After informed consent, 100 infertile couples booked for ICSI treatment were randomized into two groups. In group 1 (n = 50), patients were stimulated and monitored in Rotterdam (The Netherlands), and oocyte retrieval, ICSI procedure and embryo transfer took place in Gent (Belgium). In group 2 (n = 50), patients were stimulated, monitored and punctured in Rotterdam and the oocytes were transported in their follicular fluid in an isothermic transport box to Gent, where the ICSI procedure and the embryo transfer took place. In both groups the stimulation and monitoring regimen, puncture technique, laboratory conditions and transfer policy were identical. In both groups, the number of fertilized oocytes (7.13 ± 0.65 versus 5.53 ± 0.60, P = 0.08), the number of transferred embryos (2.36 ± 0.09 versus 2.40 ± 0.11, P = 0.87) and the embryo implantation rate [20/113 (17.7%) versus 19/103 (18.4%), P = 0.89] was similar. In group 1, the number of retrieved oocytes was higher (10.83 ± 0.95 versus 8.44 ± 0.93, P = 0.05). The total score of the embryos obtained (18.90 ± 1.73 versus 12.64 ± 1.26, P = 0.01), the number of good quality embryos (4.63 ± 0.49 versus 2.98 ± 0.38, P = 0.02), the mean score of the transferred embryos (3.32 ± 0.11 versus 2.94 ± 0.13, P = 0.05) and the number of embryos available for cryopreservation (2.70 ± 0.45 versus 1.48 ± 0.38, P = 0.03) were significantly higher in group 1. Therefore, long distance transport of oocytes prior to ICSI does not affect oocyte fertilization and embryo implantation rates, although a negative effect on embryo quality cannot be excluded.

Key words: embryo quality/ICSI/randomized study/transport–IVF

Introduction

In some countries, transport of aspirated oocytes from a clinical in-vitro fertilization (IVF) centre to a central IVF laboratory is a well established routine procedure (Jansen et al., 1986; Zarutskie et al., 1988; Talbert et al., 1991; Verhoeff et al., 1992; Balet et al., 1995; Roest et al., 1995b). Due to the increasing application of intracytoplasmic sperm injection (ICSI) the co-operation between a clinical IVF centre and a fully equipped IVF laboratory could gain further in importance. Indeed, ICSI requires highly qualified personnel and expensive laboratory equipment, which the clinical IVF centre could avoid by transport–IVF. However, there have been only few reports in the literature on transport–ICSI (De Sutter et al., 1995; Roest et al., 1995a), and all are case reports or retrospective analyses. Even though the clinical results of transport–IVF have proven its feasibility, there have been no randomized controlled trials comparing a transport-setting to ‘in-house’ IVF. Therefore, we set up a prospective randomized observational study, to investigate the possible effects of transport of the oocytes on the laboratory and clinical results of ICSI.

Materials and methods

At the Zuiderziekenhuis, Rotterdam, The Netherlands, all patients that were booked for ICSI treatment were offered the possibility to take part in the randomized prospective study. All of them had been excluded for routine IVF treatment because of extreme male infertility (<1×10⁶ spermatozoa/ml in the ejaculate and/or a morphology of <5% according to strict criteria or a recovery of <500 000 motile spermatozoa) or when a previous IVF attempt had led to total fertilization failure with at least five oocytes. After informed consent, 100 patients were randomized into two groups. A minimization procedure was performed for age (<35 or >34 years of age) and indication for ICSI (extreme oligoasthenoteratozoospermia or total fertilization failure after routine IVF).

In group 1 (n = 50), patients were stimulated and monitored in Rotterdam till the day of oocyte retrieval, which took place in Gent (Belgium). In group 2 (n = 50), both stimulation and oocyte retrieval took place in Rotterdam, after which the oocytes were transported in their follicular fluid in an isothermic transport box (Gyno Transporter; Gynotec, Malden, The Netherlands) to Gent. In both groups, the ICSI procedure and the embryo transfer took place in Gent.

In both groups the same stimulation and monitoring regimen was used. Triptorelin 0.1 mg (Decapeptyl; Ferring, Hoofddorp, The Netherlands) was administered from day 1 of a spontaneous cycle. Gonadotrophins (Humegon; Organon, Oss, The Netherlands) were started from day 3 at a dose of 225 IU/day i.m. Both triptorelin and gonadotrophins were continued until follicular maturity was obtained, as determined by transvaginal ultrasonography. Human chorionic gonadotrophin (HCG), 10 000 IU was injected i.m. (Pregnyl; Organon) 35 h before the scheduled oocyte retrieval. Transvaginal oocyte retrieval was performed in both groups under local anaesthesia. At 30 min before the puncture, patients took 10 mg of oxazepam orally (Seresta; Wyeth, Hoofddorp, The Netherlands), and received an i.m. injection of 0.5 mg of atropine and 10 mg of mibain in the thigh.
Paracervical block was performed with 10–20 ml of lidocaine 10 mg/ml (Xylocaine 1%; Astra Pharmaceutica, Rijswijk, The Netherlands). In both groups, the same single lumen follicle aspiration sets were used (Gynotec). The aspirates were collected in vials of 20 ml (Gynotec). Whenever the aspirate was contaminated with blood, 2–6 drops of heparin (5000 IU/ml) were added in order to avoid clot formation. The aspirates were not examined microscopically for the presence of oocytes but immediately sealed and placed inside the transport box without further handling. When the oocyte retrieval took place in Rotterdam, the patient’s partner travelled to Gent with the transport box containing the vials with the follicular aspirates. The distance between Gent and Rotterdam is approximately 150 km and the transport time varied from 1.5 to 2.5 h depending on the traffic conditions. The ICSI laboratory protocols followed in our centre have been described elsewhere (Dozortsev, 1996). On day 2 pregnancy rate per cycle 13/50 (26) 18/50 (36) 0.28 b

The main outcome measures of this study were fertilization rates after ICSI, embryo quality and implantation rates of embryos obtained by ICSI in the two groups.

Differences of 10% for fertilization rates and 5% for implantation rates were considered to be clinically relevant. The average fertilization score of 70% after ICSI in our centre was the starting point for calculation of the sample size. In order to be able to demonstrate a decrease in fertilization rate from 70 to 60% with a power of 80% and a double-sided alpha-error of 0.05, at least 300 oocytes had to be obtained in both groups. With an anticipated mean of seven oocytes per patient, the inclusion of 50 patients in each study group was amply sufficient for this purpose. Statistical comparisons were made with non-parametric methods (Mann–Whitney U-test and contingency table analysis). All mean values mentioned are accompanied by the standard error (SE), unless indicated otherwise.

**Results**

Due to the minimization procedure, the mean age of the patients from both groups was comparable (Table I). Six patients from group 1 and one patient from group 2 had no ovum retrieval because of a poor ovarian response. These patients were excluded from the study. In one patient from group 1, the stimulation was interrupted because of a high risk for severe ovarian hyperstimulation syndrome. In group 2, one more patient was cancelled because of destabilized diabetes mellitus type I. Therefore, a total of 48 patients in group 1 underwent oocyte retrieval, and 43 patients in group 2. In one patient from group 1, the fresh embryo transfer was cancelled for risk of severe ovarian hyperstimulation. All embryos were cryopreserved for a later transfer. This patient was excluded when pregnancy and implantation rates were calculated.

With the standard stimulation protocol as described above (mean total dose of HMG: 40.94 ± 2.63 ampoules; mean duration of stimulation: 11.67 ± 0.40 days), a mean of 10.83 ± 0.95 oocytes per patient were obtained in group 1, compared to 8.44 ± 0.93 in group 2. This difference is borderline statistically significant (P = 0.05). A mean of 9.02 ± 0.80 oocytes (83.3%) in group 1 and 7.09 ± 0.71 oocytes (84.0%) in group 2 was used for ICSI (P = 0.08). Prerequisites for performance of ICSI were an intact zona pellucida and the presence of the extruded first polar body. In group 1, 1.81 ± 0.31 oocytes (16.7%) were germinal vesicle or metaphase I oocytes and could not be used for ICSI, compared to 1.35 ± 0.30 oocytes (16.0%) in group 2 (P = 0.11). Fertilization results are listed in Table II. Embryo quality was scored 40 h after the ICSI procedure according to the scoring system described by Puissant et al. (1987). The mean number of excellent + good quality embryos (score 5 and 4) per pick-up was significantly higher in group 1 (4.63 ± 0.49 versus 2.98 ± 0.38, P = 0.02). The ratio between the number of excellent + good quality embryos and the total number of embryos was also significantly higher in group 1 (excellent + good quality embryos: 222/342 (64.9%) in group 1 versus 125/235 (53.2%) in group 2, P = 0.004). Similar results were obtained when the number of excellent quality embryos (score 5) between the two groups or the total embryo scores (sum of the individual scores of all obtained embryos) were compared (Table II). The data on the embryo transfer and quality of

<table>
<thead>
<tr>
<th>Table I. Minimized randomization</th>
<th>Group 1</th>
<th>Group 2</th>
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<tbody>
<tr>
<td>Age &lt;35 extreme OAT</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>failed IVF</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Age ≥34 extreme OAT</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>failed IVF</td>
<td>15</td>
<td>15</td>
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<tr>
<td>Total</td>
<td>50</td>
<td>50</td>
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<tr>
<th>Table II. Results</th>
<th>Group 1</th>
<th>Group 2</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>Oocytes (n)</td>
<td>10.83 ± 0.93</td>
<td>8.44 ± 0.93</td>
<td>0.05</td>
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<tr>
<td>Oocytes injected (n)</td>
<td>9.02 ± 0.80</td>
<td>7.09 ± 0.71</td>
<td>0.08</td>
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<tr>
<td>Immature oocytes (n)</td>
<td>1.81 ± 0.31</td>
<td>1.35 ± 0.30</td>
<td>0.11</td>
</tr>
<tr>
<td>1–3 PN pre-embryos/2 PN pre-embryos (n)</td>
<td></td>
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<tr>
<td>0.58 ± 0.12</td>
<td>0.51 ± 0.11</td>
<td>0.08</td>
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<td>28/433 (6.5)</td>
<td>22/305 (7.2)</td>
<td>0.71</td>
<td></td>
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<tr>
<td>Injected oocytes</td>
<td></td>
<td></td>
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<tr>
<td>5.13 ± 0.65</td>
<td>5.53 ± 0.60</td>
<td>0.08</td>
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<tr>
<td>(79.0)%</td>
<td>(78.0)%</td>
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<tr>
<td>Excellent quality embryos (n)</td>
<td>1.75 ± 0.35</td>
<td>0.67 ± 0.14</td>
<td>0.03</td>
</tr>
<tr>
<td>Excellent + good quality embryos (n)</td>
<td>4.63 ± 0.60</td>
<td>2.98 ± 0.38</td>
<td>0.02</td>
</tr>
<tr>
<td>Excellent quality embryos/total number of embryos</td>
<td>84/342 (24.6)</td>
<td>28/235 (11.9)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Excellent + good quality embryos/total number of embryos</td>
<td>222/342 (64.9)</td>
<td>125/235 (53.2)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

* Mann–Whitney U-test.
* Contingency table analysis.
* n = 48.
* n = 47.
* n = 43.
* n = 42.

Figures in parentheses are percentages.
transferred embryos can also be found in Table II. The number of embryos available for cryopreservation was significantly higher in group 1 (2.70 ± 0.45 in group 1 versus 1.44 ± 0.37 in group 2, \( P = 0.03 \)).

In group 1, 13 pregnancies were obtained compared to 18 in group 2 (pregnancy rate per cycle 36% in group 2 versus 26% in group 1, \( P = 0.28 \); pregnancy rate per transfer 43% in group 2 versus 28% in group 1, \( P = 0.14 \)). In group 1, seven of the 13 pregnancies were twin pregnancies. However, three of these were vanishing twins. All other pregnancies in group 1 were ongoing singletons. In group 2, only one twin pregnancy occurred and five of the 17 other pregnancies ended in a first trimester miscarriage. Overall, 19 out of 103 replaced embryos (18.4%) implanted in group 2, compared to 20 out of 113 (17.7%) in group 1 (\( P = 0.89 \)). The 'ongoing implantation rate' (number of evolutive gestational sacs/number of replaced embryos) was 14/103 (13.6%) in group 2 and 17/113 (15.0%) in group 1 (\( P = 0.77 \)).

Discussion

From our data it appears that transport of oocytes over a large distance is possible without compromising the clinical results of IVF/ICSI. Transport of oocytes from a clinical IVF centre to a central IVF laboratory where the fertilization procedure takes place has several potential advantages. It enables the infertile couples to undergo the major part of their IVF treatment near their home town, increasing the accessibility of IVF and decreasing travelling time and expenses. High quality IVF and \( a \ fortiori \) ICSI not only requires rather expensive laboratory equipment and skilled personnel but also a high turnover of cycles to maintain the expertise (Egbase et al., 1996). Hence, co-operation between a clinical IVF centre and a central laboratory is a form of symbiosis which can be of the greatest benefit for both centres as well as for the patient. Our data also demonstrate that a clinical IVF centre can dispense with any laboratory facilities. Indeed, in the reported transport-setting no oocyte identification, washing or incubation in culture medium was performed before transport.

Although the allocation of the patients was performed with the use of a minimization procedure for age and reason for infertility, there was a significant difference in number of oocytes obtained in the two groups, being lower in the group with oocyte transport. Although the equipment and technique of follicular puncture was identical between the two groups, the punctures were not performed by the same operators. In Rotterdam the oocyte retrievals were performed by residents from the Department of Obstetrics and Gynaecology, who rotated in the IVF programme for about 1 day every week whereas in Gent the oocyte retrievals were performed by team members of the IVF centre. Less expertise in follicular aspiration could therefore be one explanation for the lower number of oocytes obtained in Rotterdam. On the other hand, some oocytes might get lost at the time of identification of the oocytes after transport. Indeed, even when heparin is added to the follicular aspirates, blood clots are often present at the bottom of the vial, making it difficult sometimes to dissect the oocyte out of this debris. It is therefore possible that some oocytes remained trapped inside a blood clot and may have been overlooked, giving an additional explanation for the lower number of oocytes in the transport group.

Although fertilization rates and embryo implantation rates were similar in both groups, a significant difference in embryo quality was observed. This difference was evident on comparing the total embryo score per patient, the mean score per embryo, the mean number of good quality embryos per patient, the mean score per replaced embryo as well as the number of embryos available for cryopreservation. This constant finding indicates that irrespective of the number of oocytes, embryo quality might be affected by long distance oocyte transport. Even though the clinical results in terms of pregnancy rates and embryo implantation rates were not different, further investigation of the effect on embryo quality is warranted.

In conclusion, this randomized prospective study demonstrates that long distance transport of oocytes before ICSI does not influence fertilization rates, pregnancy rates and embryo implantation rates and provides a good model of co-operation between clinical IVF centres and fully equipped IVF laboratories.

References


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