A prospective randomized study comparing the outcome of in-vitro fertilization and embryo transfer following culture of human embryos individually or in groups before embryo transfer on day 2

Isabella Spyropoulou1, Christos Karamalegos2 and Virginia N.Bolton2,3

1Nuffield Department of Obstetrics and Gynaecology, John Radcliffe Hospital, Headington, Oxford and 2Assisted Conception Unit, Department of Obstetrics and Gynaecology, King’s College School of Medicine and Dentistry, Denmark Hill, London SE5 8RX, UK
3To whom correspondence should be addressed

A prospective randomized trial of in-vitro fertilization and embryo transfer was undertaken to investigate the reported beneficial effects of culturing preimplantation human embryos in groups, rather than individually. A total of 159 treatment cycles, in which the women were matched for age, basal gonadotrophin concentrations and number of previous attempts, were included in the study. Of these, 78 cycles were randomized to the ‘individual culture’ group, and 81 cycles were randomized to the ‘group culture’ group. The groups did not differ in terms of the median number of oocytes or embryos obtained per cycle. There was no statistically significant difference between the two groups in terms of treatment outcome, as assessed by pregnancies or clinical pregnancies.

Key words: co-culture/embryo/IVF/morphology/pregnancy rate

Introduction

Preimplantation mammalian embryos develop more slowly in vitro than in vivo (Bowman and McLaren, 1970; Harlow and Quinn, 1982; McKiernan and Bavister, 1994). This effect is almost certainly due to damage associated with suboptimal culture conditions, including the medium used, as well as fluctuations in pH and temperature. In early studies, very few human embryos derived by in-vitro fertilization (IVF) developed into fully expanded blastocysts in vitro (Bolton et al., 1989; Dokras et al., 1991) and, while partially attributable to inherent defects, this was cited as evidence for suboptimal culture conditions. Indeed, with modification of culture media, it is possible to obtain blastocyst formation rates of over 45% (Desai et al., 1997). It follows that any improvement of the culture system would be of benefit in terms of reducing the volume of medium in which a given number of embryos are cultured (Wiley et al., 1986; Paria and Dey, 1990; Canseco et al., 1992; Lane and Gardner 1992; Gardner et al., 1994; Kato and Tsunoda, 1994; Keefer et al., 1994). This suggests that such preimplantation embryos produce autocrine factors that promote development.

Two small studies have supported the notion of a beneficial effect of group culture in the human, in terms of increased cleavage rate and improved morphology (Moessner and Dodson, 1995), and in terms of the potential of embryos to implant and develop into viable pregnancies following embryo transfer (Almagor et al., 1996). In contrast, human embryos generated by IVF and cultured with ageing unfertilized oocytes developed less frequently to the morula stage or beyond than embryos cultured alone and, moreover, those blastocysts which did develop contained fewer cells than controls (Salahuddin et al., 1995). This suggests that factors released by degenerating, unfertilized oocytes have a detrimental effect on preimplantation embryogenesis.

The present prospective randomized study extends these earlier investigations by comparing embryo morphology and IVF outcome in 159 cycles of IVF and embryo transfer during which preimplantation human embryos were cultured either individually or in groups.

Materials and methods

Patients

Women (n = 159) undergoing therapeutic IVF at the Assisted Conception Unit of King’s College Hospital, London, UK were recruited to the study, provided they were <38 years old, had a basal (day 1–5) follicle stimulating hormone concentration of <8 IU/l, had not undergone more than two previous failed cycles of IVF and embryo transfer, and were not having intracytoplasmic sperm injection. Informed consent was not obtained since there is no firm evidence to suggest any effect of culturing embryos singly or in groups, since embryos may be cultured routinely in either condition, depending on convenience. Patients were randomized prior to ultrasound-directed follicle aspiration for oocyte collection to either individual culture (IC) or group culture (GC) of the embryos that developed following IVF.

IVF and embryo culture

Ovarian stimulation and IVF were performed as described previously (Bolton et al., 1989; Waterstone and Parsons, 1992), except that the culture medium [Earle’s balanced salt solution (EBSS) containing 0.11 mg/ml pyruvate] was supplemented with 0.001% (v/v) synthetic serum replacement (SSR) and 1% (v/v) human serum albumin (HSA; Medicult Universal IVF Medium, Medicult, Copenhagen, Denmark). Oocytes were examined at 18–20 h post insemination (hpi), and those which had developed two pronuclei (2PN) were reducing the volume of medium in which a given number of embryos are cultured (Wiley et al., 1986; Paria and Dey, 1990; Canseco et al., 1992; Lane and Gardner 1992; Gardner et al., 1994; Kato and Tsunoda, 1994; Keefer et al., 1994). This suggests that such preimplantation embryos produce autocrine factors that promote development.

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The present prospective randomized study extends these earlier investigations by comparing embryo morphology and IVF outcome in 159 cycles of IVF and embryo transfer during which preimplantation human embryos were cultured either individually or in groups.
transferred individually (IC) or in groups of 3–5 (GC) into pre-
equilibrated 20-µl drops of medium overlayed with light paraffin oil
and incubated at 37°C in an atmosphere of 5% CO₂ in air. Those
cycles in which fewer than five oocytes developed 2PN were excluded
from the study (n = 151, of which 72 would have been in the IC,
and 79 in the GC group).

Morphological scoring system
Embryos were examined at 42–44 hpi and assigned a grade between
4 and 1 according to their morphology at the level of the light
microscope, with grade 4 embryos having perfect, spherical blasto-
meres with no extracelluar fragmentation, and grade 1 embryos
having barely defined blastomers and >50% fragmentation, as
described previously (Bolton et al., 1989). Each embryo was then
given a morphological ‘score’, derived from the product of the
number of blastomers and the grade of the embryo (Steer et al.,
1992). Thus, the minimum score was ‘0’, assigned to necrotic
embryos, and the maximum score was 24, assigned to 6-cell-stage
grade 4 embryos.

Cycle outcome
The two or three cleavage-stage embryos with the highest morpho-
logical score were selected for transfer, which was performed be-
 tween 46 hpi and 49 hpi. A qualitative test for human chorionic gonado-
trophin (HCG) in urine was performed 14 days following embryo
transfer using a commercial kit (Clearview, Unipath, Bedford, UK),
and clinical pregnancy was confirmed 3 weeks later by the identifica-
tion of one or more beating fetal hearts using ultrasound.

Statistical analysis
Statistical analysis was performed to compare the two groups in
terms of implantation rate using the χ² test, and to compare all other
parameters using the non-parametric Mann–Whitney test. Significance
was set at P < 0.05 for both tests.

Results
A total of 159 treatment cycles were included in the study, of
which 78 were randomized to IC, and 81 to GC. The patients’
ages, the number of oocytes collected per cycle, the number
which developed 2PN, and the number of embryos transferred
per cycle are shown in Table I. There were no statistically
significant differences between the two groups in any of these
parameters. The outcomes of the treatment cycles are given in
Table II, again showing no significant differences between the
two groups in terms of the number of cycles resulting in the
transfer of two and three embryos, the number of each resulting
in positive HCG, and in clinical pregnancy. Implantation
data are shown in Table III. No significant differences were
observed between the two groups in terms of the number of
embryos which implanted overall, or in the number of embryos
which implanted, or led to a clinical pregnancy, in the
subgroups of patients who became pregnant, and in whom
clinical pregnancies were established.

The characteristics of the embryos cultured in each group
are given in Table IV, and show no significant differences
between the groups in the rate of cleavage or embryo score,
either in the total population of embryos or among those
selected for transfer.

Discussion
The present study compares the development of human
embryos cultured individually with those cultured in groups
of between three and five. While extending the two earlier
studies which have reported an advantageous effect of group
culture, in terms of the cleavage rate and overall morphology
of cleavage-stage embryos (Moessner and Dodson, 1995), and
in terms of the embryos’ implantation potential (Almagor et al.,
1996), the present findings fail to confirm these results.
Moreover, even when the subgroups of patients who became
pregnant are compared, thereby ruling out any negative effect
of a non-receptive uterus, the present study fails to demonstrate
any differences between the two groups in terms of implantation
rates per embryo transferred.

Although all the studies were randomized prospectively,
both the earlier studies included a relatively small number of
treatment cycles (55 and 91 respectively), compared with 159
in the present study. Moreover, while one study reported the
effect of the two culture conditions on embryogenesis in vitro
prior to embryo transfer, but not on pregnancy outcome
following embryo transfer (Moessner and Dodson, 1995), the
other reported primarily the outcome following embryo transfer
and gave only vague descriptions of embryo morphology
(Almagor et al., 1996). Indeed, in the latter study, between
three and five embryos were transferred, yet the authors do
not report whether or not there were differences between the

<table>
<thead>
<tr>
<th>Culture group</th>
<th>IC</th>
<th>GC</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of treatment cycles</td>
<td>78</td>
<td>81</td>
</tr>
<tr>
<td>Median age of patients (years; range)</td>
<td>32 (25–37)</td>
<td>32 (25–37)</td>
</tr>
<tr>
<td>Median no. oocytes (range)</td>
<td>13 (5–34)</td>
<td>15 (5–39)</td>
</tr>
<tr>
<td>Median no. 2PN embryos (range)</td>
<td>8.5 (5–22)</td>
<td>9 (5–26)</td>
</tr>
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</table>

*No significant difference between any parameters (Mann–Whitney test).
Table III. Implantation rate per embryo transferred, following individual culture (IC) or group culture (GC) of embryos prior to transfer

<table>
<thead>
<tr>
<th>Culture group</th>
<th>IC*</th>
<th>GC*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. embryos transferred</td>
<td>181</td>
<td>190</td>
</tr>
<tr>
<td>No (%) implantation sites*</td>
<td>42 (23)</td>
<td>36 (21)</td>
</tr>
<tr>
<td>No (%) gestation sacs</td>
<td>38 (21)</td>
<td>32 (17)</td>
</tr>
<tr>
<td>No (%) fetal hearts</td>
<td>37 (20)</td>
<td>29 (15)</td>
</tr>
<tr>
<td>Total no. embryos transferred in patients who became pregnant</td>
<td>75</td>
<td>61</td>
</tr>
<tr>
<td>No (%) implantation sites* in patients with positive HCG</td>
<td>42 (56)</td>
<td>36 (59)</td>
</tr>
<tr>
<td>No (%) gestation sacs in patients with positive HCG</td>
<td>38 (51)</td>
<td>32 (52)</td>
</tr>
<tr>
<td>No (%) fetal hearts in patients with positive HCG</td>
<td>37 (49)</td>
<td>29 (48)</td>
</tr>
<tr>
<td>Total no. embryos transferred in patients with clinical pregnancy</td>
<td>68</td>
<td>53</td>
</tr>
<tr>
<td>No. of fetal hearts in patients with clinical pregnancy (% of embryos transferred)</td>
<td>37 (54)</td>
<td>29 (55)</td>
</tr>
</tbody>
</table>

*These include biochemical pregnancies which are considered a single implantation site.
*No significant difference between any parameters (χ² test).

HCG = human chorionic gonadotrophin.

Table IV. Morphological characteristics of embryos at 42–44 post insemination after individual culture (IC) or group culture (GC)

<table>
<thead>
<tr>
<th>Culture group</th>
<th>IC*</th>
<th>GC*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. of embryos</td>
<td>736</td>
<td>836</td>
</tr>
<tr>
<td>No. (%) of embryos cleaved</td>
<td>677 (92)</td>
<td>787 (94)</td>
</tr>
<tr>
<td>No. of embryos transferred</td>
<td>181</td>
<td>190</td>
</tr>
<tr>
<td>Median no. of blastomeres (range)</td>
<td>2 (1–6)</td>
<td>2 (1–5)</td>
</tr>
<tr>
<td>Median no. of blastomeres in embryos transferred (range)</td>
<td>3 (2–6)</td>
<td>3 (2–5)</td>
</tr>
<tr>
<td>Median embryo score (range)</td>
<td>8 (0–24)</td>
<td>8 (0–20)</td>
</tr>
<tr>
<td>Median embryo score in embryos transferred (range)</td>
<td>12 (4–24)</td>
<td>12 (4–20)</td>
</tr>
</tbody>
</table>

*No significant difference between any parameters (Mann–Whitney test).

It is surprising that the differences seen in animal experiments (Paria and Dey, 1990) are not found in the human in the present study. The earlier studies both used Ham’s F10 medium supplemented with 15% heat-inactivated human serum for embryo culture, compared with EBSS supplemented with 0.001% SSR and 1% HSA in the present study, but it is unlikely that this would explain the different findings.

It could be argued that the use of blastocyst formation as the end-point of the animal studies may have led to artefactual results (Bavister, 1995). However, this argument is based on the fact that prolonged culture may allow any slow-cleaving embryos to ‘catch up’ with more developmentally competent, faster-cleaving embryos, thus failing to identify differences between different experimental groups. Since the mouse studies of Paria and Dey (1990) did demonstrate differences in blastocyst formation rates between different experimental groups, this argument is not valid. In terms of prolonged culture, it is more likely that the benefits of group culture identified by the animal studies are only manifested by culture beyond the 2- and 4-cell stage, and that prolonged culture of human embryos, beyond the 24 h in the present study, would demonstrate similar advantages.

This explanation cannot be invoked, however, to explain the differences between the findings of the present and previous human studies. The most marked difference in experimental design of these studies is in the volume of culture medium used. Given the beneficial effect of using smaller volumes of culture media suggested by Paria and Dey (1990), we chose to use 20-µl drops of medium, in contrast to the 1000-µl and 700-µl drops used in the studies of Moessner and Dodson (1995) and Almagor et al. (1996) respectively. Thus, it is possible that any autocrine factor(s) secreted by human embryos are already sufficiently concentrated to have a growth-enhancing effect in a drop of 20 µl containing a single human embryo, and that higher levels of the same factor(s) in the presence of additional embryos will have no effect. Indeed, the mouse studies found that the growth-enhancing effect of exogenous epidermal growth factor (EGF) was maximal at a concentration of 10 ng/ml; at 200 ng/ml, EGF did not further improve embryo development (Paria and Dey, 1990). If, however, the factor(s) secreted by a single embryo cultured in a drop of 700 µl or 1000 µl are diluted to the point of having a reduced effect, it can be seen that the presence of additional embryos would increase the concentration of the postulated factor(s), thereby restoring their stimulatory effect. Although this seems a feasible explanation, the mouse experiments upon
which this study was based used drops of 25 µl containing 1, 5 or 10 mouse embryos (Paria and Dey, 1990), and it is difficult to explain why human and mouse embryos should differ so greatly in the levels of secretion of putative autocrine stimulatory factor(s).

In conclusion, the present study fails to support the suggestion from animal experiments, and from limited data from human studies, that the development of preimplantation human embryos in vitro is enhanced when embryos are cultured in groups rather than individually. Further studies using the same experimental design but with embryos cultured in drops of larger volumes will be undertaken.

References