The culture of human cleavage stage embryos alone or in groups: effect upon blastocyst utilization rates and implantation

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SUMMARY

The effect of cleavage-stage group culture (CGC; embryos cultured in groups of three or more for the first 3 days and then individually to blastocyst) was compared to extended single embryo culture (ESC; embryos cultured individually to the blastocyst stage). While implantation and ongoing pregnancy rates were similar between groups, the blastocyst utilization rate (number of blastocysts suitable for freezing and thawing/total number of embryos cultured to Day 5 and 6) was significantly higher when embryos were cultured in CGC for women <35 yrs thereby increasing the number of embryos available for clinical use for the younger women. This strategy of group culture to Day 3 would seem an ideal protocol to capitalize on an overall embryo quality in two particular settings, namely programmes wishing to
(i) undertake Day 3 transfers, and (ii) keep embryos separate from Day 3 to Day5/6 for the purposes of selection. The culture system can also be applied to the embryos of older women without adverse effect, enabling the same system to be used for all embryos. Reproductive Biology 2010 10 3: 227–234.

Key words: human, embryo, group culture

INTRODUCTION

The development of sophisticated sequential culture media within in vitro fertilization (IVF) programmes has resulted in significant improvements in the quality of human embryos compared to those obtained in relatively simple media used originally for iv vitro culture. However, autocrine and paracrine pathways present within mammalian embryos [8] provide positive signals that are important considerations over and above the provision of better medium ingredients when designing a culture system. Accordingly, blastocyst development has been improved in animal models by modifying the basic embryo culture system in a number of ways to maximize the potential of these signals, such as minimizing the dilution of beneficial factors with the use of reduced volumes of culture medium, increasing cellular support by either the co-culture of single embryos with somatic cells, or the culture of embryos in groups [10]. The use of a group culture system to the blastocyst stage in humans is not always possible, for example when transferring embryos on Day 3 (a practice still used widely around the world; [6]), using mathematical models to predict blastocyst transfer cancellation (where individual embryos need to be monitored; [4]), or using metabolomic analysis to select the best embryo for transfer (in which embryos need to be cultured individually to allow examination of the spent medium for each embryo; [2]). However, the many events occurring within the embryo up until the 8-cell stage [3] would suggest this is an important time in the development of the embryo. The present study has therefore attempted to determine the advantage of a culture system whereby the potential benefit of embryo group culture up to Day 3 is combined with individual culture through to Day 5/6 for selection purposes.
MATERIALS AND METHODS

IVF and intracytoplasmic sperm injection (ICSI) treatment cycles with three or more zygotes grown to Day 5 and 6 between 2007 and 2009 were included in the analysis; those between March 2007 and March 2008 had embryos cultured singly to Day 3 whilst the cycles between March 2008 and April 2009 had embryos cultured in groups of 3 or more to Day 3. Both groups had the embryos cultured singly from Day 3 to Day 5/6. Cycles with ≤2 zygotes were excluded as they were not eligible for group culture, having embryo transfers selectively on Day 3. Ovarian stimulation involved the use of the GnRH agonist Lucrin (Abbott Australasia, Botany NSW 2019, Australia) in a flare protocol followed by exogenous FSH as either Puregon (Organon Australia Pty Ltd, Lane Cove NSW 2066, Australia) or Gonal-f (Merck Serono Australia Pty Ltd, Frenchs Forest NSW 2086, Australia).

Embryos were cultured in 15 μl droplets of Cook media (Cook Australia Pty Ltd, Brisbane, Australia) under oil, in Fertilization Medium (Day 0 to Day 1) followed by Cleavage Medium (Day 1 to Day 3) and then Blastocyst Medium (Day 3 to Day 6). Two different protocols of embryo culture were compared, namely:

1. extended single embryo culture (ESC), where embryos were cultured singly from Day 1 to Day 5 or 6; and
2. cleavage stage group culture (CGC), where embryos were cultured in groups of 3 to 5 embryos in cleavage media (Day 1 to Day 3) followed by single embryo culture in blastocyst media (Day 3 to Day 5).

All embryos were assessed on Day 3, 5 and 6 when available. Blastocysts were graded on a 1–3 scale, based on a modified version of the Dokras grading system [5], with Day 3 morphology being used as an arbiter in choosing a blastocyst for transfer when presented with more than one of similar grade. The blastocyst utilization rate was calculated as the number of blastocysts suitable for freezing and transfer/total number of embryos cultured to Day 5 and 6. Grade 1 and 2 embryos were considered as usable, and included expanding, expanded, hatching and fully hatched blastocysts with evident trophectoderms and inner cell masses, allowing for minor degenerative areas. The utilization rate directly correlates with embryo grading and em-
bryo quality, as only morphologically good embryos (grades 1 and 2) were transferred and frozen.

All IVF practice complied with West Australian and Australian legislation, all protocols and consent forms were approved by the Reproductive Technology Council of Western Australia, and the Quality Management System met the standards set by the Reproductive Technology Accreditation Committee of the Fertility Society of Australia. Results were expressed according to the women’s age at the time of oocyte collection, as being <35 yrs or ≥35 yrs according to the Australian and New Zealand Assisted Reproduction Database (ANZARD; [15]). Proportions were compared using the χ² test, and test results with p-values less than 0.05 were considered to be significant.

RESULTS AND DISCUSSION

The culture of groups of human embryos up to four per droplet to Day 5 is an accepted technique for growing blastocysts [7]. However, group culture is contraindicated in situations in which the progress of individual embryos is to be monitored, such as the calculation of the probability of failure of an embryo to reach the blastocyst stage [4] or the selection of blastocysts for single embryo transfer in conjunction with assessment at earlier stages [9]. Accordingly, we have not used group culture to Day 5 so that morphology and rate of development of Day 3 embryos may be used as an arbiter to select a single blastocyst for transfer when more than one of a similar grade has formed. The theoretical reasons and practical evidence of the benefit of group culture to Day 3 in the literature as mentioned earlier had prompted the present study to investigate the combination of group culture to Day 3 and then single culture thereafter in an attempt to obtain the best of both culture strategies, although it is acknowledged that this approach is not suitable for units wishing to assess pronuclear morphology and keep embryos separated after fertilization [13]. More recently, the analysis of spent medium has been proposed to select the best blastocyst giving another scenario where single embryos should be cultured from Day 3 to Day 5/6. The central laboratory
Table 1. The outcome of cycles when embryos were cultured up to Day 3 either alone (ESC) or in groups (CGC) and then singly to Day 5/6

<table>
<thead>
<tr>
<th>Parameter</th>
<th>&lt; 35 yrs old</th>
<th>≥35 yrs old</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ESC</td>
<td>CGC</td>
</tr>
<tr>
<td>Pregnancies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of transfers</td>
<td>142</td>
<td>161</td>
</tr>
<tr>
<td>positive (βhCG; %)</td>
<td>67 (47.2%)</td>
<td>75 (42.1%)</td>
</tr>
<tr>
<td>on-going (%)</td>
<td>54 (38.0%)</td>
<td>65 (40.4%)</td>
</tr>
<tr>
<td>Embryo implantation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. transferred</td>
<td>144</td>
<td>178</td>
</tr>
<tr>
<td>No. fetal hearts (%)</td>
<td>55 (38.2%)</td>
<td>74 (41.6%)</td>
</tr>
</tbody>
</table>

On-going pregnancies include all patients who presented with at least one fetal sac and one fetal heart at 7 weeks.

within the Sydney IVF group, of which Hollywood Fertility Centre is part, has already introduced metabolomics to its repertoire with embryos being cultured in groups to Day 3 and singly thereafter [12].

The culture of embryos in groups up until Day 3 in the current study did not seem to improve the functional quality of the embryos transferred compared with those embryos cultured singly, as judged by similar implantation and pregnancy rates (tab. 1). This is consistent with an earlier report where embryos were cultured alone or in groups prior to transfer on Day 2 or Day 3 [14], but contrary to a study using group culture which produced pregnancy rates more than double that of embryos grown individually [1]. However, the real benefit of early group culture in the present study was the increase in the proportion of blastocysts that could be utilized, i.e. the proportion of embryos that were suitable for transfer and freezing on Day 5 or 6. The blastocyst utilization rate was significantly higher in the group of younger women when the embryos were cultured singly (p<0.05) but no different for the older women (p=0.11), as shown in Table 2. This increase in the number of good blastocysts after a relatively short group may well
Table 2. Embryology parameters of the analyzed cycles

<table>
<thead>
<tr>
<th>Parameter</th>
<th>&lt; 35 yrs old</th>
<th>≥35 yrs old</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ESC</td>
<td>CGC</td>
</tr>
<tr>
<td>Number of women</td>
<td>143</td>
<td>193</td>
</tr>
<tr>
<td>Number of oocyte collections</td>
<td>162</td>
<td>222</td>
</tr>
<tr>
<td>Total number of oocytes</td>
<td>1733</td>
<td>2829</td>
</tr>
<tr>
<td>Total number of zygotes</td>
<td>1163</td>
<td>1685</td>
</tr>
<tr>
<td>Number of blastocysts transferred + cryopreserved</td>
<td>144 + 397</td>
<td>178 + 687</td>
</tr>
<tr>
<td>Number of usable blastocysts</td>
<td>541 (46.5%)</td>
<td>865 (51.3%)*</td>
</tr>
</tbody>
</table>

Cycles were categorized according to age of the woman at the start of treatment as those being <35yrs or ≥35yrs of age, with embryos being cultured for the first three days either singly (ESC) or in groups (CGC) and then singly to Day 5/6;

*p<0.05 compared to the ESC for the same age group
benefit programmes performing Day 3 transfers [11] by improving the overall embryo quality and increasing the number of good embryos being cryopreserved. There was no difference in the group of older women, suggesting that the grouping of embryos up to Day 3 would not have an adverse effect and the same culture system can be applied to the embryos of all women.

In summary, the present study has shown a benefit for women <35 yrs old of culturing embryos in groups of three or more up to day three even when cultured singly thereafter, with the proportion of embryos that reached the blastocyst stage that could be used for transfer or cryopreservation being increased. This strategy of group culture to Day 3 would seem an ideal protocol in two particular settings, namely programmes wishing to (i) undertake Day 3 transfers, and (ii) keep embryos separate from Day 3 to Day5/6 for the purposes of selection. The culture system can also be applied to the embryos of older women without adverse effect, enabling the same system to be used for all embryos.

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REFERENCES


