Is there any benefit from the culture of a single oocyte to a blastocyst-stage embryo in unstimulated cycles?

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BACKGROUND: The aim of the study was to test the influence of 2- and 5-day cultivation of a single oocyte on the pregnancy rate in a non-stimulated cycle. METHODS: A retrospective chart review of 391 consecutive patients undergoing IVF and intracytoplasmic sperm injection in unstimulated cycles was performed. The embryos were kept in MediCult universal IVF medium for day 2 transfers and in BlastAssist System for day 5 transfers. RESULTS: The oocyte recovery rate in the group for 2-day cultivation and in the group for 5-day cultivation was similar, being 79.4 (162/204) and 83.6% (154/187) respectively. The same is true of the fertilization rate (73.8 versus 77.7%). The blastulation rate was 52.8%. The embryo transfer rate per cycle was higher when day 2 embryos were transferred: 64.8% (105/162) compared with 35.7% (55/154) if blastocyst-stage embryos were transferred. The pregnancy rate per transferred embryo was higher when a blastocyst was transferred (40.0%) instead of a day 2 embryo (23.8%). CONCLUSION: The expected pregnancy rate calculated per embryo available on day 2 of cultivation was similar in both groups (23.8 versus 22.2%) and it was not affected by oocyte culture to the blastocyst stage.

Key words: blastocyst/embryo transfer/sequential media/single embryo/unstimulated cycle

Introduction

The premature placement after IVF to the uterus of a cleavage-stage human embryo in the early stage of development can result in impaired development (Gardner, 1998). Therefore, the implantation rate per embryo of ~20% reported in the literature remained constant for a decade. Blastocyst embryo transfer was used initially to increase the implantation rate with a reduced number of transferred embryos (Gardner et al., 1998a). The selection of blastocysts for transfer in a stimulated cycle resulted in pregnancy more frequently than if earlier stage embryos were transferred (Milki et al., 2000).

The use of blastocyst culture in human IVF cannot be considered a new idea. Human embryos have been cultured in simple culture media (Bolton et al., 1991) or in ‘co-culture’ systems (Ménézo et al., 1992) up to the blastocyst stage. Unfortunately, the implantation rates did not differ from those when an embryo was transferred to the uterus on day 2 or 3 of development, or they were worse. It was reported (Bolton et al., 1991) that in 40% of embryos it was possible to obtain blastocyst development using a simple culture medium, resulting in a pregnancy rate of only 7%.

The development of physiologically based sequential culture media followed the changing requirements of the embryo during its development. The implantation rate of human embryos cultured in this type of media was up to 50%, and was associated with high pregnancy rates of >60% when two blastocysts were transferred (Gardner et al., 1998b; Schoolcraft et al., 1999).

The effect of sequential media on the developmental potential of the resulting blastocyst and the possible influence on the implantation rate of human embryos was tested on embryos generated from stimulated cycles. The aim of our study was to test the potential benefit from the culture of a single oocyte, obtained from an unstimulated cycle, to a blastocyst-stage embryo. Such an approach eliminates the influence of blastocyst selection—as is the case in stimulated cycles—on the implantation rate per embryo transfer. So far, data on single-blastocyst transfer obtained from unstimulated cycles have not been published.

Materials and methods

All patients with aspirated oocytes from unstimulated cycles were included in the study. The patients were self-selected for unstimulated cycles from the waiting list for a stimulated cycle after they had received an invitation to participate in the study. This study was a retrospective chart review of patients undergoing IVF or intracytoplasmic sperm injection (ICSI) in unstimulated cycles between January 1999 and August 2000. The patients were divided into two groups.
Between January and October 1999, all collected oocytes were cultured for 2 days in simple medium before embryo transfer. After this period all collected oocytes were cultured in sequential culture medium for 5 days. Both groups were balanced for patient age, rate of IVF and ICSI cycles in the group and infertility characteristics. All patients signed an informed consent form to participate in the study, which was approved by the local ethics committee.

The same monitoring protocol was used before oocyte retrieval in all patients (Reljic and Vlaisavljevic, 1999).

Folliculometry was performed with a 5 MHz probe (Aloka, 1700, Tokyo, Japan). The first ultrasound examination was done on day 2 of the menstrual period. After that, follicular growth was monitored by serum oestradiol only on days 5 and 7 of the menstrual cycle. When the oestradiol concentration reached 0.49 nmol/l, the follicular diameter was measured. When the mean follicular diameter reached ≥15 mm at an oestradiol concentration of 0.49 nmol/l, the criteria for human chorionic gonadotrophin (HCG; Profasi, Serono, Aubonne, Switzerland) application were fulfilled.

The presence of urinary LH in the morning urine was checked using the Rapi Test LH (Morwell, Switzerland). A positive test on the day before HCG administration was an indication for cycle cancellation.

Follicle aspiration was done 35 h after HCG administration. Only the dominant follicle was aspirated. All follicles were flushed during oocyte retrieval. In the first hour after retrieval, the oocytes for the ICSI procedure were exposed to 80 IU/ml of hyaluronidase for 10–15 s (MediCult, Jyllinge, Denmark). The surrounding cumulus cells were stripped off the oocytes by aspiration through a pipette. Those oocytes which were in metaphase II were injected immediately after the procedure. Sperm injection was performed after sperm immobilization using polyvinylpirrolidone (PVP) (MediCult). ICSI was performed on an inverted microscope (Olympus, Japan) using Narishige micromanipulators with micropipettes (Hunter Scientific, Safron Walden, UK). The injected oocytes were rinsed and placed in a droplet of universal IVF culture medium (MediCult) covered with liquid paraffin (MediCult). Fertilization was assessed 15–18 h after insemination. On day 2 of the culture, the embryo cell number and morphology scores were registered. A numerical score from 1–4 was given, based upon blastomere morphology, number and presence of anuclear fragmentation (G1: up to 10% of fragments inside the embryo, G2: between 10–20%, G3: between 20–50% and G4: >50% of fragments). The rate of embryo development was evaluated according to the number of blastomeres. All embryos with ≥2 blastomeres on day 2 were considered to be rapidly developing embryos. Embryos with two blastomeres on day 2 were evaluated as slowly developing embryos.

In the first part of the study, all oocytes and embryos for IVF and ICSI were cultured in universal IVF medium (MediCult) and covered with liquid paraffin (MediCult). On day 2 of culture, the embryo cell number and morphology were scored. All normally fertilized embryos were transferred using a K-4000 soft catheter (Cook, Brisbane, Australia).

In the second part of the study, all embryos were cultured for 5 days in sequential BlastAssist System (MediCult) before embryo transfer. After fertilization was assessed, the embryo was placed in a new droplet of B1 medium covered with liquid paraffin (MediCult). On day 3 of culture, the embryo was transferred to a droplet of B2 medium for a further 48 h of development. On day 5 of culture, the blastocyst was assessed using the scoring system for blastocyst expansion and hatching status by Gardner and Schoolcraft (Gardner and Schoolcraft, 1999). A numerical score from 1 (the blastocoel taking up less than half the embryo volume) to 5 (hatching blastocyst, the trophectoderm starting to herniate through the zona) based upon the degree of expansion and hatching status was given to each blastocyst. Embryos that could not attain the blastocyst stage on day 5 were transferred on day 6 if the development from the compact morula stage to the blastocyst stage was completed.

During the procedure, the embryo transfer catheter was loaded directly from the culture medium covered with liquid paraffin (MediCult).

Serum oestradiol concentrations were measured by enzyme immunoassay (AxSYM Estradiol Assay; Abbott).

The luteal phase was supported by 1500 IU of HCG (Pregnyl; Organon, The Netherlands) on day 5 after oocyte retrieval.

Implantation was confirmed by determination of serum β-HCG 16 days after oocyte retrieval. Pregnancy was confirmed as clinical only in case of ultrasonic evidence of a gestation sac. Luteal support was the same for all pregnant patients in both groups. All pregnant women continued with luteal supplementation with a daily dose of 30 mg of didrogesteron (Dabroston; Duphare, The Netherlands) until week 8 of pregnancy.

The data were processed using the Statistica statistical program (StatSoft, USA). The analysis of discrete variables was done by χ² analysis and Student’s t-test was used for parametric analysis. Statistical significance was set at P < 0.05.

**Results**

In 204 cycles, a total of 162 oocytes were aspirated and cultivated in the 2-day culture system. From among 85 oocytes obtained from ICSI cycles, 81 were successfully denuded and 76 in metaphase II were injected. Fertilization occurred in 73.8% (113/153) of oocytes. The replacement of 105 embryos resulted in 25 clinical pregnancies.

### Table I. Distribution between patients with day 2 versus day 5 transfers with respect to age, diagnosis, fertilization rate and ratio of IVF/ICSI cases

<table>
<thead>
<tr>
<th>Patients (n)</th>
<th>Day 2 embryo transfer</th>
<th>Day 5 embryo transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years, mean ± SD)</td>
<td>32.2 ± 4.0</td>
<td>31.7 ± 4.4*</td>
</tr>
<tr>
<td>IVF/ICSI cases (rate)</td>
<td>0.94</td>
<td>0.83</td>
</tr>
<tr>
<td>Fertilization (%)</td>
<td>73.8</td>
<td>77.7*</td>
</tr>
<tr>
<td>Diagnosis (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>86</td>
<td>62</td>
</tr>
<tr>
<td>Tubal</td>
<td>72</td>
<td>55</td>
</tr>
<tr>
<td>Male/tubal</td>
<td>31</td>
<td>33</td>
</tr>
<tr>
<td>Unexplained</td>
<td>12</td>
<td>29</td>
</tr>
<tr>
<td>Others</td>
<td>3</td>
<td>8</td>
</tr>
</tbody>
</table>

*The differences between two groups were not significant.

### Table II. Effect of embryo grade and stage on day 2 on the implantation rate after day 2 embryo transfer

<table>
<thead>
<tr>
<th>Embryo grade and stage on day 2</th>
<th>Embryos transferred (%)</th>
<th>Embryos implanted</th>
<th>Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good G1 and G2</td>
<td>Fast (≥2 cells) 51</td>
<td>15</td>
<td>32.3</td>
</tr>
<tr>
<td>Slow (2 cells) 20</td>
<td>8</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Bad G3 and G4</td>
<td>Fast (≥2 cells) 22</td>
<td>2</td>
<td>5.9</td>
</tr>
<tr>
<td>Slow (2 cells) 12</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
The developmental potential of embryos to reach the blastocyst stage with regard to morphological grading and number of blastomeres on day 2 was higher in embryos with >2 blastomeres and a higher morphological score (Table IV). A total of 52 blastocysts developing from 90 morhologically good quality embryos resulted in 21 pregnancies (40.4%). Seventyone morphologically good quality embryos transferred on day 2 resulted in 23 clinical pregnancies (32.4%).

The expected pregnancy rate per embryo transfer of a normally fertilized embryo was calculated for the 5-day cultivation group, presuming the embryo would be transferred on day 2. This calculated pregnancy rate per cycle was not significantly higher in the 5-day cultivation group than in the 2-day cultivation group.

Discussion

The extended embryo culture includes natural embryo selection, better synchronization between the embryo and the endometrium and better assessment of the embryo implantation potential before transfer. The development of viable blastocytes is affected by the patient’s age and declines significantly in women >40 years (Pantos et al., 1999). Delayed and arrested embryonic morphological development can be the result of major genetic and chromosomal anomalies that are incompatible with cleavage. Such anomalies can be found in one-fourth of preimplantation embryos (Plachot et al., 1988).

The blastocyst transfer had a higher implantation and pregnancy rate compared with day 3 embryo transfer when special sequential culture media were used for prolonged embryo culture (Del Marek et al., 1999; Milki et al., 2000). Similar pregnancy rates, implantation and twinning rates after prolonged cultivation in sequential media were reported (Coskun et al., 2000). The findings were that day 5 transfers have no advantages over day 3 transfers. Huisman et al. did not report higher pregnancy and implantation rates after embryo selection following an extended culture period in a non-sequential medium (Huisman et al., 2000).

This study confirms previously published data showing that the transfer of blastocysts results in high pregnancy rates per embryo transferred, not traditionally obtained with the transfer of earlier-stage cleavage embryos. As a result of single-oocyte culture to a blastocyst-stage embryo, good pregnancy rates are achieved by the transfer of only one embryo even in an unstimulated cycle. In contrast to stimulated cycles, in unstimulated cycles all blastocyst-stage embryos are transferred irrespective of their quality. In stimulated cycles only the best quality blastocysts are selected and transferred. An additional difference is the high cancellation rate in unstimulated cycles, which is a consequence of embryo self-selection to attain the blastocyst stage. Reports suggest a much lower cancellation rate when blastocyst culture is applied in patients with >1 normally fertilized oocyte (Coskun et al., 2000).

The clinical pregnancy, implantation and blastulation rates
reported here are comparable to those reported in the literature related to the use of sequential media (Gardner et al., 1998b; Khorram et al., 2000). Although blastocyst culture shows the highest implantation rates in the literature, data on single-blastocyst transfers have not been published yet. When only one top-quality blastocyst was available for transfer in a stimulated cycle, the pregnancy rate was 69.6% in comparison with 86.8% when two top-scoring blastocysts were transferred (Gardner et al., 2000). The implantation rates are higher in the transfer of expanded blastocysts with visible inner cell mass than in unexpanded blastocysts (Shapiro et al., 2000).

In our study, the best results were recorded in the group where blastocysts were selected from day 2 good quality embryos. Blastocysts developed in 64.8% (48/74) of such embryos. A 50% (10/20) pregnancy rate per transfer was recorded in the group of patients with expanded, hatched blastocysts and 38.8% (7/18) among patients with unexpanded blastocysts for embryo transfer. Our data show that waiting until day 5 to transfer embryos after IVF or ICSI in unstimulated cycles will have no deleterious effect on the pregnancy rate per aspirated oocyte. The implantation rate per transfer was higher when blastocysts were transferred. The hypothetic pregnancy rate calculated from the number of two pronucleate (2PN) zygotes entering the study shows similar results in both groups (23.8 versus 22.2%). It can be speculated that a sequential medium did not increase the potential of 2PN embryos for implantation, but only supported the development from day 2 to the blastocyst stage of some embryos anticipated for implantation.

In contrast to the situation in stimulated cycles where blastocyst cultivation will ease embryo selection for transfer, in unstimulated cycles with only available embryo the procedure would select patients with a more successful IVF outcome. In unstimulated cycles a high cancellation rate before embryo transfer can be expected in contrast to stimulated cycles where blastocyst culture is applied to numerous normally fertilized oocytes. This procedure prevents some patients from waiting an additional 2 weeks for confirmation of a negative result of embryo transfer. However, there is no increase in the overall pregnancy rate per cycle.

A challenge in transfer policy in stimulated cycles—to transfer only one embryo at a time—would probably be the future method of choice to reduce the number of multiple births after IVF and ICSI. Such a strategy has not been widely tested because of the expectation of clinician and patient that it could affect a decrease in pregnancy rate per embryo transfer. However, some theoretical models for one-embryo transfer in selected groups of patients at high risk of multiple births have been discussed (Coetsier and Dhont, 1998; Strandell et al., 1999; Van Royen et al., 1999). A pregnancy rate of 29.7% was reported in a selected group of patients after day 2 or 3 one-embryo transfer (Vilska et al., 1999). These results were similar to those in programmes in which two embryos selected from many were transferred. When ≥2 blastocysts are transferred, a high percentage of multiple gestations is possible and the expected benefits of this technique, when used to reduce the multiple pregnancy rate, are lost (Milki et al., 1999, 2000).

Our study demonstrates that the implantation rate per embryo after the transfer of one embryo in a non-selected group of patients resulted in a high pregnancy rate when blastocyst-embryo transfer was performed. In all cases this embryo was the only one available for transfer. It can be supposed that in cases where >1 blastocyst is available from stimulated cycles, this approach is more effective and therefore should be adopted to avoid multiple pregnancies after the transfer of two blastocysts.

### References


### Table IV: Developmental potential of embryos with different grade and stage on day 2 to attain the blastocyst stage on day 5

<table>
<thead>
<tr>
<th>Embryo stage on day 2</th>
<th>G1,G2/&gt;2 cell</th>
<th>G1,G2/2 cell</th>
<th>G3,G4/&gt;2 cell</th>
<th>G3,G4/2 cell</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>74</td>
<td>16</td>
<td>6</td>
<td>3</td>
<td>99</td>
</tr>
<tr>
<td>Morulae* B1</td>
<td>1 (1)</td>
<td>1 (0)</td>
<td>6 (4)</td>
<td>3 (1)</td>
<td>14</td>
</tr>
<tr>
<td>B2</td>
<td>6 (2)</td>
<td>3 (1)</td>
<td>2 (1)</td>
<td>2 (1)</td>
<td>12</td>
</tr>
<tr>
<td>B3</td>
<td>6 (2)</td>
<td>3 (1)</td>
<td>2 (1)</td>
<td>2 (1)</td>
<td>12</td>
</tr>
<tr>
<td>B4</td>
<td>9 (2)</td>
<td>3 (1)</td>
<td>2 (1)</td>
<td>2 (1)</td>
<td>12</td>
</tr>
<tr>
<td>B5</td>
<td>20 (10)</td>
<td>6 (2)</td>
<td>1 (0)</td>
<td>2 (1)</td>
<td>12</td>
</tr>
<tr>
<td>Embryos transferred</td>
<td>48</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>55</td>
</tr>
<tr>
<td>Embryos implanted</td>
<td>20</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>22</td>
</tr>
</tbody>
</table>

*Transferred as blastocyst on day 6.
Day 2 versus day 5 transfers in unstimulated cycles


Received on April 30, 2001; accepted on August 10, 2001