Extended embryo culture reduces the implantation rate on day 4 and day 5 when only a maximum of three embryos are cultured beyond the pronuclear stage

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Abstract

Objectives: To study the potential of embryo transfer after 3, 4 or 5 days of embryo culture under the German embryo protection law according to which only a maximum of three zygotes are allowed to be cultured for embryo transfer.

Study design: In a prospective study, 273 patients with assisted reproductive treatment were randomly allocated for transfer on days 3, 4 or 5. Pregnancy and implantation rates were evaluated in regard to day of transfer and results were compared by Chi-square or ANOVA test.

Results: Out of 234 transfer cycles, 79 were performed on day 3, 76 on day 4 and 79 on day 5. Pregnancy and implantation rates were 41.8%/27.1% for transfer on day 3, 27.6%/14.1% for day 4 transfer and 16.5%/8.8% for transfer on day 5. These results were significantly different for pregnancy rates on day 3 versus day 5 (P < 0.001) and for implantation rates on day 3 versus day 4 (P < 0.005) and day 3 versus day 5 (P < 0.001).

Conclusions: These findings suggest that extended embryo culture is not beneficial when the option for embryo selection at later stages of development is not available.

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Keywords: Human; In vitro fertilization; Embryo transfer; Transfer on day 3, 4, 5; Sequential culture medium

1. Introduction

In most human in vitro fertilization (IVF) programmes, embryo transfer is routinely performed on day 2 or 3; however, implantation rates are low [1]. One attempt to increase the success rate of IVF might be the growth of human embryos up to the blastocyst stage and selection of the best embryos at that stage for embryo transfer. To achieve this goal, sequential culture media were developed which fulfil the differential requirements of the embryo during early preimplantation development [2,3]. Consequently, these media enabled blastocyst formation rates of up to 50% and due to extended culture those embryos with the best developmental potential were recognised more readily on day 5 [4]. Based on this approach, several studies reported higher implantation rates following day 5 transfer of selected blastocysts [5–8], whereas other studies found no beneficial effect [9–11].

In some countries, like Germany and Switzerland, selection of cleavage stage embryos is prohibited by law. Therefore, no other embryos are available for later transfer than those two or three selected at the pronuclear stage. Usually, embryo transfer in these countries is performed on day 2 or 3. The objective of this study was to assess pregnancy and implantation rates in a prospective randomized study when two or three pronuclear stage embryos were selected for extended culture and transferred on day 3 or 4 or 5. Because the German Embryo Protection Law prohibits embryo selection after extended culture, these restrictions provide an excellent option to study the implantation potential after extended culture without any further selection.
2. Material and methods

2.1. Study design

A prospective study was performed from January 2001 to May 2001 and all patients aged below 40 years who received oocyte retrieval for IVF or intracytoplasmic sperm injection (ICSI) therapy were included. For transfer on day 3, 4 or 5, we used a computer-generated randomisation list where for all patients treated within 1 week the day of transfer was the same and shifted from one week to the other.

2.2. Ovarian stimulation, gamete retrieval, in vitro fertilization, intracytoplasmic sperm injection and embryo transfer

Follicular stimulation and oocyte retrieval was performed as described [12], except that recombinant follicular stimulating hormones (FSH; Gonal F, Serono, Unterschleißheim, Germany; Puregon, Organon, Oberschleißheim, Germany) were used in addition. Sperm preparation for IVF and ICSI was performed using swim-up or a modified mini-swim-up technique, respectively, followed by insemination or intracytoplasmic sperm injection [12]. Transvaginal intrauterine transfer of a maximum of three embryos was performed with the Lisse catheter (Labotec, Göttingen, Germany). Transfer catheters were loaded first by 0.5 cm of air, followed by 40–50 μl of G2.2 medium (Vitrolife, Gothenborg, Sweden) containing the embryos and by another 0.5–0.7 cm of air [13]. Luteal phase support was performed with progesterone vaginal suppositories as described [12].

For the calculation of the pregnancy rate only cycles with proven implantation, documented by ultrasound, were considered. The implantation rate was calculated from the number of gestational sacs divided by the total number of embryos transferred.

2.3. Embryo culture

Culture of oocytes and IVF or ICSI was performed in IVF-20 (Vitrolife). On day 1, up to three pronuclear stage oocytes were randomly selected for further transfer and placed into G1.2 medium (Vitrolife), whereas supernumerary pronuclear oocytes were cryopreserved. In some cycles, a selection was performed based on pronuclear morphology [14,15]. These cycles were equally distributed between transfers on day 3 (26.6%), day 4 (23.4%) and day 5 (28.3%) (n.s.). For these cycles, there was no significant difference in regard to embryo transfer on day 3, 4 or 5 with only good prognosis pronuclear patterns (23.5% versus 26.9% versus 26.9%; n.s.), mixed prognosis pronuclear patterns (32.1% versus 31.3% versus 30.8%; n.s.) and reduced prognosis pronuclear patterns (44.4% versus 41.8% versus 42.3%; n.s.).

On day 3, embryos were placed into G2.2 medium (Vitrolife) and transferred approximately 2 h later. For transfer on day 4 or 5, embryos were placed into G2.2 medium on day 3 and cultured for another 24–48 h in G2.2 medium. Two to three hours prior to transfer, embryos were placed into a center well dish containing G2.2 medium. Due to the German Embryo Protection Law, every embryo was transferred, irrespective of its developmental progression. On day 3, the cumulative embryo score was calculated as proposed by Steer et al. [16]. As embryo selection was not possible, a blastocyst scoring system was not used.

2.4. Statistical analysis

Statistical analysis was performed with Chi-square test or ANOVA as appropriate.

3. Results

3.1. Analysis of success rates following randomisation of embryo transfer on days 3, 4 and 5

A total of 273 ovum pickup cycles were randomly allocated for transfer scheduled on day 3 (n = 90), day 4 (n = 95) or day 5 (n = 88) and a transfer was performed in 234 cycles (day 3: 79; day 4: 76; day 5: 79). The distribution of cycles with IVF or ICSI was not significantly different for the days 3 (54%, 46%), 4 (50%, 50%) and 5 (57%, 43%).
Table 2

<table>
<thead>
<tr>
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<th>Transfer cycles with at least one embryo ≥8 cells on day 3</th>
<th>Transfer cycles with all embryos &lt;8 cells on day 3</th>
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<tbody>
<tr>
<td></td>
<td>Clinical pregnancy rate per transfer cycle</td>
<td>Implantation rate</td>
</tr>
<tr>
<td>Total</td>
<td>38.8%&lt;sup&gt;a&lt;/sup&gt; (50/129)</td>
<td>22.3%&lt;sup&gt;B&lt;/sup&gt; (62/278)</td>
</tr>
<tr>
<td>Day 3</td>
<td>55.6%&lt;sup&gt;a,b,c&lt;/sup&gt; (25/45)</td>
<td>34.7%&lt;sup&gt;d,e,d&lt;/sup&gt; (34/98)</td>
</tr>
<tr>
<td>Day 4</td>
<td>36.6%&lt;sup&gt;a,e&lt;/sup&gt; (15/41)</td>
<td>18.7%&lt;sup&gt;f,d,f&lt;/sup&gt; (17/91)</td>
</tr>
<tr>
<td>Day 5*</td>
<td>23.3%&lt;sup&gt;b,c&lt;/sup&gt; (10/43)</td>
<td>12.4%&lt;sup&gt;e,d,i&lt;/sup&gt; (11/89)</td>
</tr>
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Small capitals denote significance values within a row, large capitals within a line; a,c,f,g,h,i,j,l,E,F,G,H not significant; b<sup>P </sup>< 0.005; d,k<sup>P </sup>< 0.025; D<sup>P </sup>< 0.01; A,B,C<sup><sub>E,F,G,H</sub></sup>p < 0.001.

* Blastocyst formation rates on day 5 were 44% (34/77) for cycles with at least one embryo ≥8 cells on day 3 and 2.4% (2/82) for cycles with embryos <8 cells on day 3 (<sup>P < 0.0001</sup>).

There was no significant difference in the median maternal age, the number of oocytes retrieved, the number of fertilized oocytes and the number of embryos transferred per transfer cycle between transfers on day 3, 4 or 5 (Table 1). Also the number of cycles where at least one embryo with eight cells or more was present on day 3 did not differ significantly. The mean cumulative embryo score on day 3 for transfers on day 3, 4 or 5 (50.42 ± 25.05; 49.75 ± 23.84; 45.82 ± 29.48) was not significantly different. The clinical pregnancy rate was the highest for transfers on day 3 (41.8%). It was lower, although not significantly different, for transfers on day 4 (27.6%) and significantly lower for transfers on day 5 (16.5%; <sup>P </sup>< 0.001). The implantation rates differed significantly between transfers on day 3 (27.1%) versus day 4 (14.1%; <sup>P </sup>< 0.005) and versus day 5 (8.8%; <sup>P </sup>< 0.001).

3.2. Influence of embryo development on day 3 on the success rates of transfer on days 3, 4 and 5

We further analysed the influence of embryo development on day 3 on pregnancy and implantation rates (Table 2). When the total data were analysed independent of the day of transfer, pregnancy and implantation rates were significantly higher when at least one embryo with eight cells or more was present on day 3 (38.8% and 22.3% versus 16.2% and 9.5%; <sup>P </sup>< 0.001). Similarly, pregnancy and implantation rates were significantly higher when the transfer took place on day 3 (55.6% and 34.7% versus 23.5% and 16.2%; <sup>P </sup>< 0.001). For days 4 and 5, the results were not significantly different (day 4: 36.6% and 18.7% versus 17.1 and 8.3%; day 5: 23.3% and 12.4% versus 8.3% and 4.3%).

For day 5, transfers blastocyst formation rates were 44% (34/77) for cycles with at least one embryo ≥8 cells on day 3 and 2.4% (2/82) for cycles with one or more embryos <8 cells on day 3 (<sup>P < 0.0001</sup>). For those cycles where at least one blastocyst developed until day 5, the cumulative embryo score on day 3 was significantly higher compared to cycles with no blastocysts on day 5 (81.5 ± 18.3 versus 53.4 ± 26.3, <sup>P < 0.0001</sup>).

When the data were analysed within the group of transfer cycles with at least one embryo with eight cells or more on day 3, pregnancy rates were the highest for day 3 transfers (55.6%), which was significantly different to transfer on day 5 (23.3%; <sup>P < 0.005</sup>) but not to transfer on day 4 (36.6%). Implantation rates were significantly different for day 3 (34.7%) versus day 4 and 5 (18.7 and 12.4%; <sup>P < 0.025</sup> and <sup>P < 0.001</sup>). Within the group of transfer cycles with no embryo with at least eight cells on day 3, pregnancy rates were not significantly different between days 3, 4 or 5 (23.5, 17.1 and 8.3%). Implantation rates were only significantly different between days 3 and 5 (16.2% versus 4.3%; <sup>P < 0.025</sup>) but not for day 4 (8.3%).

3.3. Success rates of transfer on day 5 in relation to embryo development

Finally, we analysed the pregnancy and implantation rates on day 5 in relation to the developmental stage reached by that day. Transfer cycles were grouped according to the most advanced embryo present in each transfer cycle on day 5 (neither morula nor blastocyst, <sup>n </sup>= 27; (34.2%); at least one morula, <sup>n </sup>= 30 (39.0%); at least one blastocyst, <sup>n </sup>= 22 (27.8%)). Transfer cycles with at least one blastocyst showed the highest pregnancy and implantation rates (36.4 and 18.4%) compared to cycles with at least one morula (13.3%, <sup>P = 0.058</sup>; and 7.2%, n.s.) and to cycles with neither morula nor blastocyst (3.7%, <sup>P < 0.005</sup>; and 1.9% <sup>P < 0.005</sup>) (Table 3).

4. Discussion

The introduction of sequential culture medium for blastocyst transfer had immediately stimulated numerous studies on the possible benefit for patients wishing to conceive a child by assisted reproduction. Several studies reported an enhanced outcome following blastocyst transfer and this was mostly attributed to better embryo selection and to improved embryo–uterine synchrony [6]. Embryo selection is a criterion which is by law not available in some countries. However, theoretically even in these countries, a benefit due to
Implantation rate 1.9% d,e (1/54) 7.2% d,f (4/56) 18.4% e,f (9/49)

Therefore, we believe that our results show that if no further selection is possible at the embryo stage, the benefit of extended culture and blastocyst transfer to the patients is doubtful. This conclusion is supported by a retrospective analysis of Kovacic et al. [28] where these authors also found no higher pregnancy and implantation rates for those transfers on day 5. This does not only allow to select those embryos which have reached the blastocyst stage but in addition enables the selection of those blastocysts which show the highest number of cells and exhibit the best morphology [5]. Under such conditions, it is a logical consequence and should be mandatory that the number of blastocysts for transfer has to be reduced in view of a maximum reduction of the multiple pregnancy rates.

Our results show that if no further selection is possible at the embryo stage, the benefit of extended culture and blastocyst transfer to the patients is doubtful. This conclusion is supported by a retrospective analysis of Kovacic et al. [28] where these authors also found no higher implantation rates after transfer on day 5 versus day 2 when only one or two embryos were available for transfer. The dramatic decrease in implantation rates which we observed in the day 5 group compared to the day 3 group is mainly due to that inability to select the best grown embryos for transfer. Because we had to transfer whatever type of embryo was present on day 5, a lot of transfer cycles were performed with embryos which were of course not competent to implant. This is supported by the fact that implantation rates for those transfer cycles on day 5 with at least one blastocyst were no longer significantly different to the ones from day 3. As the pregnancy and implantation rates after transfer on day 3 were above the average of the German IVF registry, we can hardly believe that sub-optimal culture conditions can be an explanation for the poor developmental and success rates for transfers on days 4 and 5.

In view of our data, it should also be emphasised that not all embryos with a good implantation potential on day 3 still have that potential if cultured up to day 5. This has been shown by Scott et al. [25], where embryos which developed from certain pronuclear morphology patterns showed a high implantation potential on day 3 but did not routinely form blastocysts when cultured until day 5. In our study, unfortunately, the numbers of those embryos which were derived from cycles with pronuclear scoring were too small to allow a conclusive correlation with the success rates on the different days of transfer.

In this study, pregnancy and implantation rates for those cycles where blastocysts were available on day 5 were slightly lower compared to the success rates reported in the literature [6,19,26,27]. This again can be explained by the fact that in other countries, a large number of embryos are available for selection on day 5. This does not only allow to select those embryos which have reached the blastocyst stage but in addition enables the selection of those blastocysts which show the highest number of cells and exhibit the best morphology [5]. Under such conditions, it is a logical consequence and should be mandatory that the number of blastocysts for transfer has to be reduced in view of a maximum reduction of the multiple pregnancy rates.

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Another implication of our work is that the general situation for the patients as well as for the IVF clinics is not satisfactory in countries like Germany or Switzerland. Our data show clearly that under the present understanding of the existing law, a large number of cycles remain without success. It is frustrating to foresee that a treatment has a minimum chance to be successful when only two-cell embryos are present on day 3 and no other embryos are available because they had to be frozen at the pronuclear stage and those which were used for further culture were not the ones with the best developmental potential. If an evaluation of embryonic growth on day 3 would have been possible on more than 3 embryos, the chance for a pregnancy would still have been granted. This has economic and social implications. With new policies in the health insurance system, where the patients receiving ART treatment must pay approximately 50% of the total costs on their own, couples with a healthy financial background will choose the option to get unlimited treatment abroad in those neighbouring countries, where embryo selection is possible—sometimes even at lower costs compared to Germany.

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