Similar delivery rates in a selected group of patients, for day 2 and day 5 embryos both cultured in sequential medium: a randomized study

Serena Emiliani1, Anne Delbaere, Anne-Sophie Vannin, Jamila Biramane, Miranda Verdoodt, Yvon Englert and Fabienne Devreker

Fertility Clinic, Erasme Hospital, Free University of Brussels, French Speaking, 808, Route de Lennik, B-1070 Brussels, Belgium

1To whom correspondence should be addressed. E-mail: semilian@ulb.ac.be

BACKGROUND: The existence of a real benefit of blastocyst transfer is still a matter of debate. The aim of this study was to compare, in a prospective randomized trial, the outcome of day 2 and day 5 transfer of human embryos cultured in an ‘in-house’ sequential medium.

METHODS: A total of 193 cycles from 171 patients with less than four previous IVF cycles, <39 years old and with four or more zygotes on day 1, were randomly allocated to day 2 (94 cycles) or day 5 (99 cycles) transfer. Zygotes were kept in fertilization medium until 18 h post-fertilization and then placed in a ‘glucose-free’ cleavage medium. Embryos allocated for day 5 transfer were placed in a blastocyst medium 66 h post-fertilization. Two or three embryos were replaced according to the morphology.

RESULTS: A mean (±SEM) number of 2.1 ± 0.4 and 1.9 ± 0.3 embryos were replaced on day 2 and day 5 (P < 0.001) respectively. Delivery rates per transfer were 44.1 and 37.1% [P = not significant (NS)], implantation rates were 31.4 and 29.4% (NS) and multiple delivery rates 22 and 36% (NS) for day 2 and day 5 groups respectively. Ten patients (10.1%) had no blastocysts available for transfer.

CONCLUSIONS: No clear benefits were observed using blastocyst transfer for patients aged <39 years who had had less than four previous IVF cycle attempts.

Key words: blastocyst culture/embryo transfer/IVF/sequential media

Introduction
Since the introduction of IVF in 1978, the implantation rate of human embryos still remains a limiting factor for success. Several strategies have been developed to improve embryo selection and embryo developmental potential to increase pregnancy rates without increasing the risk of multiple pregnancies (Ménézo et al., 1990). Blastocyst transfer is claimed to provide a more physiological synchronization between embryo stage and the endometrium in comparison with pronucleate or day 2–3 embryo transfer (Edwards and Beard, 1999). Furthermore, in-vitro embryo culture up to the blastocyst stage was proposed as a powerful tool to better select one or two viable embryos to replace, thereby reducing the risk of multiple pregnancies (Milki et al., 1999; Karaki et al., 2002). Despite this, embryo replacement on day 2–3 is still performed in many IVF centres for fear that prolonged in-vitro culture of embryos in sub-optimal conditions could compromise their viability, with an increased risk of having no blastocysts to replace on day 5.

Many strategies have thus been adopted to improve prolonged in-vitro embryo culture conditions as, for example, the embryo co-culture system with somatic helper cells (Ménézo et al., 1992; Oliennes et al., 1994; Schillaci et al., 1994) or embryo culture in sequential media. The composition of sequential media reflects changes in both carbohydrates and amino acid requirements during embryo development, and is thought to better support embryo growth up to the blastocyst stage (Barnes et al., 1995; Gardner et al., 1996; Jones et al., 1998a). Up to 50–55% of blastocyst development from zygotes has been reported (Gardner et al., 1998b; 2000; Jones et al., 1998a).

Although several laboratories have reported increased implantation rates following blastocyst transfer, others have failed to observe any benefit compared with cleavage stage embryo transfers. High implantation rates after blastocyst transfer were presented in comparative or retrospective studies (Alves da Motta et al., 1998; Gardner et al., 1998a; Del Marek et al., 1999; Milki et al., 1999; 2000; Balaban et al., 2001; Yoon et al., 2001), while in a series of prospective randomized trials either a benefit from blastocyst transfer (Gardner et al., 1998b; Hsieh et al., 2000; Yoon et al., 2001; Karaki et al., 2002) or similar implantation rates (Scholtes et al., 1996; Coskun et al., 2000; Plachot et al., 2000; Usunomiya et al., 2002) were observed. In all these prospective randomized trials, sequential media were only used for culture between the 8-cell embryo and blastocyst stages and not for embryos.
Table I. Composition of culture media used for blastocyst culture, the osmolality for fertilization is corrected to 280 mOsm/kg

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Fertilization (mM/l)</th>
<th>Cleavage (mM/l)</th>
<th>Blastocyst (mM/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>94.63</td>
<td>106.09</td>
<td>106.09</td>
</tr>
<tr>
<td>KCl</td>
<td>5.23</td>
<td>5.05</td>
<td>5.05</td>
</tr>
<tr>
<td>MgSO4</td>
<td>0.81</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>NaH2PO4</td>
<td>1.01</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Glucose</td>
<td>5.55</td>
<td>0.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Penicillin</td>
<td>0.17</td>
<td>0.17</td>
<td>0.17</td>
</tr>
<tr>
<td>Na-lactate</td>
<td>28.13</td>
<td>6.02</td>
<td>0.0</td>
</tr>
<tr>
<td>Na-pyruvate</td>
<td>0.33</td>
<td>0.47</td>
<td>0.47</td>
</tr>
<tr>
<td>NaHCO3</td>
<td>25.00</td>
<td>25.00</td>
<td>25.00</td>
</tr>
<tr>
<td>CaCl2·2H2O</td>
<td>1.7</td>
<td>1.7</td>
<td>1.7</td>
</tr>
<tr>
<td>Glutamine</td>
<td>0.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>NEAA</td>
<td>0.0</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>EAA</td>
<td>0.0</td>
<td>0.0</td>
<td>0.1</td>
</tr>
</tbody>
</table>

The osmolality of cleavage and blastocyst medium are 267 and 270 mOsm/kg respectively and they are not adjusted.
NEAA = non-essential amino acids; EAA = essential amino acids.

replaced at earlier stages. In only two recently published, prospective studies were sequential media used for both day 3 and day 5 transferred embryos (Rienzi et al., 2002; Van Der Auwera et al., 2002). In the first study (Van Der Auwera et al., 2002), a higher clinical pregnancy rate was observed after blastocyst replacement, while in the second one (Rienzi et al., 2002), in which morphological evaluation at day 3 and at pronuclear stage were combined for day 3 embryo selection, similar implantation potentials were observed between day 3 and day 5 embryos.

In light of these previous studies, we decided to evaluate the impact of day 2 or day 5 transfers in women aged <39 years in a prospective randomized study. Only women aged <39 years were included in the study in order to eliminate the possible interaction of maternal age on embryo development and pregnancy rate. All embryos were cultured in the same ‘in-house’ sequential medium. The use of media made in our own laboratory offers the advantage of knowing which components and in what amounts they are introduced at each stage of embryo development.

Materials and methods
This study, a prospective and randomized trial, compared day 2 versus day 5 embryo transfers, and was approved by the Ethical Board of the Erasme Hospital (Reference Number 2000/63). All the couples were informed about the study, and if they agreed to participate they were included after providing written informed consent.

Inclusion criteria
All IVF and ICSI cycles from consenting patients aged <39 years, having had not more than three previous IVF cycles and with at least four fertilized oocytes on day 1, were included. Six patients in the day 2 transfer group and one patient in the day 5 transfer group were excluded for violation of the protocols.

Randomization
The minimum number of patients to be included was calculated by the Stat Calcul software for Windows 98. Patients were randomized, for each new cycle, to receive embryo transfer on day 2 or on day 5 on the basis of their inclusion in a randomization list with permuted blocks for the two types of transfer. A total of 104 cycles with day 2 transfer and 107 cycles with day 5 were included in the study. However, 10 cycles with day 2 transfer and eight cycles with day 5 transfer were excluded from the analysis for violation of the protocols because the number of previous attempts was wrong or due to mistakes in encoding; seven and three cycles with day 2 transfer and six and two with day 5 transfer respectively.

Stimulation protocol and oocyte recovery
Ovarian stimulation was performed using GnRH analogue (buserelin acetate: Suprefact spray; Hoechst, Germany), hMG (Hume gon; Organon, The Netherlands) and hCG (Pregnyl; Organon). Oocyte retrieval was performed through vaginal puncture under ultrasound guidance 36 h after the injection of 10 000 hCG. In-vitro oocyte culture and preparation for ICSI have been described elsewhere (Emiliani et al., 1999).

Culture conditions
The composition of the sequential medium used in this study has been adapted from the composition of previous published culture media (Gardner and Lane, 1999; Devreker et al., 2001; Van den Bergh, 2001) (Table I). This medium has proven its capacity to support early embryo development. The introduction of this ‘in-house’ sequential medium reduced the embryonic fragmentation, increased the proportion of 4-cell stage embryos and significantly increased the day 2 embryo score (3.2 ± 1.41 versus 3.7 ± 1.42 respectively; P < 0.01) (Emiliani et al., 2000). These observations motivated the definitive introduction of this sequential medium for day 2 embryo culture and for the comparison of the outcome of day 2 and day 5 transfers.

Embryos were thus individually cultured in three phases: fertilization medium (modified Earle’s balanced salt solution) was used from the moment of oocyte collection until the check for pronucleus 18 h after puncture. For this, Petri culture dishes (Falcon 3001; Becton Dickinson, Belgium) were prepared 24 h prior to the oocyte collection. Each one of the dishes contained five drops of 30 µl and the drops were covered with 3 ml pre-washed mineral oil (Sigma, M8410; Sigma-Aldrich, Belgium).

After observation of the pronuclei, all the oocytes were transferred into the glucose-free cleavage medium (CLM). This part of the culture was made in large Petri dishes (Falcon 3004). For each oocyte, a row of four drops of 20 µl was made, five rows per dish, and the drops were covered with 9 ml of pre-washed oil. The first three drops were used to wash out the oocytes and the fourth drop was used for culture from day 1 to day 3 post-insemination. To minimize cross-contamination, embryos were transferred between the different drops using the Flexipet (Cook, Belgium) in a volume of 30 µl.

For culture between day 3 and day 5 after insemination, similar dishes were prepared, containing blastocyst medium (BLM). The embryos were washed and transferred as described for culture in CLM. All the products used were cell culture-tested (Sigma) except the essential and non-essential amino acids (Gibco, Life Technologies, Belgium). All media were supplemented with 0.5% (w/v) human serum albumin (Belgian Red Cross, Belgium). Culture media were prepared weekly in the IVF laboratory and tested with embryos from superovulated mice. The batch supporting <75% of mouse blastocysts after 5 days of culture was discarded. Embryo cultures were performed under a 5% CO2, 5% O2, 90% N2 atmosphere.

Embryo scoring system
This system is based on cleavage stage and embryo morphology. A score of 4 is attributed to 45 h embryos at the 2-cell stage, with regularly shaped blastomeres and no fragmentation; 2 further points...
are attributed if the 45 h embryos are at the 4-cell stage; the score is reduced by 1 point if blastomers had irregular shape, by 1 point if embryo fragmentation is <30% of the volume of the embryo and by 2 points if embryo fragmentation is >30% (Puissant et al., 1987). The selection of blastocysts for transfer is based on morphological criteria that include: dimension of the blastocoel cavity, development of the trophoblast, the inner cell mass size and shape, presence of degenerating foci or degree of blastocyst hatching (Balaban et al., 1999; Richter et al., 2001).

Embryo transfer policy

For day 2 transfer, a maximum of two embryos was transferred for all patients <35 years old and/or with less than four previous IVF attempts, except for women for whom the total score of the two best-scoring embryos was <8, for whom a third embryo was transferred, if available. Patients aged ≥35 years or with more than three previous IVF failures had two embryos replaced if the total score of the three best-scoring embryos was ≥15, while if the total score was ≤15 a third embryo was replaced, if available (Devreker et al., 1999).

For day 5 transfer, two blastocysts were replaced, if available.

For the transfer of thawed embryos, only 2 thawed embryos that cleaved or thawed blastocysts that re-expanded after a further 18 h of culture were replaced.

Statistical analysis

The data were analysed with the statistical Package for Social Sciences SPSS version 7.5 for Windows 98, under the licenses obtained by our fertility clinic from SPSS Inc. All data were checked for their normal distribution by submission to the Kolmogrov–Smirnov test and if significant non-parametric statistical analysis was applied as recommended. The χ²-test was used when necessary, with Yates' correction. P < 0.05 was considered as statistically significant.

Results

The 94 day 2 transfers (89 patients) included three cycles with a single embryo replaced, 79 cycles with two embryos replaced and in 11 cycles with three embryos replaced and one cycle with no replacement because of embryo quality.

The 99 day 5 transfers (82 patients) included 10 cycles with a single embryo replaced, 79 cycles with two embryos replaced, and 10 cycles in which there were no blastocysts available for transfer (10.1%). No significant differences were observed for: number of previous cycles, laboratory parameters and proportion of IVF/ICSI cycles (Table II); however, women with blastocyst transfers were 1 year older (P = 0.044) (Table II).

Overall, 48.3% (381/788) embryos formed blastocysts by day 5 with a well-visible inner cell mass and a blastocoel of at least half the volume of the embryo and a well-defined trophoblast. A similar number of blastocysts was observed for ICSI and IVF cycles, 52 and 46% respectively. No correlation was observed between the individual number of zygotes and the percentage of blastocysts formed on day 5 (r² = 0.003). A relationship between the stage reached by the embryos on day 2 or 3 and blastocyst formation was observed. In fact, day 2 embryos with ≥4 cells formed significantly more blastocysts compared with day 2 embryos with <4 cells [61.3% (320/521) versus to 26.7% (563/237) respectively; P < 0.0001]. 65% (250/385) of day 3 embryos with ≥8 cells reached the blastocyst stage compared with 47.0% (118/251) of day 3 embryos with ≥4 and <8 cells and compared with only 9.4% (9/96) of day 3 embryos with <4 cells (P < 0.001). Overall, the 78% (131/168) of embryos replaced at the blastocyst stage would have been chosen for a transfer on day 2.

Blastocyst formation was also related to the morphology of day 2 embryos. Embryos with poor morphology (score <4) formed significantly fewer blastocysts compared with embryos of average (score = 4) or good quality (score ≥4) 33% (97/291), 62% (163/263) or 66% (119/180) respectively (P < 0.001). Fewer embryos were replaced on day 5 in comparison with day 2 (mean ± SEM: 2.1 ± 0.4 versus 1.9 ± 0.3; P < 0.001). This difference could be explained by the fact that a higher proportion of patients had only one or no blastocysts available for transfer, in comparison with day 2 embryos (4.3 versus 20.2%; P = 0.0017).

### Table II. Patients and cycle characteristics in the two experimental groups

<table>
<thead>
<tr>
<th></th>
<th>Day 2</th>
<th>Day 5</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>89</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td>No. of cycles</td>
<td>94</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>Maternal age (mean ± SEM)</td>
<td>31 ± 3</td>
<td>32 ± 4</td>
<td>0.044</td>
</tr>
<tr>
<td>No. of previous cycles (mean ± SEM)</td>
<td>1.7 ± 0.9</td>
<td>2.0 ± 1.0</td>
<td>NS</td>
</tr>
<tr>
<td>No. ICSI cycles (%)</td>
<td>67.0</td>
<td>67.6</td>
<td>NS</td>
</tr>
<tr>
<td>No. of collected oocytes</td>
<td>11.9 ± 4.4</td>
<td>12.0 ± 4.9</td>
<td>NS</td>
</tr>
<tr>
<td>No. of replaced embryos</td>
<td>8.0 ± 3.1</td>
<td>8.1 ± 3.4</td>
<td>NS</td>
</tr>
<tr>
<td>No. of cycles with no transfer</td>
<td>2.1 ± 0.4</td>
<td>1.9 ± 0.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>No. of frozen cycles</td>
<td>1 (1.1)</td>
<td>10 (10)</td>
<td>0.016</td>
</tr>
<tr>
<td>No. of frozen embryos (mean ± SEM)</td>
<td>4.3 ± 2.4</td>
<td>3.3 ± 2.3</td>
<td>0.019</td>
</tr>
<tr>
<td>Pregnancy rate/transfer</td>
<td>46 (49)</td>
<td>39 (44)</td>
<td>NS</td>
</tr>
<tr>
<td>Delivery rate/transfer (%)</td>
<td>41 (44.1)</td>
<td>33 (37.1)</td>
<td>NS</td>
</tr>
<tr>
<td>Delivery rate/oocyte retrieval (%)</td>
<td>41 (44.1)</td>
<td>33 (33.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Cumulative delivery rate/oocyte retrieval (%)</td>
<td>47 (50)</td>
<td>36 (36)</td>
<td>0.07 (NS)</td>
</tr>
<tr>
<td>Abortion (%)</td>
<td>4 (8.5)</td>
<td>6 (15.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Multiple deliveries (%)</td>
<td>9 (22)</td>
<td>12 (36)</td>
<td>NS</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>29</td>
<td>30</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Cycles with no embryos available for transfer have been excluded from the analysis.

NS = not significant.
Delivery rates per transfer were, however, similar for the two groups, 44.1 and 37.1% for the day 2 and day 5 transfers respectively (Table II). No differences were observed between the two groups, either in the number of implantations or in the number of multiple pregnancies (Table II). Pregnancy rates were not influenced by the number of previous attempts, either for the day 2 or for the day 5 transfers. On the other hand, significantly fewer cycles had embryos available for freezing after day 5 transfer compared with day 2 transfer (53.9 versus 73.1%; \( P = 0.0012 \), Table II) and the mean number of frozen embryos was significantly lower for the group of day 5 transfers compared to day 2 transfers (3.3 ± 2.3 versus 4.3 ± 2.4; \( P = 0.019 \), Table II).

To date, 125 day 2 embryos have been thawed for 54 frozen cycles and 74 blastocysts have been thawed for 42 frozen cycles. Significantly fewer thawed blastocysts (20/74) were replaced compared with thawed day 2 embryos (57/125) (27 versus 45.6% respectively; \( P < 0.01 \)). The 57 day 2 thawed embryos were replaced in 39 cycles that resulted in six deliveries (15.4%), one miscarriage and three biochemical pregnancies. The 20 thawed blastocysts were replaced in 14 cycles that resulted in three deliveries (21.4%) and one biochemical pregnancy. Consequently, the cumulative delivery rate per oocyte retrieval was 50% (47/94) and 36.4% (36/99) for day 2 and day 5 transfer respectively (\( P = 0.07 \)).

**Discussion**

The controversies regarding blastocyst transfer have motivated our decision to compare the outcome of day 2 and day 5 transfers by a prospective, randomized trial that included women aged <39 years and who had experienced three or fewer previous IVF cycles. These criteria were chosen to reduce the number of factors that could influence blastocyst formation.

Delivery and implantation rates were similar for day 2 and day 5 transfers: 44.1 and 37.1% of delivery rate/transfer, and 31.4 and 30.9% of implantation rate respectively. Factors that could have affected blastocyst development including number of previous cycles, mean number of collected oocytes, mean number of zygotes and the proportion of ICSI cycles were similar in both groups (Table II). The difference of 1 year in age between the women of the two groups was hardly sufficient to have jeopardized the embryo development in the blastocyst transfer group.

A relationship was observed, however, between the embryo stage on day 2 or day 3 and their ability to reach the blastocyst stage. Blastocyst formation was significantly higher for day 2 embryos with \( \geq 4 \) cells or for day 3 embryos with \( \geq 8 \) cells, that gave \( \geq 60\% \) of blastocysts, whereas day 2 embryos with \( < 4 \) cells or day 3 embryos with \( < 8 \) cells developed to blastocyst only in 10–20% of the cases. To be sure that the introduction of an in-house sequential medium could not have decreased the quality of our culture condition system, this medium has been previously tested in our clinical programme. A reduction in embryonic fragmentation and an increase in cleavage rate have been observed (Emiliani et al., 2000). Furthermore, a similar sequential medium has shown that blastocyst development using an in-house medium was similar to commercial sequential media (Devreker et al., 2001).

The decrease of embryonic fragmentation is probably related to the omission of glucose during early stages of development (Quinn et al., 1995; Jones et al., 1998a; Louridas et al., 2000). The presence of glucose is one of the major components that was shown to be toxic for early embryo development (Conaghan et al., 1993; Katchdourian et al., 1996; Coates et al., 1999). This fact was attributed to the inability of the early cleavage stage embryos to metabolize glucose into energy, forcing the embryo to transform the glucose into glycogen, which is then stored, producing an increase in the osmotic value of cytoplasm. This factor produces an increase of water uptake by the cell and an increase of the ratio between cytoplasmic and nuclear volume, inducing the extrusion of cytoplasmic fragments to restore the optimal ratio (Ménézo and Guerin, 1999). It is very complex to explain the reasons for the conflicting results published to date, especially in prospective studies totally performed in sequential media. Differences in patient selection, experimental design and culture system adopted can only partially explain these discrepancies. Multiple other factors were claimed to influence the rate of blastocyst formation and the pregnancy rate. In opposition to previous studies (Gardner et al., 1998b; Jones et al., 1998b; Scholtes and Zeilmaker, 1998), no correlation was observed between the number of collected oocytes, the number of zygotes and the percentage of blastocysts between the two groups analysed. On the other hand, we did not observe a relationship between the number of zygotes and the outcome of blastocyst transfers in contrast to others (Jones et al., 1998b).

The influence of maternal age (Gardner et al., 1998b; Jones et al., 1998b; Scholtes and Zeilmaker, 1998) or of aetiology of infertility (Jones et al., 1998b) has been limited in our study by selection of patients. The relationship between embryo morphology and blastocyst developmental potential are in agreement with previous observations although the authors did not use sequential media (Rijnders and Jansen, 1998). It should, however, be admitted that if day 2 embryo score and stage are related to blastocyst formation they remain poor predictors of pregnancy. In fact the embryos considered as having the best morphology reached the blastocyst stage in ~60% of the cases only and, on the other hand, the embryos with poor morphology developed into blastocysts in ~10–20% of the cases. In a previous study (Graham et al., 2000) it was observed that only the 48% of embryos that would have been chosen for embryo transfer and/or for cryopreservation on day 2 were eventually transferred or cryopreserved at the blastocyst stage. The embryo selection based on the embryo morphology remains an indirect evaluation of embryo developmental potential either at the cleavage or at the blastocyst stage. The lack of a strong blastocyst scoring system could have jeopardized our blastocyst selection. The introduction of other criteria, such as metabolic assays, could improve the efficiency of blastocyst selection for transfer (Lane and Gardner, 1996; Khurana et al., 2000). Recently, the integration of the evaluation of pronuclear and cleavage stage to select embryos gave the same pregnancy and implantation rates for day 3 and day 5 transfer (Rienzi et al., 2002).
Resistance to the freezing–thawing process has been another proposed reason in favour of blastocyst transfer. It has been argued that reduced dimensions of blastocyst cells could help them to resist the freezing process better than earlier cleavage stage embryos. Supernumerary blastocysts were frozen in 53.9% of cycles only, whereas in 73.1% of the cycles with day 2 transfers, cleavage stage embryos were frozen. A lower number of blastocysts resisted the thawing process, however, compared with cleavage stage embryos, suggesting that blastocysts have a lower resistance to freezing and thawing as observed in previous studies (Van Der Auwera et al., 2002; Rienzi et al., 2002). In addition, more pregnancies were obtained with day 2 thawed embryos.

The risk of monozygotic twins is another factor in favour of cleavage stage embryo transfer (Rijnders et al., 1998; Da Costa et al., 2001). In our study, multiple pregnancy rates were similar for both groups although a smaller number of embryos has been replaced and no replacements of three blastocysts have been performed. However, early vaginal ultrasound at 6 weeks of gestation did not show the presence of monozygotic twins in our series, although only genetic analysis is sufficient to exclude monozygotic twins.

In conclusion, there are no advantages of offering to women aged <39 years blastocyst culture for the first four treatment cycles. Furthermore, the combination of the loss of embryos during prolonged culture (10% of the patients had no blastocysts available for transfer), the lower number of blastocysts available for freezing and the lower resistance to the thawing appeared to reduce the overall delivery rate per oocyte retrieval after blastocyst transfer (50 versus 36%, \( P = 0.07 \)), favouring the transfer of cleavage stage embryos. Although it has been argued that embryos that arrest to develop in vitro could contain chromosomal anomalies or other metabolic defects, some of these arrested embryos could have survived in in-vivo conditions if transferred earlier. All these observations are in favour of the transfer of early cleavage stage embryos.

References
Alves da Motta, E.L., Alegretti, J.R., Baracat, E.C., Olive, D. and Sera®ni, as observed in previous studies (Van Der Auwera et al., 2002; Rienzi et al., 2002). In addition, more pregnancies were obtained with day 2 thawed embryos.

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