Morula transfer as alternative to blastocyst transfer or day 3 transfer: is there a role?
Adel M. Nada, Reham F. Khalil, Ahmed Sawaf and Ahmed El-halwagy

Objective
The aim of this study was to evaluate the clinical outcomes of day 4 embryo transfers (ETs) and compare the efficacy of day 4 ET with day 5 and day 3.

Study design
All women undergoing ICSI-ET with grade I embryos only were included and divided into: group A with 1224 patients (ET day 3); group B with 159 patients (ET day 4); and group C with 84 patients (ET day 5). Luteal phase support was given to all patients by administering 800 mg cyclogest intravaginally per day for 14 days, starting from OPU day, and then quantitative β-human chorionic gonadotropin was measured in serum. Pregnant patients were closely followed up till 12 weeks postmenstrual.

Results
There was no significant difference between ET day 3 and ET day 4 regarding age, duration of infertility, and oocytes retrieved. Clinical pregnancy rate in group A was 37% and in group B 31.4% (P = 0.17) compared with group C (ET day 5) where clinical pregnancy rate was 26.2% (P = 0.39).

Conclusion
ET at the morula stage does not affect pregnancy outcome in in-vitro fertilization/ICSI cycles.

Keywords:
embryo transfer, outcome, pregnancy rates

Introduction
The majority of embryo transfers (ETs) to date have been performed on day 3 to reduce the potential risk of developmental arrest of in-vitro cultured embryos before ET. Development of sequential media has significantly improved culture conditions and allowed blastocyst transfer on day 5. Although day 5 ET provides higher clinical pregnancy outcomes with reduced risks of multiple pregnancies, it still has potential risks of developmental arrest of in-vitro fertilization (IVF) embryos. The pregnancy rate appears to be influenced by the culture environment used for the gametes. Many clinical researchers are concerned with suboptimal culture conditions for embryo development before ET. Thus, some authors have recommended day 2 or day 3 ET to avoid expected suboptimal culture conditions owing to the prolonged culture time [1–3].

However, the human embryonic genome is known to be activated at the four-cell to eight-cell stage, and early embryo-quality evaluation based on embryo morphology on day 3 cannot accurately predict pregnancy potential [4]. Because of poor prediction of early cleavage-stage embryo morphology, the number of transferred embryos needed for a successful pregnancy would be increased at day 3. However, an increased number of transferred embryos can increase the risk of multiple pregnancies [5,6].

Advances in culture media have enabled the change from early cleavage ET to blastocyst stage transfer in the practice of assisted reproductive technology [7]. Postponing ET to later stages can allow for better embryo selection and may significantly increase the implantation rate and reduce multiple pregnancies [8]. With the ability to select the most developmentally capable embryos, the number of transferred embryos can be decreased, thereby limiting high-order multiple pregnancies while maintaining an acceptable pregnancy rate [9]. However, day 5 ET at the blastocyst stage still has the potential risks of developmental arrest and reduced embryo quality in vitro, even with the most advanced sequential culture media [10].

In this present study, low pregnancy rate and high cancellation were observed if we prolong embryos in culture media to day 5; thus, we tried to transfer it to day 4 to allow for better embryo selection than day 3 and avoid embryo arrest on day 5.

Participants and methods
The study was conducted in Cairo, between 2009 and 2013, on infertile patients coming for IVF treatment option. A total of 1467 patients scheduled for ICSI-ET were divided into: group A with 1224 patients (ET day 3); group B with 159 patients (ET day 4); and group C...
with 84 patients (ET day 5). All patients were below 40 years old and were subjected to full history and physical examination: hormonal profile and pelvic ultrasound for women, and semen analysis for men. Induction of ovulation by hMG started from day 2 of menstrual cycle using agonist long protocol or antagonist protocol. With triggering ovulation there were three follicles at least greater than 17 mm in diameter. Agonist protocol was started by downregulation of the pituitary gland by using decapetyl (0.1 mg subcutaneously/day), staring from day 21 premenstrual till day of triggering, whereas antagonist protocol by cetrodite (0.25 subcutaneously) from day 6 till day of triggering. Then oocytes were retrieved under general anesthesia for all patients. ET was carried out when grade I embryos were available on days 3, 4, and 5. Luteal support was given by administering cyclogest 800 mg/day for 14 days, and β-hCG was measured in serum. Also, follow-up of pregnant patients till 12 weeks’ gestation was carried out.

**Ethics**

This study followed the principles of declaration of Helsinki and in accordance with the medical research involving human subject acts (WMO), and was approved by the medical ethical committee of Cairo University.

**Statistical analysis**

All statistical tests were conducted using a significant level of 95%. A value of $P$ less than 0.05 was considered significant. SPSS software (Statistical Package for the Social Sciences, version 20.0; SPSS Inc., Chicago, Illinois, USA) was used for statistical analysis. Data were presented as mean ± SD for continuous variables and as frequency and percentage for categorical variables. Comparison between two groups were made using $\chi^2$-test for categorical variables and unpaired Student’s $t$-test for continuous variables.

**Results**

When comparing group A (ET day 3) and group B (ET day 4) there was no significant difference observed with regard to patient age, duration of infertility, or distribution of etiological pathologies. There were no significant differences between group B (ET day 4) and group C (ET day 5) with regard to pregnancy rate, delivery of term baby, or abortion rate, with each being 31.4, 12, and 7%, respectively, in group B, and 26.2, 9.5, and 8.3%, respectively, in group C ($P = 0.39$, 0.56, and 0.68, respectively).

**Discussion**

The selection of the most viable embryo for transfer is an increasingly important aspect of IVF-ET cycles. Advances in embryo culture media have allowed a longer period of embryo culture before transfer. Many studies have reported several advantages of blastocyst transfer. Blastocyst transfer improved embryo–uterine synchrony and allowed for a greater chance of selecting suitable embryos for transfer because of their having less chromosomal abnormality [11,12].

Human embryos obtained through in-vitro techniques are routinely transferred to the uterus on day 2 or 3 when they are at the four-cell to eight-cell stage. The implantation rates of these embryos are disappointing low and range between 10 and 20% [13]. Therefore, the standard treatment is to transfer more than one embryo into the uterus to obtain a reasonable pregnancy rate. This results in a high order of multiple pregnancies and twins with increased pregnancy complications such as abortion and premature deliveries [14].

It has been suggested that transferring embryos at the blastocyst stage instead of selection at an earlier stage without knowing their developmental capability might enhance the implantation rate by a better embryo selection, thereby reducing the need to transfer more embryos [15]. The most competent embryos reaching the blastocyst stage are selected for transfer and the embryos that have arrested their development are identified and hence not transferred. However, under current culture conditions, embryo developmental arrest occurs around the eight-cell stage and formation of the blastocyst is uncommon. To overcome this difficulty, coculture of the embryos with different cell types has been used over the years [16]. These cells range from autologous cells, animal cells to cell lines. Although safety was a concern when using these cells, efficiency was not improved over the routine protocols.

It is believed that gene expression of the human embryos is switched on around the eight-cell stage immediately before compaction [4]. Therefore, nutrient requirements of embryos are different after this stage. Early embryos can grow in a simple salt solution, whereas they require more complex media after they reach the eight-cell stage. These changes also correspond to environmental changes in vivo as the embryo reaches the uterus from the fallopian tube at the stage when compaction begins. During recent years, there have been extensive studies on mammalian embryology, resulting in the development.

<table>
<thead>
<tr>
<th><strong>Table 1</strong></th>
<th><strong>D3 (n=1224)</strong></th>
<th><strong>D4 (n=159)</strong></th>
<th><strong>P-value</strong></th>
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<td><strong>Age (years)</strong></td>
<td>30.4 ± 5.2</td>
<td>30.2 ± 5.3</td>
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<td><strong>Infertility duration (?)</strong></td>
<td>6.2 ± 4.1</td>
<td>5.8 ± 4.1</td>
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<td><strong>Number of oocytes</strong></td>
<td>9.6 ± 6.3</td>
<td>9.5 ± 5.9</td>
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<tr>
<td><strong>Number of MII</strong></td>
<td>7.8 ± 5.2</td>
<td>7.8 ± 5.2</td>
<td>0.99</td>
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<td><strong>Outcome (pregnancy rate) [n (%)]</strong></td>
<td>452 (37)</td>
<td>50 (31.4)</td>
<td>0.17</td>
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<tr>
<td><strong>Abortion rate [n (%)]</strong></td>
<td>142 (11.6)</td>
<td>11 (7)</td>
<td>0.077</td>
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of two sequential media for blastocyst culture [17]. Gardner and colleagues reported in a comparative study of embryo culture media that the blastocyst formation rate was significantly enhanced (66%), with the use of new G1 and G2 media in sequence as compared with Ham’s F-10 followed by G2 media (38%). In a prospective randomized study, the use of G1 and G2 media with blastocyst transfer resulted in significantly higher implantation and similar pregnancy rates as compared with day 3 transfer in moderate to high responders [8].

However, day 5 ET at the blastocyst stage still has potential risks of cycle cancellation owing to developmental arrests and reduced embryo quality in vitro, even with the most advanced sequential culture media. Montag et al. [18] suggested that extended embryo culture is not beneficial when the option for embryo selection at later stages of development is not available. The majority of ET in IVF-ET cycles is performed with early cleavage-stage embryos or blastocysts. It seems that the transfer of day 4 embryos has rarely been attempted by assisted reproductive technology specialists even if such a transfer with the embryos that have undergone compaction and reached the morula stage may have certain advantages [19].

Day 4 transfer is not disadvantageous for the following reasons: the embryo is returned to the uterus, to an environment where it would normally reside. The embryos can also be exposed to the uterine environment for the maximum time period and be in an in-vitro environment for the minimal time period before implantation. In addition, uterine contractility is reduced at this time, all of which maximizes the potential for implantation [20]. The first report of pregnancy after transferring frozen-thawed morula/compact stage embryos was in 2001 from Tao et al. [21], and they noted a high survival and clinical pregnancy rate in frozen-thawed morula/compact stage ET cycles on day 4 [22]. In 2002, Tao et al. [21] published a retrospective study including day 4 and day 3 ET in which the authors proposed and followed a grading system for morula/compacted embryos; they reported that day 4 ET resulted in implantation rates similar to or higher than those of day 3 ET.

In the present study, the transferred embryos in day 4 showed insignificant lower pregnancy rate than day 3 transfer. When comparing day 4 and day 5, ET day 4 shows no significant differences with ET day 5 with regard to clinical pregnancy rates; however, ET day 4 still shows insignificant better pregnancy rates and less abortions (Table 2 and Fig 1).

### Table 2

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<th>D5 (n=84)</th>
<th>D4 (n=159)</th>
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<tr>
<td>Age (years)</td>
<td>31.4 ± 5</td>
<td>30.2 ± 5.3</td>
<td>0.78</td>
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<tr>
<td>Infertility duration (?)</td>
<td>5.2 ± 3.5</td>
<td>5.8 ± 4.1</td>
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<tr>
<td>Number of oocytes</td>
<td>10.6 ± 6.4</td>
<td>9.5 ± 5.9</td>
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<tr>
<td>Number of MII</td>
<td>8.4 ± 5.4</td>
<td>7.5 ± 5</td>
<td>0.12</td>
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<tr>
<td>Outcome [%]</td>
<td></td>
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<tr>
<td>Pregnancy</td>
<td>22 (26.2)</td>
<td>50 (31.4)</td>
<td>0.39</td>
</tr>
<tr>
<td>Abortion</td>
<td>7 (8.3)</td>
<td>11 (7)</td>
<td>0.68</td>
</tr>
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### References


Montag M, van der Ven K, Dorn C, van der Ven H. 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. Eur J Obstet Gynecol Reprod Biol 2006; 124:65–69.


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