Optimizing the embryo transfer technique
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Pregnancy rate is not improved by delaying embryo transfer from days 2 to 3.

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Abstract
Objective: To compare the outcome of assisted reproduction in day 2 versus day three embryo transfer.
Design: Prospective study.
Participants: A total of 927 consecutive embryo transfers for IVF and ICSI cycles including 626 embryo transfers on day 2 and 301 on day 3.
Intervention: IVF and ICSI.
Outcome measure: Clinical pregnancy rate.
Results: There is no significant difference in the pregnancy rate between ET on day 2 (50.9%) and ET on day 3 (50.5%).
Conclusion: Embryo transfer could be done on days 2 or 3 according to the convenience of the patient and the medical team.

Condensation: Embryo transfer could be done on days 2 or 3 according to the convenience of the medical team with similar results.
Introduction
Since human IVF was established as an efficient mode of treatment for infertility, many steps of the IVF procedure became standardized. However the optimum timing of embryo transfer (ET) is still debatable. In the early days of IVF, Edwards et al. (1) performed embryo transfer (ET) in the 8 – 16-cell stage, i.e. 3 or 4 days after egg collection. Later, most of the centers worldwide performed ET on day 2 when embryos are in the 4-cell stage. Lately, ET at the blastocyst stage was introduced (2).
It was reported that the first and the second cleavage divisions do not accurately predict the potential of future development and a large proportion of human embryos which arrest in vitro do so between the 4- and 8-cell stage (3). Based on this data, the idea of transferring embryos on day 3 should eliminate embryos which arrest at this sensitive stage and therefore allow selection of more viable embryos for transfer hoping that this might improve implantation and pregnancy rates (4).
The objective of this study is to compare the IVF outcome between days 2 and 3 transfer.
Materials and Methods

Patients
This is a prospective study performed at the Egyptian IVF-ET center, in Cairo, Egypt during the period from January 1998 to June 2000. A total of 927 patients who reached the stage of embryo transfer are included in the study. This represents consecutive embryo transfers including only patients during their first trial, 39 years old or less using the long GnRH agonist protocol and using only ejaculate sperm.

Patients with previous failed trials, patients above 39 years, patients who received short agonist protocol, antagonist protocols, clomiphene citrate or hMG only protocols were excluded from the study. Patients with very high or low body mass index, polycystic ovarian disease and general diseases not contradicting with performing IVF were not excluded from the study. All patients were treated by our standard GnRH long protocol, which was published before (5).

In our IVF center, we work 6 days a week with Friday as a holiday. Patients who have oocyte retrieval on Saturday, Sunday, Monday and Tuesday had their ET after 48h. Patients who have retrieval on Wednesday or Thursday had ET after 72h to avoid egg retrieval and embryo transfer on Friday.

The protocol was approved by IRB and our ethical committee. The power of the study was examined and it revealed that a sample size of 814 women provides 80% power and a two-sided significance level of 0.05 to test whether day 3 is equivalent or even superior to day 2 in an IVF/ET program. This sample size has adequate power to detect a difference of 5% in pregnancy rate/treated.

Oocyte and embryo culture
After retrieval, the oocytes were incubated in G1.2 media (Vitrolife, Motndalswaga, Goteborg) under mineral oil in tissue culture dishes (Falcon 3001) at 37°C and 5% CO₂ in air. ICSI procedure was performed about 2–3h after retrieval as described previously (6) after about 16–20h, the oocytes were checked for PN formation and the normally fertilized ones were transferred to fresh G media that was incubated overnight and no further changes of media was done till the day of embryo transfer. The embryos were cultured in groups of 3–4 in 50ml droplets of tissue culture media under mineral oil. On the day of ET, the best morphologically looking embryos were chosen. Selection of
the embryos for transfer was based on the number of blastomeres, absence of fragmentation and the most advanced stage of development. The best three embryos were transferred except in patients above the age of 37 with bad quality embryos where four embryos could sometimes be transferred. Two weeks after embryo transfer serum BhCG was measured and patients who had a positive pregnancy test were scheduled for ultrasound scan three weeks later. Clinical pregnancy was diagnosed by the presence of positive pregnancy test, ultrasound sac, echoes and pulsations.
Results
The mean age of patients was 31.7 ± 5.6 years for day 2 transfer as compared to 31.9 ± 5.2 years for day 3 transfer. There was no significant difference in the age between the two groups.
A total of 927 embryo transfers were included in the study on either day 2 (N = 626) or day 3 (N = 301). Causes of infertility in both groups are shown in Table 1.
Number of oocytes retrieved, fertilization rate, mean number of embryos transferred, number of clinical pregnancies, pregnancy rate, implantation rate and multiple pregnancies are shown in Table 2. There was no significant difference in all parameters between days 2 and 3. There were four sets of triplets in day 2 ET and two sets of triplets in day 3 ET.
The proportion of grades 1–three embryos was 27, 61 and 9 on day 2 ET versus 15, 71 and 12 on day 3 ET. The cleavage stage of embryos transferred on days 2 or 3 is shown in Fig. 1.
According to the number of embryos available for transfer, the patients were further divided into two subgroups. Subgroup A, patients with a maximum of three embryos available for transfer. Subgroup B, patients with more than three embryos available for transfer (Table 3).
There was no significant difference between the pregnancy rate in both subgroups between days 2 and 3 transfer. However, there was a significant higher PR in the subgroups with more than three embryos available for ET as compared with those who had less than three embryos available.

Statistical analysis
Statistical analysis of results was performed using the Student’s t-test and the percentage data were compared using chi-square test.
Discussion

Embryo transfer was performed either on day 2 or 3, until recently when blastocyst transfer was introduced as a possible option (2). From the available literature, it is not exactly clear if there is an advantage in delaying transfer to day 3 over day 2. Transfer of embryos to the uterus on day 3 after oocyte retrieval may be closer to the physiological time of arrival of embryo to the uterine cavity than transfer on day 2. Moreover, delaying embryo transfer would allow the selection of the most vital embryos for transfer (7).

The present study has shown that there is no significant difference in the PR between ET on days 2 or 3. Although a randomized study would have been ideal, yet there is no possibility of selection bias in selecting the day of embryo transfer as patients who had egg retrieval on Wednesdays and Thursdays had their ET on day 3, and those who had egg retrieval on other days of the weeks had their ET done on day 2. Criteria for giving hCG was fixed all through the study when the lead follicle was 19mm in diameter in the presence of three or more follicles. This meant that the day of ET was allocated at random.

Although the percentage of 4-cell stage embryos on day 2 ET was 83% as compared to the 55% 8-cell stage on day 3, this shows that embryos were arrested at 4- and 6-cell stage after extending the culture in vitro for 24h more (Fig. 1), yet this difference did not improve the pregnancy rate in day 3 over day 2 after we had the opportunity to exclude arrested embryos at 4- and 6-cell stage.

Even in patients with few embryos (three or less) there was no difference in the pregnancy rate between days 2 and 3.

Several retrospective studies have investigated this issue. Dawson et al. (4) in a retrospective study analyzed pregnancy rates in a series of patients comparing days 2 and 3 ET. There was no significant difference in the clinical PR between the two groups. The implantation rate as measured by the percentage of embryos developing to the fetal heart stage was significantly higher following transfer on day 3 (23%) than after day 2 (19%) suggesting that selection of viable embryos is improved on day 3. Furthermore, the embryos which gave rise to fetal sac significantly fewer miscarried before fetal heart stage 6% on day 3 as compared to 12% on day 2.

In a retrospective study, Carrillo et al. (8) compared day 2 with day 3 ET and found out that the implantation and pregnancy rates were
significantly higher on day 3; 24 and 44% as compared to 13 and 26% on day 2. In this study only patients with large numbers of oocytes were included.

In a large retrospective study, Huisman et al. (9) compared IVF results after day 2, 3 and 4 of ET. In general, the implantation and PR were similar in the three groups; however the implantation rate of 73 cavitating morula on day 4 was surprisingly higher (41%). The authors suggested that transfer on day 4 may give the ability to recognize embryos with very high implantation potential.

Some prospective studies compared the pregnancy rates on days 2 and 3 ET. Laverge et al. (7) in a prospective randomized study found that delaying embryo transfer by 1 day did not improve the pregnancy rate. They found a statistically lower percentage of excellent and good quality embryos on day 3 compared to day 2. However, this did not affect the pregnancy rate possibly because they were able to select good quality embryos for transfer because only patients with at least seven normally fertilized oocytes were included in the study.

In a prospective study of unselected group, patients were randomized for day 2 versus day 3 ET (10). Implantation rate was 15.8% for day 2 and 14.3 in day 3, and birth rate per ET was 18.5% for day 2 and 22.6% for day 3. There was no significant difference between both groups.

Van Os et al. (11) in a prospective randomized study of day 2 versus day 3 ET, the mean number of embryos transferred was equal in the two groups. The PR per ET was not significantly different between both groups. However, they found a significantly higher percentage of clinical abortions after ET on day 3. This is contrary to what Edwards has reported earlier (12).

Extending the culture period to beyond the time of expected activation of the embryonic genome might therefore optimize the selection of viable embryos for transfer (13). However, Extended culture may be compromised by the sub optimal embryo development in vitro compared to that in vivo. It has also been observed that a large proportion of human embryos which arrest in vitro do so between the 4- and 8-cell stage (3).

Daunson et al. (4) reported that there was no change in embryo quality between days 2 and 3 as measured by the distribution of embryo grades. However, 16% of embryos had either arrested or became
developmentally retarded between days 2 and 3 and it was therefore possible to avoid these embryos at the time of ET. The probability of selecting embryos on day 2 which will subsequently arrest in culture was calculated to be between 2 and 3%. Selection on the basis of morphology and the rate of development will result in probably selecting embryos which have not arrested if ET was delayed to day 3. Ertzeid et al. (10), randomized all patients with at least one egg fertilized to days 2 or 3 ET. There was no difference in the implantation and live birth rate. The outcome might have been different if only patients with several embryos on day 2 were included rendering better selection of embryos on day 3. They also mentioned that the rate of embryo development appears to be a more reliable indication of embryo viability than morphology. They observed a higher frequency of clinical pregnancy resulting from early cleaved two-cell embryos.

In conclusion, our data showed that although delay of in vitro culture of the embryo by 24h may eliminate arrested and poor quality embryos, yet ET on day 3 did not improve the pregnancy rate after IVF. This applied also to patients with three embryos or less, as well as patients with higher number of embryos.

It is recommended that embryo transfer could be done on either days 2 or 3 depending upon the convenience of the patients and the medical team. Our center achieved similar pregnancy rate in days 2 and 3 ET without performing ET during the weekend.
References

Table 1. Characteristics of patients in days 2 and three embryo transfer

<table>
<thead>
<tr>
<th></th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cycles</td>
<td>626</td>
<td>301</td>
</tr>
<tr>
<td>Age</td>
<td>31.7 ± 5.6</td>
<td>31.9 ± 5.2</td>
</tr>
<tr>
<td>Male factor</td>
<td>470 (75%)</td>
<td>220 (73%)</td>
</tr>
<tr>
<td>Tubal</td>
<td>94 (15%)</td>
<td>51 (17%)</td>
</tr>
<tr>
<td>Unexplained infertility</td>
<td>50 (8%)</td>
<td>21 (7%)</td>
</tr>
<tr>
<td>Others</td>
<td>12 (2%)</td>
<td>9 (3%)</td>
</tr>
</tbody>
</table>

No significant difference between all patient characteristics.

Table 2. Outcome of IVF and ICSI on days 2 and three embryo transfer

<table>
<thead>
<tr>
<th></th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>626</td>
<td>301</td>
</tr>
<tr>
<td>Number of oocytes retrieved</td>
<td>7156</td>
<td>3297</td>
</tr>
<tr>
<td>Mean number of oocytes</td>
<td>11.5 ± 3.5</td>
<td>11 ± 3.4</td>
</tr>
<tr>
<td>Fertilization rate</td>
<td>62%</td>
<td>61%</td>
</tr>
<tr>
<td>Mean number of embryos transferred</td>
<td>3.2 ± 1.1</td>
<td>3.1 ± 1.2</td>
</tr>
<tr>
<td>Number of clinical pregnancies</td>
<td>304</td>
<td>144</td>
</tr>
<tr>
<td>Clinical pregnancy rate per ET</td>
<td>50.90%</td>
<td>50.50%</td>
</tr>
<tr>
<td>Multiple pregnancy rate</td>
<td>121 (39.8%), four triplets</td>
<td>46 (31.9%), two triplets</td>
</tr>
<tr>
<td>Implantation rate</td>
<td>21.40%</td>
<td>20.60%</td>
</tr>
</tbody>
</table>

No significant difference in all parameters between the two groups.
Table 3. Pregnancy outcome in subgroup A with a maximum of three embryos available and subgroup B with more than three embryos available

<table>
<thead>
<tr>
<th>No. of pregnancies (%)</th>
<th>Day 2 (%)</th>
<th>Day 3 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subgroup A</td>
<td>39 (31)</td>
<td>19 (30.65)</td>
</tr>
<tr>
<td>Subgroup B</td>
<td>265 (56.1)</td>
<td>97 (59.9)</td>
</tr>
</tbody>
</table>

No significant difference in pregnancy in subgroups A and B between days 2 and 3 transfer.
Fig. 1. The number of blastomeres in days 2 and three embryo transfer.