Blastocyst-stage versus cleavage-stage embryo transfer in women with high oestradiol concentrations: randomized controlled trial

Eman A Elgindy a,b,*, Ahmed M Abou-Setta c, Magdy I Mostafa d

a Al-Banoon Fertility Center, Zagazig, Egypt; b Department of Obstetrics and Gynaecology, Zagazig University School of Medicine, Zagazig, Egypt; c Alberta Research Centre for Health Evidence, University of Alberta, Edmonton, Alberta, Canada; d Cairo University School of Medicine, Cairo, Egypt

* Corresponding author. E-mail address: eman_elgindy@hotmail.com (EA Elgindy).

Abstract This prospective, randomized, controlled trial tested the hypothesis that delaying embryo transfer to the blastocyst stage can increase the probability of clinical pregnancy and live birth in women with high oestradiol concentrations on the day of human chorionic gonadotrophin (HCG) undergoing intracytoplasmic sperm injection using the long protocol. A total of 200 women with oestradiol >3000 pg/ml on the HCG day with four or more good-quality, day-3 embryos were randomized in a 1:1 ratio to undergo day-3 or day-5 embryo transfer. Clinical pregnancy rates (CPR; 41% versus 59%; relative risk 0.70, 95% CI 0.52–0.93) and ongoing pregnancy/live-birth rates (35% versus 52%; relative risk 0.67, 95% CI 0.46–0.93) were lower in women undergoing cleavage-stage than blastocyst-stage embryo transfer. Using receiver operating characteristic curves, among women undergoing cleavage-stage embryo transfer, a detrimental cut-off value for not achieving pregnancy for oestradiol was 4200 pg/ml, with lower CPR and ongoing pregnancy/live-birth rates (P = 0.006 and 0.02, respectively). No detrimental cut-off value for oestradiol was identified among women undergoing blastocyst-stage embryo transfer. Delaying embryo transfer to the blastocyst stage can increase the probability of pregnancy in women with high oestradiol on the HCG day.

RBMOnline © 2011, Reproductive Healthcare Ltd. Published by Elsevier Ltd. All rights reserved.

KEYWORDS: blastocyst, cleavage-stage embryo, clinical pregnancy, embryo transfer, GnRH agonist, high oestradiol on HCG day

Introduction

Assessment of the association between oestradiol concentrations on the day of human chorionic gonadotrophin (HCG) administration and pregnancy achievement in IVF and intracytoplasmic sperm injection (ICSI) cycles has been the focus of interest for many years. The possible detrimental effect of supra-physiological oestradiol concentrations on the day...
of HCG administration has been the subject of dispute. Women with high serum oestradiol concentrations on the day of HCG administration have been reported to have unfavourable pregnancy rates (Lee et al., 2009; Simon et al., 1995, 1998), while others have failed to find this negative association (Levi et al., 2001; Sharara and McClarnock, 1999). The proposed adverse effects of high serum oestradiol include alteration in both endometrial receptivity and oocyte/embryo quality. It has also been proposed that the primary negative effect is on endometrial receptivity (Gidley-Baird et al., 1986; Hadi et al., 1994) since oocyte quality, fertilization rates, embryo cleavage (until day 2) and embryo quality were shown to be normal in women with high oestradiol (Simon et al., 1995; Yu Ng et al., 2000). Further, the implantation rate seemed to become normalized in subsequent frozen—thawed embryo transfers.

Kosmas et al. (2004), in a systematic review of retrospective studies, has shown that E2 levels do not seem to affect treatment outcome in gonadotrophin-releasing hormone (GnRH) agonist down-regulated IVF/ICSI cycles. While Valbuena et al. (2001), using an established in-vitro model for embryonic adhesion, investigated the possible deleterious effect of E2 on embryonic implantation. They showed that increasing concentrations of oestradiol had a deleterious effect on embryo adhesion. Importantly, the adhesion rate significantly decreased in the early transfer group compared with the late transfer group.

Many studies have shown that supra-physiological concentrations of steroid hormones cause impaired endometrial receptivity (Gidley-Baird et al., 1986; Hadi et al., 1994). Moreover, in cases of extreme advancement of the luteal endometrium, a transferred cleavage-stage embryo has the disadvantage of interacting with out-of-phase factors that might hinder its development to the blastocyst stage and thus its implantation (Barnes, 2000). Alternatively, blastocysts have the advantage of interacting with a less-out-of-phase endometrium, resulting in a better interaction with the implantation molecular milieu (Papanikolaou et al., 2008).

Therefore, this study postulated that blastocyst transfers might be more beneficial than cleavage-stage embryo transfer in women undergoing ICSI with high oestradiol concentrations on the day of HCG administration. First, the study chose the oestradiol cut-off value of >3000 pg/ml adopted in many studies (Lee et al., 2009; Simon et al., 1995, 1998). Importantly and in accordance with a Cochrane Review comparing blastocyst-stage versus cleavage-stage embryo transfer (Blake et al., 2007), this should be applied in women with high numbers of 8-cell embryos on day 3 in order to avoid cycle cancellation. Some studies suggested a threshold of three good embryos on the third day of embryo culture (Bungum et al., 2003), while others suggested that four or more good-quality embryos on day 3 appear to indicate that the patient will benefit from embryo transfer at the blastocyst stage and have a better chance of achieving a live delivery than with cleavage-stage embryo transfer (Papanikolaou et al., 2005).

In the current trial, the primary objective was to test the hypothesis that delaying embryo transfer to the blastocyst-stage may increase the probability of pregnancy in a selected group of women (i.e. women with high oestradiol concentrations on the day of HCG administration) undergoing their first ICSI cycle using the long agonist protocol. A secondary objective was to define a detrimental oestradiol cut-off value among cleavage-stage and blastocyst-stage transfers.

Materials and methods

Participants

This open-label, prospective, randomized, controlled trial was conducted from September 2008 to September 2010 in private and university-affiliated IVF units in Egypt. Ethical review-board approval was provided by the Al-Banoon Ethical Review Committee and informed consent was attained from all participants before study entry. The inclusion criteria was women’s age ≤35 years, with regular cycles (i.e. 24–35 days), serum day-3 FSH concentration <9.5 IU/l and antral follicle count (AFC) >6. Further, all included women were required to have oestradiol concentrations >3000 pg/ml and endometrial thickness ≥8 mm on the day of HCG administration and at least four good-quality embryos (i.e. regular symmetrical blastomeres with no fragmentation) on day 3 after retrieval (Racowsky et al., 2010).

Stimulation regimen

In all women, the long luteal-phase GnRH agonist down-regulation protocol was used. Briefly, GnRH agonist triptorelin (0.1 mg; Decapetyl; Ferring Pharmaceuticals, Switzerland) was administered daily starting from the mid-luteal phase of the pretreatment cycle. After satisfactory pituitary desensitization was achieved (i.e. endometrium <5 mm, no ovarian cysts and serum oestradiol concentration <50 pg/ml, conversion factor = 3.671 pmol/l), the dose of GnRH agonist was reduced to 0.05 mg/day and ovariian stimulation was initiated with both recombinant FSH (Puregon; MSD Pharmaceuticals, The Netherlands) and human menopausal gonadotrophin (Menogon; Ferring Pharmaceuticals, Switzerland) for all patients. Thereafter, a personalized dosage of gonadotrophin was given from day 6 of stimulation according to sequential transvaginal sonography and serum oestradiol concentrations. When at least three follicles had reached ≥17 mm in diameter, HCG (10,000 IU; Chorionon; IBSA, Switzerland) was administered. Oocytes were retrieved ~35 h after HCG administration.

ICSI and embryo culture

ICSI was performed in a standard way. Normal fertilization was assessed by the presence of two pronuclei (ZPN) 16–20 h after injection. Embryos were cultured in sequential media (Sage; Cooper Surgical, USA), in groups of four or five per 50 μl micro drop. On days 2 and 3, embryo development was evaluated according to the number of blastomeres, the percentage of fragmentation and the symmetry of the blastomeres. Participants who had at least four good-quality embryos (i.e. ≥8 regular symmetrical blastomeres with no fragmentation on day 3 and all of them had shown four or more regular symmetrical blastomeres with no fragmentation on day 2) available for transfer were candidates for the current trial.
Randomized trial of day-3 versus day-5 embryo transfer in high responders

Randomization

On day 3, the included women were randomized to undergo day-3 cleavage-stage embryo transfer (group A, n = 100) or extended culture to undergo day-5 blastocyst-stage embryo transfer, using a computer-generated block randomization. Allocation concealment was performed using 200 identical, sequentially numbered, dark-sealed envelopes, prepared by one of the investigators (MIM) and kept in the unit’s pharmacy. When the woman was eligible and agreed to participate, she was instructed to open the next available envelope to determine the group to which she was assigned. Blinding was not possible due to the nature of the study.

Extended embryo culture

Women randomized to undergo blastocyst-stage transfers had their embryos examined at 24-h intervals up to 144 h after retrieval and the cell number and developmental stage were recorded.

For classification purposes, the study initially used the system proposed by Gardner and Schoolcraft (1999). Blastocysts were given a number from 1 to 6 based on the degree of expansion and hatching status: 1. early blastocyst, the blastocoele accounting for less than one-half of the volume of the embryo; 2. blastocyst, the blastocoele occupying more than one-half of the volume of the embryo; 3. full blastocyst, the blastocoele filling the embryo completely; 4. expanded blastocyst, the blastocoele now larger than the early embryo and the zona pellucida beginning to thin; 5. hatching blastocyst, trophoderm (TE) cells beginning to herniate through the zona pellucida; and 6. hatched blastocyst, the blastocyst has completely escaped the zona pellucida. The inner cell mass (ICM) was scored as follows: A. tightly packed with many cells; B. loosely grouped with several cells; and C. very few cells. The TE was scored as follows: A. many cells forming a cohesive epithelium; B. few cells forming a loose epithelium; and C. very few large cells. Extra blastocysts were only considered for vitrification if they were regarded to be full blastocysts and onwards (grades 3–6), ICM scored A–B and TE scored A–B.

Later, the embryo-scoring system described by Racowsky et al. (2010) was used: for expansion: early, expanding, expanded, hatched; for ICM: good, fair, poor; and for TE: good, fair, poor. Generally, only cavitating blastocyst-stage embryos (i.e. embryos that had expanded sufficiently so that a distinct ICM could be identified within a well-developed blastocoele filling the embryo) were transferred.

Embryo cryopreservation

Extra embryos were vitrified according to a previously published protocol (Takahashi et al., 2005). Excess blastocysts were only considered for vitrification if they were regarded to be full blastocysts and onward (i.e. grades 3–6) (Gardner and Schoolcraft (1999)) or expanded-hatched (Racowsky et al., 2010), had ICM and TE scored A–B (i.e. good–fair). Only good-quality cleavage-stage embryos were vitrified.

Outcome measures

Clinical pregnancy rate (CPR) was the primary outcome. Defining oestradiol detrimental cut-offs among cleavage-stage and blastocyst-stage embryo transfers was the secondary outcome. In both groups, serum β-HCG tests were performed 2 weeks after embryo transfer, and transvaginal ultrasound was scheduled 3 weeks later to confirm a clinical pregnancy. CPR was defined as the number of clinical pregnancies (with positive cardiac pulsations on ultrasound) per randomized woman. Spontaneous abortion was defined as the spontaneous loss of a clinical pregnancy before 20 completed weeks of gestational age (i.e. 18 weeks after fertilization) (Zegers-Hochschild et al., 2009). Ongoing pregnancy rate was defined as the number of clinical pregnancies, continuing beyond 20 weeks of gestation expressed per randomized woman. Live-birth rate as the number of deliveries after 24 weeks of gestation with a live fetus per randomized woman.

Hormone measurements

Serum concentrations of FSH, LH, oestradiol, progesterone and β-HCG were determined using Elecsys 2010 (Roche, Germany). For FSH, the analytical sensitivity was <0.1 IU/l with total precision of 2.9%. For LH, the analytical sensitivity was 0.1 IU/l with total precision of 1.6%. For oestradiol, the analytical sensitivity was 5 pg/ml with total precision of 2.3%. For progesterone, the analytical sensitivity was 0.03 ng/ml (conversion factor = 3.18 nmol/l) with total precision of 2.4%. For quantitative β-HCG assay, the analytical sensitivity was 0.5 IU/l with total precision of 2.1%.

Statistical analysis

Data were statistically described in terms of mean ± standard deviation, frequencies and percentages, where appropriate. Comparison of quantitative variables was performed using Student’s t-test for independent samples. For comparing categorical data, the chi-squared test was performed, except when the expected frequency of events was less than five, in which case the Fisher’s exact test was used. All analyses were performed using the intention-to-treat principle.

Receiver-operating-characteristic (ROC) curve analysis was conducted to search for the most efficient cut-off values for oestradiol concentration on the day of HCG administration which could discriminate between successful and unsuccessful ICSI outcomes in women undergoing day-3 and day-5 embryo transfers. The best cut-off value was determined based on an equivalent sensitivity, specificity and the highest value of the area under the curve (AUC). Univariate and multivariate analysis models were also used to test for the preferential effect of all independent variables on CPR in each group. Relative risk, 95% confidence intervals and/or P-values are presented. A P-value <0.05 was considered to be statistically significant.

Sample size

Prior data from a retrospective study showed the CPR among women with high oestradiol undergoing day-3 and
day-5 embryo transfers to be 47% and 67%, respectively (Chen et al., 2003). Therefore, 95 women would be required to be able to reject the null hypothesis that the success rates are equal with a probability (power) of 0.8 and type-I error probability of 0.05 using the chi-squared statistic.

Results

A total of 450 women were potentially eligible for recruitment. Of these, 200 did not meet the a-priori inclusion criteria, 30 declined to participate and 20 were excluded for other reasons. On day 3 after retrieval, 200 women were randomized. Women randomized to group A (n = 100) underwent cleavage-stage embryo transfer on day 3, while participants in group B (n = 100) had extended culture to undergo blastocyst-stage embryo transfer on day 5 (Figure 1). Three women’s embryos did not reach full blastocyst development on day 5 and were left to day 6 for further culture. These three cases were included in the intention-to-treat analyses.

The two groups were balanced in respect to baseline, clinical and cycle characteristics. No differences were found regarding the women’s age, duration of infertility, basal FSH and mean antral follicle count. Number of ampoules, days of stimulation and oestradiol and progesterone concentrations on day of HCG were also comparable between the two groups. Similarly, number of retrieved and fertilized oocytes and good-quality embryos were comparable between the two groups. Even so, the number of embryos per transfer was significantly higher in group A than group B (P = 0.04) and there were more embryos available for vitrification in group A (Table 1). In contrast, the clinical and ongoing pregnancy/live-birth rates were higher in group B than in group A, while abortion and twin rates were comparable between both groups (Table 2). Two women in group A had triplet pregnancies and underwent selective embryo reduction; no adverse outcomes of the procedure were reported. Regarding the ongoing pregnancy/live-birth rate in group A, there were two losses between 21 and 24 weeks, and 35 women had an ongoing pregnancy/live birth, with 15 women pregnant and 20 women having had a documented live birth, including two preterm deliveries which resulted in one neonatal death. In group A, there were three losses between 20 and 24 weeks, and 52 women had an ongoing pregnancy/live birth, with 15 women pregnant and 37 women having had a documented live birth, including three with preterm deliveries, but no neonatal deaths.

A number of women underwent transfers using their cryopreserved embryos. Of the women in group A, 30 returned for vitrified–warmed embryo transfer. Of these, 27 underwent embryo transfer and three did not as the embryos did not survive vitrification–warming. Nine women became pregnant, with one abortion and eight ongoing pregnancies. Ten women returned for a second warming cycle. Of these, nine underwent embryo transfer with three ongoing pregnancies. Of the women in group B, 25 returned for vitrified–warmed embryo transfer. Of these, 23 underwent embryo transfer and two did not as the embryos did not survive vitrification–warming. Ten women became pregnant, with one abortion and nine ongoing pregnancies. Six women returned for a second warming cycle. Of these five, underwent blastocyst-stage embryo transfer, resulting in two ongoing pregnancies.

To assess the predictive value of serum oestradiol concentrations on the day of HCG administration on pregnancy achievement among women in group A, a ROC curve analysis was performed. The optimal detrimental cut-off value for oestradiol on the day of HCG administration was ≥4200 pg/ml for not achieving pregnancy (sensitivity 82.9%;

![Figure 1](image-url) Flowchart of participants in the study.
specificity = 50%; AUC 0.65, 95% CI 0.54–0.75; Figure 2). Women were therefore classified according to the defined cut-off value and different characteristics of the two subgroups compared (Table 3). No significant differences were found regarding the baseline characteristics; however, women with oestradiol ≥4200 pg/ml had significantly higher oestradiol and progesterone concentrations and higher numbers of retrieved and fertilized oocytes and good-quality embryos than those with oestradiol <4200 pg/ml (P < 0.001 for all comparisons). Furthermore, even though the number of embryos per transfer was comparable in the two subgroups, the participants with oestradiol <4200 pg/ml had a significantly higher CPR (P = 0.006) and ongoing pregnancy/live-birth rate (P = 0.022) than those with higher values.

Pregnant women in group A had statistically significantly lower progesterone and oestradiol compared with those who did not achieve pregnancy (P = 0.04 and P = 0.004, respectively). For participants in group B, oestradiol concentrations did not differ between pregnant women and those who did not become pregnant. Upon performing ROC curve analysis, no detrimental cut-off value for oestradiol on the day of HCG for not achieving pregnancy was apparent (Figure 3).

Multivariate regression analysis was performed for participants undergoing cleavage-stage or blastocyst-stage embryo transfer with adjustment for women’s age, duration of infertility, basal FSH, basal AFC, duration of stimulation, gonadotrophin dose, oestradiol and progesterone concentrations on the day of HCG administration, numbers of retrieved and fertilized oocytes and numbers of good-quality and transferred embryos, with no independent predictor of pregnancy identified.

Discussion

The best available evidence from randomized trials suggests that the probability of pregnancy and live births after fresh-embryo transfer is significantly higher after

Table 1  Basal, clinical and cycle characters of patients with high serum oestradiol concentrations on the day of HCG undergoing cleavage-stage and blastocyst-stage embryo transfer.

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Group A (n = 100)</th>
<th>Group B (n = 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>27.65 ± 4.12</td>
<td>28.47 ± 5.01</td>
</tr>
<tr>
<td>Duration of infertility</td>
<td>6.84 ± 3.09</td>
<td>6.22 ± 3.52</td>
</tr>
<tr>
<td>Basal FSH (IU/l)</td>
<td>6.37 ± 1.13</td>
<td>6.25 ± 1.70</td>
</tr>
<tr>
<td>AFC</td>
<td>13.60 ± 1.54</td>
<td>13.63 ± 2.00</td>
</tr>
<tr>
<td>Stimulation (days)</td>
<td>10.95 ± 1.37</td>
<td>11.28 ± 1.34</td>
</tr>
<tr>
<td>Gn ampoules</td>
<td>31.82 ± 5.69</td>
<td>32.64 ± 6.58</td>
</tr>
<tr>
<td>Oestradiol on HCG day (pg/ml)</td>
<td>3848.6 ± 630.2</td>
<td>3820.9 ± 641.3</td>
</tr>
<tr>
<td>Progesterone on HCG day (ng/ml)</td>
<td>1.69 ± 0.76</td>
<td>1.72 ± 0.86</td>
</tr>
<tr>
<td>Oocytes retrieved</td>
<td>17.37 ± 4.40</td>
<td>18.38 ± 4.72</td>
</tr>
<tr>
<td>Fertilized oocytes (2PN)</td>
<td>12.14 ± 3.45</td>
<td>13.10 ± 3.63</td>
</tr>
<tr>
<td>Good-quality embryos</td>
<td>6.73 ± 3.14</td>
<td>7.96 ± 2.92</td>
</tr>
<tr>
<td>Embryos transferreda</td>
<td>2.80 ± 0.43</td>
<td>1.97 ± 0.17</td>
</tr>
<tr>
<td>Vitrification of excess embryosb</td>
<td>95 (95)</td>
<td>75 (75)</td>
</tr>
<tr>
<td>Embryos vitrifiedb</td>
<td>4.4 ± 2.6</td>
<td>1.9 ± 1.4</td>
</tr>
</tbody>
</table>

Values are mean ± SD or n (%). Group A = day-3 cleavage-stage embryo transfer; Group B = day-5 blastocyst-stage embryo transfer.

AFC = antral follicle count; Gn = gonadotrophin; HCG = human chorionic gonadotrophin; PN = pronuclear.

aP = 0.04.
bP < 0.001.

Table 2  Cycle outcomes of patients with high serum oestradiol concentrations on the day of human chorionic gonadotrophin undergoing cleavage-stage and blastocyst-stage embryo transfer.

<table>
<thead>
<tr>
<th>Cycle outcomes</th>
<th>Group A (n = 100)</th>
<th>Group B (n = 100)</th>
<th>Relative risk (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical pregnancy</td>
<td>41/100 (41.0)</td>
<td>59/100 (59.0)</td>
<td>0.70 (0.52–0.93)</td>
<td>0.014</td>
</tr>
<tr>
<td>Abortion</td>
<td>4/41 (9.8)</td>
<td>4/59 (6.8)</td>
<td>1.44 (0.38–5.42)</td>
<td>NS</td>
</tr>
<tr>
<td>Twin pregnancy</td>
<td>8/41 (19.5)a</td>
<td>12/59 (20.3)</td>
<td>0.96 (0.43–2.14)</td>
<td>NS</td>
</tr>
<tr>
<td>Ongoing pregnancy/live birthb</td>
<td>35/100 (35)</td>
<td>52/100 (52)</td>
<td>0.67 (0.46–0.93)</td>
<td>0.015</td>
</tr>
</tbody>
</table>

Values are n/total (%). Group A = day-3 cleavage-stage embryo transfer; Group B = day-5 blastocyst-stage embryo transfer.

CI = confidence intervals; NS = not statistically significant.

aIncludes two patients who underwent selective embryo reduction to reduce triplet pregnancies to twin pregnancies.
bTwo losses between 21 and 24 weeks in the cleavage-stage embryo transfer group and three losses between 20 and 24 weeks in the blastocyst-stage transfer group.
blastocyst-stage as compared with cleavage-stage embryo transfer (Blake et al., 2007; Papanikolaou et al., 2008). However, the current study and research question is upon a more selective group of women who had oestradiol >3000 pg/ml on the day of HCG administration and four or more good-quality embryos on day 3. The study tested the hypothesis that delaying embryo transfer to the blastocyst stage may increase the probability of pregnancy in these women with high oestradiol concentrations on the day of HCG administration undergoing their first ICSI cycle using the long GnRH agonist protocol.

Although the included patient population is a very high prognosis group, women having oestradiol >3000 pg/ml on

![Figure 2](image2.png)

**Figure 2** Receiver-operating-characteristic curve for defining optimal detrimental cut-off value for oestradiol on HCG day among women undergoing day-3 cleavage-stage embryo transfer. Diagonal segments are produced as ties. HCG = human chorionic gonadotrophin.

![Figure 3](image3.png)

**Figure 3** Receiver-operating-characteristic curve for defining optimal detrimental cut-off value for oestradiol on HCG day among women undergoing day-5 blastocyst-stage embryo transfer. Diagonal segments are produced as ties. HCG = human chorionic gonadotrophin.

<table>
<thead>
<tr>
<th>Characteristics, ovarian stimulation and pregnancy outcomes</th>
<th>Oestradiol on HCG day</th>
<th>Relative risk (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>27.66 ± 4.23</td>
<td>27.63 ± 3.99</td>
<td>—</td>
</tr>
<tr>
<td>Duration of infertility (years)</td>
<td>6.80 ± 3.20</td>
<td>6.89 ± 3.80</td>
<td>—</td>
</tr>
<tr>
<td>Basal FSH (IU/l)</td>
<td>6.45 ± 1.12</td>
<td>6.22 ± 1.15</td>
<td>—</td>
</tr>
<tr>
<td>AFC</td>
<td>13.30 ± 1.20</td>
<td>13.90 ± 1.90</td>
<td>—</td>
</tr>
<tr>
<td>Stimulation (days)</td>
<td>11.02 ± 1.30</td>
<td>10.80 ± 1.40</td>
<td>—</td>
</tr>
<tr>
<td>Gn ampoules</td>
<td>33.2 ± 4.40</td>
<td>33.6 ± 4.10</td>
<td>—</td>
</tr>
<tr>
<td>Oestradiol on HCG day (pg/ml)</td>
<td>3398.8 ± 257.0</td>
<td>4582.5 ± 247.0</td>
<td>—</td>
</tr>
<tr>
<td>Progesterone on HCG day (ng/ml)</td>
<td>1.44 ± 0.45</td>
<td>2.09 ± 0.96</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Oocytes retrieved</td>
<td>14.50 ± 2.40</td>
<td>21.90 ± 2.70</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fertilized oocytes (2PN)</td>
<td>10.50 ± 2.70</td>
<td>14.90 ± 2.70</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Good-quality embryos</td>
<td>5.60 ± 2.50</td>
<td>8.70 ± 3.20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Embryos transferred</td>
<td>2.80 ± 0.37</td>
<td>2.70 ± 0.50</td>
<td>NS</td>
</tr>
<tr>
<td>Clinical pregnancy</td>
<td>32 (51.60)</td>
<td>9 (23.70)</td>
<td>2.18 (1.17–4.05)</td>
</tr>
<tr>
<td>Abortion</td>
<td>3 (9.40)</td>
<td>1 (11.10)</td>
<td>0.84 (0.09–7.16)</td>
</tr>
<tr>
<td>Ongoing pregnancy/live birtha</td>
<td>27 (43.5)</td>
<td>8 (21.05)</td>
<td>2.07 (1.05–4.07)</td>
</tr>
</tbody>
</table>

Values are mean ± SD or n (%).  
AFC = antral follicle count; CI = confidence intervals; Gn = gonadotrophin; HCG = human chorionic gonadotrophin; NS = not statistically significant; PN = pronuclear.  
aTwo losses between 21 and 24 weeks.
the day of human chorionic gonadotrophin (HCG) and who had undergone blastocyst-stage embryo transfer had higher CPR and ongoing pregnancy/live-birth rate than those undergoing cleavage-stage embryo transfer. Participants in both groups had comparable numbers of retrieved/fertilized oocytes and good-quality embryos. Moreover, women undergoing cleavage-stage embryo transfer had higher numbers of embryos transferred.

Thus the transfer of extra embryos in the day-3 group compared with day-5 group increases the probability of transferring an euploid embryo and thereby reducing the selection bias in early embryonic developmental stages (i.e. cleavage stages) (Papanikolaou et al., 2008). Even so, women with high oestradiol concentrations undergoing cleavage-stage embryo transfer fared worse. However, there were more surplus embryos available for vitrification when a cleavage-stage embryo transfer was performed. Indeed, it could be argued that combining fresh and vitrified—warmed data for both groups might increase the cumulative pregnancy rate for women with high oestradiol undergoing cleavage-stage embryo transfer. Therefore, this could be the optimal outcome for comparison in the future, as in this study a large percentage of women became pregnant and the majority who did not become pregnant during the fresh cycle have yet to undergo vitrified—warmed embryo transfer.

Importantly, two women had triplets among day-3 embryo transfers. Although they underwent fetal reduction, with no adverse outcomes, high-order multiple pregnancies have always been reported to be of great concern to assisted reproduction practitioners and society as a whole. In the current trial and others, blastocyst transfer allows better selection of embryos and high-order multiple pregnancies could be almost completely eliminated by transferring only one to two embryos in the blastocyst stage (Edwards and Beard, 1999).

Meanwhile, it appears that the benefits associated with high oestradiol concentrations in fresh ICSI cycles may be offset by the negative impact on the endometrium or other target tissues, particularly if the embryos are placed in the uterus soon after the oestradiol concentration peak for oocyte retrieval. The current data suggest that such a hypothetical effect on the endometrium may, to some extent, dissipate between day 3 and day 5. Accordingly, the beneficial effect of high oestradiol concentrations with respect to oocyte quality may become predominant when the embryos are transferred on day 5. In consistence with these results, Chen et al. (2003) reported that increasing oestradiol concentrations on the day of HCG administration were associated with improved pregnancy rates when embryo transfer was performed on day 5. However, this was a retrospective study including women with variable ovarian reserves. Only women who had good numbers of good-quality embryos on day 3 were submitted to further culture and underwent blastocyst-stage embryo transfer. It could be argued that the increase in pregnancy rates among these women could be potentially favoured by other confounding factors. Meanwhile, the current study included only women who had good numbers of good-quality embryos on day 3 and randomized to undergo either day-3 or day-5 embryo transfer. Both groups had comparable numbers of good-quality embryos with higher numbers transferred to those undergoing day-3 embryo transfers. Even so, comparable twin rate was present between the two groups. A higher implantation rate with day-5 embryos in comparison to day 3 (36%, 71/197 versus 19%, 53/280; $P < 0.001$) might explain the similar twin pregnancy rates despite the significant difference in the number of embryos per transfer. Therefore, it may be assumed that the uterine environment may be more receptive because the 2- to 3-day delay of transfer may afford the uterus more time to recover after being exposed to the peak of the super-physiological levels of E2.

It has been reported that, in GnRH-agonist cycles, endometrial biopsies taken in the preovulatory phase prior to HCG injection have shown accentuated proliferative aspects and early secretory changes even before the rise in progesterone occurs (Marchini et al., 1991). Biopsies taken on the day of oocyte retrieval show endometrial advancement in more than 90% of the cases, with no pregnancy occurring if the advancement is exceeded by 3 days (Ubaldi et al., 1997). Alternatively, Bourgain et al. (2002) reported that early luteal severe antiproliferative effects of the stimulation protocol were observed in both glandular and stromal cells when compared with natural-cycle controls; however, this difference was no longer present on later-cycle days. Moreover, Bourgain and Devroye (2003) demonstrated the absence of endometrial advanced maturation in mid-luteal phase of stimulated cycles. So, it appears that transfer of blastocysts may offer the endometrium time to recover from any negative effects caused by the exposure to high peak oestradiol concentrations.

Alternatively, regarding the concern of cancellation of embryo transfer in women subjected to extended culture, three women in the current study did not develop expanding blastocysts on day 5 and were included in the intention-to-treat analysis. Although embryos were left to day 6 and all the three cases underwent day-6 blastocyst-stage embryo transfer, these cases were excluded from group B, but included in analysis in accordance with intention to treat. Cancellation of blastocyst-stage embryo transfers in the current trial is lower than that reported in the systematic review by Papanikolaou et al. (2008). Importantly, they emphasized that if the blastulation rate of an embryology laboratory exceeds 50% of the fertilized ova (considered as a satisfactory cut-off), then the probability of a 2-pronuclei embryo not reaching the blastocyst stage due to in-vitro culture conditions decreases significantly. In the current trial, owing to the strict inclusion criteria and more than 50% blastulation rate, all cases had reached blastocyst-stage embryo transfer, whether on day 5 or 6.

Importantly, the use of oestradiol concentrations in order to predict treatment outcome is still controversial (Papageorgiou et al., 2002). Different oestradiol cut-off concentrations (Simon et al., 1995, 1998; Yu Ng et al., 2000) make interpretation of data cumbersome. The cut-off value of >3000 pg/ml adopted in the current study has been used by many investigators (Lee et al., 2009; Sharara and McClamrock, 1999; Simon et al., 1995, 1998). Other values, based on percentile curves, have been suggested (Chen
et al., 2007; Kyrou et al., 2009; Papageorgiou et al., 2002); however, there are still no uniformly accepted oestradiol percentiles. Papageorgiou et al. (2002) have used the 10th and 90th percentiles to define the type of response; with high responders having oestradiol >90th percentile. Alternatively, Kyrou et al. (2009) have used the 25th and 75th percentiles, with high responders having oestradiol >75th percentiles. An appropriate method to analyse the possible association of serum oestradiol on the day of HCG administration with the probability of pregnancy is the use of ROC curve analysis.

In the present study, a serum oestradiol concentration ≥4200 pg/ml on the day of HCG administration was identified as the most appropriate threshold for not achieving pregnancy among women undergoing cleavage-stage embryo transfer. Women having oestradiol ≥4200 pg/ml had significantly higher oestradiol and progesterone concentrations on the day of HCG and higher numbers of retrieved and fertilized oocytes and good-quality embryos than those with oestradiol <4200 pg/ml. Alternatively, the participants with oestradiol <4200 pg/ml had significantly higher CPR and ongoing pregnancy/live-birth rate than those with higher oestradiol. However, the AUC was 0.65 and the number of women having oestradiol ≥4200 pg/ml was relatively small (n = 38). Therefore, a larger-scale study needs to be conducted for further evaluation. Interestingly, in accordance with the oestradiol cut-off value determined in this study, Chen et al. (2007) evaluated 1196 GnRH agonist cycles grouped by peak oestradiol percentile distribution and the oestradiol cut-off concentration for high responders (percentile >90%) and was found to be ≥4128 pg/ml (119 cycles). They reported that high serum oestrogen concentrations were detrimental to implantation but not to the quality of oocytes, which may be due to an adverse effect on endometrial receptivity in ovarian stimulation cycles. Importantly, in the current study women undergoing day-3 embryo transfer and having oestradiol 3000–4200 pg/ml had comparable CPR to all women undergoing blastocyst-stage embryo transfer (51.6% versus 59.0%, relative risk 0.88, 95% CI 0.65–1.17). Meanwhile, those having oestradiol >4200 pg/ml had lower CPR than all women undergoing blastocyst-stage embryo transfers (23.7% versus 59.0%, relative risk 0.4, 95% CI 0.22–0.73; P < 0.001). However, although this finding might preliminarily suggest that comparable clinical outcomes could be elicited between day-3 embryo transfers with oestradiol <4200 pg/ml and blastocyst-stage embryo transfers, transferring a reduced number of embryos almost eliminates the odds of having a high-order pregnancy. Actually, although single-embryo transfer is not yet adopted by women in Egypt, a healthy singleton would be eventually the optimum outcome. To accomplish this objective, a blastocyst with the greatest potential for implantation should be selected for embryo transfer, especially in women who are good candidates for extended culture like those in the current study.

Regarding the association between high oestradiol and progesterone elevation on the day of HCG administration, it could be suggested that at least one of the mechanisms that play a role in the premature increase of plasma progesterone is linked to the high response of the ovary to ovarian stimulation. An excess in the number of follicles, and consequently an excess of proliferating granulosa cells, may lead to an increased progesterone production. Papanikolaou et al. (2009) used an arbitrarily defined detrimental cut-off for progesterone and reported that progesterone >1.5 ng/ml had an adverse effect on pregnancy outcome in women undergoing cleavage-stage, but not blastocyst-stage, embryo transfer. Moreover, when they used equal percentile analysis with four cut-off points according to progesterone measurements on the day of HCG triggering, there was a statistically significant decrease in the CPR when progesterone values were increased (>1.53 ng/ml). Performing the same analysis in the subgroup of patients undergoing single-blastocyst transfer, there was no effect of progesterone rises on the pregnancy outcome. A recent trial by this study group, among good responders using the long GnRH agonist protocol, has shown that progesterone >1.5 ng/ml on the day of HCG, as determined by the ROC curve, affects the CPR in women undergoing cleavage-stage, but not blastocyst-stage, embryo transfer (Elgindy, 2011). Therefore, it can be assumed that, in combination with the premature elevation of progesterone, the high oestradiol concentrations have a negative effect on pregnancy outcome.

In the current trial, upon doing univariate analysis, pregnant women undergoing cleavage-stage embryo transfer had statistically significant lower progesterone and oestradiol concentrations on the day of HCG administration than those who did not achieve pregnancy (P = 0.015 and P = 0.043, respectively). However, upon performing multivariate regression analysis, there was no independent predictor of pregnancy. It might be that, although the multivariate regression was done on the whole 100 cases of day-3 embryo transfers, this is still a relatively small number in relation to the large number of entered variables. Upon performing ROC curve analysis to define the detrimental cut-off value for oestradiol among women undergoing blastocyst-stage embryo transfer, the AUC calculated could not distinguish between pregnant and non-pregnant women. Interestingly, when the oestradiol detrimental cut-off value of 4200 pg/ml was applied to women undergoing blastocyst-stage embryo transfers, the CPR were comparable between women with oestradiol <4200 pg/ml and those with higher values (38/63, 60.3% versus 21/37, 51.4%; relative risk 1.06, 95% CI 0.75–1.50). Similarly, there was no statistically significant difference in the ongoing pregnancy/live-birth rates between women with oestradiol <4200 pg/ml and those with higher oestradiol (33/63, 52.4% versus 19/37, 51.4%; relative risk 1.02, 95% CI 0.69–1.51).

Accordingly, women with unusually high peak oestradiol concentrations may be more likely to benefit from delaying embryo transfer to day 5. So, on the basis of the current trial, in order to improve pregnancy rates in the case of high oestradiol concentrations at the end of ovarian stimulation, a proposed strategy could be the decision to target embryo transfer on day 5.

The potential weakness of the current trial includes the inability to blind either the women or physicians. Also, the study population included a selective high-prognosis group of young women with high oestradiol concentrations on the day of HCG administration using the long GnRH agonist protocol, which limits the generalizability of the study findings. In conclusion, delaying embryo transfer to the blastocyst stage can increase the probability of pregnancy in women
undergoing ICSI cycles using the long agonist protocol with high oestradiol concentrations on the day of HCG administration. Using ROC curve analysis, a detrimental cut-off value for oestradiol on the day of HCG is 4200 pg/ml among women undergoing cleavage-stage embryo transfer. Further, large-scale studies need to be conducted to confirm these results and to investigate differences in subgroups.

References


Papageorgiou, T., Guibert, J., Goffinet, F., Patrat, C., Fulla, Y., Janssens, Y., Zorn, J.R., 2002. Percentile curves of serum oestradiol levels during controlled ovarian stimulation in 905 cycles stimulated with recombinant FSH show that high oestra-

dong is not detrimental to IVF outcome. Hum. Reprod. 17, 2846–2850.


Valbuena, D., Martin, J., de Pablo, J.L., Remohi, J., Pellicer, A., Simon, C., 2001. Increasing levels of oestradiol are deleterious to embryonic implantation because they directly affect the embryo. Fertil. Steril. 76, 962–968.

Yu Ng, E.H., Yeung, W.S., Yee Lan, L.E., So, W.W., Ho, P.C., 2000. High serum oestradiol concentrations in fresh IVF cycles do not

Declaration: The authors report no financial or commercial conflicts of interest. Trial registration number: ACTRN1261100028909.

Received 14 January 2011; refereed 4 August 2011; accepted 4 August 2011.