Single top quality embryo transfer as a model for prediction of early pregnancy outcome

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BACKGROUND: Single embryo transfer (particularly of a top quality embryo) is an excellent model to correlate embryo quality in terms of morphological criteria to early pregnancy. We investigated whether this model could provide us with more information on what happens after implantation in the first trimester of pregnancy.

METHODS: The outcome of 370 consecutive single top quality embryo transfers in patients younger than 38 years was analysed for pregnancy and first-trimester pregnancy loss (FTPL) before 13 weeks of gestation. Analysis was done on each cohort of embryos from which the transferred top quality embryo was selected. Serum HCG levels were measured on day 8 and day 12 after day 3 embryo transfer. The HCG index was calculated as the level of HCG on day 12/HCG on day 8.

RESULTS: The pregnancy rate after single top quality embryo transfer was 51.9%. This was independent of the patients’ age. FTPL, however, appeared to be age dependent: 15.4% for the whole group, 9% in patients younger than 30 years and 19% in patients above 30 years. The pregnancy rate was 50% in IVF cycles and 52% in ICSI cycles; FTPL was 19% in IVF cycles and 10% in ICSI cycles. Multiple regression analysis showed that these differences originated from age differences between both populations rather than from technique-related factors. An HCG level >45 IU/l on day 12 was predictive for ongoing pregnancy with 75.6% sensitivity and 100% specificity; an HCG index >3.5 similarly predicted ongoing pregnancy with 72.3% sensitivity and 100% specificity.

CONCLUSIONS: These data show that embryo selection for transfer on day 3 can be used as an excellent tool for prediction of pregnancy but not for prediction of FTPL. The pregnancy rate of a single top quality embryo is not related to age, whereas FTPL is age dependent.

Key words: assisted reproduction/early pregnancy/single embryo transfer/top quality embryo

Introduction

Assisted reproductive technology (ART) has focused on increasing the pregnancy rate, predominantly by increasing the quality of embryos transferred. A combination of improved selection criteria (Van Royen et al., 1999, 2001, 2003) and improved culture conditions is of paramount importance to optimize the selection of the embryo with the highest implantation potential. After implantation, however, an important percentage of pregnancy loss is encountered. Fifteen to 20% of all recognized pregnancies fail to progress beyond the first trimester. These figures are irrespective of the way in which the pregnancies are conceived, either spontaneously (Nybo Andersen et al., 2000) or after ART (Liu et al., 1988). Winter et al. (2002) found that early pregnancy loss prior to 6–7 weeks of pregnancy was related to poor embryo quality.

We have been interested to know what happens to highly competent embryos after implantation in the first trimester, particularly because a direct link to embryo quality can be made.

What happens to the embryo after it has been transferred in the uterus remains largely unknown. The only outcome variables after embryo transfer are detection of HCG in serum and urine, and ultrasonography later in pregnancy. The predictive value of a single serum HCG level has been shown after assisted conception (Zayed et al., 2001; Homan et al., 2002).

In this study, we used single embryo transfer of a top quality IVF/ICSI embryo as a model to calculate the predictive value of variables such as HCG measurements with regard to early pregnancy outcome.

Materials and methods

Subjects

From January 1998 until August 2002, we performed 379 single top quality embryo transfers, accounting for 22.6% of all transfers. There
were nine single embryo transfers in patients of 38 years of age or older which were omitted from the analysis. Analysis was done on the remaining 370 single top quality embryo transfers. There were 150 (40.4%) patients ≤30 years of age, 161 (43.6%) patients >30 and ≤35, and 59 (16.0%) patients were >35 and ≤38. The mean age of the patients was 30.9 ± 3.6 (SD) years. A total of 166 (44.9%) ICSI cycles and 204 (55.1%) IVF cycles were performed. Male infertility was present in 48.9%, female infertility in 27% and mixed infertility in 12.7% of cycles. The cause of infertility was unknown in 11.4% of cycles.

**Treatment protocol**

Patients were treated with the long GnRH agonist desensitization protocol, starting in the midluteal phase with 6 × 100 µg of buserelin (Suprefact, Hoechst, Germany) intranasally for a period of 3 weeks. Thereafter, gonadotrophin stimulation (Metrodin HP or Gonal-F; Serono, Geneva, Switzerland) was initiated. When at least three mature follicles with a diameter of 18 mm were present, 10 000 IU of HCG (Profasi; Serono, Geneva, Switzerland) was given. The oocyte pick up was performed exactly 37 h later under vaginal ultrasound guidance.

**Embryo quality assessment**

Approximately 16–19 h after insemination/injection, normal fertilization was checked. Two pronuclear oocytes were cultured in Ménézo B2 medium. The next day (40–43 h after insemination/injection), embryos were separated and transferred to Medi-Cult M3 medium for a further 24 h culture. Every embryo was scored for the total number of cells, and the presence of anuclear fragments and multinucleated blastomeres on day 2 and day 3.

An embryo was considered a top quality embryo if there were four or five blastomeres on day 2 and seven or more blastomeres on day 3 with <20% of fragments and the total absence of multinucleated blastomeres at any stage of early cleavage (Van Royen et al., 1999). Supernumerary embryos were frozen.

**Embryo transfer technique**

All transfers were performed on an out-patient basis using a Wallace embryo transfer catheter (Sims Portex Ltd, Hythe, Kent, UK) consisting of an inner and outer catheter.

**Luteal phase**

In all cycles, the luteal phase was supported with 200 mg of buserelin and 200 mg of progesterone (Utrogestan, Besins, Belgium) administered vaginally.

**HCG analysis**

HCG analysis in serum was performed routinely on day 8 and day 12 after embryo transfer. There were 192 conception cycles; in 173 cases, HCG values on both day 8 and 12 after embryo transfer were obtained. βHCG was measured in serum with an immunometric assay (Vitros ECI, Ortho-clinical diagnostics NV, Beerse, Belgium). Calibration of the standards was performed against the WHO third international standard 75/537.

**Definitions**

Pregnancy was defined as two increasing values of HCG ≥5 IU/l. First-trimester pregnancy loss (FTPL) was a pregnancy leading to pregnancy loss prior to 13 weeks of gestation (i.e. biochemical pregnancy, clinical miscarriage and ectopic pregnancy). Ongoing pregnancy was defined as a pregnancy reaching beyond 12 weeks of pregnancy.

**Statistical analysis**

Statistical analysis was performed using SPSS software. Differences between continuous variables were analysed using the Student’s t-test; differences between percentages were analysed using the χ² test. The predictive value of HCG was determined using receiver operator curves (ROCs) to calculate sensitivity and specificity. Multiple regression analysis was used to investigate the contribution of age and fertilization method in the outcome. A P-value <0.05 was considered statistically significant.

**Results**

A total of 370 single top quality embryo transfers in patients younger than 38 years of age resulted in 192 pregnancies (51.9%). Thirty cycles (8.1%) ended in a biochemical pregnancy, four (1.1%) cycles ended in an ectopic pregnancy, 23 (6.2%) cycles ended in a clinical miscarriage and 135 (36.5%) cycles resulted in ongoing pregnancies. A total of 57 (29.7%) pregnancies were lost.

We analysed whether pregnancy rate and FTPL were related to age (Table I). The pregnancy rate after single top quality embryo transfer was independent of age. FTPL, however, occurred significantly less often in women ≤30 than in patients >30 years of age (P = 0.01).

The impact of the fertilization method is shown in Table II. There were 108 (52.9%) pregnancies after IVF versus 84 (50.6%) after ICSI (NS). FTPL occurred in 40 (19.6%) IVF cycles and in 17 (10.2%) ICSI cycles, which was significantly different (P = 0.011).

Multiple regression analysis was performed to determine the influence of age and type of fertilization method on FTPL. Age above 30 years had an odds ratio (OR) of 2.4 [95% confidence interval (CI) = 1.2–4.9] while IVF treatment had an OR of 1.8 (95% CI = 0.9–3.6) for FTPL. It could be concluded that the differences in FTPL were related to age, rather than to the technique used.

Analysis of the cohort of embryos from which the top quality embryo was selected is summarized in Table III. The total number of embryos, the number of top quality embryos and the number of cryopreserved embryos was calculated per cycle.

**Table I.** Evolution of 192 pregnancies after single top quality embryo transfer related to age

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Pregnancies</th>
<th>Ongoing pregnancies</th>
<th>FTPL</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤30, n = 150</td>
<td>78 (52%)</td>
<td>64 (42.7%)</td>
<td>14 (9.3%)</td>
</tr>
<tr>
<td>&gt;30–≤35, n = 161</td>
<td>82 (50.9%)</td>
<td>50 (31.1%)</td>
<td>32 (19.9%)</td>
</tr>
<tr>
<td>&gt;35–&lt;38, n = 59</td>
<td>32 (54.3%)</td>
<td>21 (35.6%)</td>
<td>11 (18.7%)</td>
</tr>
</tbody>
</table>

FTPL = first trimester pregnancy loss.

**Table II.** Evolution of 192 pregnancies after single top quality embryo transfer and IVF or ICSI

<table>
<thead>
<tr>
<th>Method</th>
<th>Pregnancies</th>
<th>Ongoing pregnancies</th>
<th>FTPL</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVF n = 204</td>
<td>108 (52.9%)</td>
<td>68 (33.3%)</td>
<td>40 (19.6%)</td>
</tr>
<tr>
<td>ICSI n = 166</td>
<td>84 (50.6%)</td>
<td>67 (40.4%)</td>
<td>17 (10.2%)</td>
</tr>
</tbody>
</table>

FTPL = first trimester pregnancy loss.
The number of embryos did not differ between cycles which resulted in a pregnancy and those which did not. However, there were significantly more top quality embryos and cryopreserved embryos in the pregnant patient group than in the patient group that did not become pregnant. Further analysis (Table IV) showed significantly more top quality embryos and cryopreserved embryos in cycles ending in FTPL than in cycles resulting in ongoing pregnancy.

No difference was noted in the number of embryos, the number of top quality embryos and the number of cryopreserved embryos between ICSI and IVF. No relationship was observed between these parameters and patients’ age (data not shown).

We analysed whether HCG follow-up could predict the risk of FTPL after transfer of a top quality embryo. In 184 patients receiving a top quality embryo, the HCG value on day 8 after transfer could be analysed; the mean value was 20.5 IU/l (±10.7 SD). For 180 patients, the mean HCG value on day 12 was 153.3 IU/l (±90.8 SD). To evaluate the level of HCG rise the HCG index was calculated as HCG on day 12/HCG on day 8. The mean value for this parameter was 8.0 (±4.5 SD).

The predictive value for ongoing pregnancy after transfer of one top quality embryo was calculated using ROCs. A cut-off value for HCG ≥ 45 IU/l predicts a sensitivity of 75.6% for ongoing pregnancy after transfer of a top quality embryo with 100% specificity (Figure 1). A cut-off value for the HCG index (HCG day 12/day 8) ≥ 3.5 predicts a sensitivity of 72.3% for ongoing pregnancy after transfer of a top quality embryo with 100% specificity (Figure 2).

**Discussion**

The pregnancy rate after single top quality embryo transfer is independent of patients’ age if she is younger than 38 years. FTPL, however, is significantly less for patients <30 (9%) than for those >30 years of age (19%). The age-related risk for FTPL compares with the risk of spontaneous abortion calculated for the whole population of Denmark (Nybo Andersen et al., 2000). This study shows that the risk for spontaneous abortion is 11% in the age group between 20 and 29 years, 15% between 30 and 34 years and 25% between 35 and 39 years. These figures indicate that day 3 embryo scoring gives an excellent estimate of the prerequisites for an embryo to implant in the days after the transfer. It cannot, however, fully predict the competence for ongoing pregnancy in the first trimester.

Analysis of the cohort of embryos (number of embryos, number of top quality embryos and number of cryopreserved embryos) from which the top quality embryo for transfer was selected shows that there are significantly more top quality and cryopreserved embryos in the pregnant patient group.

| No. of embryos | 8.5 ± 5.1 | 9.2 ± 4.7 | 0.152 |
| No. of top quality embryos | 2.7 ± 2.2 | 3.2 ± 2.2 | 0.013 |
| No. of cryopreserved embryos | 2.2 ± 2.6 | 2.8 ± 2.4 | 0.021 |

**Table III.** Analysis of the cohort of embryos from which the top quality embryo was selected (n = 370)

<table>
<thead>
<tr>
<th>Non-conception</th>
<th>Cycles with pregnancy</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of embryos</td>
<td>8.5 ± 5.1</td>
<td>9.2 ± 4.7</td>
</tr>
<tr>
<td>No. of top quality embryos</td>
<td>2.7 ± 2.2</td>
<td>3.2 ± 2.2</td>
</tr>
<tr>
<td>No. of cryopreserved embryos</td>
<td>2.2 ± 2.6</td>
<td>2.8 ± 2.4</td>
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</table>

**Table IV.** Analysis of the cohort of embryos in cycles with pregnancy (n = 192) after single top quality embryo transfer

<table>
<thead>
<tr>
<th>Ongoing pregnancy</th>
<th>FTPL</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of embryos</td>
<td>9.1 ± 4.3</td>
<td>9.4 ± 5.4</td>
</tr>
<tr>
<td>No. of top quality embryos</td>
<td>3.0 ± 2.0</td>
<td>3.8 ± 2.6</td>
</tr>
<tr>
<td>No. of cryopreserved embryos</td>
<td>2.5 ± 2.1</td>
<td>3.4 ± 2.8</td>
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</tbody>
</table>

FTPL = first trimester pregnancy loss.

Figure 1. HCG on day 12: area under ROC = 0.806 (P < 0.001).

Figure 2. HCG index = HCG day 12/day 8: area under ROC = 0.698 (P < 0.001).
and Strandell et al. (2000). The patient population that receives a single top quality embryo transfer is a selected group of patients which represents 22.6% of all transfers. This ‘top’ group of patients seems to produce a homogenous cohort of embryos after ovarian stimulation.

The implantation potential of a top quality embryo can be derived from analysis of HCG levels in serum. Both the individual value of HCG level on the twelfth day after embryo transfer and the dynamics of the HCG increase, calculated by the HCG index (day 12/day 8), are excellent predictors for ongoing pregnancy. The combination of both parameters does not improve the predictive value. Poikkeus et al. (2002) evaluated HCG levels on day 12 after fresh day 2 embryo transfers and frozen transfers; a cut-off value for HCG of 76 IU/l predicts a viable pregnancy with a sensitivity of 80% and specificity of 82%.

It is becoming more and more clear that not only the genetic make-up of the oocyte but also the integrity of the meiotic spindle is pivotal to early embryogenesis. Recent hypotheses point to the presence of two distinct mechanisms of embryo wastage in early embryo development. The first is chaotic mosaicism which can be considered a non-nuclear (chromosomal) mechanism of early embryo wastage. The second is non-disjunction which affects early embryo development through a nuclear (chromosomal) mechanism (Wilding et al., 2003). Therefore, improvement of the success rate in IVF/ICSI should be directed to making the best possible embryo selection in order to improve the pregnancy rate and to decrease fetal wastage. It has been shown by Munné et al. (1999) that aneuploidy screening in preimplantation embryos does not improve the implantation rate but reduces the embryo loss after implantation.

The question of whether subfertile couples are more prone to pregnancy loss remains unsolved. No such association has been reported despite the chance of over-representation in this group (Macklon et al., 2002).

A history of FTPL following IVF has been reported to be a positive factor to predict future success with IVF treatment (Levy et al., 1991; Templeton et al., 1996; Croucher et al., 1998). We have also shown that patients experiencing FTPL had significantly more top quality and cryopreserved embryos than patients with an ongoing pregnancy and thus should not be considered a poor prognosis group.

It is likely that the events following the onset of implantation are determined predominantly by the genetic quality of the embryos. We have shown that with the use of morphological embryo selection criteria, a pregnancy rate of 51.9% can be achieved with top quality embryos. However, post-implantation wastage is still 15.4%. These data indicate that embryo selection gives an excellent estimation of the chance of becoming pregnant but cannot predict FTPL.

References


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