Predictors of ongoing implantation in IVF in a good prognosis group of patients

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BACKGROUND: The aim of this study was to investigate whether, in a large randomized trial, it is possible to identify specific maternal and/or embryo variables that could independently correlate with ongoing implantation in IVF/ICSI. METHODS: In a Scandinavian study, 661 women were randomized to elective single embryo transfer or double embryo transfer. Women aged <36 years undergoing their first or second IVF cycle and with at least two good quality embryos were eligible. Only one cycle per subject was included. In the present study, cycles with 0 or 100% ongoing implantation (n = 520) were analysed regarding maternal and embryo variables. RESULTS: In this selected study group, the ongoing implantation rate was 195/734 (26.6%). In the univariate analysis, first IVF cycle, conventional IVF as fertilization method and 4-cell embryos showed a statistically higher ongoing implantation rate than did second IVF cycle, ICSI and non-4-cell embryos. In the multivariate analysis the same variables correlated independently to ongoing implantation. In addition, ovarian sensitivity correlated independently to ongoing implantation. CONCLUSION: This information should be used when selecting the number of embryos for transfer with the overall aim to reduce the rate of multiple births while maintaining a satisfactory birth rate.

Key words: embryo selection/ongoing implantation/prediction of IVF outcome/randomized controlled trial

Introduction

In assisted reproduction, a major problem is the high multiple pregnancy rate which correlates both to the number of transferred embryos and their quality (Staessen et al., 1992; Steer et al., 1992; Hu et al., 1998; Strandell et al., 2000). Currently, in Sweden and the other Nordic countries, only one or two embryos are transferred in order to decrease the multiple births and thereby the risks for the children born after IVF (Bergh et al., 1999; Ericsson and Källén, 2001; Schieve et al., 2002; Strömberg et al., 2002; Helmerholst et al., 2004; Jackson et al., 2004; Wennerholm and Bergh, 2004). In order to learn how to choose an optimal embryo for transfer, it is imperative to increase our knowledge about the embryo’s potential to implant.

The scoring of cleavage stage embryos includes evaluation of the grade of fragmentation, blastomere size, multinucleation, cytoplasmatic appearance and number of blastomeres per embryo (Puissant et al., 1987; Steer et al., 1992; Pelinick et al., 1998; Van Royen et al., 1999, 2001; Hardarson et al., 2001). The quality of the embryo has been suggested to correlate to oocyte and zygote morphology, e.g. appearance of the cytoplasm, pronuclei and polar bodies (Scott and Smith, 1998; Ebner et al., 2000). The embryo cleavage rate has also been found to be an indicator of embryo quality, with 4-cell embryos being optimal on day 2 and 8-cell embryos on day 3 (Ziebe et al., 1997; Van Royen et al., 2001). Early first cleavage has been found to positively correlate with a higher pregnancy rate (Shoukir et al., 1997; Sakkas et al., 1998; Lundin et al., 2001; Salumets et al., 2003; Van Montfoort et al., 2004). In a recent study (Ziebe et al., 2003) embryo morphology was shown to correlate with chromosomal status of the embryo. Apart from embryo quality, other known variables predicting pregnancy in IVF are female age, number of previous IVF cycles and presence of tubal infertility (Strandell et al., 2000).

The aim of this study was to investigate whether, in a large, randomized trial, it is possible, in a consolidated material, to identify retrospectively specific parental and/or embryo variables that could independently predict ongoing implantation in IVF.

Materials and methods

In a prospective, controlled, randomized trial including 661 women in Scandinavia the patients were randomized to eSET or DET (Thurin et al., 2004). From this study, fresh cycles with 0 or 100%...
ongoing implantation \((n = 520)\), including 734 embryos transferred on day 2, were selected and analysed for the dependent variable ongoing implantation.

The following variables were checked for independence: female age, body mass index, one previous IVF cycle, duration of infertility, tubal infertility, numbers of aspirated, fertilized and cleaved oocytes/embryos, number of good quality embryos, the number of IU of FSH per oocyte retrieved, number of blastomeres, blastomere size, grade and localization of fragments, rate of early cleavage and method of fertilization.

Women aged \(<36\) years, undergoing their first or second IVF or ICSI cycle and with at least two good quality embryos, were eligible for the randomized trial. Only one cycle per patient was included. Reasons for infertility included tubal factor, endometriosis, hormonal factor, unexplained infertility and male factor. A total of 71.5\% had primary infertility and 28.5\% had secondary infertility.

Each clinic was allowed to follow its local stimulation protocol. The women were treated using a stimulation protocol including down regulation with a GnRH agonist in a long protocol. Stimulation was performed with recombinant FSH (Gonal-F; Serono, Switzerland; Puregon; Organon, The Netherlands). Oocytes were retrieved 36–38 h following hCG using transvaginal sonographically guided puncture. Fertilization was performed by conventional IVF or ICSI, following standard techniques. Commercial culture media were used according to local routines. Embryo transfer was performed 2 days after oocyte retrieval. Luteal support was given with progesterone, either intramuscularly or vaginally.

Embryos on day 2 were considered to be good quality if having 4–6 blastomeres and if \(<20\%\) fragmentation was observed. According to the protocol, embryos for transfer were selected depending on cleavage rate and grade of fragmentation. Multinucleated embryos were generally not transferred. Elective SET was defined as a cycle where there were at least two good embryos to choose from and a single embryo was transferred. Pregnancy was defined as a positive test in urine or serum 2 weeks after embryo transfer. Ongoing implantation rate was defined as number of gestational sacs with a fetus with heart activity, detected with sonography in gestational week 7–8, per number of transferred embryos.

The study was performed between May 2000 and October 2003. The ethical committees of the participating clinics approved the study. Both public and private clinics participated.

**Statistics**

Distributions of the variables are given as means and SD. In the univariate analysis, Student’s \(t\)-test was used for continuous variables and Fisher’s exact test for dichotomous variables.

The \(P\)-values for embryo variables were adjusted in a conservative direction since some women contributed with more than one embryo. An adjusted variance was achieved by multiplying the variance by \(n_2/n_1\) where \(n_1\) = number of patients and \(n_2\) = number of embryos.

For multivariate purposes, logistic regression analysis was performed and included variables with \(P < 0.1\) in the univariate analysis. The embryo variable 4-cell embryos was chosen instead of number of blastomeres.

All significance tests were two-tailed and \(P < 0.05\) was considered significant. SPSS 11.5 and SAS software version 8.2 were used for computing the statistics.

**Results**

In the group presented in this study, consisting of cycles where all or none of the transferred embryos implanted, the ongoing implantation rate was 195/734 (26.6\%).

In the univariate analysis of maternal variables (Table I), women performing their first IVF cycle showed a significantly higher ongoing implantation rate of 120/405 (29.6\%) compared to those performing their second IVF cycle having an implantation rate of 23/115 (20.0\%) \((P = 0.041)\). No significant differences were found for any infertility cause in the univariate analysis. Neither did primary or secondary infertility differ in the univariate analysis.

In the univariate analysis of embryo variables (Table II), conventional IVF as fertilization method resulted in a significantly higher ongoing implantation rate than ICSI \((P = 0.008)\). The implantation rate in the conventional IVF group was 130/416 (31.3\%) and in the ICSI group 66/318 (20.8\%). A significantly higher rate of implantation was also found for 4-cell embryos compared to non-4-cell embryos on day 2 after oocyte retrieval, 182/650 (28.0\%) versus 13/84 (15.5\%) \((P = 0.041)\). The mean number of IU of FSH per oocyte retrieved was 170.99 (SD = 151.68) in cycles, resulting in an ongoing implantation versus cycles which did not \((P = 0.064)\).

In the multivariate analysis (Table III) the variables first IVF cycle \((P = 0.013)\), IVF as method of fertilization \((P = 0.007)\), 4-cell embryos \((P = 0.045)\) and number of IU of FSH per oocyte retrieved \((P = 0.012)\) were independently predictive of an ongoing implantation.

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**Table I.** Maternal variables; univariate analysis

<table>
<thead>
<tr>
<th></th>
<th>Ongoing implantation ((n = 143))</th>
<th>Not ongoing implantation ((n = 377))</th>
<th>(P)</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>30.7 (3.0)</td>
<td>30.9 (3.1)</td>
<td>0.45</td>
<td>0.976</td>
<td>0.917–1.039</td>
</tr>
<tr>
<td>Body mass index (kg/m(^2))</td>
<td>24.5 (4.6)</td>
<td>24.6 (4.6)</td>
<td>0.93</td>
<td>0.998</td>
<td>0.857–1.039</td>
</tr>
<tr>
<td>No. of previous IVF cycles</td>
<td>0.16 (0.37)</td>
<td>0.24 (0.43)</td>
<td>0.041</td>
<td>0.594</td>
<td>0.359–0.983</td>
</tr>
<tr>
<td>Infertility duration (years)</td>
<td>3.8 (1.8)</td>
<td>3.8 (1.9)</td>
<td>0.76</td>
<td>1.016</td>
<td>0.915–1.128</td>
</tr>
<tr>
<td>Tubal infertility (% of women)</td>
<td>18.9</td>
<td>19.4</td>
<td>0.90</td>
<td>1.032</td>
<td>0.632–1.685</td>
</tr>
<tr>
<td>No. of aspirated oocytes</td>
<td>12.8 (5.3)</td>
<td>12.9 (5.6)</td>
<td>0.82</td>
<td>0.790</td>
<td>0.532–1.174</td>
</tr>
<tr>
<td>No. of fertilized oocytes</td>
<td>8.6 (3.7)</td>
<td>8.7 (3.7)</td>
<td>0.38</td>
<td>1.023</td>
<td>0.972–1.077</td>
</tr>
<tr>
<td>No. of cleaved embryos</td>
<td>8.2 (3.4)</td>
<td>7.9 (3.6)</td>
<td>0.34</td>
<td>1.031</td>
<td>0.973–1.084</td>
</tr>
<tr>
<td>No.of good quality embryos</td>
<td>4.7 (2.5)</td>
<td>4.5 (2.3)</td>
<td>0.45</td>
<td>1.031</td>
<td>0.952–1.115</td>
</tr>
<tr>
<td>No. of embryos transferred</td>
<td>1.4 (0.5)</td>
<td>1.4 (0.5)</td>
<td>0.24</td>
<td>0.790</td>
<td>0.532–1.174</td>
</tr>
<tr>
<td>FSH (IU) per oocyte retrieved</td>
<td>170.0 (120.8)</td>
<td>197.3 (151.7)</td>
<td>0.063</td>
<td>0.998</td>
<td>0.997–1.000</td>
</tr>
</tbody>
</table>

Values are mean (SD) unless otherwise stated.

OR = odds ratio; CI = confidence interval.
Table II. Embryo variables; univariate analysis (adjusted P-values)

<table>
<thead>
<tr>
<th></th>
<th>Ongoing implantation</th>
<th>Not ongoing implantation</th>
<th>P</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 196)</td>
<td>(n = 538)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Method of fertilization (% IVF embryos)</td>
<td>66.3</td>
<td>53.2</td>
<td>0.008</td>
<td>0.576</td>
<td>0.384–0.864</td>
</tr>
<tr>
<td>No. of blastomeres*</td>
<td>4.1 (0.37)</td>
<td>4.2 (0.6)</td>
<td>0.069</td>
<td>0.663</td>
<td>0.426–1.032</td>
</tr>
<tr>
<td>4-Cell embryos (%)</td>
<td>93.4</td>
<td>86.8</td>
<td>0.041</td>
<td>1.210</td>
<td>1.007–1.452</td>
</tr>
<tr>
<td>Blastomere size (% even sized blastomeres)</td>
<td>91.0</td>
<td>91.1</td>
<td>0.26</td>
<td>1.357</td>
<td>0.726–2.537</td>
</tr>
<tr>
<td>Grade of fragmentation (% embryos with &lt; 10% fragmentation)</td>
<td>58.7</td>
<td>55.8</td>
<td>0.554</td>
<td>0.888</td>
<td>0.599–1.316</td>
</tr>
<tr>
<td>Localization of fragments (% embryos with localized fragments* )</td>
<td>65.8</td>
<td>72.5</td>
<td>0.259</td>
<td>1.372</td>
<td>0.792–2.378</td>
</tr>
<tr>
<td>Rate of early cleavage (% embryos*)</td>
<td>29.3</td>
<td>28.4</td>
<td>0.880</td>
<td>1.043</td>
<td>0.560–1.816</td>
</tr>
</tbody>
</table>

a Mean (SD).

b Calculated on the 111/320 embryos where this variable was evaluated.

*Calculated on the 116/327 embryos where this variable was evaluated.

OR = odds ratio; CI = confidence interval.

Table III. Multivariate analysis: variables that are independently predictive of ongoing implantation

<table>
<thead>
<tr>
<th></th>
<th>Adjusted P</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>First IVF cycle</td>
<td>0.013</td>
<td>1.975</td>
<td>1.153–3.385</td>
</tr>
<tr>
<td>IVF as method of fertilization</td>
<td>0.007</td>
<td>1.751</td>
<td>1.160–2.644</td>
</tr>
<tr>
<td>4-Cell embryos</td>
<td>0.045</td>
<td>1.209</td>
<td>1.004–1.456</td>
</tr>
<tr>
<td>FSH per oocyte retrieved</td>
<td>0.013</td>
<td>0.998</td>
<td>0.996–0.999</td>
</tr>
</tbody>
</table>

OR = odds ratio; CI = confidence interval.

Discussion

In this study, we have included embryo variables as well as maternal variables in a consolidated analysis for prediction of implantation. Earlier studies have only taken embryo variables into account (Staessen et al., 1992; Giorgetti et al., 1995; Ziebe et al., 1997; Van Royen et al., 2001), although it is well known that maternal variables such as maternal age and rank of the IVF cycle have a large influence on implantation (Templeton and Morris, 1996; Strandell et al., 2000). However, in a recent Belgian study, maternal age was considered as well as embryo variables (De Neubourg et al., 2004), showing that the pregnancy rate after single embryo transfer was independent of maternal age in women aged <38 years.

Another strength of this present study is that only cycles with 0 or 100% implantation are included.

It might be taken for granted that twins born after DET always derive from two different embryos. Even if most twins are heterozygotic, it is known that the rate of monozygotic twinning is higher after IVF/ICSI (Abusheika et al., 2000) than after spontaneous conception. The rate of monozygotic twinning in this study is not known. However, according to Weinberg's law (Weinberg et al., 1902) the estimated number of monozygotic twins in this cohort ought to be zero.

Four variables correlated independently with ongoing implantation; first treatment cycle of IVF/ICSI, IVF as fertilization method, transfer of 4-cell embryos on day 2, and ovarian sensitivity.

Women undergoing their first IVF cycle had a significantly higher ongoing implantation than women undergoing their second cycle. Similar findings (but not significant) were noted for the total randomized study of 661 subjects where ongoing implantation rate was 32.3% for first cycle patients and 26.5% for second cycle patients (P = 0.127) (Thurin et al., 2004). These findings are in line with the large observational study on >36,000 cycles in the UK, where the live birth rate in the first cycle was 14% and then a steady decline was seen for each following cycle (Templeton and Morris, 1996).

The fact that the implantation rate after IVF was significantly higher than after ICSI might be due to paternal characteristics, i.e. cause of infertility. At least when ICSI is used for severe male factor infertility, lower fertilization and implantation rates have been found (Tournaye et al., 1995; Devroey et al., 1996; Silber et al., 2003). Another reason might be that ICSI is a more invasive method, exposing the oocytes to a more detrimental environment during the procedure, thus possibly impairing embryo quality. The finding of a lower implantation rate after ICSI is in accordance with international data (Society for Assisted Reproductive Technology and the American Society for Reproductive Medicine, 2004) where the pregnancy per transfer was slightly less for ICSI than for IVF (36.5 versus 40.4%). For ESHRE the values were almost identical (28.7% for ICSI and 28.4% for IVF) (Nyboe-Andersen et al., 2004). In a study comparing fertilization methods for non-male infertility, IVF was found to have numerically better pregnancy outcome (OR 1.44; 95% CI 0.95–2.21) although not statistically significant (Van Rumste et al., 2004).

In our study a vast majority of the ICSI cycles were performed because of male infertility, only 22/258 of the ICSI cycles were performed for non-male factor infertility. In the male infertility group, patients with obstructive azoospermia needing percutaneous epididymal sperm aspiration (PESA)/testicular sperm aspiration (TEA) were included, but not patients with non-obstructive azoospermia needing TESA/testicular sperm extraction (TESE).

In the present study, 4-cell embryos (n = 650) implanted to a significantly greater degree than non-4-cell embryos (n = 84). These results are in accordance with several previous studies that have shown that 4-cell embryos implant at a higher rate than embryos with both fewer or more cells on day 2 (Giorgetti et al., 1995; Ziebe et al., 1997; Van Royen et al., 2001). The reason for this may be that a synchronized cleavage rate correlates to an optimal embryo quality. However, in a recent study, no correlation was found between the rate of aneuploidy and cleavage rate (Ziebe et al., 2003).
It might therefore be speculated that cleavage rate is mainly associated with embryo metabolism, thereby affecting implantation rate. Ovarian sensitivity, assessed as number of FSH IU per oocyte retrieved, correlated independently with ongoing implantation. This variable was recently suggested by Holte et al. (2004) in a prediction model for minimizing twins after IVF. In addition, in a recent Danish study the starting dose of FSH was significantly lower in cycles resulting in an ongoing implantation (Popovic et al., 2004). The amount of FSH per oocyte retrieved is a variable which is easy to calculate and can be performed for each IVF cycle. It is thus of high practical value.

Body mass index (BMI) showed a wide variation between subjects in this study, range 17–49 kg/m² (mean 24.6; SD 4.6). There was no significant difference in the univariate analysis of BMI between women with ongoing implantation and not ongoing implantation, either when BMI was assessed as a continuous variable or as a group variable (< 19, 19–24, 25–29, ≥ 30 kg/m²). This is in contrast to earlier studies showing that obesity is a negative predictor for IVF (Wittemer et al., 2000; Nichols et al., 2003; Fedorcsak et al., 2004).

Early first cleavage has in several earlier studies been found to correlate significantly to ongoing pregnancy and/or birth (Shoukri et al., 1997; Sakkas et al., 1998; Lundin et al., 2001; Salumets et al., 2003). This could not be corroborated in this study. The main reason for this may be that early first cleavage was only scored at some of the participating clinics.

It has been shown that embryos with large differences in cell sizes have higher rates of chromosomal abnormalities and lower implantation rate (Hardarson, 2001; Van Montfoort, 2004); however, the latter was not found in the present study.

A possible general explanation for not identifying these previously discussed embryo variables as predictors of implantation could be the multicentre design. The analyses are based on results from 11 Nordic centres. It is plausible that different laboratories do not score all embryo variables in an exactly similar way and the inter-laboratory differences may be higher than anticipated. Most other studies of embryo variables have been performed as single centre studies.

Of importance is that this study was performed with only good prognosis women, i.e. those having at least two 4-cell embryos with < 20% fragmentation on day 2. Among these patients, already selected by embryo morphology and receiving good quality embryos at transfer, it might be more difficult to identify further variables predicting implantation. This could explain why factors known from other studies to predict pregnancy did not seem to be of prognostic value in our study group. The differences seen here might thus be ultimate differences, those that remain when the other maternal and embryo factors are optimal, while for example early first cleavage and blastomere size may have a larger impact in a more general patient population where both good and poor prognosis patients are included.

In conclusion, fertilization method with conventional IVF, first IVF cycle, 4-cell embryos and ovarian sensitivity have been shown to be independent predictors of ongoing implantation in IVF. This information should thus be used when selecting the number of embryos for transfer with the overall aim of reducing the rate of multiple births while maintaining a satisfactory birth rate. The recommendation to a woman aged < 36 years, performing her first treatment with conventional IVF, having 4-cell embryos on day 2 after oocyte retrieval and considering ovarian sensitivity, should be single embryo transfer.

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References
The cumulative embryo score: a predictive embryo scoring technique to select the optimal number of embryos to transfer in an in-vitro fertilization and embryo transfer programme. Hum Reprod 7,117–119.

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