Experience with the elective transfer of two embryos under the conditions of the German embryo protection law: results of a retrospective data analysis of 2573 transfer cycles

M.Ludwig1,3, B.Schöpper1, A.Katalinic2, R.Sturm1, S.Al-Hasani1 and K.Diedrich1

1Department of Gynecology and Obstetrics and 2Department of Social Medicine, Medical University of Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany
3To whom correspondence should be addressed

The German embryo protection law (Embryonenschutzgesetz, ESchG) does not allow embryo selection. Therefore, only as many oocytes at the pronuclear stage (PN), as are planned to be transferred, are allowed to be cultured. It is not known whether, under these conditions, it is possible to reduce the number of embryos for transfer without a corresponding reduction of the overall pregnancy rate (PR). We retrospectively analysed 2573 consecutive transfer cycles following either in-vitro fertilization (IVF) or IVF/ intracytoplasmic sperm injection. Out of these cycles, 234, 329 and 792 were performed with one, two, and three embryos respectively, because only that number was available (non-elective transfer). Another 123 and 1095 transfer cycles were performed with two and three embryos, respectively, which were selected from a higher number of PN oocytes (elective transfer). The clinical ongoing PR were 3.9, 9.1 and 17.7% respectively for the groups with non-elective transfer of 1, 2 and 3 embryos, and 22.0 and 22.5% for the groups with elective transfers with two and three embryos, respectively. There was no statistically significant difference in PR between the two elective embryo transfer groups up to the age of 40 years. The multiple pregnancy rate was reduced by 7.9%. The reduction of the number of embryos transferred from three to two can be performed even under the conditions of the ESchG without an effect on the overall PR.

Key words: elective embryo transfer/German embryo protection law/multiple pregnancy rates/pronuclear stage selection

Introduction

The avoidance of high order multiple pregnancies is one of the most important aims in assisted reproduction (Cohen 1998). It is accepted that elective transfer of two qualitatively excellent embryos results in the same pregnancy rate compared to the elective transfer of three embryos – with a significant reduction in high order multiple pregnancy rate (Staessen et al., 1993; Staessen et al., 1995; Fujii et al., 1998; Templeton and Morris, 1998).

The basis of these ideas is the ability to select the embryos with the best morphological quality and the best chance of implantation. This opinion is shared by most but not all who work in the field of reproductive medicine (Yaron et al., 1997). Another way to reduce multiple birth rate is to perform selective feticide, if multiple pregnancies have been established. This may result in good outcomes (Yaron et al., 1998; Antsaklis et al. 1999) but is a frank ethical problem.

The German embryo protection law (Embryonenschutzgesetz, ESchG) does not allow embryo selection, but only selection at the pronuclear stage. Furthermore, only as many pronuclear stage oocytes are allowed to be selected as are planned to be transferred in the same cycle. This means that after pre-selection of, for example, three pronucleated oocytes, these three must be transferred on the same or the subsequent day. A second selection process is not allowed. The idea of this law was to avoid ethical problems related to cryopreservation of surplus embryos or wastage of embryos, because these have – according to the German law – the status of individual persons. Non-selected pronuclear stages have to be discarded or are allowed to be cryopreserved for a subsequent transfer. The number of embryos transferred in one cycle is limited to three (Keller et al., 1992; Ludwig and Diedrich, 1999). The consequence of this law is that the actual pregnancy rates per embryo transfer achieved in the year 1997 in Germany following in-vitro fertilization (IVF) (24.4%) and intracytoplasmic sperm injection (ICSI) (23.3%) according to the national IVF registry (Deutsches IVF Register, 1998) were somewhat lower compared to the rest of the world. For example, in the USA, where the embryo transfer policy is less strict and embryo selection can be performed routinely, the pregnancy rate per embryo transfer is as high as 33% and the delivery rate per embryo transfer is 27.5% [Society for Assisted Reproductive Technology (SART), 1998]. The penalty, however, is a multiple pregnancy rate in the range of up to 40% (SART, 1998).

Until now there have been no data presented regarding the question of what pregnancy rates can be expected when only two embryos are transferred after selection at the pronuclear stage.

Therefore, it is still not known whether, under the conditions of the embryo protection law, there is the possibility to avoid multiple pregnancies by electively transferring only two embryos without reduction of the overall pregnancy rate. To answer this question, we performed a retrospective data analysis.

Materials and methods

Study design and definitions

Data from 2573 consecutive IVF and IVF/ intracytoplasmic sperm injection cycles, which resulted in an embryo transfer, were retrospec-
induction was performed using 10,000 IU human chorionic gonadotropin (hCG). Clinical pregnancy rates in the different groups (Table I), it is remarkable that there is a considerable risk of twins (20%), triplets (3%), and maybe quadruplets due to monozygotic twinning, if three instead of two embryos are replaced. In particular, women <35 years of age were counselled in this way. However, the counselling was non-directive, and it was left up to the patients to decide whether they wanted to have two or three embryos replaced, if a selection was possible.

According to the ESchG, either two or three oocytes were selected, wherever possible, 16–20 h after insemination or ICSI. The remaining were cryopreserved at the pronuclear stage (Al-Hasani et al., 1996). If patients refused to have their oocytes cryopreserved, these were discarded at the pronuclear stage after the selection process.

PN stages were selected according to the decision of the embryologists. No special selection criteria or scores were used. Only in some cycles at the end of 1998 was the score of Scott and Smith (1998) used.

**Ovarian stimulation**

Ovarian stimulation was performed according to the long luteal protocol in most cases, using a gonadotrophin releasing hormone (GnRH) agonist depot formulation and either human recombinant follicle stimulating hormone (rFSH) or human menopausal gonadotrophin (HMG). IVF and/or ICSI was done according to our centre’s procedure as previously described (Al-Hasani et al., 1996, 1999). Ovulation induction was performed using 10,000 IU human chorionic gonadotrophin (HCG) i.m.

Luteal phase support was given using either several HCG administrations, vaginal progesterone or vaginal progesterone combined with HCG.

**Cumulative embryo score**

A previously published cumulative embryo score (Steer et al. 1992) was slightly modified, since we do not use four but three different degrees of embryo quality. The individual score of a single embryo was calculated after multiplication of the number of its blastomeres and the degree of its quality (1: fair; 2: moderate; 3: ideal). The cumulative embryo score for those which were transferred was then calculated as the sum of the single embryo scores.

**Statistical analysis**

Differences between subgroups were analysed with the Kruskal–Wallis test and Mann–Whitney test. Z-tests were used for contingency tables. Statistical significance was assumed at the 5% level. Analyses were performed with SPSS 8.0 software (SPSS, Chicago, IL, USA).

**Results**

In all, 2849 cycles were performed during the study period. Of these, 2573 resulted in an embryo transfer (90.3%). No transfer cycles were excluded from the analysis. The data of all studied patients are shown in Table I, and the age distribution is shown in Figure 1 and Figure 2.

There were 234, 329, and 792 cycles done with one (group 1), two (group 2), and three embryos (group 3) respectively because only that number of PN/embryos was available; 123 and 1095 cycles were done with elective transfer of two (group 4) and three embryos (group 5) respectively, as well as cryopreservation of the supernumerary PN. Percentages of primary and secondary infertility as well as percentage of ICSI cycles in all five groups were comparably high (Table I).

Mean patient age showed a tendency to decrease from group 1 through 5, which is also shown in Figure 2 ($P < 0.05$).

Clinical pregnancy rates were 5.5, 12.2, 23.1, 25.2 and 28.6% and clinical ongoing pregnancy rates were 3.9, 9.1, 17.7, 22.0 and 22.5% for groups 1, 2, 3, 4 and 5, respectively. Clinical ongoing singleton pregnancy and multiple pregnancy rates, as well as abortion rate, were analysed according to patient age (Figure 3). Results for different threshold values are shown in Table II and demonstrate similar pregnancy rates up to the age of 40 years, if elective embryo transfer can be performed. There was no statistically significant difference between the two groups. Among patients in the older age groups ($\geq 35$ years) it appeared that the pregnancy rate was higher in the elective three embryo groups than the two embryo groups but this was not significant.

In groups 4 and 5 the mean number ($\pm$ SD) of the cryopreserved pronuclear stage oocytes was $4.16 \pm 3.16$ and $3.04 \pm 3.13$ respectively. This demonstrates that a mean of one PN stage oocyte less was cryopreserved in the group with three embryos transferred compared to the one with two embryos transferred.

Figure 4 illustrates the oocyte number and cumulative embryo score in relation to the number of retrieved and transferred embryos. When this figure is compared with the pregnancy rates in the different groups (Table I), it is remarkable that the oocyte number shows a closer correspondence to the increasing pregnancy rates from group 1 through 5, than does the cumulative embryo score.

**Discussion**

The elective transfer of two embryos under the conditions of the ESchG, with selection at the pronuclear stage, is an opportunity for most patients to avoid higher order multiple pregnancies. Doing this, we could avoid 4% twin, 2.9% triplet and 1.0% quadruplet pregnancies (by comparison of groups 4 and 5 in Table I), the latter due to monozygotic twinning after transfer of three embryos.

This is the first analysis which shows that reduction of the number of transferred embryos is an option in Germany which would not reduce pregnancy rates in cases where selection of pronuclear stage oocytes is possible. This is important because until now, it has not been demonstrated that such a procedure to reduce multiple pregnancy rates would not also reduce total pregnancy rates.

The pregnancy rates in the non-elective transfer groups were consistently lower compared with the elective transfer groups. This might be explained by the fact that more patients included in the non-elective transfer group had a low response to stimulation treatment or other factors which determine a lower
Elective transfer of two embryos in Germany

Figure 1. Age distribution within the studied population of 2573 patients.

Table I. Patient characteristics and treatment outcome according to the number of retrieved and transferred embryos. Values are either percentages or mean ± SD

<table>
<thead>
<tr>
<th></th>
<th>Non-elective embryo transfer</th>
<th>Elective embryo transfer</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 1</td>
<td>Group 2</td>
<td>Group 3</td>
</tr>
<tr>
<td>Embryos transferred (n)</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Cycles (n)</td>
<td>234</td>
<td>329</td>
<td>792</td>
</tr>
<tr>
<td>Age (years)</td>
<td>34.76 ± 5.68</td>
<td>34.08 ± 4.92</td>
<td>33.10 ± 4.92</td>
</tr>
<tr>
<td>Cycles (n)</td>
<td>234</td>
<td>329</td>
<td>792</td>
</tr>
<tr>
<td>Primary infertility (%)</td>
<td>72.1</td>
<td>67.1</td>
<td>67.3</td>
</tr>
<tr>
<td>ICSI cycles (%)</td>
<td>75.2</td>
<td>76.6</td>
<td>79.0</td>
</tr>
<tr>
<td>ICSI/TESE (%)</td>
<td>5.1</td>
<td>7.6</td>
<td>10.5</td>
</tr>
<tr>
<td>IVF cycles (%)</td>
<td>17.9</td>
<td>14.3</td>
<td>9.8</td>
</tr>
<tr>
<td>Oocytes retrieved</td>
<td>4.83 ± 4.07</td>
<td>5.94 ± 3.87</td>
<td>8.67 ± 4.24</td>
</tr>
<tr>
<td>Cumulative embryo score</td>
<td>8.34 ± 4.57</td>
<td>17.32 ± 8.03</td>
<td>27.97 ± 10.71</td>
</tr>
<tr>
<td>Two-pronuclear oocytes (n)</td>
<td>1.00 ± 0.00</td>
<td>2.00 ± 0.00</td>
<td>3.00 ± 0.00</td>
</tr>
<tr>
<td>Pregnancy rate (%)</td>
<td>5.5 (13)</td>
<td>12.2 (40)</td>
<td>23.1 (183)</td>
</tr>
<tr>
<td>Abortion rate (%)</td>
<td>30.8 (4)</td>
<td>25.0 (10)</td>
<td>23.5 (43)</td>
</tr>
<tr>
<td>% twins (n)</td>
<td>–</td>
<td>17.5</td>
<td>18.5</td>
</tr>
<tr>
<td>% triplets (n)</td>
<td>–</td>
<td>17.5 (7)</td>
<td>15.8 (29)</td>
</tr>
<tr>
<td>Clinical ongoing pregnancy rate (%)</td>
<td>3.9</td>
<td>9.1</td>
<td>17.7</td>
</tr>
</tbody>
</table>

aAge significantly decreased in patient groups from left to right of the table (P < 0.05).
bSignificantly different (P < 0.05).

Intracytoplasmic sperm injection using ejaculated spermatozoa.

No. of oocytes retrieved significantly increased in patient groups from left to right of the table (P < 0.05).

No. of metaphase II oocytes and cumulative embryo score significantly increased in patient groups undergoing non-elective transfer from left to right of the table (P < 0.05).

Values in parentheses are numbers of pregnancies (1), abortions (2), twins (3), triplets (4) and quadruplets (5).

ICSI = intracytoplasmic sperm injection; MESA = micro-epididymal sperm aspiration; TESE = testicular sperm extraction; IVF = in-vitro fertilization.

success rate. This is also reflected by the apparently higher age of these patients (Table I and Figure 2).

Interestingly, despite counselling each patient upon the possible risks of multiple pregnancies, the patients who have electively three embryos replaced are younger compared to those who have electively two embryos replaced. This might show that younger patients are prepared to take a higher risk of multiple pregnancy compared to older patients, to achieve a pregnancy. This is in accordance with a recent evaluation of patients’ concerns regarding multiple pregnancies in the USA, where the positive pregnancy test alone is the sign of success, irrespective of whether it is a singleton or multiple pregnancy (Faber 1997). It is astonishing that 50% of patients find a triplet pregnancy an acceptable outcome, and 20% even accept a quadruplet pregnancy.

The question remains: up to which age can the number be...
reduced without a reduction of pregnancy rates? Table II shows similar pregnancy rates, independent of elective transfer of two or three embryos, up to the age of 40 years. These data must be carefully interpreted, since this study was not done prospectively and not randomized. Therefore, the group of older patients having only two embryos replaced might represent patients who were thought to have had a better chance to conceive, compared to those who were counselled to have three embryos replaced.

This is confirmed by the data shown in Figure 3. In patients >35 years of age, there was a clear decline in pregnancy rates (Figure 3) with a similar decline in multiple pregnancy rates but an increase in abortion rates, as shown also by others (Roseboom et al. 1995; Svendsen et al. 1996). A similar age limit was also chosen by another group of workers (Staessen et al., 1993, 1995) two embryos were transferred electively when (i) patients were <37 years old, (ii) patients were in their first or second treatment cycle, and (iii) a large cohort of good embryos (at least two good quality embryos) were available with supernumerary embryos for freezing. Pregnancy rates of 48.3 and 41.3% were reported after IVF and ICSI respectively, after elective two embryo transfer, and rates of 43.7 and 47.6% after IVF and ICSI respectively after elective three embryo transfer.

Cohen (1998) also reported the results of a logistic regression analysis of factors linked to multifetal pregnancies, based on FIVNAT data, and found that both a higher number of embryos replaced as well as the maternal age were correlated (P < 0.01) with the multiple pregnancy rates (Cohen, 1998).

Devreker et al. reported on two different protocols to reduce multiple pregnancy rates. In the years 1994 up to 1996 they only transferred two embryos in those cycles in which at least three excellent embryos were present. Thus they reduced their high multiple pregnancy rate from 45.6 to 27.7%, without reduction of the overall pregnancy rate (Devreker et al., 1999). Subsequently this group changed its strategy and transferred two embryos in all patients who were (i) <35 years of age, (ii) were in their first or second treatment cycle, and (iii) had at least two embryos with good morphological quality. They reduced their multiple pregnancy rate by that procedure to 7.3% after elective transfer of two embryos, and to 10.5% after elective transfer of three embryos. The overall pregnancy rate remained the same.

### Table II. Pregnancy rates (%) depending on the elective transfer of either two or three embryos and on patient age

<table>
<thead>
<tr>
<th>Threshold age (years)</th>
<th>Patient age (years)</th>
<th>Elective embryo transfer of</th>
<th>2 embryos</th>
<th>3 embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>&lt;30</td>
<td>15.6 (5/32)</td>
<td>30.8 (116/376)</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>&gt;30</td>
<td>28.6 (26/91)</td>
<td>26.0 (197/719)</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>&lt;35</td>
<td>28.2 (22/78)</td>
<td>29.7 (243/819)</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>&gt;35</td>
<td>20.0 (9/45)</td>
<td>25.4 (70/276)</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>&lt;40</td>
<td>27.1 (30/111)</td>
<td>28.8 (303/1053)</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>&gt;40</td>
<td>8.3 (1/12)</td>
<td>23.8 (10/42)</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2. Median age and lower and upper quartile in the different groups.

Figure 3. Singleton and multiple pregnancy rates and abortion rates depending on patient age. The three parts of each bar together comprise the total clinical pregnancy rate.
Elective transfer of two embryos in Germany

When the group of non-elective two (group 2) versus non-elective three (group 3) embryo transfers as well as the non-elective (groups 1, 2 and 3) versus elective (groups 4 and 5) embryo transfer groups were compared to each other. As shown in Figure 4, the cumulative embryo score (Figure 4b) does not seem to have much influence on the pregnancy rate achieved: it was similar in the group of non-elective two embryo transfer (ongoing pregnancy rate: 9.1%) and elective two embryo transfer (ongoing pregnancy rate: 22.0%). On the contrary, the number of oocytes retrieved (Figure 4a), which was similar in both elective transfer groups but higher in the elective compared to the non-elective transfer groups, was more closely associated with the ongoing pregnancy rate.

To conclude, there seems to be a certain age limit above which the elective transfer of two instead of three embryos will lead to a reduction of the overall pregnancy rate. Since most studies were done retrospectively, however, and the elective transfer of two or three embryos was not randomized (which would be ethically difficult to do) it is not possible to define the exact age limit. An empirical threshold value of 35 years of age might be chosen if the results from this study as well as others are respected.

Therefore considering the conditions of the ESchG, it is recommended to transfer three instead of two embryos in patients /H11022/35 years of age, if the patient has been informed about both the high multiple pregnancy risk as well as the possibility of a reduction in overall pregnancy rate if the number of embryos transferred is reduced to two.

Two developments in recent years may improve the outcome of IVF cycles still more, with an excellent possibility of reducing multiple pregnancy rates. One approach is the culture of blastocysts in sequential media (Gardner and Lane, 1997; Gardner et al., 1998a,b). Using this approach, single blastocysts can be transferred with a high implantation rate of ~25%, obviating the need for multiple transfer. Since this approach is based on a natural selection process of embryos in culture, it cannot be applied under the current conditions of the ESchG, which allows only pre-selected oocytes at the pronuclear stage to be cultured. The idea of this self-selection process of human embryos in vitro has also been proposed to be effective by delaying the embryo transfer from day 2 to day 3 (Dawson et al., 1995). Scholtes et al. (1998) recently showed, that the rate of blastocyst transfers on day 5 depends more on the number of oocytes retrieved, and consequently on the number of oocytes fertilized, than on female age. This can also be simply explained by the possibility of self-selection of the most viable embryos. These approaches, inevitably, must lead to higher pregnancy rates in other centres worldwide compared with those in Germany.

Others have reported on a threshold age of 42 years to reduce the number of embryos transferred, and achieved good results regarding the overall pregnancy rate (Fujii et al., 1998).

Recently, a large cohort of IVF cycles from the UK has been analysed regarding the question of which factors might influence multiple pregnancy rates (Templeton and Morris 1998). A total of 44 236 IVF cycles was evaluated in 25 240 patients who were registered by the Human Fertilisation and Embryology Authority from August 1991 up to April 1995. Only those patients who had fewer than four oocytes fertilized gained from transfer of three instead of two embryos. However, these patients also had a higher risk of multiple pregnancies. Furthermore, the patients aged ≥40 years with fewer than four oocytes fertilized had a significantly higher chance of achieving pregnancy when three embryos were transferred compared with two embryos transferred. The multiple pregnancy rate in this group remained unchanged. If, however, more than four oocytes were available, neither pregnancy nor multiple pregnancy rates were significantly changed.

To conclude, there seems to be a certain age limit above which the elective transfer of two instead of three embryos will lead to a reduction of the overall pregnancy rate. Since most studies were done retrospectively, however, and the elective transfer of two or three embryos was not randomized (which would be ethically difficult to do) it is not possible to define the exact age limit. An empirical threshold value of 35 years of age might be chosen if the results from this study as well as others are respected.

Therefore considering the conditions of the ESchG, it is recommended to transfer three instead of two embryos in patients ≥35 years of age, if the patient has been informed about both the high multiple pregnancy risk as well as the possibility of a reduction in overall pregnancy rate if the number of embryos transferred is reduced to two.

Two developments in recent years may improve the outcome of IVF cycles still more, with an excellent possibility of reducing multiple pregnancy rates. One approach is the culture of blastocysts in sequential media (Gardner and Lane, 1997; Gardner et al., 1998a,b). Using this approach, single blastocysts can be transferred with a high implantation rate of ~25%, obviating the need for multiple transfer. Since this approach is based on a natural selection process of embryos in culture, it cannot be applied under the current conditions of the ESchG, which allows only pre-selected oocytes at the pronuclear stage to be cultured. The idea of this self-selection process of human embryos in vitro has also been proposed to be effective by delaying the embryo transfer from day 2 to day 3 (Dawson et al., 1995). Scholtes et al. (1998) recently showed, that the rate of blastocyst transfers on day 5 depends more on the number of oocytes retrieved, and consequently on the number of oocytes fertilized, than on female age. This can also be simply explained by the possibility of self-selection of the most viable embryos. These approaches, inevitably, must lead to higher pregnancy rates in other centres worldwide compared with those in Germany.

Another promising approach might be the selection of pronuclear stage oocytes using quality criteria, which help to predict the cleavage rate, quality of the embryos and implantation rate (Scott and Smith, 1998). With the help of this quality scoring system, it might be possible to increase pregnancy rates still further, for older patients as well, who have more
pronucleated oocytes than are planned to be transferred. The future will show how this scoring system will help to improve overall pregnancy rates in Germany.

References


Received on May 24, 1999; accepted on October 15, 1999

324