

# Frozen embryo transfers: implications of clinical and embryological factors on the pregnancy outcome

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**BACKGROUND:** Frozen embryo transfers are characterized by impaired pregnancy outcome and increased incidence of pregnancy loss as compared with fresh IVF/ICSI embryo transfers. In this study, we performed a retrospective analysis of clinical and embryological factors that potentially influence the outcome of frozen embryo transfer. **METHODS:** We reviewed the outcome of 1242 frozen embryo transfers with respect to the age of the woman, the method of fertilization, embryo quality before and after freezing and the number of embryos transferred. **RESULTS AND CONCLUSIONS:** The pregnancy (positive hCG) and clinical pregnancy rates were 25.8 and 21.1%, respectively. A total of 107 (33.3%) of the 321 pregnancies identified by a positive hCG test miscarried either before (18.4%) or after (15%) the clinical recognition of gestational sac(s). The delivery rate for the frozen embryo transfers analysed was 17.2%. Our data revealed that the delivery rate after frozen embryo transfer was dependent on both the woman's age and the quality of embryos transferred, at the same time being unaffected by IVF/ICSI treatment. In addition, the increased woman's age at IVF/ICSI treatment was identified as the only parameter elevating the biochemical pregnancy rate, whereas the clinical abortion rate was found to be unrelated to the clinical or embryological parameters studied.

*Key words:* abortion/embryo quality/frozen embryo transfer/IVF

## Introduction

Frozen embryo transfer was for the first time successfully accomplished 20 years ago (Zeilmaker *et al.*, 1984). During the following developments, frozen embryo transfer has become an important component of assisted reproductive technology (ART), bringing two important contributions to the field: it provides the means to reduce the number of transferred embryos, thereby diminishing the risk of multiple pregnancies (Tiitinen *et al.*, 2001), and it allows maximizing of the cumulative pregnancy rate per oocyte retrieval (Bergh *et al.*, 1995). Recently, European data were published on ART for 2001 (Andersen *et al.*, 2005). According to this report, almost 42 000 frozen embryo transfers are performed annually in Europe, with ~16% clinical pregnancy rate reported (Andersen *et al.*, 2005). The pregnancy outcome of frozen embryo transfer is known to be dependent on multiple clinical and embryological factors, including the age of the woman at IVF/ICSI treatment (Schalkoff *et al.*, 1993; Wang *et al.*, 2001), the method of oocyte fertilization used (Van Steirteghem *et al.*, 1994), the developmental stage of embryos at freezing (Salumets *et al.*, 2003), the embryo quality before freezing (Schalkoff *et al.*,

1993), the extent of embryo damage after thawing (Edgar *et al.*, 2000) and the resumption of post-thaw blastomere divisions (Van der Elst *et al.*, 1997).

The monitoring of ART outcome has, however, demonstrated that the pregnancy rate in frozen embryo transfer cycles is ~40% less compared to fresh IVF/ICSI cycles (Andersen *et al.*, 2005). In addition, the chance of live birth following frozen embryo transfer is further reduced by the increased incidences of pregnancy wastage (Aytoz *et al.*, 1999). Biochemical pregnancy and clinical abortion rates of 15–20% and 20–25%, respectively, have been reported after the transfers of cryopreserved embryos (Kowalik *et al.*, 1998; Aytoz *et al.*, 1999; Van den Abbeel *et al.*, 2000; Salumets *et al.*, 2003). Although the reasons for impaired pregnancy and elevated spontaneous abortion rates following frozen embryo transfer are not completely understood, they are most likely caused by the damage to embryos occurring during the freezing and thawing procedures (Edgar *et al.*, 2000). Considering the importance of frozen embryo transfer in ART, the main objective of the present study is to offer a comprehensive analysis of clinical and embryological factors that may influence the delivery rate after frozen embryo

transfer, with special attention paid to the analysis of the parameters that may confer an increased risk for experiencing the pregnancy loss following frozen embryo transfer.

## Materials and methods

### Study period and patients

In this study, we included patients undergoing IVF/ICSI treatment at the Infertility Clinic of the Family Federation of Finland in Helsinki from 1993 to 2001 with a frozen embryo transfer between 1997 and 2001. In total, the data analysis covered the evaluation of 420 single frozen (SF) embryo transfers and 822 double frozen (DF) embryo transfers with 1400 IVF- and 664 ICSI-derived embryos.

### IVF and ICSI procedures

IVF/ICSI procedures used at our clinic remained essentially unchanged during 1993–2001 and have been extensively described elsewhere (Söderström-Anttila *et al.*, 1996; Salumets *et al.*, 2002). Briefly, the patients underwent pituitary down-regulation with a GnRH agonist commenced in the mid-luteal phase of the previous menstrual cycle. When suppression was achieved, ovarian stimulation was performed using either human menopausal or recombinant FSH. When two or more follicles reached the size of  $\geq 17$  mm in diameter, hCG was administered, and transvaginal oocyte retrieval was performed 36 h later. Oocytes were incubated in Universal IVF medium (MediCult *a/s*, Copenhagen, Denmark). Insemination or microinjection was carried out 4–6 h after oocyte retrieval. Oocytes were checked for the presence of pronuclei and polar bodies 16–18 h after insemination or ICSI. Zygotes were transferred to fresh Universal IVF medium and were cultured in open-culture system for the following 24 h. The quality of embryos was evaluated 44–48 h after insemination or ICSI based on the number of blastomeres, the degree of fragmentation and the equality of the blastomeres. Embryo morphology was scored as follows: grade 1, no fragments and equal blastomeres; grade 2,  $<20\%$  fragments and equal blastomeres; grade 3A, no fragments and unequal blastomeres or 20–35% fragments, irrespective of the equality of the blastomeres; grade 3B, 35–50% fragments, irrespective of the equality of the blastomeres and grade 4,  $>50\%$  fragments, irrespective of the equality of the blastomeres. Embryos exhibiting morphology grades of 1–3A were considered suitable for freezing. Fresh embryo transfer and cryopreservation were performed 44–48 h after insemination or ICSI.

### Embryo freezing

Cryopreservation was carried out using automated Kryo 10 series II biological freezer (Planer Products Ltd, Sunbury-on-Thames, UK) following a slow freeze protocol using 1,2-propanediol (PROH) (Sigma, Saint Louis, MO, USA) as a cryoprotectant (Lassalle *et al.*, 1985). The freezing solution was prepared in phosphate-buffered saline (PBS) (Gibco, Life Technologies, Paisley, UK) supplemented with 20% v/v human serum (Finnish Red Cross, Helsinki, Finland). Embryos were first incubated in 1.5 M PROH freezing solution at room temperature for 10 min, and then in 1.5 M PROH and 0.2 M sucrose (Sigma) freezing solution at room temperature for 10 min. During the last incubation, one to three embryos were loaded into plastic ministraws (0.25 ml, Paillette Souple, Industrie de la Médecine Vétérinaire, Machelen, Belgium), and the freezing program was executed as follows: embryos were placed in the freezing machine at 18°C, cooled at  $-2^\circ\text{C}/\text{min}$  to  $-8^\circ\text{C}$ , held at  $-8^\circ\text{C}$  for manual seeding (10 min), cooled at  $-0.3^\circ\text{C}/\text{min}$  to  $-30^\circ\text{C}$ , and then at  $-30^\circ\text{C}/\text{min}$  to  $-150^\circ\text{C}$ , before being plunged into liquid nitrogen.

### Embryo thawing and replacement

The straws with frozen embryos were removed from liquid nitrogen, exposed to room temperature (30 s) and immersed in a water bath at

$30^\circ\text{C}$  (30 s). Thawed embryos were first incubated in a series of decreasing PROH concentrations (1 M for 5 min and 0.5 M for 5 min) in the thawing solution (0.2 M sucrose and 20% v/v human serum in PBS, at room temperature), next in the thawing solution only (10 min, at room temperature) and finally in sucrose-free thawing solution at  $37^\circ\text{C}$  (10 min), before transferring to culture media.

Frozen embryo transfer was performed either in a natural or in down-regulated hormone replacement cycle. Vaginal micronized progesterone (Lugesteron®, Leiras, Finland) was used for luteal support. Embryos were accepted for transfer if they retained  $\geq 50\%$  of blastomeres intact after thawing. Irrespective of the number of embryos thawed, a maximum of two embryos were selected for transfer either on the day of embryo thawing ( $n = 1053$ ) or after 24 h of culture ( $n = 189$ ).

### Pregnancy outcome of frozen embryo transfer

A positive serum hCG test ( $\geq 10$  IU/l) conducted 16 days after frozen embryo transfer confirmed pregnancy. The clinical pregnancy was documented by the presence of gestational sac(s) with or without fetal heartbeat on transvaginal sonography at 6–7 weeks of gestation. The early pregnancy loss (biochemical pregnancy) was recorded if no embryonic sac was noted during ultrasound examination of a woman with a positive hCG test. Frozen embryo transfer resulted in a clinical abortion if pregnancy loss occurred between the clinical detection of a pregnancy and the 22nd week of gestation. Finally, the delivery was defined as birth at a gestational age of more than 22 weeks.

The pregnancy (positive hCG) rate was determined as a ratio between the number of positive hCG tests and the total number of frozen embryo transfers. The clinical pregnancy rate was obtained by dividing the number of clinical pregnancies with the total number of embryo transfers. In the calculation of implantation rate, the number of gestational sacs was divided with the number of frozen embryos transferred. The biochemical pregnancy rate was presented as the number of early pregnancy losses from the total number of positive hCG tests, whereas the clinical abortion rate was estimated as the proportion of the clinical spontaneous abortion to the total number of clinical pregnancies. Delivery rate was defined as a ratio between deliveries and embryo transfers and the twin delivery rate was described as the number of twin deliveries per total number of births.

### Statistical analysis

The aim of the statistical analysis was to evaluate the influence of several clinical and embryological factors on the delivery rate of frozen embryo transfer. At our clinic, a maximum of two frozen embryos are transferred. As a consequence, the incidence of twin deliveries remains low, making the analysis of clinical and embryological parameters favouring twin deliveries unachievable. The assessment of parameters possibly related to the delivery rate of frozen embryo transfer was accomplished by using both the univariate and the multivariate analysis by employing the statistical software package SAS System for Windows version 8.01 (SAS Institute, Cary, NC, USA). The impacts of the following factors were investigated: woman's age at IVF/ICSI treatment, the number of embryos transferred (DF embryo transfer versus SF embryo transfer), the method of fertilization (IVF versus ICSI), embryo morphology before cryopreservation (grade 1 or 2 versus grade 3A), blastomere number before freezing (four or more versus two or three blastomeres) and cryosurvival after thawing (intact versus partially damaged embryos). However, because of the low number of frozen embryo transfers with embryos cultured for additional 24 h before transfer, these procedures were only studied by univariate statistics. The assessment of our data did not reveal any differences in frozen embryo transfer pregnancy outcome between the grade 1 and grade 2 embryo transfers. Consequently, these frozen embryo transfers were combined into a single group for statistical analysis.

The univariate analysis of collected data was accomplished using chi-square test, Fisher's exact test and *t*-test. The mean values were given with SDs, and *P* values of  $\leq 0.05$  were considered to indicate statistical significance. The multivariate analysis by backward logistic regression analysis was carried out to formulate and evaluate statistical models describing and predicting the individual influence of several clinical and embryological factors on the success of frozen embryo transfer. Odds ratios (ORs) with 95% confidence intervals (CIs) were presented. The multivariate statistical approach was conducted exclusively on SF embryo transfers ( $n = 420$ ) and DF embryo transfers ( $n = 245$ ) with embryos demonstrating identical quality. In addition, the standardization and exponential transformation of patients' age were used in the construction of statistical models to more accurately simulate the impact of age on frozen embryo transfer pregnancy outcome.

## Results

### Overall results

During the study period, 3133 embryos were thawed in 1287 cycles, with 79.3% of the survival rate. A total of 2064 embryos were transferred in 1242 (96.5% of all cycles) frozen embryo transfers. The average number of embryos transferred was 1.7 in 420 SF embryo transfer and 822 DF embryo transfer cycles. The mean age ( $\pm$ SD) of a woman at IVF/ICSI treatment was  $34 \pm 4.3$  years. All frozen embryo transfers resulted in 321 (25.8%) positive hCG tests. However, 59 (18.4%) pregnancies miscarried before the clinical recognition of gestational sac(s). Ultrasonography revealed 262 clinical pregnancies. In 228 (87%) and 34 (13%) of clinical pregnancies, one and two gestational sacs, respectively, were observed. The clinical pregnancy and implantation rates were 21.1% (262/1242) and 14.3% (296/2064), respectively. Of the 296 embryonic sacs, 58 (19.6%) were aborted. The spontaneous abortion rate per clinical pregnancy was 18.3% (48/262). The delivery rate was 17.2% (214/1242) with 11.2% ( $n = 24$ ) of twin deliveries.

### The univariate analysis of factors affecting the results of frozen embryo transfer

The statistical analysis indicated that the pregnancy (positive hCG), clinical pregnancy and delivery rates were significantly higher after DF embryo transfer than they were after SF

embryo transfer (Table I). However, the method used for fertilization of oocytes was found not to be associated with the pregnancy outcome of frozen embryo transfer (Table I). In the evaluation of embryo quality, both the morphological appearance and the number of blastomeres before cryopreservation were assessed. These two embryo characteristics were both clearly related to the pregnancy outcome of frozen embryo transfer (Table II). Transferred embryos exhibiting superior morphological quality (grade 1 or 2) were more likely to produce clinical pregnancies (implantation rate of 16.8%) than embryos possessing poorer (grade 3A) quality (implantation rate of 12.4%,  $P < 0.05$ ). The statistical differences in the success of frozen embryo transfer were also observed between the transfers with embryos possessing four or more and two to three blastomeres before cryopreservation. Frozen embryo transfers with embryos having four or more blastomeres were associated with markedly higher positive hCG, clinical pregnancy, implantation and delivery rates compared with frozen embryo transfers with embryos possessing fewer cells. The cryosurvival of embryos after thawing was also identified as a crucial factor determining the success of frozen-thawed embryo transfers (Table II). The embryo transfers with fully intact embryos resulted in a considerably better efficacy of frozen embryo transfer treatment than transfers with partially damaged embryos. The observed pregnancy results for embryo transfers with intact and partially damaged thawed embryos were as follows: positive hCG rate (27.1 versus 17.1%,  $P < 0.05$ ); clinical pregnancy rate (22.8 versus 12.2%,  $P < 0.05$ ) and delivery rate (18.7 versus 9.8%,  $P < 0.05$ ). According to the univariate statistical analysis, the most significant factor in predicting the success of frozen embryo transfer was the resumption of mitosis after thawing (Table II). The embryos that accomplished blastomere divisions during 24 h post-thaw culture exhibited significantly better developmental potential than embryos that failed to develop. The frozen embryo transfers with cleaved embryos showed clearly better positive hCG (36 versus 12.5%,  $P < 0.05$ ), clinical pregnancy (31.2 versus 3.1%,  $P < 0.0001$ ), implantation (22.1 versus 2.2%,  $P = 0.001$ ) and delivery (24.8 versus 3.1%,  $P < 0.01$ ) rates than non-cleaved embryo transfers. However, the univariate statistical analysis revealed that the biochemical pregnancy and clinical

**Table I.** The univariate statistical analysis of clinical factors influencing the pregnancy outcome of frozen embryo transfer

| Parameter   | Number of embryos transferred |                               | Method of fertilization |                   |
|---|-------------------------------|-------------------------------|-------------------------|-------------------|
|   | Double frozen embryo transfer | Single frozen embryo transfer | IVF                     | ICSI              |
| Mean age in years $\pm$ SD                                  | 34.0 $\pm$ 4.1                | 34.0 $\pm$ 4.6                | 34.3 $\pm$ 4.1***       | 33.4 $\pm$ 4.5*** |
| Number of embryo transfers                                  | 822                           | 420                           | 846                     | 396               |
| Number of embryos transferred                               | 1644                          | 420                           | 1400                    | 664               |
| Average number of embryos transferred $\pm$ SD              | 2                             | 1                             | 1.7 $\pm$ 0.5           | 1.7 $\pm$ 0.5     |
| Number of positive hCG tests (% per embryo transfer)        | 231 (28.1)*                   | 90 (21.4)*                    | 223 (26.4)              | 98 (24.7)         |
| Number of biochemical pregnancies (% per positive hCG test) | 39 (16.9)                     | 20 (22.2)                     | 41 (18.4)               | 18 (18.4)         |
| Number of clinical pregnancies (% per embryo transfer)      | 192 (23.4)**                  | 70 (16.7)**                   | 182 (21.5)              | 80 (20.2)         |
| Number of gestational sacs (% per embryo transfer)          | 226 (13.7)                    | 70 (16.7)                     | 206 (14.7)              | 90 (13.6)         |
| Number of clinical abortions (% per gestational sac)        | 48 (21.2)                     | 10 (14.3)                     | 43 (20.9)               | 15 (16.7)         |
| Number of deliveries (% per embryo transfer)                | 154 (18.7)*                   | 60 (14.3)*                    | 147 (17.4)              | 67 (16.9)         |

\* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.005$ .

**Table II.** The univariate statistical analysis of embryological factors influencing the pregnancy outcome of frozen embryo transfer

| Parameter   | Morphological grade      |                          | Number of blastomeres    |                         | Cryosurvival             |                          | Resumption of mitosis    |                       |
|---|--------------------------|--------------------------|--------------------------|-------------------------|--------------------------|--------------------------|--------------------------|-----------------------|
|   | 1 or 2                   | 3A                       | ≥4                       | 2 or 3                  | Intact                   | Partially damaged        | Cleaved                  | Non-cleaved           |
| Mean age in years ± SD                                      | 33.8 ± 4.3               | 33.9 ± 4.4               | 34.1 ± 4.4               | 33.7 ± 4.1              | 34.0 ± 4.3               | 34.3 ± 4.5               | 34.0 ± 4.1               | 33.6 ± 4.7            |
| Number of embryo transfers                                  | 548                      | 403                      | 592                      | 368                     | 927                      | 82                       | 125                      | 32                    |
| Number of embryos transferred                               | 820                      | 662                      | 934                      | 566                     | 1481                     | 117                      | 199                      | 45                    |
| Average number of embryos transferred ± SD                  | 1.5 ± 0.5 <sup>†††</sup> | 1.6 ± 0.5 <sup>†††</sup> | 1.6 ± 0.5                | 1.5 ± 0.5               | 1.6 ± 0.5 <sup>***</sup> | 1.4 ± 0.5 <sup>***</sup> | 1.6 ± 0.5                | 1.4 ± 0.5             |
| Number of positive hCG tests (% per embryo transfer)        | 150 (27.4)               | 91 (22.6)                | 180 (30.4) <sup>†</sup>  | 74 (20.1) <sup>††</sup> | 251 (27.1) <sup>*</sup>  | 14 (17.1) <sup>*</sup>   | 45 (36.0) <sup>*</sup>   | 4 (12.5) <sup>*</sup> |
| Number of biochemical pregnancies (% per positive hCG test) | 29 (19.3)                | 19 (20.9)                | 35 (19.4)                | 11 (14.9)               | 40 (15.9)                | 4 (28.6)                 | 6 (13.3) <sup>*</sup>    | 3 (75.0) <sup>*</sup> |
| Number of clinical pregnancies (% per embryo transfer)      | 121 (22.1)               | 72 (17.9)                | 145 (24.5) <sup>**</sup> | 63 (17.1) <sup>**</sup> | 211 (22.8) <sup>*</sup>  | 10 (12.2) <sup>*</sup>   | 39 (31.2) <sup>†††</sup> | 1 (3.1) <sup>††</sup> |
| Number of gestational sacs (% per embryo transferred)       | 138 (16.8) <sup>*</sup>  | 82 (12.4) <sup>*</sup>   | 164 (17.6) <sup>**</sup> | 70 (12.4) <sup>**</sup> | 235 (15.9)               | 11 (9.4)                 | 44 (22.1) <sup>†</sup>   | 1 (2.2) <sup>†</sup>  |
| Number of clinical abortions (% per gestational sac)        | 26 (18.8)                | 15 (18.3)                | 28 (17.1)                | 14 (20.0)               | 44 (18.7)                | 2 (18.2)                 | 9 (20.5)                 | 0 (0)                 |
| Number of deliveries (% per embryo transfer)                | 99 (18.1)                | 60 (14.9)                | 122 (20.6) <sup>**</sup> | 50 (13.6) <sup>**</sup> | 173 (18.7) <sup>*</sup>  | 8 (9.8) <sup>*</sup>     | 31 (24.8) <sup>**</sup>  | 1 (3.1) <sup>**</sup> |

\* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.0005$ ; <sup>†</sup> $P \leq 0.001$ ; <sup>††</sup> $P \leq 0.0005$ ; <sup>†††</sup> $P \leq 0.0001$ .

abortion rates after frozen embryo transfer were unrelated to the embryo quality before and after cryopreservation.

**The multivariate analysis of factors affecting the results of frozen embryo transfer**

The results of the multivariate statistics on factors predicting the frozen embryo transfer pregnancy outcome are summarized in Table III. According to our analysis, the woman’s age is the major factor contributing to the pregnancy outcome of frozen embryo transfer. The effects of patients’ age at IVF/ICSI treatment on ORs for different aspects of frozen embryo transfer outcome for women at certain age groups compared with those with a mean age of 33.9 years are depicted in Figure 1.

On the basis of our data, the age of the woman at IVF/ICSI treatment was observed to be an important factor in the multivariate model predicting the positive hCG rate following frozen embryo transfer. Besides the marked age effect, the multivariate analysis of collected data also revealed the principal roles of the number and the quality of frozen embryos transferred in determining the positive hCG rate of frozen embryo transfer. The OR calculated for the positive hCG test for DF embryo transfer was 1.67 (95% CI 1.16–2.41) compared with that of SF embryo transfer. In addition, the frozen embryo transfers with embryos exhibiting superior embryo morphology (OR 1.56; 95% CI 1.07–2.28) and having four or more blastomeres before cryopreservation (OR 2.05; 95% CI 1.38–3.04) also resulted in elevated pregnancy rates than frozen embryo transfers with poorer quality embryos. The detailed analysis of different clinical and embryological factors that could possibly influence the probability for pregnancy loss before the clinical recognition of gestational sac(s) displayed that the biochemical pregnancy rate was solely determined by the woman’s age at embryo freezing. The calculated ORs for the biochemical pregnancy rate for women 5 years younger and 5 years older than women of the mean age were 0.79 (95% CI 0.67–0.98) and 2.08 (95% CI 1.21–3.56), respectively.

The multivariate model derived from our data consisted of three variables related to the clinical pregnancy rate of frozen embryo transfer. The woman’s age, the number of embryos replaced and the overall quality of frozen embryos transferred were all identified as being independently and strongly related to the clinical pregnancy rate of frozen embryo transfers, whereas the delivery rate after frozen embryo transfer was only dependent on the woman’s age and the quality of embryos transferred. Among the variables incorporated into the multivariate regression models, the number of blastomeres in embryos before freezing was observed to have the highest impact on the clinical pregnancy and delivery rates of frozen embryo transfer. The ORs for clinical pregnancy and delivery rates between the frozen embryo transfers with embryos possessing four or more and less than four blastomeres were 1.96 (95% CI 1.27–3.02) and 2.01 (95% CI 1.28–3.18), respectively. It also became evident from our data that none of the clinical and embryological factors studied influenced the probability for spontaneous abortion after the clinical recognition of pregnancy.



demonstrated no differences between the delivery rates of elective SF embryo transfers (28.6%) and DF embryo transfers (25.7%).

The question of whether the method of fertilization could influence the pregnancy outcome of frozen embryo transfer has been examined in several studies by comparing the developmental potential of frozen-thawed early cleavage stage embryos obtained either through conventional IVF or through ICSI procedure (Van Steirteghem *et al.*, 1994; Kowalik *et al.*, 1998; Mandelbaum *et al.*, 1998; Simon *et al.*, 1998). This concern is justified, as the breaching of zona pellucida and the penetration of oolemma by injection may potentially disturb the embryo cryopreservation by interfering with the movement of water and cryoprotectants across the cell membranes during the freezing and thawing processes. Mandelbaum *et al.* (1998) studied 185 ICSI- and 809 IVF-frozen embryo transfer cycles with embryos frozen 2 or 3 days after fertilization and found no differences between the groups in terms of clinical pregnancy rates. Other studies have also shown similar results (Van Steirteghem *et al.*, 1994; Kowalik *et al.*, 1998). To the contrary, in a retrospective study comparing the pregnancy outcome between 83 IVF- and 204 ICSI-frozen embryo transfers, a significantly higher clinical pregnancy rate was achieved in IVF (32.5%) than that in ICSI (20%) (Simon *et al.*, 1998). However, the lower pregnancy rate in the ICSI group compared to IVF was attributed to the poorer quality of embryos transferred (Simon *et al.*, 1998). In the present study, the frozen embryo transfers with embryos cryopreserved 2 days after oocyte retrieval resulted in comparable clinical pregnancy rates for IVF (21.5%) and ICSI (20.2%) treatments. In summary, the findings of our study and those of several others (Van Steirteghem *et al.*, 1994; Kowalik *et al.*, 1998; Mandelbaum *et al.*, 1998) further support the conception that the micromanipulation does not compromise the developmental potential of frozen-thawed cleavage stage embryos.

The quality criteria utilized in the selection of embryos for cryopreservation vary substantially between the different ART programmes. In most studies, the supernumerary day 2 embryos have been frozen if they possessed at least two blastomeres and contained  $\leq 20\%$  of anucleated fragments (Ziebe *et al.*, 1998; Van den Abbeel *et al.*, 2000). In this study, all day 2 embryos with fragmentation occupying up to one-third of the embryo's volume were considered eligible for freezing. In accordance with previous studies (Schalkoff *et al.*, 1993; Kondo *et al.*, 1996), our findings reconfirmed the vital role of the embryo quality in the success of frozen embryo transfer. The multivariate analysis of our data demonstrated that better embryo morphology and faster blastomere cleavage rate were independently associated with improved delivery rate after frozen embryo transfer. However, the relatively high delivery rates after frozen embryo transfers with moderate quality (grade 3A) embryos (14.9%) and embryos having two to three blastomeres (13.6%) observed in the present study warrant cryopreservation of non-top-quality embryos as well to achieve the maximum cumulative delivery rate per oocyte retrieval. Embryo selection for frozen embryo transfer can be further improved by the culture of thawed embryos for additional 24 h before transfer. The previous studies, as well as the present

one, demonstrate that the transfer of cleaved embryos can significantly increase the delivery rate per replacement (Van der Elst *et al.*, 1997; Ziebe *et al.*, 1998).

Our analysis revealed that 33.3% (107/321) of the pregnancies identified by a positive hCG test miscarried either before (18.4%, 59/321) or after (15.0%, 48/321) the clinical recognition of a gestational sac(s). The preclinical abortion rate of 18.4% is in line with several previous reports demonstrating biochemical pregnancy rates of 15–20% after frozen embryo transfer (Kowalik *et al.*, 1998; Aytoz *et al.*, 1999). Only a few studies have undertaken a comprehensive analysis of the risk factors for early pregnancy loss of either IVF/ICSI (Winter *et al.*, 2002) or frozen embryo transfer (Aytoz *et al.*, 1999) conceptions. The multivariate analysis performed in the present study revealed that the patients' age at IVF/ICSI treatment is the only parameter showing statistically significant positive association with the biochemical pregnancy rate after frozen embryo transfer (Figure 1). Our results are consistent with those of Winter *et al.* (2002) showing relatively constant biochemical pregnancy rate for women up to the age of 40 years and subsequent escalation of the incidence of early pregnancy loss. It is generally accepted that this unfavourable trend is primarily caused by ageing of the oocytes, leading to the sharply increased prevalence of meiotic non-disjunctions and chromosomal abnormalities (Dailey *et al.*, 1996). There has been some concern that ICSI may be associated with an elevated occurrence of early pregnancy loss after frozen embryo transfer (Van Steirteghem *et al.*, 1994). However, the results of this study showing similar biochemical pregnancy rates for both IVF and ICSI conceptions agree with other more recent reports with similar results (Kowalik *et al.*, 1998; Aytoz *et al.*, 1999).

Limited data exist regarding the relationship between the embryo quality and the occurrence of early pregnancy loss following frozen embryo transfer (Van den Abbeel *et al.*, 1997). Our results of multivariate analysis show an absence of association between the biochemical pregnancy rate and embryo quality before cryopreservation. However, this conclusion should be considered with caution because it supposedly depends on the quality criteria used in the selection of embryos for freezing and the protocol applied in the embryo cryopreservation. The univariate evaluation of our data revealed a difference, albeit not statistically significant, in terms of biochemical pregnancy rate between the frozen embryo transfers with intact embryos (15.9%) and partially damaged embryos (28.6%). The lower incidence of early pregnancy loss after frozen embryo transfers with survived embryos (17.3%) compared to partially damaged embryos (42.9%) has also been shown previously (Van den Abbeel *et al.*, 1997). All these reports indicate that the cryodamage of replaced embryos is the primary culprit for increased incidence of early pregnancy losses observed after frozen embryo transfers. Furthermore, three of four pregnancies ended before the ultrasonographic detection of a gestational sac(s) in the group of frozen embryo transfers with embryos that failed to resume blastomere division during the 24 h of post-thaw culture. The calculated biochemical pregnancy rate of 75% for non-cleaved embryo transfers was substantially higher than that observed for frozen embryo transfers with cleaved embryos (13.3%). However, this finding should

be verified by further studies because of the low number of pregnancies evaluated.

The studies focusing on the obstetric outcome of conceptions achieved by frozen embryo transfer have reported 20–25% spontaneous abortions from all clinically ascertained gestations (Kowalik *et al.*, 1998; Aytoz *et al.*, 1999; Van den Abbeel *et al.*, 2000). We report comparable spontaneous abortion rate of 18.3% for 262 clinical frozen embryo transfer-pregnancies. Additionally, the statistical processing of our data uncovered that none of the clinical and embryological parameters evaluated were associated with the incidence of pregnancy loss occurring between the ultrasonographic determination of gestational sac(s) and the 22nd week of pregnancy. This finding is contradictory to other studies that have linked the elevated clinical abortion rate with advanced maternal age (Tummers *et al.*, 2003) and ICSI (Aytoz *et al.*, 1999). We found a similar positive association between the woman's age at IVF/ICSI and the clinical abortion rate, but this trend was statistically not significant. Comparable clinical spontaneous abortion rates for IVF (20.9%) and ICSI (16.7%) pregnancies reported in this study also agree with others (Kowalik *et al.*, 1998) and indicate the relative safety of ICSI treatment.

In conclusion, this study provides a comprehensive analysis of clinical and embryological factors putatively related to the pregnancy outcome after frozen embryo transfer. To the best of our knowledge, this report is the first attempt at scrutinizing the parameters most likely to influence the pregnancy wastage after frozen embryo transfer. The detailed analysis of our data revealed that the delivery rate after frozen embryo transfer was dependent on both the woman's age and the quality of embryos transferred, although being unaffected by IVF/ICSI procedure. Our results demonstrated that 33.3% of the pregnancies identified by a positive hCG test miscarried either before or after the clinical recognition of gestational sac(s). The age of a woman at the time of oocyte retrieval was identified as the only parameter associated with biochemical pregnancy rate, whereas the clinical abortion rate was found to be unrelated to the clinical and embryological parameters studied.

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