Obstetric outcome of pregnancies after the transfer of cryopreserved and fresh embryos obtained by conventional in-vitro fertilization and intracytoplasmic sperm injection

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This study reports the obstetric outcome of pregnancies obtained after the transfer of cryopreserved or fresh embryos where the initial procedure was standard in-vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). Pregnancies obtained after frozen IVF (n = 245) or frozen ICSI (n = 177) were compared with a control group of pregnancies after fresh embryo transfer in standard IVF (n = 245) and ICSI (n = 177) cycles were selected as controls. The controls were matched according to maternal age, parity and date of embryo transfer. In the standard IVF group, the biochemical pregnancy rates in the cryopreserved and fresh groups were 18.8 and 9.8% respectively (P < 0.01). In the ICSI group, the biochemical pregnancy rates in the cryopreserved and fresh groups were 16.4 and 6.8% respectively (P < 0.01). The miscarriage rates were comparable between the cryopreserved and fresh groups. However, in the frozen ICSI group the miscarriage rate (26.0%) was significantly higher than in the frozen conventional IVF group (13.1%) (P = 0.001). The frequencies of preterm deliveries, infants with very low birthweight and intrauterine deaths were similar in the groups. The low birthweight rates in the frozen IVF (16.1%) and ICSI (12.1%) groups were significantly lower than those in the fresh IVF (32.2%) and ICSI (32.7%) groups (P < 0.001). The major malformation rates in the frozen IVF (2.4%) and ICSI (2.9%) groups were not different from the major malformation rates in the fresh IVF (4.5%) and ICSI (2.4%) groups. In conclusion, the cryopreservation process had no negative impact on the outcome of pregnancies over 20 weeks of gestation. Long-term follow-up studies are needed in order to prove the safety of the freezing–thawing process.

Key words: cryopreserved embryos/in-vitro fertilization/intracytoplasmic sperm injection/pregnancy outcome

Introduction

Successful cryopreservation of mammalian embryos was described in 1972 (Whittingham et al., 1972). The first successful pregnancy from a frozen human embryo was described in 1983 (Trounson and Mohr, 1983). Later, clinical results of pregnancies and births after cryopreservation were published in larger series (Mohr et al., 1985; Van Steirteghem and van den Abbeel, 1990).

Cryopreservation can provide an increased cumulative pregnancy rate while decreasing the risk of multiple gestations and the risk of ovarian hyperstimulation syndrome (Trounson, 1986; Bergh et al., 1995). Cryopreservation also provides an additional chance of pregnancy in a treatment cycle which does not involve full ovarian stimulation and oocyte retrieval. All these advantages have contributed to widespread incorporation of cryopreservation techniques into IVF programmes.

Analyses of the data generated from embryo banking in the mouse and a wide variety of other species have shown that embryo freezing is a safe and effective method in animal breeding and that there is no evidence to suggest genetic alteration, increased birth defects or any other abnormality, even when embryos are exposed to high levels of ionizing radiation (Ashwood-Smith, 1986; Glenister et al., 1986). However, it has been reported (Duliois et al., 1995) that cryopreservation of mouse embryos, without being severely detrimental, may have delayed effects.

The safety of in-vitro fertilization (IVF) with or without micromanipulation has been evaluated in large studies (MRC Working Party On Children Conceived By In-Vitro Fertilisation, 1990; Rizk et al., 1991; Doyle et al., 1992; Rufat et al., 1994; Bonduelle et al., 1996, 1998; Olivennes et al., 1997). It has been reported that perinatal outcome of pregnancies obtained after IVF is less favourable than those obtained spontaneously (Rizk et al., 1991; Doyle et al., 1992). However, none of these studies has prospectively compared the outcome of IVF children on a long-term basis.

Few studies have evaluated obstetrical outcome in pregnancies resulting from cryopreservation of embryos obtained from standard IVF (Frydman et al., 1989; Sutcliffe et al., 1995; Olivennes et al., 1996; Wennerholm et al. 1997). So far, only early outcome has been analysed in studies reporting on the obstetric outcome of pregnancies obtained after transfer of frozen embryos generated by intracytoplasmic sperm injection (ICSI) (Van Steirteghem et al., 1994; Al Hasani et al., 1996; Hoover et al., 1997; Macas et al., 1998).

In this retrospective study, we evaluated early (<20 weeks of gestation) and late (≥20 weeks of gestation) obstetric outcome in pregnancies obtained after the transfer of cryopreserved embryos generated by conventional IVF and ICSI. We also compared these outcomes with that of pregnancies obtained after fresh embryo transfer after standard IVF and ICSI.
Materials and methods

Two hundred and forty-five pregnancies obtained after the transfer of cryopreserved embryos generated by conventional IVF and 177 pregnancies conceived after the transfer of frozen embryos generated by ICSI during the period from May 1986 to July 1997 were included in the study. As controls, 245 pregnancies resulting from standard IVF with fresh embryos and 177 pregnancies resulting from ICSI with fresh embryos were selected. The groups were matched according to maternal age ±6 months, parity and date of embryo transfer ±1 year. Pregnancy outcome for singletons and twins was compared separately. In the ICSI group, only cycles where ejaculated spermatozoa were used were selected. Pregnancies obtained after transfer of frozen embryos resulting from an oocyte donation programme were not included in this study.

The obstetrical data were acquired through a specific questionnaire which was filled in and sent back to our centre by the patient or her gynaecologist or paediatrician. Where the data were incomplete, additional information was requested personally by the research nurse.

Each patient attending the infertility clinic was informed about the specific aspects of the treatment. On this occasion, in the ICSI group, the patients were also asked if they would be willing to undergo a prenatal diagnosis, i.e. amniocentesis and chorionic villus sampling if pregnancy ensued. All of them were advised to take part in a prospective follow-up programme for the children (Bonduelle et al., 1994, 1998).

The description of the ICSI procedure has been published previously (Van Steirteghem et al., 1995). Supernumerary embryos were cryopreserved during previous conventional IVF or ICSI attempts. Embryos with <20% of their volume filled with anucleate fragments were cryopreserved by a slow freezing protocol with dimethyl sulphoxide.

The majority of the embryos were frozen at the three- to four-cell stage. The freezing and thawing procedure has been described in detail previously (Van den Abbeel et al., 1997). After thawing, only embryos with a maximum of 50% blastomere damage were transferred. Frozen–thawed embryos were replaced in natural; stimulated or artificial cycles.

Pregnancy was diagnosed when a rise in serum human chorionic gonadotrophin (HCG) concentration was observed on two consecutive occasions from 11 days after embryo transfer. Serum HCG measurement were performed twice weekly through the luteal phase. If in the seventh week of gestation a sac was visualized by ultrasonography, the pregnancy was considered clinical. If there was no sac, the pregnancy was considered biochemical. The diagnosis of intrauterine pregnancy was confirmed by laparoscopy. Gestational age was calculated as the time between the beginning of the last menstrual period and the date of birth of the child. The last menstrual period was calculated by adding 15 days to the date of embryo transfer.

Abortion was defined as pregnancy loss before 20 weeks of gestational age and preterm delivery was defined as delivery of a live-born or stillborn infant before 37 weeks of gestational age. A live-born or stillborn infant weighing <2500 g at birth was considered a low-birthweight infant. A live-born or stillborn infant weighing <1500 g at birth was considered a very-low-birthweight infant. The death of a fetus of at least 20 weeks gestation before delivery was defined as intrauterine death.

Major malformation was defined as that which generally causes functional impairment or requires surgical correction.

The χ² test or Fisher’s exact probability test was used to compare the percentages and the unpaired t-test or Wilcoxon test was used to compare the samples in different groups.

Results

The mean (±SD) maternal age in the cryopreserved and fresh embryo groups after standard IVF was 32.6 ± 4.1 years and the mean maternal age in the cryopreserved and fresh embryo groups after ICSI was 32.7 ± 4.2 years. The mean (±SD) numbers of embryos transferred in the cryopreserved and fresh groups after conventional IVF were 2.3 ± 0.8 and 2.5 ± 0.6 respectively. The mean (±SD) numbers of embryos transferred in the frozen and fresh groups after ICSI were 2.8 ± 0.8 and 2.8 ± 0.7 respectively. The differences between groups were not statistically significant.

In the frozen IVF group, the biochemical pregnancy rate was 18.8% (46/245), in the fresh IVF group it was 9.8% (24/245) (P < 0.01). In the frozen IVF group, 32 pregnancies ended in abortion (13.1%). In the controls, the abortion rate was also 13.1% (Table I). In the frozen embryo group, 13 (5.3%) ectopic pregnancies occurred and in the control group seven (2.9%). In each group, one pregnancy was terminated because of trisomy 21. In the frozen ICSI group, 29 out of 177 pregnancies were biochemical (16.4%). In the fresh ICSI group, 12 pregnancies were biochemical (6.8%) (P < 0.01).

The abortion rates in the frozen and fresh embryo groups were 26.0 (46/177) and 18.6% (33/177) respectively (P = 0.13) (Table I). In the frozen ICSI group, five (2.8%) ectopic pregnancies occurred and three pregnancies were terminated (two singletons with Klinefelter and a twin pregnancy with 47,XXX and 47,XY,+21). No pregnancy was terminated in the control group.

In the study group after conventional IVF, 153 pregnancies continued over 20 weeks of gestation (62.4%). One hundred and twenty-six of these pregnancies were singletons (82.4%) and 27 were twins (17.6%). In the controls, 181 pregnancies continued over 20 weeks of gestation (73.9%). One hundred and twenty-four pregnancies were singletons (68.5%) and 56 were twins (30.9%) (Table I). In the control group, one pregnancy was terminated at 25.3 weeks of gestation because of severe oligohydramnios. In the study group after ICSI, 94 (53.1%) out of 177 pregnancies continued over 20 weeks of gestation and in the controls 128 (72.3%) out of 177 pregnancies continued over 20 weeks of gestation (P < 0.001). In the study group the rates of singleton and twin pregnancies were 84.0 and 16.0% respectively. In the controls 68.8% of pregnancies were singleton and 31.2% of pregnancies were twin (Table I).

The ratios of the number of babies beyond 20 weeks of gestation to the number of embryos transferred in the frozen IVF, fresh IVF, frozen ICSI and fresh ICSI groups were 32.5 (180/554), 37.7 (235/624), 22.4 (109/487) and 34.1% (168/474) respectively. In the frozen ICSI group, this ratio was significantly lower than the other groups (P < 0.001).

Mean (±SD) gestational age and mean weight at birth for singletons in the frozen embryo group after standard IVF were 38.7 ± 2.0 weeks and 3322.0 ± 569.4 g respectively. The same parameters for twins were 35.3 ± 3.8 weeks and 2381.1 ± 631.1 g respectively. In the controls, mean gestational age and mean weight at birth for singletons were 38.6 ± 2.2 weeks and 3239.5 ± 609.9 g. For twins, mean gestational age at birth was 34.8 ± 3.8 weeks and mean birthweight was 2279.9 ± 715.6 g (Table II).
In the frozen embryo group after ICSI mean (±SD) gestational ages at birth for singletons and twins were 38.5 ± 2.5 and 36.2 ± 2.3 weeks respectively. In the same group mean birthweights for singletons and twins were 3231.4 ± 629.7 and 2567.5 ± 521.8 g. In the fresh embryo group after ICSI mean (±SD) gestational ages at birth for singletons and twins were 38.6 ± 2.5 and 35.5 ± 2.4 weeks respectively. Mean birthweights for singletons and twins were 3288.1 ± 475.2 and 2380.2 ± 490.4 g. No significant difference was found between the groups (Table II).

Pregnancy outcome over 20 weeks of gestation and birth characteristics in the frozen embryo groups and in the controls are summarized in Table III. The preterm delivery (<37 weeks of gestation) rates in the frozen and fresh IVF groups were 19.6 and 27.2% respectively ($\chi^2$, NS). The preterm delivery rates in the frozen and fresh ICSI groups were 21.3 and 25.0% respectively ($\chi^2$, NS). For singleton pregnancies, in the frozen IVF group 122 birthweights were available, in the fresh IVF group 123, in the frozen ICSI group 75 and in the fresh ICSI group 88. For twin pregnancies, in the frozen IVF group 52 birthweights were available, in the fresh IVF group 107, in the frozen ICSI group 24 and in the fresh ICSI group 80. The low-birthweight rates and the very-low-birthweight rates in the frozen IVF group were 16.1 and 2.9% respectively. In the fresh IVF group, the same parameters were 32.2 and 6.5% respectively. In the frozen ICSI group, the rate of fetuses with low birthweight was 12.1% and the rate of fetuses with very low birthweight was 4.0%. In the fresh ICSI group, 32.7% of the fetuses were with low birthweight and 2.4% were with very low birthweight. The frequencies of infants with low birthweight in the frozen IVF and ICSI groups were significantly lower than those in the fresh IVF and ICSI groups (Table III). In the frozen IVF group two fetuses and in the fresh IVF group 3 fetuses died in utero, while in the fresh ICSI group one fetus died in utero. The perinatal mortality rates were not calculated because of lack of follow-up of some children in the different groups.

Major malformations up to the age of 1 year are shown in Table IV. The rates of loss to follow-up in the frozen IVF, fresh IVF, frozen ICSI and fresh ICSI groups were 5.2, 4.7, 4.6 and 1.2% respectively. In the frozen IVF, four out of 165 infants (2.4%) and in the fresh IVF 10 out of 224 infants (4.5%) had major malformations. In the frozen ICSI group, major malformations were found in three of the 104 infants (2.9%). In the fresh ICSI group, four of the 166 infants (2.4%) had major malformations.

**Discussion**

In the present study, we analysed obstetrical outcome of pregnancies obtained after cryopreserved embryo transfer where the initial procedures were conventional IVF ($n = 245$) or ICSI ($n = 177$). We also compared this outcome to that of pregnancies obtained after fresh embryo transfer after standard IVF ($n = 245$) or ICSI ($n = 177$). Since the subgroup of frozen ICSI patients who received testicular spermatozoa was too small for statistical analysis, the results of only those cycles where ejaculated spermatozoa were used are reported. The use of testicular spermatozoa requires further study.

The controls were matched with respect to maternal age,
A. Aytoz et al.

Table III. Pregnancy outcome ≥20 weeks of gestation and birth characteristics in the cryopreserved and fresh embryo groups after standard in-vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI)

<table>
<thead>
<tr>
<th></th>
<th>Cryopreserved standard IVF</th>
<th>Fresh standard IVF</th>
<th>Cryopreserved ICSI</th>
<th>Fresh ICSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Singletons</td>
<td>126</td>
<td>124</td>
<td>79</td>
<td>88</td>
</tr>
<tr>
<td>Preterm deliveries</td>
<td>14 (11.1)</td>
<td>16 (12.9)</td>
<td>12a (15.2)</td>
<td>4b (4.5)</td>
</tr>
<tr>
<td>Low birthweight</td>
<td>11 (9.0)</td>
<td>10 (8.1)</td>
<td>5 (6.7)</td>
<td>4 (4.5)</td>
</tr>
<tr>
<td>Very low birthweight</td>
<td>0</td>
<td>3 (2.4)</td>
<td>2 (2.7)</td>
<td>0</td>
</tr>
<tr>
<td>Intrauterine death</td>
<td>2 (1.6)</td>
<td>1 (0.8)</td>
<td>0</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td>Twins</td>
<td>27 (54 fetuses)</td>
<td>56 (111 fetuses)</td>
<td>15 (30 fetuses)</td>
<td>40 (80 fetuses)</td>
</tr>
<tr>
<td>Preterm deliveries</td>
<td>16 (59.3)</td>
<td>33 (58.9)</td>
<td>8 (53.3)</td>
<td>28 (70.0)</td>
</tr>
<tr>
<td>Low birthweight</td>
<td>17a (32.7)</td>
<td>64a (59.8)</td>
<td>7a (29.1)</td>
<td>51a (63.8)</td>
</tr>
<tr>
<td>Very low birthweight</td>
<td>5 (9.6)</td>
<td>12 (11.2)</td>
<td>2 (8.3)</td>
<td>4 (5.0)</td>
</tr>
<tr>
<td>Intrauterine death</td>
<td>0</td>
<td>2 (1.8)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>153 (180 fetuses)</td>
<td>180 (235 fetuses)</td>
<td>94 (109 fetuses)</td>
<td>128 (168 fetuses)</td>
</tr>
<tr>
<td>Preterm deliveries</td>
<td>30 (19.6)</td>
<td>49 (27.2)</td>
<td>20 (21.3)</td>
<td>32 (25.0)</td>
</tr>
<tr>
<td>Low birthweight</td>
<td>28a (16.1)</td>
<td>74a (32.2)</td>
<td>12a (12.1)</td>
<td>55a (32.7)</td>
</tr>
<tr>
<td>Very low birthweight</td>
<td>5 (2.9)</td>
<td>15 (6.5)</td>
<td>4 (4.0)</td>
<td>4 (2.4)</td>
</tr>
<tr>
<td>Intrauterine death</td>
<td>2 (1.1)</td>
<td>3 (1.3)</td>
<td>0</td>
<td>1 (0.6)</td>
</tr>
</tbody>
</table>

ab Values are significantly different (P < 0.05); cd Values are significantly different (P < 0.01); ef Values are significantly different (P < 0.001); \( \chi^2 \) test.

In the fresh standard IVF group one twin pregnancy was reduced to singleton pregnancy.

*In the cryopreserved IVF, fresh IVF, cryopreserved ICSI and fresh ICSI groups, there were 122, 123, 75 and 88 birthweights available respectively.

*In the cryopreserved IVF, fresh IVF, cryopreserved ICSI and fresh ICSI groups, there were 52, 107, 24 and 80 birthweights available respectively.

Table IV. Liveborn children with major malformations until 1 year of age

<table>
<thead>
<tr>
<th>Major malformations</th>
<th>Cryopreserved standard IVF</th>
<th>Fresh standard IVF</th>
<th>Cryopreserved ICSI</th>
<th>Fresh ICSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. with malformation</td>
<td>4 (2.4)</td>
<td>10 (4.5)</td>
<td>3 (2.9)</td>
<td>4 (2.4)</td>
</tr>
<tr>
<td>Flexed thumbs, congenital naevus</td>
<td>Valvar aortic stenosis</td>
<td>Neurorhabdomatosis</td>
<td>Cleft lip and palate (n = 2)</td>
<td>Sacral lipoma</td>
</tr>
<tr>
<td>Ichthyosis</td>
<td>Amniotic band</td>
<td>Hypospadias (n = 2)</td>
<td>Urethral valve</td>
<td>Hydrocephaly</td>
</tr>
<tr>
<td>Ichthyosis</td>
<td></td>
<td>Strabismus (n = 3)</td>
<td>Pes equinovarous</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Truncus arteriosus</td>
<td>Vesico-ureteral reflux</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cryptorchidism</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ingual hernia, cryptorchidism</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Posterior urethral valve</td>
<td>(with left hydrourephosis)</td>
<td></td>
</tr>
</tbody>
</table>

There were no significant differences between the groups (Fisher’s exact probability test).

Values in parentheses are percentages.

parity and date of embryo transfer. In the frozen embryo groups after standard IVF and ICSI, the biochemical pregnancy rates were 18.8 and 16.4% respectively (not significantly different). In the fresh conventional IVF and ICSI groups, the biochemical pregnancy rates were 9.8 and 6.8% respectively (not significantly different). The frequencies of biochemical pregnancies in the frozen embryo groups were significantly higher than those in the fresh embryo groups (P < 0.01) (Table I).

Frydman reported biochemical pregnancy rates of 14% after transfer of cryopreserved embryos where the initial procedure was conventional IVF (Frydman et al., 1989). In that study, 1,2-propanediol was used as cryoprotectant and there was no control group. In a recent study from FIVNAT, lower biochemical pregnancy rates have been reported after transfer of frozen (9%) and fresh (8.5%) embryos where the original treatment was standard IVF (FIVNAT, 1994). Similar biochemical pregnancy rates have been obtained in the fresh and frozen transfer groups (6.9 versus 5.9%) after conventional IVF (Macas et al., 1998). However, after ICSI, the biochemical pregnancy rate in the frozen group (28.6%) was four times higher than in the fresh group (7.3%) (Macas et al., 1998). It is difficult to explain the high subclinical pregnancy rates in our study groups. The policy of selection of embryos for transfer and for freezing or the method of measuring HCG probably causes different results between centres. Although the quality of embryos transferred was not analysed in this study, a recent study from our centre has reported a biochemical pregnancy rate of 17.3% even after the transfer of fully intact embryos (Van den Abbeel et al., 1997). The impact of the freezing–thawing process on the development of embryonic disc and on implantation needs further investigation.

In the standard IVF groups the abortion rates were the same (13.1%) in the frozen and fresh groups. In the ICSI groups, the frequencies of abortions were 26.0 and 18.6% in the frozen and fresh embryo groups respectively (P = 0.13) (Table I). The miscarriage rate in the frozen embryo transfer group where the initial procedure was ICSI (26.0%), was significantly higher than that in the frozen embryo transfer group where the initial procedure was standard IVF (13.1%) (P = 0.001).

In the literature, data comparing pregnancy losses following standard IVF and ICSI are limited (Al Hasani et al., 1996; Hoover et al., 1997; Macas et al., 1998). In these studies, embryos were cryopreserved at the pronuclear stage. In our study, however, embryos were cryopreserved at an early cleavage stage. Similar pregnancy loss rates in the frozen
The frequencies of infants with very low birthweight and of intrauterine deaths were comparable for all the groups.

In conclusion, this study analysing obstetric outcome of pregnancies obtained after the transfer of cryopreserved and fresh embryos where the initial cycles were conventional IVF and ICSI showed significantly higher biochemical pregnancy rates in the frozen embryo groups than in the fresh embryo groups. The frequency of abortions in the ICSI frozen group was significantly higher than in the standard IVF frozen group. However, once the fetuses reached 20 weeks of gestation, no pathological features were found in the frozen groups in terms of prematurity, low-birthweight, very-low-birthweight or intrauterine death rates as compared with children born after the transfer of fresh embryos. The major malformation rates were similar in the groups and did not vary from the incidence in the general population (National Perinatal Statistics Unit and the Fertility Society of Australia, 1992). Long-term follow-up studies are needed in order to prove the safety of freezing–thawing processes in assisted reproduction techniques.

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A. Aytoz et al.


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