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# Obstetric and perinatal outcome of children conceived from cryopreserved embryos

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**The main purpose of this study was to evaluate the obstetric and neonatal outcome of children conceived from cryopreserved embryos. The medical records of 270 infants (163 singletons, 98 twins and nine triplets) were reviewed and compared with two control populations of children born after in-vitro fertilization (IVF) with fresh embryos and children born after spontaneous pregnancies. The controls were matched according to maternal age, parity, plurality and date of delivery. In the cryopreserved group the gestational age at delivery for singletons was 279 ± 13 days with birthweight 3476 ± 616 g; for twins gestational age was 257 ± 19 days with birthweight 2574 ± 560 g; for triplets gestational age was 228 ± 3 days with birthweight 1752 ± 183 g. The incidence of preterm birth (< 37 weeks gestation) was 5.6% for singletons, 44.9% for twins and 100% for triplets. Seven children had major malformations (2.7%) and perinatal mortality occurred in two children (8‰). Gestational age at delivery, birthweight, the incidence of malformations and the perinatal mortality were comparable with the two control groups both for singletons and twins. Significantly more singletons in the cryopreserved group were delivered by Caesarean section compared with the spontaneous group. The number of infants with low Apgar score (<7 at 5 min) and the number of infants admitted to neonatal intensive care units were similar in the cryopreserved and spontaneous groups. In conclusion, the cryopreservation process did not seem to adversely influence fetal development and no increased perinatal risk was found.**

*Key words*: cryopreserved embryos/in-vitro fertilization/malformation/obstetric outcome

## Introduction

The first successful pregnancy after transfer of cryopreserved human embryos was described in 1983 by Trounson *et al.* and the first birth after cryopreservation was reported in 1984 (Zeilmaker *et al.*). The technique has rapidly been incorporated into most in-vitro fertilization (IVF) programmes and a great number of children have been born.

The cryopreservation technique offers several advantages in an IVF programme. The current use of ovulation induction regimens yields a high number of oocytes which can be fertilized. Today, in order to reduce the number of multiple births, most IVF clinics in Scandinavia transfer maximally two pre-embryos. Supernumerary embryos can be frozen, stored and used in subsequent cycles without ovarian hyperstimulation and leading to an improvement in overall pregnancy rate (Bergh *et al.*, 1995a). Severe ovarian hyperstimulation syndrome (OHSS) during ovulation induction is a serious complication. Since it has been observed that pregnancy will aggravate OHSS and also prolong its duration, freezing of all embryos and delaying transfer by one or more cycles may decrease the rate and severity of OHSS (Navot *et al.*, 1992; Wada *et al.*, 1993) and still yield a reasonably high delivery rate (Bergh *et al.*, 1995b). The cryopreservation will also increase the possibility of having more than one child after IVF without having additional ovarian stimulation and oocyte retrieval.

The pregnancy rate after cryopreservation–thawing depends on the freezing programme used, the cleavage stages of the embryos at freezing, the quality of the frozen embryos and the survival rate of the blastomeres after thawing (Lasalle *et al.*, 1985; Hartshorne *et al.*, 1990). Synchronization between the development of the endometrium and embryo replacement is also very important (Cohen *et al.*, 1988) as is the number of frozen–thawed embryos transferred. Pregnancy and birth rates per transfer have for recent years been reported to be 25–30% and 15–20%, respectively, for embryos cryopreserved at the pronuclear stage or at early cleavage stages (Testart *et al.*, 1987; Sathanandan *et al.*, 1992).

Cryopreservation and thawing involve important cellular changes but until recently there were no reports of adverse effects in mammalian pre-embryos. Previous studies in animals suggest that freezing is not mutagenic, and the background radiation of frozen pre-embryos does not produce abnormal offspring (Whittingham *et al.*, 1977). A recent French investigation of adult mice obtained by transfer of cryopreserved pre-embryos (Dulioust *et al.*, 1995) indicated certain differences in morphological and behavioural features. The authors concluded that embryo cryopreservation, without being severely detrimental, might have certain adverse effects on the offspring.

Previous studies on the obstetric outcome of IVF pregnancies have reported an increase in adverse perinatal outcome with high frequencies of preterm birth, low birthweight, small for gestational age offspring and perinatal deaths (Wennerholm *et al.*, 1991; Tan *et al.*, 1992; Tanbo *et al.*, 1995; Gissler *et al.*, 1995). This increase is largely related to the high rate of multiple pregnancies, which account for 20–30% of all pregnancies in most IVF programmes compared with 1–2%

in the general population. The major risk in multiple pregnancies is the increased likelihood of preterm delivery. Twin pregnancies represent the majority of multiple pregnancies observed after IVF. The frequency of preterm birth in twin pregnancies varies between 40 and 50%. In addition, low birthweight and intrauterine growth retardation are more frequent, resulting in a higher perinatal mortality rate. Some recent publications have stressed the fact that singleton pregnancies after IVF have a more adverse perinatal outcome than spontaneous pregnancies (Tanbo *et al.*, 1995; Olivennes *et al.*, 1993). The underlying factors are not fully understood but the infertility status and/or ovarian stimulation may play a role.

Many studies have shown that the incidence of major congenital malformations in standard IVF pregnancies is comparable with population-based estimates (Rizk *et al.*, 1991; Medical Research International, Society for Assisted Reproductive Technology and The American Fertility Society, 1992; Rufat *et al.*, 1994; FIVNAT, 1995).

Today there are limited studies available on the obstetric and paediatric outcome of pregnancies after cryopreservation (Frydman *et al.*, 1989; Wada *et al.*, 1994; Sutcliffe *et al.*, 1995a,b; Heijnsbroek *et al.*, 1995; Olivennes *et al.*, 1996).

The aim of the present study was to evaluate the safety of the cryopreservation technique and to follow up a complete cohort of children conceived following the replacement of cryopreserved embryos and compare their obstetric and neonatal outcome both with children conceived after IVF with fresh embryos and with children conceived spontaneously.

## Material and methods

The study population included the total cohort of births between June 1990 and July 1995 after IVF with cryopreserved–thawed embryos at the Department of Obstetrics and Gynecology, Sahlgrenska University Hospital and the Fertility Center Scandinavia, Carlanderska Hospital, Göteborg, Sweden. All live-born and all infants stillborn after more than 28 weeks gestation were included. Two hundred and seventy children were born after 215 pregnancies: 163 singletons, 98 twins and nine triplets. The frequency of multiple birth was 24.2%.

After parental consent was obtained the medical records were reviewed. The parents of three singletons declined to participate in the study but declared that the pregnancies and deliveries had been uncomplicated with normal gestational lengths and birthweights of the infants. These three children were excluded from the study. Thus the study population consisted of 212 deliveries and 267 infants.

Data on the medical and obstetrical history of the mothers was obtained from medical records at the IVF clinics and data on pregnancy complications, obstetric and perinatal data were retrieved from the delivery records from the obstetric departments where the patients were delivered.

As controls, two different groups were chosen. One group consisted of births after IVF with fresh embryos, and was selected from the IVF register at the two IVF clinics. The other control group consisted of spontaneous pregnancies identified from the delivery books at three hospitals, Sahlgrenska University Hospital and the East Hospital in Göteborg and from one smaller hospital on the west coast of Sweden, Uddevalla Hospital. The groups were matched according to maternal age, ±5 years, parity, plurality and date of delivery.

For triplets a comparison was made with spontaneous triplets from the Swedish Medical Birth Register (SMBR) between 1990 and 1995, as no matching spontaneous control group could be identified at the three hospitals. Since all mothers of the triplets in the cryopreserved and standard IVF groups were nulliparous, only triplets born by nulliparous women in the SMBR were included. As controls, 47 sets of spontaneous triplets (141 children) were identified from the SMBR. The triplets are excluded for group comparison and discussed separately.

Ovarian hyperstimulation was used before oocyte collection. Standard procedures for oocyte retrieval, embryo culture and transfer were described earlier in detail (Wikland *et al.*, 1983; Wikland *et al.*, 1994). All embryos were frozen at early cleavage stages using propanediol as cryoprotectant (Lasalle *et al.*, 1985). The duration of embryo cryostorage varied between 1 month and 2 years.

The majority of cryopreserved embryos were supernumerary during a previous IVF attempt. In a few cases embryos were frozen because of the risk of ovarian hyperstimulation. The frozen–thawed embryos were replaced in natural cycles in 82% and in stimulated cycles in 18% of cases. The fresh embryos were all replaced in stimulated cycles. The mean number of embryos replaced was 2.6 both for the cryopreserved and for the fresh embryo group.

For IVF pregnancies gestational age was calculated from the day of embryo replacement which was defined as day 16 of the cycle. All spontaneous pregnancies had an early ultrasound between 16 and 18 weeks gestation for assessment of gestational age.

Pregnancy-induced hypertension was defined as follows: transient hypertension-repeated blood pressure (BP) measurements of ⩾140/90 mmHg not associated with proteinuria after 20 weeks of gestation; mild pre-eclampsia: repeated BP measurements of ⩾140/90 mmHg with proteinuria of ⩾0.3 g/day after 20 weeks gestation; severe pre-eclampsia: repeated BP measurements of ⩾160/110 mmHg and proteinuria of ⩾4 g/day after 20 weeks gestation.

Preterm premature rupture of the membranes (PPROM) was defined as rupture of the membranes without contractions before 37 weeks gestation. Major malformation was defined as a condition requiring surgical correction or causing functional impairment.

Ethical approval was obtained at the outset of the study.

### *Statistics*

Paired comparisons were made between the cryopreserved and the fresh embryo IVF groups, between the cryopreserved and the spontaneous groups and between the fresh embryo IVF and the spontaneous groups for all parameters listed in the tables.

Paired *t*-test was used for comparisons between the groups. If the correlation coefficient between the values of the two individuals in the matched pair was low, an unpaired *t*-test or Mann–Whitney *U*-test was used according to the instructions in the program (In Stat 2.01 GraphPad Software, San Diego, California, USA). For comparisons of proportions Fisher's exact test or McNemar's test for paired observations was used. Two-tailed tests were used. $P < 0.05$ was considered statistically significant. Only statistically significant values are given in the tables.

With a sample size of 160 women with singleton pregnancies in each group it was possible to detect a difference in birthweight of ⩾180 g, assuming an $\alpha$ of 0.05, two-tailed test, and a $\beta$ of 0.20 (80% power).

## Results

In our freezing programme the survival rate (defined as ⩾50% of the blastomeres viable after thawing) was 78%. The pregnancy rate per embryo replacement was 21%. Spontaneous abortion occurred in 23% and ectopic pregnancy in 4% of

**Table I.** Maternal characteristics

| | Cryopreserved ($n$ = 209) | Standard IVF ($n$ = 209) | Spontaneous ($n$ = 209) |
|---|---|---|---|
| Age (years; mean ± SD) | 33.6 ± 3.3 | 34.0 ± 3.1[a] | 33.0 ± 3.8[b] |
| Primigravida (%) | 34[c] | 37 | 45[d] |
| Nulliparous (%) | 69 | 69 | 69 |
| Smokers (%) | 18 | 17 | 20 |
| Infertility duration (years; mean) | 6.5 | 6.9 | 0 |
| range | (1–20) | (1–17) | |

[a] versus [b]: $P$ = 0.002, Mann–Whitney test.
[c] versus [d]: $P$ = 0.030, McNemar's test.

cases. Seventy-three per cent of the pregnancies resulted in a birth of at least one infant constituting the study population. Maternal characteristics of the study population and the control groups are shown in Table I.

The mean age of the mothers was 33.6, 34.0 and 33.0 years in the study group, the standard IVF group and the spontaneous group respectively. The age difference between the standard IVF group and the spontaneous group was statistically significant ($P$ = 0.002, Mann–Whitney test). Sixty-nine per cent of the women in all groups were nulliparous. Among the parous women, 45% in the cryopreserved group and 34% in the standard IVF group had at least one previous IVF child. Thirty-four per cent of the women in the cryopreserved group, 37% in the standard IVF group and 45% in the spontaneous group were primigravida. The difference between the cryopreserved and the spontaneous group was statistically significant ($P$ = 0.030, McNemar's test). A higher rate of ectopic pregnancies in the IVF groups was the main reason for the difference. In all groups 23% were twin pregnancies.

The smoking habits were similar for the three groups; the frequency of daily smokers was 18%, 17% and 20% in the cryopreserved, the standard IVF and the spontaneous groups respectively. A tubal factor was the main reason for infertility in both the cryopreserved and standard IVF group, 71% and 75% respectively. Other reasons for infertility were endometriosis, unexplained infertility, an ovulatory disorder or a male factor. The mean duration of infertility was 6.5 years (range 1–20) for the cryopreserved group and 6.7 years (range 1–17) for the standard IVF group.

The complications during pregnancy for the three groups are shown in Table II. The total number of hypertensive complications was similar in the groups. The standard IVF group showed numerically more pregnancies with severe pre-eclampsia, which was not statistically significant.

Only women in whom preterm labour and haemorrhage necessitated hospitalization are included in Table II. Preterm labour and PPROM occurred more frequently in the IVF groups than in the spontaneous group. The difference was significant for PPROM in the cryopreserved and the standard IVF group versus the spontaneous group. Haemorrhage occurred more often in the IVF group compared to the cryopreserved group. Half of the haemorrhages occurred in the first trimester.

The mean gestational age for singletons was similar for all

**Table II.** Complications during pregnancy for cryopreserved, standard in-vitro fertilization (IVF) and spontaneous groups

| | Cryopreserved ($n$ = 209) | | Standard IVF ($n$ = 209) | | Spontaneous ($n$ = 209) | |
|---|---|---|---|---|---|---|
| | $n$ | % | $n$ | % | $n$ | % |
| Hypertension | 15 | (7.2) | 16 | (7.7) | 13 | (6.2) |
| Transient hypertension | 6 | (2.9) | 2 | (1.0) | 5 | (2.4) |
| Mild pre-eclampsia | 5 | (2.4) | 7 | (3.3) | 6 | (2.9) |
| Severe pre-eclampsia | 4 | (1.9) | 7 | (3.3) | 2 | (1.0) |
| PPROM | 18[a] | (8.6) | 19[b] | (9.1) | 7[c] | (3.3) |
| Haemorrhage | 8[d] | (3.8) | 21[e] | (10.0) | 10 | (4.8) |
| Preterm labour | 25 | (12.0) | 21 | (10.0) | 18 | (8.6) |
| Gestational diabetes | 5 | (2.4) | 3 | (1.4) | 0 | (0) |

[a] versus [c]: $P$ = 0.037; [b] versus [c]: $P$ = 0.019; [d] versus [e]: $P$ = 0.020 (McNemar's test).
PPROM = preterm premature rupture of the membranes.

**Table III.** Gestational age of pregnancies following cryopreservation, standard IVF and spontaneous pregnancies

| | Cryopreserved | | Standard IVF | | Spontaneous | |
|---|---|---|---|---|---|---|
| | $n$ | % | $n$ | % | $n$ | % |
| Singleton: | ($n$ = 160) | | ($n$ = 160) | | ($n$ = 160) | |
| >37 weeks | 151 | (94.4) | 142 | (88.7) | 151 | (94.4) |
| 32–36 weeks | 7 | (4.3) | 18 | (11.3) | 8 | (5.0) |
| 28–31 weeks | 2 | (1.3) | 0 | (0) | 1 | (0.6) |
| <28 | 0 | (0) | 0 | (0) | 0 | (0) |
| Days (mean ± SD) | 279 ± 13 | | 277 ± 14 | | 280 ± 12 | |
| Twin: | ($n$ = 49) | | ($n$ = 49) | | ($n$ = 49) | |
| >37 weeks | 27 | (55.1) | 29 | (59.2) | 34 | (69.4) |
| 32–36 weeks | 18 | (36.7) | 12 | (24.5) | 11 | (22.4) |
| 28–31 weeks | 4 | (8.2) | 6 | (12.2) | 3 | (6.1) |
| <28 weeks | 0 | (0) | 2 | (4.1) | 1 | (2.1) |
| Days (mean ± SD) | 257 ± 19 | | 254 ± 26 | | 260 ± 21 | |

the groups (Table III). Although the number of singleton preterm deliveries (<37 weeks gestation) was higher in the IVF group, this difference was not statistically significant and no delivery occurred very preterm (<32 weeks gestation) in this group. The mean gestational age for twin pregnancies was also similar.

The distribution in birthweight is presented in Table IV. The mean birthweights for singletons were comparable in all three groups. Standard IVF twins had a significantly lower birthweight compared to spontaneous twins. No other significant differences were found.

The mode of delivery is shown in Table V. For singletons, the cryopreserved group showed the highest frequency of Caesarean sections (CS), 26.3%, which was significantly higher than in the spontaneous group. More than half of the operative procedures were classified as emergency CS. For twins, the differences between the groups were less and 40.8% of twins in the cryopreserved group were delivered with CS.

No difference was seen in Apgar score at 5 min. The mean Apgar score was 9.7 ± 0.7, 9.6 ± 1.2 and 9.7 ± 1.1 for the cryopreserved, standard IVF and spontaneous groups

**Table IV.** Birthweight (g) for pregnancies following cryopreservation, standard IVF and spontaneous pregnancies

| | Cryopreserved | | Standard IVF | | Spontaneous | |
|---|---|---|---|---|---|---|
| | *n* | % | *n* | % | *n* | % |
| Singleton: | (*n* = 160) | | (*n* = 160) | | (*n* = 160) | |
| >2500 | 152 | (95.0) | 148 | (92.5) | 154 | (96.3) |
| 1000–2499 | 7 | (4.4) | 12 | (7.5) | 6 | (3.7) |
| <1000 | 1 | (0.6) | 0 | (0) | 0 | (0) |
| SGA<–22% | 7 | (4.4) | 9 | (5.6) | 6 | (3.7) |
| Mean birthweight ± SD | 3476 ± 616 | | 3407 ± 637 | | 3459 ± 523 | |
| Twin: | (*n* = 98) | | (*n* = 98) | | (*n* = 98) | |
| >2500 | 58 | (59.2) | 56 | (57.1) | 67 | (68.4) |
| 1000–2499 | 40 | (40.8) | 38 | (38.8) | 28 | (28.6) |
| <1000 | 0 | (0) | 4 | (4.1) | 3 | (3.1) |
| SGA<–22% | 21 | (21.4) | 20 | (20.4) | 21 | (21.4) |
| Mean birthweight ± SD | 2574 ± 560 | | 2441 ± 666[a] | | 2673 ± 647[b] | |

[a] versus [b]: $P = 0.014$, unpaired *t*-test.
SGA = small for gestational age (< –2SD) according to the Swedish reference values for birth weight (Marsal *et al*., 1996).

**Table V.** Mode of delivery for pregnancies following cryopreservation, standard IVF and spontaneous pregnancies

| | Cryopreserved | | Standard IVF | | Spontaneous | |
|---|---|---|---|---|---|---|
| | *n* | % | *n* | % | *n* | % |
| Singleton: | (*n* = 160) | | (*n* = 160) | | (*n* = 160) | |
| Vaginal spontaneous | 98 | (61.3) | 109 | (68.1) | 118 | (73.8) |
| Vaginal, operative | 20 | (12.5) | 15 | (9.4) | 16 | (10.0) |
| Caesarean | 42[a] | (26.3) | 36 | (22.5) | 26[b] | (16.3) |
| Elective | 19 | (11.9) | 17 | (10.6) | 7 | (4.4) |
| Emergency | 23 | (14.4) | 19 | (11.9) | 19 | (11.9) |
| Twin: | (*n* = 49) | | (*n* = 49) | | (*n* = 49) | |
| Vaginal spontaneous | 27 | (55.1) | 28 | (57.1) | 24 | (48.9) |
| Vaginal, operative | 2 | (4.1) | 1 | (2.0) | 2 | (4.1) |
| Caesarean | 20 | (40.8) | 20 | (40.8) | 23 | (46.9) |
| Elective | 8 | (16.3) | 6 | (12.2) | 7 | (14.3) |
| Emergency | 12 | (24.5) | 14 | (28.6) | 16 | (32.6) |

[a] versus [b]: $P = 0.038$ (McNemar's test).

respectively. Low Apgar score (<7 at 5 min) occurred in two infants in the cryopreserved group, in 10 in the IVF group and six in the spontaneous group. Only one infant in the IVF group and two in the spontaneous group had abnormal Apgar score (<7) at 10 min.

Perinatal death occurred in two infants in the cryopreserved group; one infant with trisomy 13 died soon after delivery and one twin died *in utero* at 35 weeks gestation without any obvious medical reason. In the IVF group, one twin born at 29 weeks gestation died after delivery due to severe intrauterine growth retardation and prematurity. The perinatal mortality in the spontaneous group consisted of one baby with lethal achondroplasia which died of respiratory insufficiency and one twin with fulminating neonatal sepsis born at 32 weeks gestation. The perinatal mortality was 8/1000, 4/1000 and 8/1000 for the cryopreserved, the standard IVF and the spontaneous groups respectively.

Major malformations are shown in Table VI. In the cryopreserved group, seven infants had major malformations; one

**Table VI.** Children with major malformation

| | Cryopreserved (*n* = 258) | Standard IVF (*n* = 258) | Spontaneous (*n* = 258) |
|---|---|---|---|
| Major malformation: | *n* = 7 (2.7%) | *n* = 8 (3.1%) | *n* = 8 (3.1%) |
| | Trisomy 13 | Trisomy 21 | Achondroplasia |
| | Trisomy 21 | Translocation[a] | VSD |
| | Trisomy 21 | VSD | VSD |
| | VSD | VSD | ASD |
| | ASD | ASD | ASD |
| | Cleft palate+ | ASD | ASD |
| | hypospadia | Hypospadia | Aplasia of one ear |
| | Limb malformation | Omfalocele | Omfalocele |

[a]Unbalanced translocation; VSD = ventricular septal defect; ASD = atrial septal defect.

lethal trisomy 13, two with trisomy 21, one with ventricular septal defect with a small shunt, one with atrial septal defect, one with cleft palate and hypospadia and one with only one hand. None of the infants with cardiac malformations needed surgery. No therapeutic abortion because of malformation or chromosomal abnormality was performed in the cryopreserved group. Eight infants in both the standard IVF and the spontaneous groups had major malformations. Minor malformation consisting of subluxation of the hips, positional talipes, haemangiomas, congenital naevi, pre-auricular tags and undescended testes occurred in 12 babies in the cryopreserved group, in six babies in the standard IVF group and in 11 babies in the spontaneous group. The differences were not statistically significant.

For singletons, the mean stay at the neonatal intensive care units (NICU) was 2.0 (0–82), 3.1 (0–60) and 1.2 (0–43) days for the cryopreserved, the standard IVF and the spontaneous groups respectively. The mean stay at NICU differed significantly ($P = 0.011$, paired *t*-test) between twins in the cryopreserved group (9.0 days, range 0–50) versus twins in the standard IVF group (14.7 days range 0–78). Spontaneous twins had a mean stay at NICU of 8.1 (0–120) days.

The proportion of boys:girls was 123:135, 136:122 and 131:127 in the cryopreserved, the standard IVF and the spontaneous groups respectively. The differences were not statistically significant.

Data for the triplets are shown in Table VII. The mean maternal age was 32.0 years, 34.0 years and 30.2 years for the cryopreserved, the standard IVF and the spontaneous triplet groups from SMBR respectively. All women were nulliparous. Gestational age and birthweight were comparable for the cryopreserved and spontaneous groups. No major malformation or perinatal death occurred in the cryopreserved group. In the standard IVF group, one child had a cleft palate and three children from two pregnancies died from extreme prematurity. In the SMBR group the perinatal mortality was 64/1000 (nine children).

## Discussion

This study was designed to follow up the total cohort of children conceived from cryopreserved embryos from the first

**Table VII.** Outcome for triplets following cryopreservation, standard IVF and spontaneous pregnancies from the Swedish Medical Birth Register (SMBR)

| | Cryopreserved ($n$ = 9) | Standard IVF ($n$ = 9) | SMBR ($n$ = 141) |
|---|---|---|---|
| Maternal age (years, mean) | 32.0 | 34.0 | 30.2 |
| (range) | (28.0–36.0) | (34.0–34.0) | (18–38) |
| Gestational age (weeks, mean) | 32.6 | 29.6 | 31.4 |
| (range) | (32.3–33.0) | (26.0–32.1) | (23–37) |
| Birthweight (g, mean) | 1752[a] | 1253[b] | 1599 |
| (range) | (1515–2015) | (665–1810) | (400–3050) |
| Major malformation | 0 | 1 | 4 |
| Perinatal mortality [$n$ (%)] | 0 (0) | 3 (33) | 9 (6.4) |

[a] versus [b]: $P = 0.014$ (Mann–Whitney test).

delivery, 1990 to June 1995. Our purpose was to evaluate both the possible influence of the cryopreservation technique and the role of IVF on the obstetric and perinatal outcome. Children conceived following standard IVF and children conceived spontaneously were chosen as controls. Infertile couples differ from the general population in several aspects; age, parity, previous obstetric history and socio-economic factors. It is also well known that infertility treatment such as ovulation induction and IVF will yield a much higher frequency of multiple pregnancies.

In this study the obstetric and perinatal data are presented. All children were located. The medical records from three singletons could not be obtained since the parents declined to participate. The parents agreed, however, to a telephone interview and reported that the pregnancies and deliveries had been uncomplicated and that the children were in good health. In the present study the controls were matched with respect to maternal age, parity, multiplicity and to some extent the locality (city versus provincial town). Age, parity and especially multiplicity contribute to preterm birth, low birthweight and an increased perinatal mortality.

The incidences of preterm birth, low birthweight and small for gestational age offspring in the cryopreserved group were comparable with those in the control groups and also with that in the general Swedish population for singletons, twins and triplets (SMBR). The mean gestational age was similar for singletons and twins, both in the study and in the control groups.

In an earlier retrospective study (Wada *et al.*, 1994) the outcome of 283 births conceived following cryopreservation was compared with 961 births from fresh embryos with respect to birth characteristics and perinatal mortality. The groups were comparable with respect to maternal age but parity was not mentioned. The mean gestational age and the mean birthweight of singleton, twin and triplet births did not differ significantly between the groups. The incidence of low birthweight and preterm delivery was lower in the cryopreserved twin group than in the fresh embryo twin group. Our results regarding birthweight were better for infants from cryopreserved embryos than from fresh embryos, although the difference was not statistically significant. Most fresh embryos are replaced in stimulated cycles and frozen–thawed embryos are replaced in natural cycles, 82% in our study and 49% in Wada's study. A possible adverse affect of hyperstimulation, yielding for example elevated concentrations of relaxin with increased risk of preterm birth, has been discussed (Bell *et al.*, 1987; Platek *et al.*, 1997).

In a French study of children conceived from cryopreserved embryos (Olivennes *et al.*, 1996) the incidence of preterm birth was 14.7% for singletons. However, half of these preterm births (6/11) occurred during the 36th week of gestation.

This study confirms that the frequency of CS is higher among IVF singletons than in the general Swedish population, which is 11% (Wennerholm *et al.*, 1991; Tanbo *et al.*, 1995; Wennerholm *et al.*, 1996). Spontaneous singletons had a higher frequency of CS than in the general population, indicating that maternal age and parity influences the rate of CS. In the absence of obvious differences in pregnancy complications, the different rates of CS in the two IVF singleton groups and the spontaneous singleton group suggest that psychological factors may be involved.

Standard IVF twins spent significantly more days in neonatal care units than twins in the cryopreserved group due mainly to a lower mean birthweight and associated problems. The perinatal mortality was comparable for the groups, although the number of children in each group was too small to allow an analysis of mortality. The perinatal mortality was lower than the mortality rate reported in our first study on standard IVF pregnancies (Wennerholm *et al.*, 1991).

Previous studies of children from cryopreserved embryos have not shown any increased risk of major or minor malformations (Sutcliffe *et al.*, 1995b). In most studies, embryos were cryopreserved at the early cleavage stage using propanediol as cryoprotectant or at the blastocyst stage using glycerol as cryoprotectant (Frydman *et al.*, 1989; Wada *et al.*, 1994; Sutcliffe *et al.*, 1995b). In the study presented by Wada *et al.* (1994) a major malformation was reported in 1% of the children in the cryopreserved group and 3% in the standard IVF group. According to the authors a possible explanation for the reduction in the incidence of congenital malformations could be related to the freezing–thawing technique. Since some embryos are lost during the freezing–thawing procedure the technique may eliminate defective embryos. An intriguing speculation from the results in our study is that more male than female embryos might be lost during the freezing–thawing procedure, resulting in predominance of female infants born.

In our study the number of major malformations was similar in the groups and the incidence of 2.7% of major malformation in the cryopreserved group did not vary from the incidence in the general Swedish population. However, in all these studies the number of patients is small and much larger numbers are needed to evaluate the risk of malformations.

Triplets constitute an obstetrically high risk group, mainly due to the increased risk of preterm birth. Triplets from cryopreserved embryos did not show any difference in gestational age or birthweight compared with spontaneous triplets but did of course differ from singletons and twins. No perinatal mortality occurred in the cryopreserved group, but three infants died from extreme prematurity in the standard IVF group. Today, most IVF clinics in Sweden are aware of the high risk

with triplets or higher order births and thus transfer only two embryos. Two of the three triplet gestations in the cryopreserved group were delivered in 1991. The third triplet occurred after the replacement of only two embryos. The corresponding standard IVF triplet pregnancy also occurred after replacement of two embryos. No embryo reduction was performed in the cryopreserved group.

In this study the total multiple birth frequency was 24.2%; 22.8% were twin pregnancies and 1.4% were triplets. Over the period of study the incidence of triplet pregnancies was reduced but the incidence of twin pregnancies has essentially remained unchanged. Today there is much evidence of an increased risk also for twin pregnancies compared with singletons. Although the majority of twins and triplets are free of major handicap they have increased incidences of developmental disability, cerebral palsy, mental retardation, sensory impairments, language delays, learning disability and attention and behavioural problems compared with singletons (Allen, 1995; Tanbo *et al*., 1996). A future concern is how to further reduce the number of multiple pregnancies without decreasing the pregnancy rate.

In conclusion, this study of the total cohort of children conceived from cryopreserved embryos did not show any major pathological features compared with children conceived from fresh embryos or with children conceived spontaneously. Neither the cryopreservation process nor the IVF technique seemed to adversely influence fetal or infant development during pregnancy and the early neonatal period. In an ongoing study the same cohort of children is being followed up until 18 months of age to allow further evaluation of growth, development and morbidity.

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