This paper provides an overview of the effects of cryopreservation on obstetric outcome and child development. Cryopreservation of embryos has no apparent negative impact on perinatal outcome and early infant development. The available data do not indicate an elevated congenital malformation rate. Intracytoplasmic sperm injection and cryopreservation is comparable with conventional in-vitro fertilization and cryopreservation. It remains unclear if embryo freezing poses long-term risks to children so conceived. There are several potential advantages of oocyte freezing which, however, is still a research procedure. Key words: children/cryopreservation/embryos/obstetric outcome/oocytes

Introduction
In the early 1970s, Whittingham and colleagues demonstrated that mammalian embryos could survive freezing to temperatures as low as −269°C and become normal fertile offspring after thawing and transfer to the uterus (Whittingham et al., 1972). The first human pregnancy following cryopreservation of an 8-cell embryo was reported in 1983 (Trounson and Mohr, 1983). This pregnancy ended in a stillbirth after 24 weeks of gestation. Zeilmaker and co-workers described the first live birth after embryo cryopreservation in 1984 when a set of twins was born (Zeilmaker et al., 1984) and more births were soon reported from England and Australia. Since then, embryo cryopreservation has been practised extensively and is now routine in most IVF programmes. The latest world report showed that >80 000 embryos were frozen and >4000 pregnancies were conceived with this technique during 1995 (De Mouzon and Lancaster, 1997).

Embryo cryopreservation provides several clinical advantages, e.g. (i) reducing the risk of multiple pregnancies by limiting the number of embryos replaced without destroying excess embryos, (ii) offering the possibility for supernumerary embryos to be frozen, stored and used in subsequent cycles without ovarian stimulation, leading to an improved overall pregnancy rate (Bergh and Hamberger, 1995), (iii) decreasing the potential risk of ovarian hyperstimulation syndrome (OHSS), which is aggravated by pregnancy (Wada et al., 1993), and (iv) creating the possibility of having more than one child after IVF without undergoing additional cycles of ovarian stimulation and oocyte retrieval.

Several embryo-freezing methods have been developed using glycerol, dimethylsulphoxide (DMSO) or 1,2 propanediol (PROH) as cryoprotectants. Propanediol was introduced as a cryoprotectant for pronucleate and early cleavage stages, whereas the other agents are mainly used for later cleavage stages.

The pregnancy rate after cryopreservation and thawing depends on the freezing programme used, the cleavage stages of the embryos when frozen, the quality of the frozen embryos and the survival rate of the blastomeres after thawing (Lassalle...
Children born after cryopreservation

The synchronization of endometrial development and embryo replacement is also important (Cohen et al., 1988), as is the number of frozen-thawed embryos transferred. Some studies have reported rates of pregnancy and childbirth per transfer at 25–30% and 15–20%, respectively, for embryos cryopreserved at the pronuclear or early cleavage stages (Testart et al., 1987; Sathanandan et al., 1992). The higher pregnancy rate after transfer of embryos when they are cryopreserved at the pronuclear stage rather than cleavage stage may be only a consequence of embryo selection since only those cleaving following thawing are normally transferred. However, one disadvantage with cryopreservation of embryos at the pronuclear stage is that fewer embryos are then available from which to select for fresh embryo transfer.

Freezing of human embryos raises at least two concerns in addition to those raised by IVF in general. The first is a possible increased risk to the embryo as a result of the freezing-thawing process itself. The safety of freezing human embryos was largely unquestioned until the publication of a recent report of subtle morphological, developmental and behavioural changes in mice born after the transfer of frozen-thawed embryos (Dulioust et al., 1995). This work was subjected to serious methodological criticism (Testart, 1998); however, additional authors have expressed reservations about the potential long-term risks to children born after cryopreservation as embryos (Beale, 1996; Winston, 1996). The efficacy and safety of extended cryopreservation has not been conclusively demonstrated in the literature. There are case reports of successful outcomes after embryo cryopreservation for 7.5 and 8 years respectively (Go et al., 1998; Ben-Ozer and Ver mesh, 1999) but also reports of decreased embryo survival and a trend towards pregnancy rate reduction with extended cryopreservation (Testart et al., 1987; Lin et al., 1995).

The second concern raised by freezing is that it offers not only advantages but also introduces options that may or may not be harmful, since it provides a reversible cessation of development. The technical possibilities generated by freezing of human embryos raise major legal, ethical and social issues such as authority over stored embryos, posthumous use, inheritance rights and family relations after embryo donation.

This article reviews the literature concerning the follow-up of pregnancies and children conceived from frozen embryos and oocytes with special reference to perinatal outcome, malformations, growth and development.

Definitions

Preterm birth: birth occurring before completion of the 37th week of gestation. High birthweight: >4500 g; low birthweight: <2500 g. Perinatal mortality: the sum of stillborns and deaths of liveborns during the first 7 days of life per 1000 infants born.

Perinatal outcome in children born after cryopreservation as embryos

Children born after cryopreservation as embryos are prone to risks that are well known in connection with IVF pregnancies in general. The perinatal outcome of pregnancies following IVF differs from that following spontaneous conception, mainly due to the high incidence of multiple pregnancies. Most countries report an incidence of multiple births of between 20 and 30% following assisted conception, resulting in 30–45% of all IVF babies being born as multiple birth babies, compared with 2–3% in the general population (De Mouzon and Lancaster, 1997). The major inherent risk in multiple pregnancy is the increased likelihood of preterm delivery with consequent low birthweight and a higher perinatal mortality and morbidity.

A recent retrospective registry study of all IVF births in Sweden between 1982 and 1995 showed that an IVF baby has a 20-fold increased risk of being born in a multiple birth, compared with the general population (Bergh et al., 1999). In this study, 23.9% of the IVF deliveries were twins and 2.8% were triplets. The corresponding figures in the general population were 1.15 and 0.02% respectively. Complications in pregnancy and neonatally observed after IVF were almost entirely due to multiple pregnancies, the rates being as follows: preterm birth (deliveries) twins: 47.3%, singleton 11.2%; low birthweight (children): twins 43.8%, singletons 8.9%, respectively. However, IVF singletons also ran a significantly increased...
risk of preterm birth and low birthweight compared with the general population, although part of this increased risk might be explained by different maternal characteristics such as higher age, lower parity and the infertility per se in the IVF group.

Hospital-derived data

The first report on obstetric outcome after transfer of cryopreserved human embryos was authored by Frydman and co-workers (Frydman et al., 1989). Fifty consecutive pregnancies were analysed and 31 babies were born. One pregnancy was terminated at 22 weeks of gestation because of a major limb malformation. One singleton child was born preterm (preterm birth in singleton gestations: 4%). There was a high incidence of breech presentation at term in singleton pregnancies (12%).

Another small, descriptive study from one clinic in the Netherlands analysed the obstetric outcome of the first 30 women who conceived after transfer of cryopreserved embryos (Heijnsbrook et al., 1995). A low prematurity rate (4%) and a high breech presentation rate (14%) was found in singleton pregnancies in this study as well. The birthweight tended to be above average, with 45% of singletons weighing more than the 75th percentile of weight for gestation. Minor malformations were found in two infants, consisting of clubfeet and an undescended testicle.

A detailed retrospective study from the Bourn Hall Clinic in the UK has been published (Wada et al., 1994). All consecutive births following embryo cryopreservation between 1985 and 1991, resulting in 283 babies, were analysed and compared with all babies born after conventional IVF with fresh embryos (n = 961). No difference was found in maternal age or multiple birth rate between the two groups. The outcome for singletons was similar but significantly fewer twins in the cryopreserved group were delivered preterm and had a low birthweight compared with the conventional IVF group.

Wennerholm et al. evaluated the obstetric outcome in a complete cohort of children born after cryopreservation at two Swedish IVF clinics (Wennerholm et al., 1997). In that study, 258 infants were matched for maternal age, parity, single or twin pregnancy and date of delivery with an equal number of infants born after conventional IVF and spontaneously conceived infants. The obstetric outcome is summarized in Table I. Only one significant difference emerged: twins in the conventional IVF group had a significantly lower mean birthweight compared with spontaneously conceived twins.

National registry data

The French National Registry (FIVNAT, 1996) is the largest existing IVF pregnancy database. However, it covers ~80% of recovery attempts but only ~55% of pregnancy outcomes.

More than 15 000 embryo transfers following freezing and nearly 100 000 transfers of fresh embryos were compared (FIVNAT, 1996). Significant differences were found for several parameters. First, the pregnancy rate was lower after transfer of one frozen embryo. However, from the time of implantation, pregnancies initiated with frozen embryos were no longer at a disadvantage compared with those resulting from fresh embryos. The frequency of spontaneous abortions was similar. Singleton pregnancies after cryopreservation were subject to fewer medical problems such as hospitalization, pre-eclampsia and preterm labour.

Preterm birth in singletons occurred in 8.2% and 9.1% in the cryopreserved and fresh embryo transfer groups, respectively (not significant). Singleton babies born after cryopreservation had a higher mean birthweight, and fewer infants were small for gestational age. One simple explanation could be the fact that more women were nulliparous in the fresh embryo transfer group.

The outcome of different methods was also analysed in the Swedish IVF birth cohort study (Bergh et al., 1999). Although they only represented a small proportion of all IVF singleton babies (8.2%), the 270 singletons from the cryopreserved group had a more favourable outcome with lower odds ratios (OR) for preterm birth and low birthweight (OR 0.59; 95% CI, 0.36–0.97, and OR 0.45; 95% CI, 0.25–0.82, respectively), compared with fresh-cycle IVF, after stratification for year of birth, maternal age and parity.

Malformations

Table II summarizes the outcome concerning malformations, according to the World Report (De
Children born after cryopreservation

Table I. Perinatal characteristics of children born after cryopreservation as embryos, in comparison with children born after conventional IVF and spontaneous conception (Wennerholm et al., 1997)

<table>
<thead>
<tr>
<th></th>
<th>Cryopreserved</th>
<th>Conventional IVF</th>
<th>Spontaneous</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Singletons (n)</td>
<td>160</td>
<td>160</td>
<td>160</td>
<td></td>
</tr>
<tr>
<td>Gestational age (mean ± SD)</td>
<td>279 ± 13</td>
<td>277 ± 14</td>
<td>280 ± 12</td>
<td>NS</td>
</tr>
<tr>
<td>Preterm birth (%)</td>
<td>5.6</td>
<td>11.3</td>
<td>5.6</td>
<td></td>
</tr>
<tr>
<td>BW (mean ± SD)</td>
<td>3476 ± 616</td>
<td>3407 ± 637</td>
<td>3459 ± 523</td>
<td></td>
</tr>
<tr>
<td>LBW &lt;2500 g (%)</td>
<td>4.6</td>
<td>5.6</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>SGA (%)</td>
<td>4.4</td>
<td>5.6</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>Twins (twin pairs) (n)</td>
<td>49</td>
<td>49</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>Gestational age (mean ± SD)</td>
<td>257 ± 19</td>
<td>254 ± 26</td>
<td>260 ± 21</td>
<td>NS</td>
</tr>
<tr>
<td>Preterm birth (%)</td>
<td>44.9</td>
<td>40.8</td>
<td>30.6</td>
<td></td>
</tr>
<tr>
<td>Twins (infants)</td>
<td>98</td>
<td>98</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>BW (mean ± SD)</td>
<td>2574 ± 560</td>
<td>2441 ± 666</td>
<td>2673 ± 647</td>
<td>0.014a</td>
</tr>
<tr>
<td>LBW &lt;2500 g (%)</td>
<td>40.8</td>
<td>42.9</td>
<td>31.6</td>
<td></td>
</tr>
<tr>
<td>SGA (%)</td>
<td>21.4</td>
<td>20.4</td>
<td>21.4</td>
<td></td>
</tr>
<tr>
<td>All infants (n)</td>
<td>258</td>
<td>258</td>
<td>258</td>
<td></td>
</tr>
<tr>
<td>Perinatal mortality/1000</td>
<td>8</td>
<td>4</td>
<td>8</td>
<td>NS</td>
</tr>
<tr>
<td>Major malformation (%)</td>
<td>2.7</td>
<td>3.9</td>
<td>3.5</td>
<td>NS</td>
</tr>
</tbody>
</table>

aConventional IVF versus spontaneous group.
BW = birthweight; LBW = low birthweight; SGA small for gestational age (-2SD) according to the Swedish reference levels (Marsál et al., 1996).

Table II. Malformation rate in children born after cryopreservation as embryos in comparison with conventional IVF and intracytoplasmic sperm injection (ICSI)

<table>
<thead>
<tr>
<th>Author/year/country</th>
<th>Study period</th>
<th>Cryopreservation</th>
<th>IVF % children with malformation</th>
<th>ICSI % children with malformation</th>
</tr>
</thead>
<tbody>
<tr>
<td>De Mouzon and Lancaster (1997), World Report</td>
<td>1995</td>
<td>2005</td>
<td>2.0</td>
<td>2.1</td>
</tr>
<tr>
<td>SART (1999), USA</td>
<td>1996</td>
<td>1457a</td>
<td>1.9</td>
<td>1.8</td>
</tr>
<tr>
<td>Westergaard et al. (1999), Denmark</td>
<td>1994-95</td>
<td>105</td>
<td>2.9</td>
<td>4.9</td>
</tr>
<tr>
<td>Bergh et al. (1999), Sweden</td>
<td>1982-95</td>
<td>451</td>
<td>4.7</td>
<td>5.5</td>
</tr>
</tbody>
</table>

aDeliveries.

Mouzon and Lancaster, 1997) and available national registry data; the American Society for Reproductive Medicine/Society for Assisted Reproductive Technology (SART, 1999), the recently published Danish registry study (Westergaard et al., 1999) and the Swedish registry study (Bergh et al., 1999). The malformation rate after cryopreservation seems comparable to that after conventional IVF and intracytoplasmic sperm injection (ICSI), although the number of infants born after cryopreservation is rather small in the Danish and Swedish studies. A small but significantly increased risk of malformations was found in the whole Swedish IVF birth cohort compared with the general population (relative risk 1.39, 95% CI, 1.25–1.54). The malformation rate did not differ between children conceived from conventional IVF, ICSI or cryopreservation in that study (Bergh et al., 1999).

Wada et al. found a significantly lower rate of malformations in the cryopreserved group and the authors suggested a possible selection of better
embryos with the cryopreservation procedure as an explanation for this observation (Wada et al., 1994).

**Postnatal growth and development in children born after cryopreservation as embryos**

There are only three studies focusing on follow-up beyond the postnatal period on children born after cryopreservation. In the first study which has been published in two papers, Sutcliffe and co-workers made a detailed assessment of 91 children conceived after cryopreservation and a control group of 83 normally conceived children; the groups were matched for age, sex and social class but not for multiple birth (Sutcliffe et al., 1995a,b). The proportion of multiple birth babies was high in the study group (68 singletons, 20 twins and three triplets), and as a consequence they were more prone to perinatal problems, such as preterm birth and low birthweight. Sutcliffe and colleagues made a very careful assessment of malformations and found no statistically significant differences between the groups. The rate of major congenital malformations was 3.3% and 2.4% and the rate of minor malformations was 31.9% and 21.7%, in the cryopreserved and spontaneous groups, respectively. The children's mental development was assessed with the Griffith scale. Both groups scored above normal (= 100). Analysis of the Griffith scale subquotients revealed a small but significant difference in hearing and speech, the cryopreserved group scoring lower than the spontaneous group (107 versus 112).

Olivennes and co-authors presented a French follow-up study of children conceived from cryopreserved embryos (Olivennes et al., 1996). This group was able to follow up 89 of the complete cohort of 93 infants with assessments of the children's development by questionnaire. In children aged 1-9 years, no abnormalities of height and weight development were noted and only one child had a malformation (a short ureter) at follow-up. The questionnaire included various questions on the psychomotor development of children <5 years old. Psychomotor delay was observed in one singleton infant born prematurely. Scholastic performance was assessed in children by ranking them in their school class. Only one out of 45 children >5 years was considered by his parents to have learning difficulties (although he was in a normal class) and several of the children were at the head of the class. This study lacked a control group but the authors concluded that there were no major pathological features in the study group, referring to data from the general population.

Wennerholm et al. analysed the postnatal development in the previously mentioned cohort of children born after cryopreservation (n = 255 surviving infants) (Wennerholm et al., 1998). At the age of 18 months, the health of each child, beginning from birth, was evaluated, using the records from the paediatric health centres. Four infants (1.5%) dropped out of the study. In the cryopreserved group, 17% of the children were classified as having a chronic disease, a figure that was comparable to the other groups. Most of the children had only mild or moderate disability, with atopic manifestations dominating in all groups. The growth curves for length from birth to 18 months of age, based on the current Swedish charts, showed that there was no difference between the groups, neither for singletons nor twins. The only child with cerebral palsy in this study was one of triplets.

The authors summarized that normal obstetric outcome and normal development, growth and health during early infancy was observed in children born after cryopreservation, when compared with matched control groups. However, minor handicaps, learning difficulties and attention and perception disorders cannot be ruled out at this early age. It is also likely that minor problems might escape detection, if this study design is used.

In all these studies, the power to detect differences in adverse outcome is low, e.g. with a cerebral palsy incidence of 2.4/1000 children (Hagberg et al., 1996), only large increased risks can be detected with sufficient power.

**Outcome of pregnancies after the replacement of cryopreserved, ICSI-derived embryos**

It might be speculated that the damage inflicted on the zona pellucida during the ICSI procedure sensitize the embryo to the chemical and physical changes associated with freezing and thawing, resulting in a higher chance of a negative impact on the outcome of pregnancies derived from ICSI.
Indeed, a higher incidence of clinical abortions (30.7%) after cryopreservation of ICSI embryos, compared with the incidence after ICSI with fresh embryos (11.5%), was reported by the Brussels group (Wisanto et al., 1996). Other groups have not reported any difference in abortion rate between fresh and frozen ICSI embryos (Palermo et al., 1996; Wennerholm et al., 2000). The Brussels group also reported similar pregnancy rates per transfer, preclinical pregnancy loss and clinical miscarriage rates after cryopreservation of embryos obtained from ICSI and IVF, respectively (Kowalik et al., 1998).

The perinatal data from ICSI singleton pregnancies, obtained from two IVF clinics in Sweden during a 5 year period, are shown in Table III (Wennerholm et al., 2000). The cryopreserved singleton group consisted of 120 babies (103 derived from ICSI using ejaculated spermatozoa, six from epididymal and seven from testicular spermatozoa). Perinatal outcome in the cryopreserved ICSI group was comparable to ICSI and fresh embryo transfer. The ICSI group consisted of a total of 1192 ICSI infants, of which 158 originated from cryopreserved embryos. Five major malformations (3.2%) occurred in the cryopreserved group, similar to the rate in the ICSI fresh embryo transfer groups.

In a small series, Bonduelle et al. observed two abnormal karyotypes (XXY, Klinefelter) in 34 performed procedures (5.9%), probably representing a statistical variation on the higher risk of sex chromosome aberrations found in ICSI pregnancies (Bonduelle et al., 1998).

Children born after cryopreservation of oocytes

Cryopreservation of oocytes could be an alternative to embryo cryopreservation. Early results for survival, fertilisation and cleavage were disappointing, leading to sporadic pregnancies only for more than 10 years. However, the introduction of ICSI has greatly improved clinical efficiency.

The cryopreservation of human oocytes offers an alternative solution to the ethical and legal problems related to embryo storage. Furthermore, it might be one method of preserving the reproductive capability of women who risk its loss due to impending premature ovarian failure, pelvic disease, surgery or cancer treatment. Today, only a handful of births after oocyte cryopreservation have been reported. Initially, concern was expressed about the risk of spindle disorganization and the induction of aneuploidy (Sathananthan et al., 1988). The children reported so far have been healthy but they are few in number. Porcu and co-workers from Italy have reported six healthy babies after cryopreservation of oocytes (Porcu et al., 1998). A case report exists of a birth following cryopreservation of immature human oocytes (Tucker et al., 1998) and there are also recent reports on ongoing pregnancies after ICSI with testicular (Porcu et al., 1999a) and epididymal spermatozoa injected into frozen-thawed human oocytes (Porcu et al., 1999b).

Cryopreservation of ovarian tissue

Cryopreservation of ovarian tissue and transplantation may be a future option to preserve female fertility especially in young women undergoing curative chemotherapy or radiotherapy for malignant diseases. Due to their more undifferentiated state and small volume, the immature oocytes found in primordial follicles might be more tolerant to freezing and thawing than mature oocytes. There is also the advantage of numbers, since hundreds of follicles can be obtained with an ovarian biopsy or thousands

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Table III: Obstetric outcome in intracytoplasmic sperm injection singletons according to sperm origin and the replacement of frozen-thawed embryos (Wennerholm et al., 2000)

<table>
<thead>
<tr>
<th>Sperm origin</th>
<th>Ejaculated (n = 593)</th>
<th>Epididymal (n = 37)</th>
<th>Testicular (n = 23)</th>
<th>Cryopreserved (n = 120)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preterm birth (%)</td>
<td>9.3</td>
<td>10.8</td>
<td>8.7</td>
<td>3.3</td>
</tr>
<tr>
<td>LBW (%)</td>
<td>8.1</td>
<td>5.4</td>
<td>17.4</td>
<td>4.2</td>
</tr>
<tr>
<td>High BW (%)</td>
<td>3.4</td>
<td>5.4</td>
<td>0</td>
<td>6.7</td>
</tr>
<tr>
<td>SGA (%)</td>
<td>6.6</td>
<td>10.8</td>
<td>4.3</td>
<td>5.0</td>
</tr>
</tbody>
</table>

LBW = low birthweight; BW = birthweight; SGA = small for gestational age (<-2 SD) according to the Swedish reference levels (Marsal et al., 1996).
if an ovariectomy is performed. Successful transplantation of cryopreserved ovarian cortical tissue into castrated ewes was first performed by Gosden and colleagues (Gosden et al., 1994). Return of oestrus cycles was observed and some animals regained fertility and lambs were born. Recently, a successful transplantation of cryopreserved autologous ovarian tissue into a previously oophorectomized woman with non-malignant disease was reported (Oktay et al., 1999), showing that this technique might be practicable in humans and thus has the potential to become an alternative treatment in assisted reproductive technology in the future.

**Conclusions**

The advantages of embryo cryopreservation must be weighed against its known and potential disadvantages. The greatest risk in assisted reproductive treatments today is no doubt the high frequency of multiple birth pregnancies. If elective one-embryo transfer is to be the method of choice in the future, good cryopreservation and thawing techniques will be mandatory. The transfer of frozen-thawed embryos has no apparent negative impact on obstetric outcome, compared with fresh embryo transfer. The reported frequency of birth defects is not increased but the fact that the power to detect any difference in malformation rate is very low in most studies must be taken into consideration. Large registry studies with appropriate control groups will be required. There is even less information available on the subsequent health and development of children born after cryopreservation as embryos, but the existing data indicate normal development, health and growth during early infancy. So far, long-term paediatric development is apparently not a cause for concern, but the few studies performed are small. The evidence is thus limited and we cannot be absolutely certain that there are no long-term consequences of embryo cryopreservation in humans.

**Acknowledgement**

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