BACKGROUND: An estimated 3.5 million children have been born to date using assisted reproduction technologies. We reviewed the data in order to evaluate current knowledge of medical outcome for IVF/ICSI children born after cryopreservation, slow freezing and vitrification of early cleavage stage embryos, blastocysts and oocytes.

METHODS: A systematic review was performed. We searched the PubMed, Cochrane and Embase databases from 1984 to September 2008. Inclusion criteria for slow freezing of early cleavage stage embryos were controlled studies reporting perinatal or child outcomes. For slow freezing and vitrification of blastocysts and oocytes, and vitrification of early cleavage stage embryos, case reports on perinatal or child outcomes were also included. Three reviewers independently read and evaluated all selected studies.

RESULTS: For early cleavage embryos, data from controlled studies indicated a better or at least as good obstetric outcome, measured as preterm birth and low birthweight for children born after cryopreservation, as compared with children born after fresh cycles. Most studies found comparable malformation rates between frozen and fresh IVF/ICSI. For slow freezing of blastocysts and for vitrification of early cleavage stage embryos, case reports on perinatal or child outcomes were also included. Three reviewers independently read and evaluated all selected studies.

CONCLUSION: Data concerning infant outcome after slow freezing of embryos was reassuring. Properly controlled follow-up studies of neonatal outcome are needed after slow freezing of blastocysts and after vitrification of early cleavage stage embryos, blastocysts and oocytes. In addition, child long-term follow-up studies for all cryopreservation techniques are essential.

Key words: cryopreservation / slow freezing / vitrification / pregnancy outcome / birth defect

Introduction

Infertility has now been internationally recognized as a public health issue (Fathalla, 2002) and assisted reproduction technologies (ART) are increasingly used to overcome it. An estimated 3.5 million children have been born to date after ART (ICMART, 2008). The first child after embryo freezing was born in 1984 (Zeilmaker et al., 1984) and the first child after oocyte freezing was born as early as in 1986 (Chen, 1986), but the technique remained clinically dormant until recently.

A growing proportion of the children born after ART (estimated by ICMART to about 25% worldwide (ICMART, 2008), and in some countries such as Finland and Australia up to 40%), are now born after cryopreservation of either cleavage stage embryos, in the majority of cases, or of blastocysts or oocytes. The current trend of transferring fewer embryos has resulted in more embryos being available for freezing. Oocyte freezing has been more frequently used owing to improved survival of cancer patients, as well as for legal reasons in some countries. As these developments are fairly recent, less is known about the safety of the techniques in terms of neonatal outcome and child follow-up.

The health of children born after ART has always been of concern, and increased risks have been identified, such as higher risks of survival of cancer patients, as well as for legal reasons in some countries. As these developments are fairly recent, less is known about the safety of the techniques in terms of neonatal outcome and child follow-up.

The health of children born after ART has always been of concern, and increased risks have been identified, such as higher risks of
prematurity and its sequelae, and of birth defects (Nygren et al., 2007). The risks identified to date do not seem to be associated with the techniques per se, but rather as being attributable to parental characteristics and from clinical policies of transferring more than one embryo at the time, causing highly increased proportions of multiple pregnancies and deliveries. Recently concerns about possible imprinting problems associated with these techniques have also been raised (Amor and Halliday, 2008).

With the current introduction and increased use of a new freezing technique, vitrification, the risks may also be different due to a theoretically different risk profile (e.g. differences in concentrations of potentially toxic cryoprotectants) as compared with the currently dominant slow-freezing technique.

These developments prompted us to review and evaluate current knowledge of medical outcomes for IVF-children born after slow-freezing or vitrification and thawing of embryos or oocytes.

Materials and Methods

Search strategy

We searched Medline, Embase and the Cochrane Database of Systematic Reviews from 1984 until September 12, 2008.

The following key words were used.

Early cleavage stage embryos

('Cryopreservation'[Mesh] OR cryopreserve* OR freeze* OR froze* OR unfroz* OR vitrificat*) AND ('Reproductive Techniques, Assisted'[Mesh] OR ART OR assisted reproductive techn* OR IVF OR ICSI OR in-vitro fertilization OR in vitro fertilization OR fertilization in vitro OR intracytoplasmic sperm injection) AND ('Treatment Outcome'[Mesh] OR 'Pregnancy Outcome'[Mesh] OR anom* OR malform* OR abnormal* OR birth defects OR congenital abnormalities OR child development OR birth* OR pregnanc* OR children OR child* OR infants) AND (comparative study OR cohort OR retrospective) AND (control OR compar*) AND ((Humans[Mesh]) AND (English[lang])).

Blastocysts/oocytes

('Cryopreservation'[Mesh] OR cryopreserve* OR freeze* OR froze* OR unfroz* OR vitrificat*) AND ('Reproductive Techniques, Assisted'[Mesh] OR ART OR assisted reproductive techn* OR IVF OR ICSI OR in-vitro fertilization OR in vitro fertilization OR fertilization in vitro OR intracytoplasmic sperm injection) AND ('Treatment Outcome'[Mesh] OR 'Pregnancy Outcome'[Mesh] OR anom* OR malform* OR abnormal* OR birth defects OR congenital abnormalities OR child development OR birth* OR pregnanc* OR children OR child* OR infants) AND ('Oocytes'[Mesh] OR 'Blastocyst'[Mesh] OR oocyte* OR blastocyst*) AND ((Humans[Mesh]) AND (English[lang])).

We searched PubMed September 12, 2008 for additional references such as advance access publication using ‘cryopreservation OR vitrification’ as key words together with ‘English’ and ‘added to PubMed the last 180 days’ as limitations.

We searched the reference lists of identified articles manually for additional references. We tried to contact authors whenever appropriate. Guidelines for meta-analysis and systematic reviews of observational studies were followed (Stroup et al., 2000).

Independent searches were performed by three researchers (slow freezing: AL and UBW; vitrification: VSA) and two librarians. Three independent reviewers (AL, UBW, VSA) performed the abstract screening.

Disagreements were resolved by discussion and consensus. A pre-designed proforma was used to collate data.

Inclusion/exclusion criteria

Early cleavage stage embryos

All studies published in the English language reporting perinatal or child outcomes for singletons or multiples after cryopreservation with slow freezing or vitrification were included.

We excluded studies without a control group (fresh IVF/ICSI or Naturally conceived) except for studies of vitrification, due to the importance of this new method. In case of double publication the latest one was used.

Studies of outcome measures after freezing that did not separate singleton and multiple births were excluded except for birth defects. Studies of cryopreserved embryos that did not separate outcome measures in children after using donor and non-donor oocytes were excluded.

Blastocysts and oocytes

All studies published in the English language including case reports of perinatal or child outcomes for singletons or multiples after cryopreservation with slow freezing or vitrification were included.

We excluded reports of neonatal outcome after slow-frozen and vitrification of oocytes and blastocysts obtained after in vitro maturation cycles. We also excluded all studies reporting ongoing pregnancies or deliveries that did not mention the health of the infants born. In case of double publications the latest one was used.

Outcome measures

(i) preterm birth (defined as delivery < 37 weeks of gestation)
(ii) very preterm birth (defined as < 32 weeks of gestation)
(iii) low birthweight (defined as birthweight < 2500 g)
(iv) very low birthweight (defined as birthweight < 1500 g)
(v) small for gestational age
(vi) perinatal mortality (stillbirth and early neonatal mortality within 7 days)
(vii) late neonatal mortality 7–28 days
(viii) infant mortality
(ix) birth defects
(x) chromosome abnormalities
(xi) childhood growth
(xii) childhood mental development
(xiii) childhood morbidity and cancer

Selection of studies

Early cleavage stage embryos

A total of 437 articles and reports were identified from the systematic search. Forty-six articles and reports studied child outcome after cryopreservation. Of these studies only 21 fulfilled all inclusion criteria and are listed in Table I. Twenty-three were excluded. Five studies were double publications (Bonduelle et al., 1996; Wennerholm et al., 1997; Aytou et al., 1999; Bergh et al., 1999; Van den Abbeel et al., 2000) and three had no control group (Frydman et al., 1989; Heijnsbroek et al., 1996; Ollennes et al., 1996). Five studies from the Australia and New Zealand Assisted Reproduction Database (ANZARD) reported accumulated data for singleton and multiples and no data for birth defects (Dean and Sullivan, 2003; Bryant et al., 2004; Wang et al., 2006, 2007; Waters et al., 2006). Ten Society for Assisted Reproductive Technology (SART) reports were excluded (SART, 1990, 1991, 1992, 2001b, a, b, 2004, 2007; Wright et al., 2005, 2006, 2007); four had no perinatal data and six presented perinatal outcome data for cryopreservation but with non-donor and donor oocytes not separated. Four additional articles...
<table>
<thead>
<tr>
<th>Reference, country</th>
<th>Design</th>
<th>Study period</th>
<th>Participants</th>
<th>Outcome measures included in this systematic review</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wada et al. (1994), UK</td>
<td>Retrospective hospital based cohort study</td>
<td>1985–1991</td>
<td>185 cryo singletons, 86 cryo twins (43 deliveries), 12 cryo triplets (4 deliveries), 592 fresh IVF singletons, 288 fresh IVF twins (144 deliveries), 81 fresh IVF triplets (27 deliveries)</td>
<td>Preterm birth, low birthweight, stillbirth, perinatal mortality, major birth defects</td>
<td>Both early cleavage stage embryos and blastocysts. Cryoprotectant: propanediol and glycerol (blastocysts)</td>
</tr>
<tr>
<td>Sutcliffe et al. (1995a,b), UK</td>
<td>Retrospective hospital based cohort study</td>
<td>Children born 1989–1994, follow up cryo 25.08 ± 12.86 months, controls 29.19 ± 14.63 months.</td>
<td>68 cryo singletons, 20 cryo twins, 3 cryo triplets, 83 controls (81 naturally conceived singletons and 2 naturally conceived twins)</td>
<td>Preterm birth, mental and neurological development Major and minor birth defects</td>
<td>Control group not matched for paternal age and multiplicity No details of methods of cryopreservation</td>
</tr>
<tr>
<td>Westergaard et al. (1999), Denmark</td>
<td>Population based register study</td>
<td>1994–1995</td>
<td>Total 2245 IVF children (1913 IVF, 180 ICSI, 105 cryo, 47 oocyte donation), 2245 naturally conceived controls</td>
<td>Birth defects</td>
<td>Multiplicity not given for different ART techniques. Preterm birth, low birthweight, perinatal mortality analyzed for total ART population No details of methods of cryopreservation Very low birthweight only analyzed for the total ART population No details of methods of cryopreservation</td>
</tr>
<tr>
<td>Schieve et al. (2002), USA</td>
<td>Population based register study</td>
<td>1996–1997</td>
<td>Total 42 463 children (8.9% cryo, non donor oocyte, 78.0% fresh, non donor oocyte) 18 408 singletons, 18 399 twins, 5127 triplets</td>
<td>Low birthweight</td>
<td>Questionnaire, 73.4% response rate (IVF, ICSI, cryo together, 70.4% for cryo). Birthweight and birth length analyzed but not gestational age or low birthweight. Mental development and birth defects only analyzed for the total ART population No details of methods of cryopreservation</td>
</tr>
<tr>
<td>Nakajo et al. (2004), Japan</td>
<td>Retrospective questionnaire cohort study</td>
<td>Children born 1995–2003, follow up at age, 3, 6 and 9 months, 1, 1.5 and 2 years</td>
<td>105 cryo (74 singletons, 28 twins, 3 triplets), 406 ICSI (217 singletons, 165 twins, 24 triplets), 120 IVF (64 singletons, 44 twins, 12 triplets)</td>
<td>Growth</td>
<td>No details of methods of cryopreservation</td>
</tr>
<tr>
<td>Study</td>
<td>Type of Study</td>
<td>Dates</td>
<td>Study Details</td>
<td>Findings</td>
<td>Additional Notes</td>
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<tr>
<td>Wang et al. (2005), Australia</td>
<td>Retrospective cohort study</td>
<td>1996–2000</td>
<td>Total 17 724 (5120 cryo, 12 604 fresh) 11 556 singletons, 5513 twins, 657 triplets/+</td>
<td>Preterm birth, low birthweight</td>
<td>No details of methods of cryopreservation</td>
</tr>
<tr>
<td>Kallen et al. (2005a), Sweden</td>
<td>Population based register study</td>
<td>1982–2001</td>
<td>Total 16 280 infants (1055 cryo IVF, 419 cryo ICSI, 10 228 fresh IVF, 4545 fresh ICSI)</td>
<td>Preterm birth, low birthweight</td>
<td>Mortality rates analyzed for singletons and twins combined and therefore not included in Table III. Neonatal complications analyzed for fresh and cryo together and therefore not included in this study.</td>
</tr>
<tr>
<td>Kallen et al. (2005b), Sweden</td>
<td>Retrospective cohort study</td>
<td>Median follow up 5.5 years of age for all IVF children</td>
<td>10 088 singleton deliveries, 3006 twin deliveries, 147 triplet/s/ + deliveries</td>
<td>Birth defects</td>
<td>No details of methods of cryopreservation additional data from EpC (2009, Personal communication)</td>
</tr>
<tr>
<td>Olson et al. (2005), USA</td>
<td>Retrospective cohort study</td>
<td>1989–2002</td>
<td>Total 1462 IVF infants (236 IVF, 476 ICSI, 335 cryo, 415 ZIFT), 8422 naturally conceived controls, 343 intrauterine insemination</td>
<td>Major birth defects at 1 year of age</td>
<td>Number of singletons and twins not given. No comparison with naturally conceived controls or intrauterine insemination No details of methods of cryopreservation</td>
</tr>
<tr>
<td>Desai et al. (2007), USA</td>
<td>Retrospective hospital based cohort study</td>
<td>2002–2006</td>
<td>11 deliveries</td>
<td>Birth defects</td>
<td>Only cryoloop vitrification of day 3 embryos Cryoprotectant: DMSO, ethylene glycol, sucrose</td>
</tr>
<tr>
<td>Shih et al. (2008), Australia</td>
<td>Retrospective register study</td>
<td>1978–2005</td>
<td>3708 cryo IVF and ICSI births, 4406 fresh IVF and ICSI births</td>
<td>Birth defects, low birthweight, Z score, perinatal death, birth defects</td>
<td>Subanalysis of singleton first ART births Some data could be included in Wang et al. (2005) Embryos cryopreserved on day 2 (rarely day 3 or 6) Cryoprotectant: propanediol</td>
</tr>
<tr>
<td>Belva et al. (2008), Belgium</td>
<td>Prospective hospital based cohort study</td>
<td>1983–2006</td>
<td>547 cryo ICSI children (multiple birth rate 29.8%, (29.3% twins, 0.5% triplets)) 390 cryo IVF children (27.2% multiple birth rate, (25.7% twins, 1.5% triplets)), 2840 fresh ICSI (multiple birth rate 47.2% (43% twins, 4.0% triplets)), 2955 fresh IVF (multiple birth rate 47.3% (42.3% twins, 4.9% triplets, 4 quadruplets))</td>
<td>Preterm birth, low birthweight, very low birthweight, perinatal death, birth defects, chromosome abnormalities</td>
<td>Comparison of cryo ICSI (1993–2006) and cryo IVF (1986–2006) to fresh ICSI (1991–1999) and fresh IVF (1983–1999), respectively Embryos cryopreserved on day 1–6 Cryoprotectant: propanediol, DMSO, or glycerol (day 5/6 embryos)</td>
</tr>
<tr>
<td>Rama Raju et al. (2008), India</td>
<td>Retrospective hospital based cohort study</td>
<td>2005–2007</td>
<td>89 cryo IVF/ICSI infants (50 singletons, 36 twins, 3 triplets) 216 fresh IVF/ICSI infants (118 singletons, 92 twins, 6 triplets)</td>
<td>Birth defects</td>
<td>Preterm birth, low birthweight, very low birthweight, pregnancy complications, Apgar score, admission to neonatal intensive care units, analyzed for singleton and multiples together Cryoloop vitrification Embryos cryopreserved on day 3 Cryoprotectant: ethylene glycol</td>
</tr>
<tr>
<td>Balaban et al. (2008), Turkey</td>
<td>Retrospective study</td>
<td>2006–2007</td>
<td>8 deliveries (6 singletons and 2 twins)</td>
<td>Health</td>
<td>Cryoloop vitrification Embryos cryopreserved on day 3. Cryoprotectant: ethylene glycol and propanediol</td>
</tr>
</tbody>
</table>

DMSO: dimethylsulphoxide; ART: assisted reproduction technology.
identified from other sources were included (Desai et al., 2007; Balaban et al., 2008; Belva et al., 2008; Rama Raju et al., 2008), giving 25 included studies (Table I).

Blastocysts and oocytes

A total of 990 studies were identified from the systematic search. Of these studies only 12 fulfilled the inclusion criteria for blastocysts (Choi et al., 2000; Yokota et al., 2001; Rosenlund et al., 2002; Quintans et al., 2003; Sills et al., 2003; Son et al., 2003; Smith et al., 2005; Takahashi et al., 2005; Hiraoka et al., 2006, 2007; Mukaida et al., 2006; Parriego et al., 2007) and 30 for oocytes (Kuleshova et al., 1999; Porcu et al., 2000; Quintans et al., 2002; Fosas et al., 2003; Katayama et al., 2003; Yoon et al., 2003; Bonni et al., 2004; Kan et al., 2004; Notrica et al., 2004; Azambuja et al., 2005; Chen et al., 2005, 2008; Kuwayama et al., 2005; Kyono et al., 2005; Levi Setti et al., 2005, 2006; Li et al., 2005; Tjer et al., 2005; La Sala et al., 2006; Montag et al., 2006; Antinori et al., 2007; Bianchi et al., 2007; Bonni et al., 2007; De Geyter et al., 2007; De Santis et al., 2007; Gook et al., 2007; Konc et al., 2007; Yang et al., 2007; Chian et al., 2008; Greco et al., 2008). Two longer reports including more than 100 deliveries concerning slow freezing of blastocysts did not report any outcome data on the children (Veeck et al., 2004; Kosasa et al., 2005). Data from one large vitrification of blastocysts study (Mukaida et al., 2003) was included in a later study by Takahashi et al. (2005) and was therefore excluded here. Another small study did not report any outcome data (Vanderzwalmen et al., 2002). Two small series by Bonni including 12 and 4 live births, respectively, did not report outcome data (Bonni et al., 2006a, b). Case reports included in later retrospective reports were also excluded.

Methodological quality of included studies

Early cleavage stage embryos

We selected studies with a control group except for vitrification. Most studies had fresh IVF/ICSI cycles as control groups. Three studies included naturally conceived children as controls (Sutcliffe et al., 1995b; Wennnerholm et al., 1998; Westergaard et al., 1999; Sutcliffe, 2000; Olson et al., 2005). One study also compared outcomes for children born after cryo ICSI versus cryo IVF (Belva et al., 2008).

Many studies, including the large population based registry studies, did not report the method of cryopreservation used (Table I, comments). Since slow freezing of early cleavage stage embryos has been the main freezing procedure in most clinics and countries, these studies were included in the part of this review analysing outcomes after this type of cryopreservation.

Two studies used only vitrification (Desai et al., 2007; Rama Raju et al., 2008). Both used cryoloop vitrification on day 3 embryos. Desai et al. (2007) used dimethylsulphoxide (DMSO) and ethylene glycol as cryoprotectant agents; Rama Raju et al. (2008) used ethylene glycol. Here, only birth defects as outcome measure were used from these studies.

In other studies reporting the method of cryopreservation, a variety of cryopreservation protocols were used, sometimes also changing during the study period. Most of these studies included only freezing of day 2 or 3 embryos. One study reported freezing of embryos from day 1 to 6 (Belva et al., 2008). The type of cryoprotectant differed between the studies (propanediol, DMSO, glycerol).

Outcome measures, such as birth defects, were difficult to interpret and compare between studies, owing to differences in the definition of birth defects, how data were collected, inclusion or exclusion of miscarriages and stillbirths, and the method of assessment and selection of control group. SART collected data on neonatal abnormalities from 1991 to 1997: the definition of abnormalities was described as ‘structural and functional abnormalities’. This may include some conditions not generally considered as birth defects, and exclude others. Details regarding how programs obtained the information were not collected and not standardized. Owing to these problems, SART did not report birth defects after 1997. No statistical analysis was presented in the SART reports.

The preterm birth rate for cryo singletons varied between 9.2 and 12.0%. Most studies reported cryo IVF and cryo ICSI together and only two studies reported these methods separately and found that the preterm birth rates between the cryo IVF group and the cryo ICSI group were comparable (Kallen et al., 2005a; Belva et al., 2008). The corresponding preterm birth rate for fresh IVF/ICSI singletons varied between 7.4 and 14%. In the Swedish (Kallen et al., 2005a) and the Australian (Wang et al., 2005; Shih et al., 2008) studies the preterm birth rate in singletons was significantly lower for children born after cryopreservation than in children born after fresh cycles, although in the rest of the studies no significant differences in preterm birth rates were observed.

For twins, the preterm birth rates for cryo IVF/ICSI infants varied between 33 and 62% as compared with 47.6 and 61.3% for fresh IVF/ICSI twins. The Belgian study (Belva et al., 2008) found a significantly higher preterm birth rate for frozen IVF versus fresh IVF twins, although no significant difference was found for ICSI twins. In

Slow freezing and vitrification of blastocysts and oocytes

We searched for and included studies with data on health aspects of the children born. Most studies were small and the information regarding neonatal outcome was scanty. In only one study was the neonatal outcome compared with a control group (frozen versus fresh blastocyst transfer) (Takahashi et al., 2005). The data of mean birthweight of the babies was usually either not separated between singletons and multiples (Choi et al., 2000; Son et al., 2003; Takahashi et al., 2005) or was not mentioned at all. Many of the publications were case reports.

The frozen/thawed oocytes were sometimes fertilized with frozen spermatozoa, for example frozen ejaculated sperm (Notrica et al., 2004), frozen epididymal sperm (Azambuja et al., 2005), frozen testicular sperm (Levi Setti et al., 2005) or the embryo was frozen after use of a frozen oocyte and frozen sperm (Gook et al., 2007). There were also reports of surrogacy treatment (Yang et al., 2007) and oocyte recipients (Fosas et al., 2003). Different cryopreservation protocols and different vitrification methods and solutions were used.

To date, there are no follow-up studies of the growth and long-term health of children born after slow freezing/vitrification of blastocysts and oocytes.

Results

Early cleavage stage embryos

Preterm birth for singletons and twins

Data on preterm birth rate were reported in 6/7 studies including approximately 11 000 children born after cryopreservation and 37 000 children born after fresh IVF/ICSI (Table II).

The preterm birth rate for cryo singletons varied between 9.2 and 12.0%. Most studies reported cryo IVF and cryo ICSI together and only two studies reported these methods separately and found that the preterm birth rates between the cryo IVF group and the cryo ICSI group were comparable (Kallen et al., 2005a; Belva et al., 2008). The corresponding preterm birth rate for fresh IVF/ICSI singletons varied between 7.4 and 14%. In the Swedish (Kallen et al., 2005a) and the Australian (Wang et al., 2005; Shih et al., 2008) studies the preterm birth rate in singletons was significantly lower for children born after cryopreservation than in children born after fresh cycles, although in the rest of the studies no significant differences in preterm birth rates were observed.

For twins, the preterm birth rates for cryo IVF/ICSI infants varied between 33 and 62% as compared with 47.6 and 61.3% for fresh IVF/ICSI twins. The Belgian study (Belva et al., 2008) found a significantly higher preterm birth rate for frozen IVF versus fresh IVF twins, although no significant difference was found for ICSI twins. In
<table>
<thead>
<tr>
<th>Reference, country</th>
<th>Cryo infants (IVF/ICSI)</th>
<th>Fresh infants (IVF/ICSI and/or NC)</th>
<th>Cryo singletons PTB N (%)</th>
<th>Fresh singletons PTB N (%)</th>
<th>OR/AOR (95% CI) and/or P-value</th>
<th>Cryo singletons LBW N (%)</th>
<th>Fresh singletons LBW N (%)</th>
<th>OR/AOR (95% CI) and/or P-value</th>
<th>Cryo twins PTB N (%)</th>
<th>Fresh twins PTB N (%)</th>
<th>OR/AOR (95% CI) and/or P-value</th>
<th>Cryo twins LBW N (%)</th>
<th>Fresh twins LBW N (%)</th>
<th>OR/AOR (95% CI) and/or P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wada et al. (1994) UK</td>
<td>IVF: 177 singletons, 78 twins</td>
<td>IVF: 527 singletons, 262 twins</td>
<td>19/158 (12%)</td>
<td>67/494 (14%)</td>
<td>P &gt; 0.05</td>
<td>13/177 (7%)</td>
<td>68/527 (13%)</td>
<td>P &gt; 0.05</td>
<td>12/36 (33%)</td>
<td>72/125 (58%)</td>
<td>P &lt; 0.05</td>
<td>30/78 (38%)</td>
<td>140/262 (53%)</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Sutcliffe et al. (1995a), UK</td>
<td>68 singletons, 20 twins, 3 triplets</td>
<td>81 NC singletons, 2 NC twins</td>
<td>7/68 (10.3)</td>
<td>6/82 (7.4)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Schieve et al. (2002) USA</td>
<td>42 463 infants (8.9% cryo, 78.0% fresh)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>(10.5)</td>
<td>NA</td>
<td>(13.6)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>(49.5)</td>
<td>NA</td>
<td>(56.0)</td>
<td>NA</td>
</tr>
<tr>
<td>Wang et al. (2005), Australia</td>
<td>5120 singletonb</td>
<td>81 NC singletons, 2 NC twins</td>
<td>7/68 (10.3)</td>
<td>6/82 (7.4)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Kallen et al. (2005a, b), Sweden</td>
<td>IVF: 10 228b</td>
<td>ICSI: 4545b</td>
<td>NA</td>
<td>NA</td>
<td>0.69 (0.50–0.95)</td>
<td>NA</td>
<td>NA</td>
<td>0.49 (0.02–0.75)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Shih et al. (2008), Australia</td>
<td>2387 cryo IVF/ICSI singleton first births; 429 sets of IVF/ICSI twins</td>
<td>3110 fresh IVF/ICSI singleton first births; 825 sets of IVF/ICSI twins</td>
<td>NA (9.2)</td>
<td>NA (12.3)</td>
<td>1.4 (1.1–1.6)</td>
<td>NA (6.5)</td>
<td>NA (11.6)</td>
<td>1.9 (1.6–2.4)</td>
<td>&lt;0.001</td>
<td>206/431 (48)</td>
<td>428/828 (52)</td>
<td>NS</td>
<td>184/431 (43)</td>
<td>418/835 (50)</td>
</tr>
<tr>
<td>Beva et al. (2008), Belgium</td>
<td>384 IVF (281 singletons, 98 twins)</td>
<td>2920 IVF (1523 singletons, 1251 twins)</td>
<td>34/281 (11.9)</td>
<td>140/1523 (9.0)</td>
<td>1.38 (0.92–2.05)</td>
<td>20/281 (7.1)</td>
<td>121/1523 (7.9)</td>
<td>0.64 (0.54–1.45)</td>
<td>62/98 (62.0)</td>
<td>600/1251 (47.6)</td>
<td>1.77 (1.17–2.69)</td>
<td>49/98 (50.0)</td>
<td>568/1251 (45.1)</td>
<td>1.21 (0.80–1.81)</td>
</tr>
<tr>
<td>+0.05</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

AOR: adjusted odds ratio; LBW: low birthweight; NA: not available; NC: naturally conceived; OR: odds ratio; PTB: preterm birth.

*Include one set of twins.

1Singletons and multiples.

*Cryo embryos ¼ 1.0, OR (99% CI), adjusted for maternal age, parity, cause of infertility (male or female), number of embryos transferred, type of embryos and type of procedure.

*Fresh IVF embryos ¼ 1.0, adjusted for year of birth, maternal age, parity, smoking and years of involuntary childlessness.

*Combined low birthweight (<5000 g). Numbers of births differs from those mentioned in methods section.

*Fresh ICSI embryos ¼ 1.0.
contrast, in an earlier study from the UK (Wada et al., 1994), the preterm birth rate was significantly higher for twins from fresh IVF than cryo IVF cycles. In all other studies no significant difference was found.

No significant differences in preterm birth rates for either singleton or twins between cryo ICSI or cryo IVF were observed (Belva et al., 2008).

Low birthweight for singletons and twins

Low birthweight rate was reported in 6/7 studies including approximately 15,000 children (Table II). The low birthweight rate among singletons after cryo IVF/ICSI varied between 6.2 and 10.5% and for fresh IVF/ICSI cycles between 7.2 and 13.6%. In the Australian (Wang et al., 2005; Shih et al., 2008) and Swedish (only IVF and not ICSI) (Kallen et al., 2005a) studies the low birthweight rate in singletons was significantly lower for cryo children compared with children born after fresh cycles. The other studies reporting on low birthweight in singletons did not observe any significant difference (Wada et al., 1994; Belva et al., 2008). No difference in low birthweight rate was demonstrated between cryo IVF and cryo ICSI (Belva et al., 2008).

In the Australian study (Shih et al., 2008) singleton first babies born after fresh embryo transfer had a lower mean birthweight (3.3%; 95% confidence interval (CI); 2.3–4.3%) and a lower mean z score (0.233 standard deviations; 95% CI 0.181–0.285) than those born after cryopreservation.

Low birthweight rate among twins from frozen IVF/ICSI cycles varied from 38 to 50% as compared with 45.1–56.0% for children from fresh IVF/ICSI cycles. Three studies found significantly lower low birthweight rates for twins from frozen cycles compared with fresh cycles (Wada et al., 1994; Wang et al., 2005; Shih et al., 2008) (Table II).

Very low birthweight

Very preterm birth was not reported in any study whereas very low birthweight was reported in one study (Belva et al., 2008). No significant difference was found for singletons between frozen and fresh cycles (IVF: odds ratio (OR) 0.39; 95% CI 0.09–1.61, ICSI: OR 1.06; 95% CI 0.43–2.63) although for IVF twins from frozen cycles a significantly higher rate of very low birthweight was observed as compared with children from fresh cycles (OR 2.18; 95% CI 1.21–3.92). No difference in very low birthweight rate was found between cryo IVF and cryo ICSI (Belva et al., 2008).

Mortality

Perinatal mortality was reported in only two studies (Wada et al., 1994; Shih et al., 2008) (Table III). No difference in perinatal mortality between singletons and twins from fresh and frozen cycles was found in the UK study (Wada et al., 1994), although a significantly higher rate of perinatal mortality was observed for singletons from fresh versus frozen cycles in the Australian study (Shih et al., 2008). No data were found for late neonatal or infant mortality.

Birth defects and chromosome abnormalities

Seventeen studies have reported on birth defects (Table IV). The malformation rate in frozen cycles for all IVF/ICSI (singletons and multiples) varied between 0.7% (Wada et al., 1994) and 8.6% (Kallen et al., 2005b). The corresponding figures for fresh IVF/ICSI varied between 0.7% (SART, 1998) and 8.7% (Kallen et al., 2005b). Only two studies have separated malformation rates for IVF and ICSI (Kallen et al., 2005b; Belva et al., 2008). In the Swedish study no significant difference was found between frozen IVF or frozen ICSI as compared with fresh IVF or fresh ICSI when adjusting for year of birth, maternal age and number of infants born. In the Belgian study from 2008, significantly more cryo ICSI children had birth defects (6.4%) as compared with children born after fresh ICSI (3.4%) or cryo IVF (3.1%, OR 2.15; 95% CI 1.10–4.20). In the total cryo group (IVF + ICSI) a significantly higher rate of malformations was also found as compared with the total fresh group (OR 1.42; 95% CI 1.03–1.93). However, no significant difference was found for cryo IVF versus fresh IVF (3.1 versus 3.8%). No adjustments were made for maternal factors in the Belgian study (Belva et al., 2008).

Only four studies reported on birth defects in singletons and twins separately. In the Belgian study only cryo ICSI singletons as compared with fresh ICSI singletons had significantly higher malformation rates. In the study of Olson a higher malformation rate was found only in IVF/ICSI twins as compared with fresh IVF/ICSI twins (Olson et al., 2005). The other two studies found no differences between the groups (Sutcliffe et al., 1995a; Shih et al., 2008).

The three studies reporting on children born after vitrification included 99 infants and 11 deliveries (Desai et al., 2007; Balaban et al., 2008; Rama Raju et al., 2008). No significant difference in birth defects was seen as compared with fresh IVF cycles in the study by Rama Raju et al. (2008).

Only one study reported the rate of chromosome aberrations (Belva et al., 2008). Overall, the pre- and post-natal rate of chromosome aberrations in cryo ICSI as compared with fresh ICSI were similar (OR 1.27; 95% CI 0.66–2.44). Further, the rate of de novo chromosome abnormalities showed no significant difference (OR 1.96; 95% CI 0.92–4.14). No results were reported for cryo IVF versus fresh IVF.

Growth

Two studies reported on growth (Wennerholm et al., 1998; Nakajo et al., 2004). In the Swedish study 255 children from cryopreserved embryos were matched with 255 children born after IVF with fresh embryos and 252 children from spontaneous pregnancies (Wennerholm et al., 1998). The children were followed up to 18 months, and growth was normal and similar between groups. In the questionnaire study by Nakajo, the response rate was 73.4% (483/658) and included 343 children born after ICSI, 78 born after IVF and 81 after cryopreservation (Nakajo et al., 2004). The children were followed up to 2 years. For singletons, growth was similar to that in naturally conceived controls irrespective of ART method. The growth of IVF, ICSI and cryo multiples was significantly delayed as compared with naturally conceived singletons but had caught up by the age of 6 months after which it was similar.

Childhood morbidity and mental development

In the Kallen register study of 16,280 children born after IVF with a median follow-up time of 5.5 years, 1474 children were born after cryopreservation (Kallen et al., 2005c). Three of the cryo children had a cancer diagnosis (2 central nervous system malignancies and 1 histocytosis), while 1.94 were expected (EpC, 2009, personal communication). In the other Swedish study (Wennerholm et al., 1998)
### Table III  Perinatal mortality of singletons and twins after transfer of frozen and fresh IVF and ICSI early cleavage stage embryos

<table>
<thead>
<tr>
<th>References, country</th>
<th>Cryo infants (IVF/ICSI)</th>
<th>Fresh infants (IVF/ICSI and/or NC)</th>
<th>Cryo singletons stillbirth, n (%)</th>
<th>Fresh singletons stillbirth, n (%)</th>
<th>OR/AOR (95% CI) and/or P-value</th>
<th>Cryo singletons PNM, n (%)</th>
<th>Fresh singletons PNM, n (%)</th>
<th>OR/AOR (95% CI) and/or P-value</th>
<th>Cryo twins stillbirth, n (%)</th>
<th>Fresh twins stillbirth, n (%)</th>
<th>OR/AOR (95% CI) and/or P-value</th>
<th>Cryo twins PNM, n (%)</th>
<th>Fresh twins PNM, n (%)</th>
<th>OR/AOR (95% CI) and/or P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wada et al. (1994), UK</td>
<td>IVF: 177 singleton; 78 twins</td>
<td>IVF: 527 Singleton; 262 twins</td>
<td>0</td>
<td>NA</td>
<td>2/185 (10.8)</td>
<td>8/592 (13.5)</td>
<td>P &gt; 0.05</td>
<td>0</td>
<td>NA</td>
<td>4/86 (46.5)</td>
<td>11/288 (38.2)</td>
<td>P &gt; 0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shih et al. (2008), Australia</td>
<td>2387 cryo IVF/ICSI singleton first births; 429 sets of IVF/ICSI twins</td>
<td>3110 Fresh IVF/ICSI singleton first births; 835 sets of IVF/ICSI twins</td>
<td>NA</td>
<td>NA</td>
<td>NA (10)*</td>
<td>NA (18)*</td>
<td>0.027</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Belva et al. (2008), Belgium</td>
<td>390 IVF (284 singletons, 100 twins)</td>
<td>2955 IVF (1556 singletons, 1250 twins)</td>
<td>5/284</td>
<td>6/1556</td>
<td>2.41 (1.91–3.05)b</td>
<td>NA</td>
<td>12/1553c</td>
<td>d</td>
<td>2/100</td>
<td>30/1289c</td>
<td>NA</td>
<td>50/1289c</td>
<td>d</td>
<td></td>
</tr>
<tr>
<td></td>
<td>547 ICSI (384 singletons, 160 twins)</td>
<td>2840 ICSI (1499 singletons, 1228 twins)</td>
<td>2/384</td>
<td>18/1499</td>
<td>2.11 (1.73–2.57)c</td>
<td>NA</td>
<td>19/1513c</td>
<td>f</td>
<td>1/160</td>
<td>26/1259c</td>
<td>NA</td>
<td>30/1259c</td>
<td>f</td>
<td></td>
</tr>
</tbody>
</table>

PNM perinatal mortality.

*Includes stillbirths >19 weeks of gestation and neonatal deaths (within 28 days of birth).

Fresh IVF embryos = 1.0.

Data from Bonduelle et al., Hum Reprod 2002.

Personal communication M. Bonduelle: perinatal mortality for singletons and twins: cryo IVF versus fresh IVF 3% versus 2.3% (70/2995) (P = 0.41).

Personal communication M. Bonduelle: perinatal mortality for singletons and twins: cryo ICSI versus fresh ICSI 2.3% versus 1.9% (54/2889) (P = 0.48).
<table>
<thead>
<tr>
<th>Reference, country</th>
<th>Cryo infants (IVF/ICSI and/or NC)</th>
<th>Fresh infants (IVF/ICSI and/or NC)</th>
<th>Cryo singletons birth defects, n (%)</th>
<th>Fresh singletons birth defects, n (%)</th>
<th>OR/ AOR (95% CI)/ P-value</th>
<th>Cryo twins/ multiples birth defects, n (%)</th>
<th>Fresh twins birth defects, n (%)</th>
<th>OR/ AOR (95% CI)/ P-value</th>
<th>Cryo all (singletons and multiples), birth defects, n (%)</th>
<th>Fresh all (singletons and multiples), birth defects, n (%)</th>
<th>OR/AOR (95% CI)/ P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SART (1993), USA</td>
<td>431 deliveries, 79% singletons</td>
<td>3215 deliveries, 70% singletons</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>SART (1994), USA</td>
<td>619 deliveries, 78% singletons</td>
<td>4206 deliveries, 67% singletons</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Wada et al. (1994), UK</td>
<td>IVF: 177 singletons, 78 twins</td>
<td>IVF: 527 singletons 262 twins</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>2/283 (0.7)</td>
<td>32/961 (3.3)</td>
<td>&gt;0.05</td>
<td>NA</td>
</tr>
<tr>
<td>Sutcliffe et al. (1995b), UK</td>
<td>68 singletons, 20 twins, 3 triplets</td>
<td>81 NC singletons, 2 NC twins</td>
<td>Major 2/68 (2.9) Minor 22/68 (32.4)</td>
<td>Major 2/81 (2.5) Minor 18/81 (22.2)</td>
<td>1.2 (0.2–8.7) Minor 1.7 (0.8–3.5)</td>
<td>Major 1/23 (4.3%) Minor 7/23 (30.4)</td>
<td>Major 0 _</td>
<td>1.8</td>
<td>2.3</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>SART (1995), USA</td>
<td>791 deliveries, 70% singletons</td>
<td>5103 deliveries, 66% singletons</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>SART (1996), USA</td>
<td>1076 deliveries, 76% singletons</td>
<td>4912 deliveries, 64% singletons</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>2.6</td>
<td>2.7</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>De Mouzon and Lancaster (1997)</td>
<td>2005 IVF: 19 869 ICSI: 3325</td>
<td>4654 deliveries, 64% singletons; ICSI: 1185 deliveries, 64% singletons</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>2.0</td>
<td>IVF 2.1, ICSI 2.7</td>
<td>NA</td>
</tr>
<tr>
<td>SART (1998), USA</td>
<td>1136 deliveries, 77% singletons</td>
<td>IVF: 6754 deliveries, 64% singletons; ICSI: 1185 deliveries, 64% singletons</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>1.0</td>
<td>IVF 0.7, ICSI 1.1</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>SART (1999), USA</td>
<td>1457 deliveries, 73% singletons</td>
<td>IVF: 6379 deliveries, 60% singletons, ICSI: 3632 deliveries, 62% singletons</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>1.9</td>
<td>IVF 1.8, ICSI 1.8</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>
Westergaard et al. (1999), Denmark
- 105a IVF, 180 ICSI, 47 EDa, 2245 NCa
- IVF: 94/1913 (4.9); ICSI: 3/18 (1.7); ED: 7/47(14.9); NC: 103 /2245 (4.6)

SART (2000), USA
- 1719 deliveries, 74% singletons
- IVF: 7353 deliveries, 60% singletons; ICSI: 4949 deliveries, 63% singletons
- IVF: 10 228a; ICSI: 4530a
- 81/1055 (7.7); 392/4530 (8.7)

Kallen et al. (2005b), Sweden
- IVF: 1055a; ICSI: 419a
- IVF: 10 228a; ICSI: 4530a
- 832/10 228 (8.1); 392/4530 (8.7)

Olson et al. (2005), USA
- 335a
- 236 IVFa; 476 ICSIa; 415 ZIFTa
- 8/335a (2.4)

Desai et al. (2007), USA
- 11 deliveries
- 3110 fresh IVF/ICSI first births; 825 sets of IVF/ICSI twins
- 2.3

Shih et al. (2008), Australia
- 2387 cryo IVF/ICSI singleton first births; 429 sets of IVF/ICSI twins
- 2.6

Belva et al. (2008), Belgium
- 390 IVF (284 singletons, 106 multiples)
- 2955 IVF (1556 singletons, 1399 multiples)
- 6/284 (2.1) 49/1556 (3.1) 0.67 (0.28–1.56)a

Rama Raju (2008), India
- 89 cryo IVF/ICSI infants (50 singletons, 36 twins, 3 triplets)
- 216 fresh IVF/ICSI infants (118 singletons, 92 twins, 6 triplets)
- 6/106 (5.7) 63/1399 (4.5) 1.27 (0.54–3.01)c

Children born after cryopreservation of embryos or oocytes

**Notes:**
- ED oocyte donation.
- *Singletons and multiples.
- Fresh IVF = 1.00, adjusted for year of birth, maternal age and number of infants in birth.
- Fresh IVF embryos = 1.0.
- Fresh ICSI embryos = 1.0.
the prevalence of chronic disease at 18 months of age was similar in the cryopreserved, fresh IVF and naturally conceived groups.

Only one study reported on mental development and clinical neurological assessment including squint and hearing loss (Sutcliffe et al., 1995b). In the UK study 91 cryo children and 83 naturally conceived controls had their development assessed (using the Griffiths’s scales of mental development) at the ages of 25 and 29 months, respectively. The authors concluded that they only found small differences and that the overall development of cryo children was not a cause for concern.

Slow freezing and vitrification of blastocysts

Although there are several studies reporting on up to 60 ongoing pregnancies and deliveries after slow freezing of blastocysts, we could identify only four case reports presenting some outcome data on a total of five children (Rosenlund et al., 2002; Quintans et al., 2003; Sills et al., 2003; Smith et al., 2005). Birthweight of the singletons was 2832–3005 g and that of one set of dizygotic twins 1304 g/1559 g. Only one of these children had been karyotyped, with a normal result (Rosenlund et al., 2002).

Four retrospective studies and four case reports have presented information on 252 children born after vitrification of blastocysts (Choi et al., 2000; Yokota et al., 2001; Son et al., 2003; Takahashi et al., 2005; Hiraoka et al., 2006; Mukaida et al., 2006; Hiraoka et al., 2007; Parriego et al., 2007). Data for these studies are presented in Supplementary Table S1. Takahashi et al. (2005) presented neonatal data on 147 infants born after vitrification using cryoloop. There were no statistical differences in the mean gestational age, birthweight, preterm birth rate, or congenital birth defect rates as compared with fresh blastocyst transfer. The frequency of preterm deliveries was 18.5% and that of low birthweight was 43.5% among all children born (Takahashi et al., 2005).

Slow freezing and vitrification of oocytes

Altogether 22 papers, including 11 case reports and a total of 148 children, presented at least some information on neonatal health of children born after slow freezing of oocytes (Porcu et al., 2000; Quintans et al., 2002; Fosas et al., 2003; Kan et al., 2004; Borini et al., 2004; Notrica et al., 2004; Azambuja et al., 2005; Chen et al., 2005; Levi Setti et al., 2005, 2006; Li et al., 2005; Tjer et al., 2005; La Sala et al., 2006; Montag et al., 2006; Bianchi et al., 2007; Borini et al., 2007; De Geyter et al., 2007; De Santis et al., 2007; Gook et al., 2007; Konc et al., 2007; Yang et al., 2007; Greco et al., 2008). Data for these studies are presented in Supplementary Table S2. In only three of the 11 retrospective studies, and in 9 of 11 case reports, were data on birthweight of the children presented and it was consistently found to be within normal ranges. Karyotype examination was carried out in about one fourth of the children and it was normal in all cases. In most studies the only information given about children was ‘healthy’.

After vitrification of oocytes there was neonatal data on 221 infants (Kuleshova et al., 1999; Katayama et al., 2003; Yoon et al., 2003; Kuwayama et al., 2005; Kyono et al., 2005; Antinori et al., 2007; Chen et al., 2008; Chian et al., 2008). Data for these studies are presented in Supplementary Table S3. In the largest study, Chian et al. (2008) reported data on 200 children. The mean birthweight was 2920 g for singletons and 2231 g for multiples. The low birthweight rate among singletons was 18% and among multiples 80%. The premature delivery rate was 26% for singletons and 71% for multiple pregnancies. The incidence of congenital malformations was 2.5%. There was no control group.

Discussion

The overall aim of this systematic review was to summarize data on children conceived and delivered using ART with frozen, thawed embryos or oocytes and to compare this data with children born from fresh IVF and/or children born after spontaneous conception.

For early cleavage embryos, data from controlled studies (Kallen et al., 2005a; Wang et al., 2005; Shih et al., 2008) indicate a better or at least as good obstetric outcome, measured as preterm birth and low birthweight /very low birthweight for cryo children as compared with children born after fresh cycles. However, a recent register study using data from the New Zealand Assisted Reproduction Technology Database and published only as an abstract (Wang et al., 2008) showed that transferred fresh blastocysts and thawed cleavage embryos, cultured and transferred as blastocysts had a higher rate of ‘the best perinatal outcome’ (defined as the rate of live born singletons with a birthweight ≤2500 g and a survival more than 28 days) compared with all other types of transferred embryos. A recent study from Belgium (Belva et al., 2008) including 547 cryo ICSI and 390 cryo IVF children showed that cryo ICSI twins had significantly higher preterm birth and very low birthweight rates than twins from fresh ICSI, although other obstetric outcomes were comparable. Furthermore, a higher rate of malformations was noticed for cryo ICSI as compared with fresh ICSI, and also as compared with cryo IVF. Large registry studies are needed to address infrequent outcomes, such as birth defects. In population based registry studies from Sweden (Kallen et al., 2005b), Australia (Shih et al., 2008) and the USA (SART, 1993, 1994, 1995, 1996, 1998, 1999, 2000) no differences in malformation rates were found between cryo children and children from fresh transfer although the US studies did not distinguish between singletons and twins. In addition, two recent large registry studies, published only as abstracts, one from Denmark (Pinborg et al., 2008) and one from France (Royere et al., 2006) showed no difference in malformation rates between cryo children and children born after fresh transfer. The Belgian finding of a higher malformation rate in cryo ICSI children is an observation that warrants further attention, but it might be attributable to other factors. Time period for recruitment differed between cryo ICSI children and children born after fresh transfer and might have introduced a bias, as discussed by the authors. Only three small studies included children from spontaneous conception as controls (Sutcliffe et al., 1995a; Wennerholm et al., 1998; Westergaard et al., 1999; Sutcliffe, 2000). No differences were found in neonatal outcome or malformation rate, although it should be borne in mind that the number of children born after cryopreservation was small.

For growth, childhood morbidity and mental development the data are limited, with few differences found between cryo children and children born after fresh transfer, and indicating no causes for concern.

The reason for better outcome for children born after cryopreservation as compared with children born after fresh transfer in most studies is not known. Mechanisms that have been discussed include
adverse effect of hormone stimulation in fresh cycles and selection of women as well as embryos. Embryos surviving freezing and thawing might be of better quality than fresh embryos, and this may have a positive influence on child outcome.

Vitrification as a method for freezing has increased greatly in use in recent years, particularly for freezing of blastocysts and oocytes. Better post-thaw survival rates and encouraging pregnancy rates as compared with slow freezing of embryos have been demonstrated (Loutradi et al., 2008). Most studies after vitrification of blastocysts are case reports or small case series without data on infant outcome. We identified only four retrospective studies and four case reports, which included information on 252 children. The largest study (Takahashi et al., 2005) showed no differences in obstetric outcomes for children born after vitrified blastocysts compared with children born after fresh blastocysts but a preterm birth rate of 18.5% and low birthweight rate of 43.5% among all children in the vitrified group is worth noting.

Data on children born after freezing of oocytes, both slow freezing and vitrification, is also sparse. We found some neonatal information on a total of 148 children born after slow freezing of oocytes and 221 children born after vitrification of oocytes. The very limited data published about the children showed birthweights within the normal range. The largest study on vitrified oocytes (Chian et al., 2008) including 200 children found a preterm birth rate of 26% among singletons, which is two to three times higher than that reported for slow freezing of embryos.

In conclusion, cryopreservation of oocytes and spare embryos has gained increased importance in recent years, concomitant with the introduction of single embryo transfer and the increased demand to preserve oocytes for future use. Slow freezing of embryos has been used for 25 years and data concerning infant outcome seems reassuring with even higher birthweights and lower rates of preterm and low birthweights than children born after fresh IVF/ICSI. For the newly introduced technique of vitrification of blastocysts and oocytes, very limited data have been reported on obstetric and neonatal outcomes. This emphasizes the urgent need for properly controlled follow-up studies of neonatal outcome and a careful assessment of evidence currently available before these techniques are added to daily routines as discussed in a recent editorial in Human Reproduction (Van Steirteghem, 2008). In addition, long-term child follow-up studies are needed for all cryopreservation techniques.

Supplementary material
Supplementary data are available at http://humrep.oxfordjournals.org/.

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References


Bianchi V, Coticchio G, Distratiss V, Di Giusto N, Flamigni C, Borini A. Differential sucrose concentration during dehydration (0.2 mol/l) and rehydration (0.3 mol/l) increases the implantation rate of frozen human oocytes. Reprod Biomed Online 2007;14:64–71.


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