Commentary

Obstetric and perinatal outcome in 200 infants conceived from vitrified oocytes

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Abstract

Cryopreservation of oocytes by vitrification is a promising new technique for assisted human reproduction. Any new technical development must be accompanied with data concerning obstetric and perinatal outcome. This study analysed the obstetric and perinatal outcomes in 165 pregnancies and 200 infants conceived following oocyte vitrification cycles in three assisted reproduction centres. The results indicate that the mean birth weight and the incidence of congenital anomalies are comparable to that of spontaneous conceptions in fertile women or infertile women undergoing in-vitro fertilization treatment. These preliminary findings may provide reassuring evidence that pregnancies and infants conceived following oocyte vitrification are not associated with increased risk of adverse obstetric and perinatal outcomes.

Keywords: birth defect, birth weight, pregnancy, oocytes, vitrification

The development of an effective oocyte cryopreservation system will have a significant impact on clinical practice of reproductive medicine and assisted reproductive technology. An effective oocyte cryopreservation programme would be a useful treatment option for women who are at risk of losing their ovarian function because of pelvic diseases, surgery or radio/chemotherapy (Lobo, 2005). An effective oocyte cryopreservation programme will also benefit infertile couples with moral or religious objections about cryopreservation of embryos. In addition, a successful oocyte cryopreservation protocol would facilitate the logistics of coordinating egg donors with recipients.

Since the first live birth reported from frozen-thawed eggs (Chen, 1986), there have been limited numbers of live births achieved. Conventional slow-freezing method results relatively low egg survival and pregnancy rates (Oktay et al., 2006). Therefore, oocyte cryopreservation using the conventional slow-freezing method is considered an experimental procedure because of the inconsistent survival and pregnancy rates and the limited number of live births achieved (Practice Committee of American Society of Reproductive Medicine, 2004; Ethics Committee of American Society of Reproductive Medicine, 2005). In the last few years, modified slow-freezing methods (Fabbri et al., 2001; Stachecki and Cohen, 2004; Borini et al., 2004, 2006; Bianchi et al., 2005; Coticchio et al., 2005) and vitrification protocols (Kuleshova et al., 1999; Kuleshova and Lopata, 2002; Yoon et al., 2003; Chian et al., 2004, 2005; Kuwayama et al., 2005; Kyono et al., 2005; Koutlaki et al., 2006) have improved the survival rate of oocytes post-thawing. However, it has been indicated that, although the introduction of elevated dehydrating sucrose concentrations (Yang et al., 1998; Fabbri et al., 2001; Borini et al., 2004, 2006; Levi-Setti et al., 2006) and the use of sodium-depleted cryopreservation solution (Stachecki et al., 1998; Quintans et al., 2002; Boldt et al., 2003, 2006; Stachecki and Cohen, 2004; Azambuja et al., 2005) during slow-freezing procedure seems to increase the survival and fertilization rates of oocytes post-thawing, there is no well-controlled evidence of improved clinical outcome.

Recent advances in vitrification methods have led to egg survival rate over 85% and the pregnancy rates comparable to those achieved with fresh eggs (Katayama et al., 2003; Chian et al., 2005; Kuwayama et al., 2005; Lucena et al., 2006; Selman et al., 2006; Antinori et al., 2007; Yoon et al., 2007). The application of vitrification techniques to human oocytes has been questioned, indicating that it is in the absence of basic biological studies that address safety issues (Gook and Edgar, 2007). Indeed, reviewing current vitrification protocols found that relatively higher concentrations of cryoprotectants have been used to vitrify the oocytes, and the cytotoxicity of cryoprotectants and the osmotic changes in vitrification solutions are the main concern (Fahy, 2007). Recent animal model studies indicated that not only the survival rates of vitrified oocytes and blastocysts have improved but also the fertilization rate of vitrified oocytes has increased post-warming (Chian et al., 2004; Huang et al., 2007a; Larman et al., 2007).

More recently, it has been demonstrated that less damage was detected in the vitrified oocytes compared with slow-freezing oocytes in terms of meiotic spindle integrity and chromosome alignment (Huang et al., 2007a). Interestingly, it has been also found that vitrification of in-vitro matured oocytes results in high survival rates, normal meiotic spindle and chromosome alignment and no increased incidence of aneuploidy (Huang et al., 2007a).
Nevertheless, at the moment, the clinical efficiency and safety issue of vitrified oocytes cannot be precisely assessed because of the lack of well-controlled clinical trials (Gook and Edgar, 2007). Although it is important to assess viability and abnormality of the vitrified oocytes via embryology parameters (De Santis et al., 2007), the most important parameter to evaluate is the rate of healthy live births resulting from vitrified oocytes. However, there have been no data reported on obstetric and perinatal outcome for infants conceived with vitrified eggs.

The obstetric and perinatal outcomes in 165 pregnancies (200 infants) conceived from the vitrified eggs in three fertility treatment centres in Canada, Colombia and Mexico are reported here. For vitrification, mature eggs retrieved from women following controlled ovarian stimulation were first suspended in equilibration medium containing mixture of 7.5% ethylene glycol and 2-propanediol (PROH) or dimethylsulfoxide (DMSO) for 5 min and transferred to vitrification medium (15% ethylene glycol and PROH or 15% ethylene glycol and DMSO, plus 0.5 M sucrose) for 45–60 s at room temperature. The eggs were then loaded onto the McGill Cryoleaf or Cryotop and immediately plunged into liquid nitrogen for storage. After the eggs had been vitrified between 1 to 13 months, the eggs were warmed and inseminated by intracytoplasmic sperm injection, and the resulting embryos were transferred. The following outcome measures were analysed: pregnancy complications, number of multiple pregnancies, mode of delivery, gestational age at delivery, birth weight, Apgar scores and incidence of congenital anomalies.

As shown in Table 1, there was a total of 165 women with a mean age of 34 ± 0.5 years who achieved pregnancy following

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All pregnancies &lt;br&gt; (n = 165)</th>
<th>Singleton pregnancies &lt;br&gt; (n = 137)</th>
<th>Multiple gestation pregnancies &lt;br&gt; (n = 28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean gestational age &lt;br&gt; (weeks + days)</td>
<td>37 ± 1</td>
<td>37 ± 3</td>
<td>35 ± 5</td>
</tr>
<tr>
<td>No. of deliveries at &lt;br&gt;34–37 weeks (%)</td>
<td>46 (30)</td>
<td>30 (22)</td>
<td>16 (57)</td>
</tr>
<tr>
<td>No. of deliveries at &lt;br&gt;&lt;34 weeks (%)</td>
<td>10 (6)</td>
<td>6 (4)</td>
<td>4 (14)</td>
</tr>
<tr>
<td>Birth weight (mean ± SEM) (g)</td>
<td>2784 ± 37</td>
<td>2920 ± 37</td>
<td>2231 ± 55</td>
</tr>
<tr>
<td>No. of LBW (%)</td>
<td>68 (34)</td>
<td>24 (17)</td>
<td>44 (74)</td>
</tr>
<tr>
<td>No. of VLBW (%)</td>
<td>4 (2)</td>
<td>1 (0.7)</td>
<td>3 (5)</td>
</tr>
<tr>
<td>Median Apgar score at 1 min</td>
<td>8</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Median Apgar score at 5 min</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

**Incidence of congenital anomalies**

<table>
<thead>
<tr>
<th>Anomaly</th>
<th>All newborns &lt;br&gt; (n = 200)</th>
<th>Singleton newborns &lt;br&gt; (n = 141)</th>
<th>Multiple gestation newborns &lt;br&gt; (n = 59)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biliary atresia</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Club foot</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Skin hemangioma</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Ventricular septal defect</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total (%)</td>
<td>5 (2.5)</td>
<td>2 (1.4)</td>
<td>3 (5.1)</td>
</tr>
</tbody>
</table>

LBW = low birth weight, 1500–2500 g; VLBW = very low birth weight, <1500 g.
SEM = standard error of mean
embryo transfer. The multiple pregnancy rate was 17% (26 sets of twins and two of triplets). The Caesarean section rate in singleton pregnancies was 37% (n = 51), compared with 96% in those with multiple pregnancies (n = 27). The mean birth weight was 2920 ± 37 g for singletons and 2253 ± 55 g for multiples. The incidence of congenital anomalies in this cohort of newborns was 2.5% (two ventricular septal defects, one biliary atresia, one club foot and one skin hemangioma), which is comparable to that of spontaneous conceptions in fertile women or infertile women undergoing in-vitro fertilization treatment (Tan et al., 1992).

These preliminary findings may provide reassuring evidence that pregnancies and infants conceived following egg vitrification are not associated with increased risk of adverse obstetric and perinatal outcomes.

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