Does the culture medium used during IVF treatment affect twin birth weight discordance?

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Perinatal outcome of singletons after IVF or ICSI

Systematic review (17 studies; 14 matched controls)
Comparison of natural and assisted conceptions

- Preterm delivery (37 wk) RR 2.0 (1.8-2.3)
- Low birth weight (< 2.5 kg) RR 1.7 (1.5-1.9)
- Very low birth weight (< 1.5 kg) RR 3.0 (2.0-4.4)
- SGA (birth weight <10th centile for gestation) RR 1.5 (1.4-1.7)
- Perinatal mortality RR 1.7 (1.3-2.6)

Possible causes for the worse perinatal outcome after IVF/ICSI

- Specific feature of the subfertile population
- Influence of hormonal stimulation
- Influence of in-vitro culture
In vitro-culture

- Large offspring syndrome is seen after exposure of bovine and ovine embryos to a variety of unusual environments prior to the blastocyst stage
- Main characteristic is increased fetal growth; newborns frequently have a 2-fold or more increase in weight
- Most culture systems with which the LOS has been associated, involved the use of serum

Young et al., Rev Reprod 1998

Effect of serum on fetal weight

- Culture of preimplantation mouse embryos in a chemically defined medium (M16) with or without calf serum
- Comparison between fetuses of the two different groups dissected from both uterine horns from the same recipient female at E14

Khosla et al., Biology of Reproduction 2001

Aim of present study

Effects of in vitro culture on human birth weight
- Singleton pregnancies
- Twin pregnancies
Twin discordance

- In the present study, we examine the effect of the culture medium used during IVF treatment on twin birth weight discordance.

- Birth weight discordance in twins is associated with adverse perinatal outcome (Amaru et al., Obstet Gynecol 2004; Branum et al., Obstet Gynecol 2003; Demissie et al., Obstet Gynecol 2002).

- Defined as >25% difference in birth weight of the 2 children.

In-vitro culture

- For our internal quality control program our IVF lab uses 2 distinct culture media systems for the IVF/ICSI procedure.

- Therefore, we are able to compare the influence of different culture media on the perinatal outcome of pregnancies.

M&M


- Culture media used:
  - Vitrolife (Göteborg, Sweden): IVF-50 and G1 version 3
  - Cook (Brisbane, Australia): K-SIFM and K-SICM

- Assignment:
  - Strictly alternating
  - Day before OPU
  - By lab technicians, unaware of patient characteristics or treatment cycles.
Exactly the same ovarian stimulation, fertilization, culture and embryo transfer procedures were used in both groups

Dichorionic twin pregnancies with live-born children after fresh embryo transfer following IVF/ICSI

Results

Vitrolife
- 716 ovum pick ups
- 198 births with liveborn children
- 178 singletons

Cook
- 717 ovum pick ups
- 153 births with liveborn children
- 129 singletons

Maternal and paternal height, weight, BMI, age and smoking habits (≥10 sigarettes/day) were similar between the study groups

<table>
<thead>
<tr>
<th></th>
<th>Vitrolife (n=20)</th>
<th>Cook (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycles with ICSI (% of total)</td>
<td>10 (50%)</td>
<td>16 (67%)</td>
</tr>
<tr>
<td>Maternal age in years (± SEM)</td>
<td>33.3 (± 0.9)</td>
<td>32.7 (± 1.0)</td>
</tr>
<tr>
<td>Gestational age in weeks (± SEM)</td>
<td>36.3 (± 0.6)</td>
<td>36.5 (± 0.5)</td>
</tr>
</tbody>
</table>
Results

Mean birth weight of 88 children of 44 twin pregnancies resulting from cultures in Vizualice or Cook

<table>
<thead>
<tr>
<th></th>
<th>Vizualice</th>
<th>Cook</th>
</tr>
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<tbody>
<tr>
<td>Mean</td>
<td>2.645 ± 0.72</td>
<td>2.65 ± 0.66</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>0.65</td>
<td>0.68</td>
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<td>NS</td>
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Results

Mean birth weight of 88 children adjusted for gestational age and gender (t-score)

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<thead>
<tr>
<th></th>
<th>Vizualice</th>
<th>Cook</th>
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<tbody>
<tr>
<td>Mean</td>
<td>-1.0 ± 1.3</td>
<td>-0.11 ± 1.4</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>1.2</td>
<td>1.4</td>
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Results

Mean weight difference between both children of 44 twin pregnancies in both study groups

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<thead>
<tr>
<th></th>
<th>Vizualice</th>
<th>Cook</th>
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<tbody>
<tr>
<td>Mean</td>
<td>0.47 ± 0.25</td>
<td>0.49 ± 0.25</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>0.25</td>
<td>0.25</td>
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<tr>
<td>NS</td>
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</tbody>
</table>
Results

| Number of discordant twins in both study groups (≥25% difference in birth weight) |
|---------------------------------|---|---|---|
|                                 | Vitrolife | Cook |
| p<0.05 Fisher’s exact test      | 14        | 6   |

Discussion

- To our knowledge, this is the first study evaluating the influence of culture medium on growth discordance in twin pregnancies.
- Imprinted genes play key roles in the control of fetal growth. Altered imprinting can cause growth defects.
- Several animal studies have established a relationship between embryo culture and disrupted expression of imprinted genes.

Discussion

- In vitro culture of preimplantation mouse embryos affects the expression of imprinted genes.

Khosla et al., Biology of Reproduction 2001
Discussion

- Considering increasing numbers of IVF/ICSI children worldwide (~ 46,000 in Europe in 2004*), research to optimize culture media for IVF procedures is very important.
- Larger studies are needed
- Our results indicate a significant relationship between the type of medium used to culture embryos during the first few days after fertilization and twin birth weight discordance.

* Nyboe Anderson et al., Hum Reprod 2008

Cause

Disturbed epigenetic mechanisms?