Maternal Diabesity, Oocyte Quality and Reproductive Outcomes

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Financial Disclosure

◊ Member, Scientific Advisory Board of Ovascience

Global obesity trends among adults
Obesity and Women

◊ Over 35% of all reproductive age women in the US are obese and about 1/3 of them have some degree of glucose intolerance=Diabesity
◊ Diabesity poses specific health related consequences
◊ Diabetes, CVD, Cancer
◊ Ovulatory disorder
◊ Longer times to conception even if young and ovulatory
◊ Adverse reproductive outcomes

Obesity and Reproductive Outcomes

<table>
<thead>
<tr>
<th>Variable</th>
<th>Study/Reference</th>
<th>Odds Ratio (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obesity</td>
<td>Flegal et al.</td>
<td>3.2 (1.04–10.6)</td>
<td>0.03</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Robker et al.</td>
<td>1.6 (1.2–2.1)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Obesity and Reproductive Outcomes

<table>
<thead>
<tr>
<th>Congenital anomaly</th>
<th>Overweight</th>
<th>Obesity</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neural tube defects</td>
<td>1.2 (1.04–1.39)</td>
<td>1.8 (1.1–2.9)</td>
<td>0.001</td>
</tr>
<tr>
<td>Cardiac septal defect</td>
<td>1.1 (0.9–1.3)</td>
<td>1.8 (1.1–2.2)</td>
<td>0.001</td>
</tr>
<tr>
<td>Cleft lip and palate</td>
<td>1.0 (0.87–1.19)</td>
<td>1.2 (1.03–1.4)</td>
<td>0.02</td>
</tr>
<tr>
<td>Isolated Anal atresia</td>
<td>1.1 (0.9–1.3)</td>
<td>1.4 (1.1–1.9)</td>
<td>0.008</td>
</tr>
<tr>
<td>Craniofacial anomalies</td>
<td>1.2 (0.9–1.6)</td>
<td>1.7 (1.2–2.5)</td>
<td>0.01</td>
</tr>
<tr>
<td>Diaphragmatic hernia</td>
<td>0.6 (0.2–1.5)</td>
<td>0.8 (0.3–2.1)</td>
<td>0.02</td>
</tr>
<tr>
<td>Sacral agenesis</td>
<td>0.9 (0.3–2.4)</td>
<td>1.1 (0.4–2.9)</td>
<td>0.02</td>
</tr>
<tr>
<td>Hypertelorism</td>
<td>1.2 (0.9–1.7)</td>
<td>1.6 (1.1–2.9)</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Cesarean section rates: 13.5%
Obesity and Offspring Outcomes

Obesity and Adverse Pregnancy Outcomes: Proposed Mechanisms

Clinical evidence supporting oocyte as target:

- Failure to achieve a live birth increases with higher BMI, significantly with the use of autologous oocytes ($P < 0.0001$), and to a greater extent among women < 35 years of age ($P < 0.0001$).

- Higher BMI is associated with an increased failure to achieve a clinical intrauterine gestation; this risk was overcome with the use of DONOR OOCYTES.

Luke et al., Hum Reprod 2011
Maternal metabolism and the oocyte: Animal Model Data

Abnormal maternal physiology and the oocyte: Type 1 diabetes

- Animal models of Type 1 diabetes
  - Smaller oocytes, impaired maturation, increased granulosa cell apoptosis
  - Poor reproductive outcomes: growth restriction and congenital anomalies
  - Abnormal mitochondria morphology, mtDNA copy number, spindle defects/chromosome misalignment

Wang Q et al, Mol Endo 2009
Ratchford AM et al., A J Phys Endo Metab; 2007
Chang AS, et al, Endocrinology; 2005
Moley KH et al, J Reprod Fertil; 1991
Wang Q et al, PLoS ONE, 2010

Mitochondrial Ultrastructure of Diabetic Oocyte

Wang et al., 2009, Mol Endo, 23:1603-12
Metabolic Dysfunction in Diabetic Oocyte

Increased mtDNA Content in Diabetic Oocyte

Disrupted Mitochondrial Distribution in Diabetic Oocyte
Meiotic Defects in Diabetic Oocyte

Spindle/Chromosome (Defects: 19% vs. 5% Ctrl)

Aneuploidy in diabetic oocytes

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Min</th>
<th>Number of Cells</th>
<th>Nploidy</th>
<th>Aneuploidy</th>
<th>PMBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>General</td>
<td>43</td>
<td>43 (100.0%)</td>
<td>2 (4.7%)</td>
<td>0 (0.0%)</td>
<td></td>
</tr>
<tr>
<td>Diabetic</td>
<td>27</td>
<td>29 (100.0%)</td>
<td>10 (34.5%)</td>
<td>4 (13.8%)</td>
<td></td>
</tr>
</tbody>
</table>

*ns/PMBC: p=0.05; n=0.01; doubled; *p<0.001 versus controls.

Summary: Maternal diabetes causes

- Structural, spatial and metabolic dysfunction of mitochondria in oocytes;
- Spindle defects and chromosome misalignment result in aneuploid embryos;
- These defects in oocytes probably contribute to the reproductive problems experienced by type I diabetic women.
How does maternal diabetes exert its effects on the oocyte?

Maternal diabetes adversely impacts cumulus cells contributing to poor oocyte quality

Our evidence
- Glucose transporter expression and transport: Decreased
- Mitochondria status in cumulus cells: Abnormal
- Gap junction communication: Impaired

Wang et al, PLoS ONE, 2010

New data using fluorescent tagged glucose analog NDBG

Wang et al, Endocrinology 2012

Decreased live 6-NBDG uptake in diabetics

Wang et al, Endocrinology 2012
Live imaging to determine the link between glucose uptake, ATP and spindle formation

6-NBDG uptake was significantly lower and correlated with decreased ATP in the same oocyte

Strong correlation between decreased glucose uptake and abnormal spindles in diabetics only
Conclusion of diabetes work

- Maternal diabetes affects oocyte and cumulus cell via metabolic changes in part due to communication difficulties
- COCs are adversely affected by the diabetic environment which directly affects oocyte ATP and spindle formation
- This may be the cause of poor pregnancy outcome in these patients as well as others with high rates of poor outcomes

This may include oocyte from obese, PCOS and/or aged women

Obesity and Reproductive Outcomes: A High Fat Diet (HFD) Model of Obesity

| TABLE 1. Characteristics of female mice on a regular diet vs. a high-fat diet after 16 weeks of feeding |
|---------------------------------------------------|---------------------------------------------------|
|          | Regular diet | High-fat diet |
|          | (n = 100)    | (n = 100)     |
| Weight (g) | 22 ± 0.3     | 26 ± 0.3     |
| Cholesterol | 0.47 ± 0.17  | 0.93 ± 0.15  |

Results are expressed as mean ± SD.

*P < 0.05 vs. control.

*P < 0.01 vs. control.
Oocyte Size and Maturation

- Increased apoptosis: TUNEL nuclei—17.6 ± 1.2% Control vs 59.64 ± 2.2% Obese
- Smaller size: Significantly smaller diameter in GV and MII oocytes from Obese vs Control
- Delay to maturation: Significantly lower % of oocytes reaching GVBD in Obese vs Control

Obesity: altered oocyte mitochondria structure

Abnormal mitochondrial protein expression related to biogenesis and fission:

Altered Drp1 localization
Meiotic Spindle and Chromosomal Abnormalities

**Increased aneuploidy in oocytes from obese mice:**

How does obesity impact cumulus oocyte complex?
Obesity: altered cumulus cell mitochondria structure

Increased apoptosis in cumulus cells of Obese Mice

Obesity leads to insulin-resistance in cumulus cells
Embryo transfer studies

- Blastocysts have decreased IGF1R expression
- Transferred blastocyst stage embryo from obese to non-obese

Blastocyst transfer studies from HFD to control

<table>
<thead>
<tr>
<th>CRL Embryo</th>
<th>Placenta Diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>HFD</td>
<td>*</td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>HFD</td>
<td>*</td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
</tbody>
</table>

*P<0.001

Luzzo et al, unpublished

Abnormal ventricle and choroid plexus development

10/10 dams
87 pups

4/10 mice
27 pups

Luzzo et al, unpublished
Embryo transfer studies

- One-cell zygote transfer from obese to non-obese mouse

One cell zygote transfer—fetal growth lag

Collaboration with Sasson and Simmons from UPenn

Smaller pups at birth and 3 weeks of age
Obesity and Offspring Growth

• At 13 weeks pups from obese mice: glucose intolerance, increased cholesterol, and higher body fat %.
• Suggests early development of metabolic syndrome

Conclusions

✦ Maternal diabetes has adverse effects on pregnancy as early as the oocyte maturation step

✦ Energy metabolism of the oocyte is compromised, possibly due to metabolic perturbation in the cumulus cells

Conclusions

✦ Energy depletion results in abnormal spindle formation and chromosome misalignment which may manifest as miscarriages in diabetic women
✦ Mitochondrial dysfunction may carry over to the next generation resulting in malformations, growth retardation and metabolic syndrome in the offspring
Summary

Maternal metabolic derangement

Cumulus Cells

Mitochondria

TCA Metabolism

Apoptosis

Transfer

Energy source

Meiotic defects

TCA Metabolism

Mitochondrial Dysfunction

Imprinting disorder

Oocyte

Anomalously abnormal mitochondria

Metabolic dysfunction

Embryo

Reproductive Problems

Long term effects on offspring

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Qiang Wang
Maggie Chi

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An intracellular pathway for glucose transport into mouse oocytes

Wang et al, AJP Endo Metab, 2012

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CB blocks glucose transport into CC, not oocytes

Wang et al, AJP Endo Metab, 2012

CBX blocks transport into oocyte, not CC

Wang et al, AJP Endo Metab, 2012

Oocyte glucose levels are increased in diabetic oocytes

Wang et al, AJP Endo Metab, 2012
Conclusions

Wang et al, AJF Endo Meth, 2012