The Intrafollicular Environment: Effects on Ovulation Induction Outcome

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Oocyte Developmental Competence

- Ability of the oocyte to complete meiosis and undergo fertilization, embryogenesis and term development
- Requires nuclear and cytoplasmic maturation
- Acquired gradually throughout follicular development through complex endocrine and paracrine mechanisms

Schramm RD et al. Hum Reprod 1999;14:2544
Thomas FH et al. Reprod Biol Endocrinol 2006;4:19
Factors Affecting Oocyte Development

- **Endocrine factors** interact to create a changing intrafollicular environment, in which a proper sequence of events dictates oocyte development.

- **Paracrine interactions** control energy utilization, follicle growth and oocyte development.

- The **oocyte** controls its own developmental fate by regulating granulosa cell proliferation, differentiation and steroidogenesis.

Schramm RD et al. Hum Reprod 1999;14:2544
Thomas FH et al. Reprod Biol Endocrinol 2006;4:19
Reproductive Abnormalities and the Intrafollicular Environment

- Polycystic ovary syndrome (Hyperandrogenism)
- Adiposity-dependent insulin resistance (hyperinsulinemia)
- Diminished ovarian reserve (hypoestrogenism/elevated FSH)
Elevated serum free androgen levels predict a reduced ongoing pregnancy rate.

Testosterone treatment of immature mouse oocytes impairs both maturation *in vitro* and subsequent embryonic development.

Insulin resistance also lowers the conception rate.

Impaired fertilization of PCOS oocytes can occur without gross chromosomal abnormalities or nuclear immaturity.

After IVF with ICSI, increased miscarriage rate can occur in lean PCOS patients.

Controlling for PCOS, insulin resistance also increases the risk for miscarriage after IVF.

High miscarriage rate in obese PCOS patients follows transfer of normal-appearing embryos into a surrogate uterus.

Maturation and Development of Human Oocytes

**Maturation**

![Bar chart showing maturation rates of human oocytes with E2-BSA and BSA](chart1)

**Development**

![Bar chart showing development rates of human oocytes with E2-BSA and BSA](chart2)

Tesarik J et al. JCEM 1995;80:1438
Steroid Effects on the Human GV Oocyte

- E2 supports a nongenomic, calcium-mediated mechanism of cytoplasmic maturation in the immature oocyte.

- The E2/androgen ratio to which oocytes are exposed during follicle growth affects the quality of mature human oocytes obtained through IVF.

- PCOS oocytes obtained from hyperandrogenic follicles and matured in vitro have impaired embryonic development.

- Pregnancy outcome by IVF is related more to the E2/androgen ratio than to the absolute amount of E2 in the follicle.

<table>
<thead>
<tr>
<th></th>
<th>Normal (N=30)</th>
<th>PCOS (N=11)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>bioLH (ng/mg)</td>
<td>0.5±0.3</td>
<td>0.4±0.2</td>
<td>0.7</td>
</tr>
<tr>
<td>17OHP₄ (ng/mg)</td>
<td>11.4±4.0</td>
<td>12.2±4.1</td>
<td>0.6</td>
</tr>
<tr>
<td>DHEA (ng/mg)</td>
<td>0.03±0.02</td>
<td>0.07±0.1</td>
<td>0.06</td>
</tr>
<tr>
<td>A₄ (ng/mg)</td>
<td>0.2±0.07</td>
<td>0.9±1.7</td>
<td>0.006</td>
</tr>
<tr>
<td>T (pg/mg)</td>
<td>26.8±12.3</td>
<td>65.1±65.4</td>
<td>0.001</td>
</tr>
<tr>
<td>DHT (pg/mg)</td>
<td>21.9±17.7</td>
<td>30.1±24.1</td>
<td>0.2</td>
</tr>
<tr>
<td>E₂ (ng/mg)</td>
<td>3.5±2.0</td>
<td>5.0±2.6</td>
<td>0.1</td>
</tr>
<tr>
<td>iFSH (ng/mg)</td>
<td>4.0±2.0</td>
<td>2.7±1.0</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Foong SC et al. JCEM 2006;91:2327
Normal follicles | PCOS follicles

**Lactate** (mMol/L)

- **R² = 0.55**
- **P < 0.001**

- **R² = 0.72**
- **P = 0.004**

**Glucose** (mMol/L)

- **R² = 0.29**
- **P < 0.007**

- **R² = 0.55**
- **P < 0.025**

**P4** (uMol/L)

- **R² = 0.29**
- **P < 0.007**

- **R² = 0.55**
- **P < 0.025**
Human Metaphase II Oocyte
Wood J et al. JCEM 2007;92:705
Bidirectional Cumulus-Oocyte Signaling

- Growth
- Maturation
- Proliferation
- Expansion
- Steroidogenesis

Growth factors
- Energy Substrate
- Androgen
- Progesterone
- IGF-I/insulin

Oocyte
Cumulus cell

FSH
LH

GDF-9
BMP-15
The Cumulus-Oocyte Complex

- Coordinates follicle development with oocyte maturation.
- Provides energy substrate for oocyte meiotic resumption.
- Regulates oocyte transcription.
- Promotes nuclear and cytoplasmic maturation of the oocyte.

Sugiura K et al. Reprod Fertil Devel 2005;17:667-674
Androgen Receptor mRNA Expression in Follicles of IVF Patients
Insulin Levels in Follicles of IVF Patients

Phy J et al. JCEM 2004;89:3561
Dumesic DA et al. JCEM 2007;92:1438
IR mRNA Expression in Follicles of IVF Patients

Normal PCOS

28S

5.5

5.0

4.5

4.0

3.5

3.0

2.5

2.0

1.5

MG CC MG CC MG CC MG CC

IRA IRB IRA IRB

Numb e 3.5 3.0 2.5 2.0

Phy J et al. J CEM 2004;89:3561
Effect of Insulin on Mouse Oocyte Developmental Competency

Diminished Ovarian Reserve

- Reduced IVF pregnancy outcome independent of age that accompanies decreased oocyte numbers and increased amounts of FSH administered.

- Mechanisms include intrafollicular endocrine abnormalities, increased aneuploidy and inherent oocyte defects.

Gleicher N et al. Fertil Steril in press
Correlation Between Amounts of FSH Administered and Intrafollicular FSH Levels

P < 0.01
Intrafollicular Hormone Levels in Normal IVF Patients Based Upon Response to FSH

<table>
<thead>
<tr>
<th></th>
<th>High Responders (N=13)</th>
<th>Low Responders (N=10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>E₂ (ng/mg)</td>
<td>3.5 ± 1.7</td>
<td>2.2 ± 1.0</td>
<td>0.01</td>
</tr>
<tr>
<td>P (ng/mg)</td>
<td>197.5 ± 94.2</td>
<td>258.9 ± 136.1</td>
<td>0.0003</td>
</tr>
<tr>
<td>T (pg/mg)</td>
<td>28.8 ± 11.0</td>
<td>19.8 ± 4.3</td>
<td>0.002</td>
</tr>
<tr>
<td>17-OHP (ng/mg)</td>
<td>12.0 ± 4.3</td>
<td>10.6 ± 4.1</td>
<td>0.9</td>
</tr>
<tr>
<td>A (ng/mg)</td>
<td>0.2 ± 0.1</td>
<td>0.1 ± 0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>DHT (pg/mg)</td>
<td>24.5 ± 17.1</td>
<td>16.4 ± 3.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Insulin (µU/mg)</td>
<td>0.1 ± 0.04</td>
<td>0.1 ± 0.07</td>
<td>0.3</td>
</tr>
</tbody>
</table>

All data expressed as mean ± SD

Foong, SC et al. Fertil Steril 2005;83:1377-83
Increased FSH Availability

■ Animal studies
  ● Increases glucose utilization by cumulus-oocyte complexes.
  ● Accelerates oocyte maturation *in vitro* and increases chromosomal abnormalities.
  ● Induces chromosomal abnormalities *in vivo* after superovulation with FSH.

■ Human studies
  ● Increases embryo aneuploidy *in vivo* following ovarian stimulation for IVF.

Growth Differentiation Factor 9 (GDF-9); Bone Morphogenetic Protein 15 (BMP-15)

- Expressed by oocytes during early follicle development.
- Important for follicle growth beyond the primary stage.
- Involved with granulosa cell proliferation, differentiation and steroidogenesis.

Hreinsson JG et al. JCEM 2002; 87:316
Fortune JE. Am Reprod Sci 2003; 78:135
Growth Differentiation Factor 9 (GDF-9); Bone Morphogenetic Protein 15 (BMP15)

- In follicles of IVF patients, BMP-15 levels
  - positively correlate with E2 levels and negatively correlate with FSH concentrations,
  - predict oocyte fertilization and embryo development.

- In PCOS oocytes, GDF-9 mRNA levels are reduced in preantral and early antral follicle development.

Filho FLT et al. J Clin Endocrinol Metab 2002;87:1337-1344
Conclusion

- PCOS, obesity and diminished ovarian reserve induce their own intrafollicular abnormalities.
- These abnormalities may affect the oocyte directly or indirectly through cumulus-oocyte signaling.
- The degree to which the oocyte controls its own developmental fate remains unclear.
- Endocrine/paracrine factors and genes interact in women to promote oocyte developmental competence.
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