Developmental Programming: Impact of Prenatal Androgenization on Nonhuman and Human Primates

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Dec 13, 2011
Gender-Related Issues in Medicine
Economic Burden of PCOS in U.S. > $4 Billion Annually:

<table>
<thead>
<tr>
<th>Condition</th>
<th>Annual costs (in millions)</th>
<th>% of total costs</th>
</tr>
</thead>
<tbody>
<tr>
<td>For Initial Evaluation</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>99</td>
<td>2.3</td>
</tr>
<tr>
<td>For Treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Menstrual dysfunction</td>
<td>1,350</td>
<td>30.9</td>
</tr>
<tr>
<td>Infertility</td>
<td>533</td>
<td>12.2</td>
</tr>
<tr>
<td>Type 2 DM</td>
<td>1,766</td>
<td>40.4</td>
</tr>
<tr>
<td>Hirsutism</td>
<td>622</td>
<td>14.2</td>
</tr>
<tr>
<td>Total</td>
<td>4,370</td>
<td>100</td>
</tr>
</tbody>
</table>

DM = diabetes mellitus

Azziz 2005
Definition of PCOS
1990 National Institute of Health and Human Development Conference on PCOS

Hyperandrogenism and chronic anovulation, excluding other endocrinopathies (e.g., virilizing tumors, nonclassical congenital adrenal hyperplasia, hyperprolactinemia, and Cushing's syndrome)

Zawadzki 1992
Definition of PCOS
2003 Revised Rotterdam Consensus
(2 out of 3 parameters)

1. Clinical/biochemical hyperandrogenism
2. Oligo- or anovulation
3. Polycystic ovaries

(excluding other endocrinopathies [e.g., virilizing tumors, nonclassical congenital adrenal hyperplasia, hyperprolactinemia, and Cushing's syndrome])
PCOS Phenotypes
2003 Revised Rotterdam Consensus

Androgen Excess

Severe 75%

Ov PCOS 14%

Ovulatory Dysfunction

Polycystic ovaries

Mild PCOS 11%

Abbott 2009

Ov = ovulatory
Origins of PCOS
The “Two Hit” Hypothesis

- Genetic or epigenetic factors reset reproductive and metabolic trajectories in early life, while
- Environmental influences later in life affect the severity of the adult PCOS phenotype.

Dumesic 2007
<table>
<thead>
<tr>
<th>Location</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spain</td>
<td>6.5%</td>
</tr>
<tr>
<td>Southeastern US</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>6.5%</td>
</tr>
<tr>
<td>White</td>
<td>8.0%</td>
</tr>
<tr>
<td></td>
<td>4.0%</td>
</tr>
<tr>
<td>Lesbos, Greece</td>
<td>6.8%</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>8.0%</td>
</tr>
<tr>
<td>Australia</td>
<td>8.7%</td>
</tr>
</tbody>
</table>

## Prevalence Among Mothers and Sisters of PCOS Probands

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Mothers</th>
<th>Sisters</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCOS</td>
<td>24%</td>
<td>32%</td>
</tr>
<tr>
<td>PCOS</td>
<td>8%</td>
<td>16%</td>
</tr>
<tr>
<td>PCOS</td>
<td></td>
<td>22%</td>
</tr>
<tr>
<td>Hyperandrogenism only</td>
<td></td>
<td>24%</td>
</tr>
</tbody>
</table>

Heritability of PCOS

- Twin studies show a strong genetic contribution to PCOS.
- Sons of PCOS women have higher body weight from infancy and develop insulin resistance and dyslipidemia as adults.
- Androgen overproduction is a feature of long-term cultured PCOS theca cells.

Genes Implicated with PCOS

- fibrillin-3 (FBN3)
- 17β-hydroxysteroid dehydrogenase type 6 (HSD17B6)
- pro-opiomelanocortin (POMC)
- activin receptor type-2A (ACVR2A)
- feminization-1 homolog b (FEM1B)
- small glutamine-rich tetratricopeptide-containing protein alpha (SGTA)
- GWAS-identified susceptibility foci, chromosomes 2 and 9

**PCOS may be an inherited disorder, with several genetic variants, each contributing a moderate effect, combined with risk-increasing lifestyle and environmental factors.**

GWAS = genome-wide association study

Evolutionary Genetics of PCOS

- Origins of PCOS may have begun in Paleolithic hunter-gatherer communities, in which environmental stress favored survival of individuals with the greatest capacity for energy storage to endure prolonged episodes of privation (the so-called “thrifty genotype”).

- PCOS phenotypic expression is likely the product of many different genes, with potentially different profiles in different populations.

Azziz 2011
Hypothalamic Sensitivity to Steroid Negative Feedback on LH Secretion

Controls

PCOS

LH = luteinizing hormone; P4 = progesterone

Pastor 1998
### PCOS-like Phenotype: Prenatally Androgenized (PA) Female Rhesus Monkeys and Sheep

<table>
<thead>
<tr>
<th></th>
<th>PA monkeys Early-treated</th>
<th>PA monkeys Late-treated</th>
<th>PA Sheep</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ovarian Hyperandrogenism</strong></td>
<td>Yes</td>
<td>Yes</td>
<td>Ovarian androgen upregulation</td>
</tr>
<tr>
<td><strong>Anovulation</strong></td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Enlarged polyfollicular ovaries</strong></td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>LH hypersecretion</strong></td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Reduced steroid negative feedback on LH</strong></td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Impaired embryonic development</strong></td>
<td>Yes</td>
<td>Yes</td>
<td>Impaired fertility</td>
</tr>
</tbody>
</table>

Dumesic D et al. 2007
Effect of PCOS on Body Fat Distribution

BMI = body mass index

Holte, 1994
Metabolic Syndrome (MBS) in 49 Adolescent PCOS Patients

- Adjusting for BMI, the odds of PCOS girls having MBS are:
  - 4.5 fold higher than that of girls in the normal population and
  - 3.8 fold higher of each quartile increase in plasma unbound testosterone.

Coviello 2006, Dumesic 1998
### PCOS-like Abnormalities in Prenatally Androgenized (PA) Female Rhesus Monkeys

<table>
<thead>
<tr>
<th></th>
<th>PA monkeys Early-treated</th>
<th>PA monkeys Late-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visceral obesity</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Insulin resistance</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>β cell impairement</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Glucose intolerance</td>
<td>Yes</td>
<td>yes</td>
</tr>
<tr>
<td>Increased type 2 Diabetes</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

Abbott D et al. 2007
Serum Insulin Levels in Dams and Female Fetuses

(a) Dams

(b) Fetuses

Backtransformed means ± 95% CI

TP = testosterone propionate; CI = confidence interval

Abbott 2010
40% of female human fetuses have elevated serum androgen levels.

Midgestational human fetal ovaries have 17 alpha hydroxylase and genes encoding steroidogenesis.

Umbilical vein testosterone levels are elevated in female infants born to mothers with PCOS.

Midgestational human fetal ovaries also have insulin, IGF-I/-II and steroid receptors.

Endocrine Antecedents to PCOS

- Elevated serum leptin levels in *newborns* of PCOS women positively correlate with birth weight and maternal BMI at mid-gestation.

- Elevated serum AMH levels occur in *infant daughters* of PCOS women.

- Enlarged ovaries and hyperinsulinemia exist in *female children* of PCOS women, along with LH hypersecretion and androgen excess in puberty.

- Premature adrenarche occurs in some groups of *PCOS girls*.

Environmental Factors: Nutrition

Obesity influences the adolescent PCOS phenotype by unmasking or amplifying symptoms of hyperinsulinemia and hyperandrogenism in susceptible individuals.

McCartney 2007, Franks 2008
Testosterone (pg/mL) and SHBG (nmol/liter) levels in relation to Tanner stage for obese and normal girls. Testosterone (T) and SHBG levels increase with Tanner stage, while insulin levels decrease. McCartney 2007

T = testosterone; SHGB = sex hormone-binding globulin
**Environmental Influences: Nutrition**

- LH → P450c17 → Androgens
- Insulin ↔ SHBG
- SHBG → Free T
- 5 alpha-reductase
- Androgen Excess → DHT

P450c17 = cytochrome P450 enzyme 17α-hydroxylase; DHT = dihydrotestosterone

Classification of Ovarian Follicles

**Preantral Growth**

- Primordial follicle
- Fully grown oocyte

**Gonadotropin-dependent Growth**

- Primary follicle
- Secondary follicle
- Graafian follicle
- Theca interna

**Classification**

- Primordial follicle
- Primary follicle
- Secondary follicle
- Early tertiary follicle
- Fully grown oocyte

**Gonadotropin**

- Gonadotropin-dependend growth

**Terms**

- Follicle
- Oocyte
- Theca interna
Ovarian Changes in Testosterone-Treated Adult Female Rhesus Monkeys

Vendola et al., J Clin Invest, 1998; 101: 2622-9
Mechanisms of Follicular Arrest in PCOS
(Small Antral Follicle Stage)

- Elevated levels of 5a-reduced androgens
  - Increased 5a-reduced androgen levels inhibit granulosa cell proliferation and aromatase activity \textit{in vitro}.

- Hyperinsulinemia
  - Insulin excess induces premature follicle luteinization, arresting cell proliferation and follicle growth.

- Abnormalities of TGF-\textbeta superfamily members
  - Increased AMH inhibits FSH-induced recruitment and follicle growth.

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
PCOS & IVF Pregnancy Outcome

- Impaired fertilization of PCOS oocytes occurs without gross chromosomal abnormalities or nuclear immaturity.
- Despite more oocytes with lower fertilization, PCOS and non-PCOS patients have similar rates of pregnancy & life birth/started IVF cycle.
- IVF patients with PCOS have a livebirth rate/IVF cycle no better than that of normal women despite increased numbers of oocytes retrieved.
- High miscarriage rate in obese PCOS patients follows transfer of normal-appearing embryos into a surrogate uterus.

Heijnen E et al. 2006; Cano F et al. 1997; Tian L et al. 2007; Sengoku K et al. 1997
Steroid Levels in Size-Matched Follicles of Normal Women and PCOS Patients

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Normal</th>
<th>PCOS</th>
<th>n</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2 (nmol/l)</td>
<td>311 (5-3939)</td>
<td>64 (14-328)</td>
<td>22</td>
<td>0.009</td>
</tr>
<tr>
<td>P (nmol/l)</td>
<td>318 (65-1347)</td>
<td>258 (94-718)</td>
<td>22</td>
<td>NS</td>
</tr>
<tr>
<td>AD (nmol/l)</td>
<td>3990 (1700-7250)</td>
<td>6165 (2919-14025)</td>
<td>22</td>
<td>0.005</td>
</tr>
<tr>
<td>T (nmol/l)</td>
<td>125 (10-390)</td>
<td>173 (23-455)</td>
<td>22</td>
<td>NS</td>
</tr>
</tbody>
</table>

Effects of 1 μM Estradiol/1μM Androstenedione on Intracellular Calcium in Human Germinal Vesicle Oocytes

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.

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

- Oocytes obtained from hyperandrogenic PCOS follicles and matured \textit{in vitro} have impaired embryonic development.

Tesarik J, Hum Reprod Update 1997;3:95
Barnes FL et al. Fertil Steril 1996;65:1151
Yding Andersen, \textit{JCEM} 1993;77:1227
# Intrafollicular Hormone Levels in Normal Women and PCOS Patients Undergoing IVF

<table>
<thead>
<tr>
<th>Hormone</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 et al. JCEM 2006;91:2327
PCA component 1
PCA component 2

Wood et al. JCEM 2007;92:705
Metabolic Abnormalities in Human Follicular Fluid

- Intrafollicular insulin levels are positively correlated with BMI and fasting serum insulin levels on the day of oocyte retrieval.
- Follicle fluid insulin levels are elevated in women with impaired glucose tolerance.
- Total free fatty acid levels in follicular fluid vary inversely with follicle development and oocyte quality.

Hyperinsulinemia: A Link Between PCOS and Obesity
Insulin Sensitivity in PCOS

* $p<0.001$ Ob vs Nob

** $p<0.001$ PCOS vs NL

Dunaif A 1997
# Adipocyte Size vs. Insulin Sensitivity in PCOS

(31 Age- and BMI-matched NL and PCOS women)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>PCOS</th>
<th>Controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m^2)</td>
<td>24.8 ± 4.8</td>
<td>24.7 ± 4.9</td>
<td>NS</td>
</tr>
<tr>
<td>SC abdominal adipose volume (liters)</td>
<td>3.7 ± 1.8</td>
<td>3.8 ± 2.0</td>
<td>NS</td>
</tr>
<tr>
<td>Visceral adipose volume (liters)</td>
<td>0.9 ± 0.5</td>
<td>0.8 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>GDR (mg/kg X min)</td>
<td>11.0 ± 3.0</td>
<td>13.0 ± 4.1</td>
<td>0.01</td>
</tr>
<tr>
<td>SC abdominal adipocyte volume (picoliter)</td>
<td>590 ± 187</td>
<td>536 ± 166</td>
<td>0.04</td>
</tr>
</tbody>
</table>

SC abdominal adipocyte size inversely correlated with insulin sensitivity

Manneras-Holm 2011
Adipose Tissue Expandability Theory

Maximum adipose expansion

Body Weight

Insulin Sensitivity

Proper adipocyte number, size, location and function

Improper adipocyte number, size, location or function

De Zegher 2009
Adipose tissue

Adipocytes

Adipose stem cells (ASCs)

Adipose tissue

Macrophage (ATM)

Self-renewal

ASCs

Preadipocytes

Small lipid drops

Cell differentiation during adipogenesis

Adipogenesis

Fat storage

Inflammation

Immature cells

Mature cells

Big cells

Cell death

ATMs

Signaling pathways involved in adipogenesis

ASCs

BMP4

Preadipocytes

Wnt and BMP4/2

Adipocytes
Effect of T without and with BMP4 on adipogenesis

A  

**PPARγ**

Fold change in gene expression

Unt  T  BMP4  BMP4 + T

*  **  ***

B  

**C/EBPα**

Fold change in gene expression

Unt  T  BMP4  BMP4 + T

**  ***
Deficiency of adipocyte lipid storage as one of the major cause of PCOS?
Il finito
Effect of T and DHT in adipogenesis

** p< 0.0001  (vs control)

* p< 0.03      (T+F vs T)

** p< 0.0001  (vs control)

* p< 0.03      (T+F vs T)

** p< 0.0001  (vs control)

* p< 0.02      (T+F vs T)
Effect of T in BMP4
# Effect of Body Mass On Pregnancy Outcome by IVF

<table>
<thead>
<tr>
<th>Body Mass Index</th>
<th>No. of women</th>
<th>% achieving ≥ one pregnancy&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underweight</td>
<td>&lt;20</td>
<td>441</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.65-1.01)</td>
</tr>
<tr>
<td>Moderate</td>
<td>20-24.9</td>
<td>1910</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Overweight</td>
<td>25-29.9</td>
<td>814</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.68-0.97)</td>
</tr>
<tr>
<td>Obese</td>
<td>30-34.9</td>
<td>304</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.57-0.95)</td>
</tr>
<tr>
<td>Very obese</td>
<td>≥35</td>
<td>117</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.32–0.95)</td>
</tr>
</tbody>
</table>

<sup>a</sup><sup>p</sup> <0.001

Wang JX et al. 2000
Change in PPARg, C/EBPa and C/EBPb gene expression

* p<0.01, T or DHT vs. untreated (unt); ** p<0.05, T plus F vs. T alone
Clomiphene-Resistant PCOS Patients Receiving Low-Dose FSH Stimulation

- Elevated serum free androgen levels predict a reduced ongoing pregnancy rate.
- Testosterone treatment of immature mouse oocytes impairs maturation *in vitro* and embryonic development.
- Insulin resistance also lowers the conception rate.

Immunofluorescence of cells in human adipose

S-100(+) adipocytes
CD14(+) adipose tissue macrophages
DAPI(+) nuclear stain
### Pregnancy outcome of IVM in PCOS

<table>
<thead>
<tr>
<th>PCOS definition</th>
<th>% mature</th>
<th>% fertilized</th>
<th>Embryo development ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=11 NL</td>
<td>78</td>
<td>43</td>
<td>92</td>
</tr>
<tr>
<td>N=9 HA+MI+PCO</td>
<td>62</td>
<td>26</td>
<td>78</td>
</tr>
</tbody>
</table>

*In vitro* matured oocytes from PCOS women showed lower rates of maturation, fertilization and embryo development than those of normal women.

**NL** = normal

Barnes 1996
Effects of E2 on Intracellular Calcium in Human Germinal Vesicle Oocytes

Tesarik J et al. JCEM 1995;80:1438
PCOS & IVF Pregnancy Outcome

- After IVF/ICSI, increased miscarriage rate can occur in lean PCOS patients.
- Insulin resistance increases miscarriage risk after IVF, controlling for PCOS.

Effect of Insulin on Murine Oocyte Developmental Competency

State evidence for heritability of PCOS.

Explain how obesity alters the PCOS phenotype.

State hormonal mechanisms of impaired folliculogenesis in PCOS.

Propose lipotoxicity as a link between metabolic and reproductive dysfunction in PCOS.
Interplay between Insulin and Androgen in Impaired Follicular Growth in PCOS

- Number of 2-5 mm follicles positively correlates with serum testosterone levels (recruitment),

- Number of 6-9 mm follicles negatively correlates with fasting serum testosterone and insulin levels/BMI (selection).

- Hyperandrogenism is associated with excessive early follicular growth (enhanced recruitment) that will not progress to the dominant stage (impaired selection) due to androgen excess and/or hyperinsulinemia

Jonard 2003
Transcriptional Cascade Controlling Adipocyte Differentiation

PPAR gamma
C/EBP alpha

Siersbaek 2011
Sonographic Criteria for PCO

- Presence of 12 or more follicles in each ovary measuring 2-9 mm in diameter, and/or
- Increased ovarian volume (> 10 ml)
- Only one ovary fitting this definition is sufficient to define PCO.
- Does not apply to women taking OCPs
- If evidence of a dominant follicle (> 10 mm) or a corpus luteum, scan should be repeated next cycle.

OCPs = oral contraceptive pills

Hum Reprod 2004