Endocrine control of female reproductive function

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Female reproductive organs in human

Ovary contains:
- stromal matrix (connective tissue, nerves, lymphatic and blood vessels)
- smooth muscle fibres
- follicles
- corpora lutea
- corpora albicans
- surface epithelium, overlaying tunica albuginea

Essential Reproduction
Fig. 8.1
The follicle is the functional unit of the ovary

Growth independent of external regulation

Regulated by FSH and LH from pituitary

FOLLICULOGENESIS (growth and development of the follicle) accompanies and supports OOGENESIS (growth and maturation of the oocyte)
Primordial follicle pool and lifetime fertility

Cells in ovarian follicles

Primordial follicle (Non-growing)

Preantral follicle
Cells in ovarian follicles

Early antral follicle

- Blood vessel
- Fluid accumulation
- Membrana propria
- Zona
- Oocyte
- Granulosa
- Theca interna
- Theca externa
- Stroma

Late antral follicle

- Antrum
- Mural granulosa cells
- Follicular antrum
- Cumulus
- Oocyte
Preovulatory follicle & ovulation

Corpus luteum/ corpus albicans
Regulators of follicle growth and recruitment

Primordial to preantral stages:

- Gonadotropin INDEPENDENT i.e. no exogenous factors
- Intraovarian/paracrine growth factors and cytokines important
- Balance of stimulatory (activation/recruitment) and inhibitory (quiescence/apoptosis) factors
  - Anti Mullerian Hormone (AMH) produced by granulosa cells of larger follicles *inhibits* primordial follicle recruitment (Amh<sup>-/-</sup>)
  - LIF and Kit-ligand *promote* primordial follicle growth
Regulators of follicle growth and recruitment

Early antral and beyond:

- Gonadotropin DEPENDENT i.e. FSH and LH
- Granulosa and theca cells acquire FSHR and LHR
- Inhibin-Activin-Follistatin axis (TGFβ Family)
  - ve and +ve feedback to anterior pituitary of FSH synthesis/secretion
When do the different follicle types arise during the menstrual cycle?

<table>
<thead>
<tr>
<th>Morphology</th>
<th>Day of menstrual cycle</th>
<th>Diameter (mm)*</th>
<th>FSH/LH receptors present?</th>
<th>Oestrogen in peripheral blood (pmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preantral</td>
<td>Throughout</td>
<td>&lt;0.5</td>
<td>-</td>
<td>NA</td>
</tr>
<tr>
<td>Very early antral</td>
<td>Throughout</td>
<td>&lt;2</td>
<td>-</td>
<td>NA</td>
</tr>
<tr>
<td>Early antral</td>
<td>1–6</td>
<td>2–7</td>
<td>+</td>
<td>&lt;20</td>
</tr>
<tr>
<td>Expanding antral†</td>
<td>6–10</td>
<td>7–10</td>
<td>+</td>
<td>100–200</td>
</tr>
<tr>
<td>Expanded antral</td>
<td>10–12</td>
<td>10–20</td>
<td>+</td>
<td>200–400</td>
</tr>
<tr>
<td>Preovulatory</td>
<td>13–14</td>
<td>20–25</td>
<td>+</td>
<td>800±§</td>
</tr>
</tbody>
</table>

*Recent advances in ultrasound technology now make it possible to monitor these final stages of follicular growth in the conscious subject, and thereby to ascertain how near the follicles are to ovulation.
†In naturally cycling women a single dominant follicle emerges at this point and only it grows thereafter.
§10³–10⁴ higher oestrogen concentrations within the follicular fluid itself.
NA, not applicable.
Comparison of follicular development across different species

<table>
<thead>
<tr>
<th>Species</th>
<th>Preantral phase (days)</th>
<th>Antral phase (days)</th>
<th>Preovulatory phase (hours)</th>
<th>Luteal phase (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>6–10</td>
<td>3–4</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>Human</td>
<td>77–85*</td>
<td>8–12</td>
<td>30–36</td>
<td>12–15</td>
</tr>
<tr>
<td>Sheep</td>
<td>NK</td>
<td>4–5</td>
<td>22</td>
<td>14–15</td>
</tr>
<tr>
<td>Cow</td>
<td>NK</td>
<td>c.10</td>
<td>40</td>
<td>18–19</td>
</tr>
<tr>
<td>Pig</td>
<td>NK</td>
<td>c.10</td>
<td>41</td>
<td>15–17</td>
</tr>
<tr>
<td>Horse</td>
<td>NK</td>
<td>c.10</td>
<td>40</td>
<td>15–16</td>
</tr>
</tbody>
</table>

*Also includes very early antral development (see Table 8.2).
NK, not known.
Steroid hormone production in the ovary

Unlike male, hormones produced in a cyclic manner, gonadotrophin controlled

- Cyclic, gonadotropin-controlled production for episodic release of gametes
- Estrogen dominance prior to ovulation – FOLLICULAR PHASE
- Progesterone dominance after ovulation – LUTEAL PHASE
Hypothalamic-pituitary-gonadal axis

Gonadotrophs - same cell produces LH and FSH

FSH = follicle stimulating hormone
LH = luteinising hormone

[Ch 55, Boron and Boulpaep, Medical Physiology (2017) 3rd Ed]
Relationship between GnRH pulse rate and production of FSH or LH

- Fast pulse of GnRH = LH
- Slow pulse of GnRH = FSH
- Continuous infusion of GnRH = decrease in FSH and LH secretion.
- Pulsatile secretion = normal FSH and LH secretion.
- Pulse is more important than amplitude.

Activin, follistatin and inhibin in the ovary

- Opposing effects on FSH release only
  - **Activin**: +ve
    - Increases FSH binding and FSH-induced aromatization (i.e. production of estrogen)
    - Participates in androgen synthesis, *enhancing* action of LH in the ovary.
  - **Follistatin**: -ve
    - Inhibits FSH release
    - Binding and bio-neutralisation of activin
  - **Inhibin**: -ve
    - Suppresses FSH (although FSH stimulates inhibin secretion = negative feedback)
    - Reduced by GnRH and enhanced by IGF-1
Reminder - cells in ovarian follicles

Early antral follicle

Late antral follicle

Essential Reproduction 7th Edition 2013
2-cell 2-gonadotropin hypothesis

Early antral follicles

At this stage, only TC have LHR
Only GCs develop FSHR
TC supply androstenedione to GCs
which, in the presence of FSH,
express aromatase (CYP19), to
convert androgens to estrogens

↑ androgens from theca fuels
massive increase in estrogen which
further stimulates GC proliferation
Positive feedback loop = E2 surge

GCs acutely FSH and estrogen responsive, therefore rapid growth

Adapted from Johnson, M. H. (2007). Essential Reproduction, Sixth edn
Late antral or preovulatory follicles

2-cell 2-gonadotropin hypothesis

$\uparrow$ estrogens acts with FSH to stimulate LHR expression on granulosa cells but NOT cumulus cells; also increases LH pulses from the pituitary. Therefore, LH surge $\rightarrow$ increased production of progesterone = luteinisation

Progestosterone + PGR = ovulation!

Adapted from Johnson, M. H. (2007). Essential Reproduction, Sixth edn (Massachusetts, Blackwell Publishing)
- Cycles impact on whole body
- Rising E2 increases pituitary responsiveness to GNRH resulting in the LH surge that precedes ovulation.
- FSH peak precedes LH surge
- P steadily increases to become dominant during the luteal phase

- Why cycles?? Dual roles of the female genital tract.

1. Transport of gametes to site of fertilisation
2. Implantation and growth of fetus

Martini, Fundamentals of Anatomy & Physiology (2006) 7th Edition Fig. 28.26
Summary of ovarian structure and folliculogenesis

• Follicle is the functional reproductive unit of the ovary
• Folliculogenesis is a highly structured and controlled sequence of developmental stages
• Control of follicle growth:
  ➢ Early stages of development are gonadotropin independent
  ➢ Later stages respond to FSH & LH
Summary of steroid production and ovarian cycles

- Estrogen dominance prior to ovulation
- Progesterone dominance after ovulation
- Progesterone/PGR critical for ovulation
- Theca cells produce androgens
- Granulosa cells convert androstenedione to estrogen in presence of FSH
- Surge of estrogen results in LH surge, ovulation and luteinisation of granulosa cells to produce progesterone
- Ovarian cycle patterns conserved across species – lengths differ
What happens when the endocrine control of female reproductive function is altered?

Can lead to endocrine disorders associated with ovulatory dysfunction and disrupted menstrual cycles.
Polycystic ovary syndrome (PCOS)

PCOS is the most common endocrine disorder of women in their reproductive years.

PCOS is a complex, heterogeneous disorder with reproductive, endocrine, metabolic and psychological features.

Defined in 1935 by Stein and Leventhal
- polycystic ovaries in infertile, overweight women with menstrual dysfunction

A lifelong condition that impacts systems across the body.

Worldwide prevalence of 10%

Present in 12-18% of women of reproductive age in Australia
Clinical features of PCOS

**Endocrine traits:**
- Hyperandrogenism
- LH hypersecretion

**Reproductive traits:**
- Menstrual disturbances, anovulation
  - infertility
- Arrested follicle maturation
- Polycystic ovaries
- Pregnancy complications
  - ↑ risk miscarriage, gestational diabetes

**Metabolic traits:**
- Hyperandrogenism
- LH hypersecretion
- Menstrual disturbances, anovulation
  - infertility
- Arrested follicle maturation
- Polycystic ovaries
- Pregnancy complications
  - ↑ risk miscarriage, gestational diabetes
Clinical features of PCOS

**Endocrine traits:**
- Hyperandrogenism
- LH hypersecretion

**Hirsutism**

**Reproductive traits:**
- Menstrual disturbances, anovulation
  - infertility
- Arrested follicle maturation
- Polycystic ovaries
- Pregnancy complications
  - ↑ risk miscarriage, gestational diabetes

**Metabolic traits:**
- Metabolic syndrome
- Obesity
- Insulin resistance
- Impaired glucose tolerance

Increased risk of developing:
- Cardiovascular disease
- Hepatic steatosis
- Type-2 diabetes
How do you diagnose PCOS?

Rotterdam, AE-PCOS and NIH diagnostic criteria’s.

1st internationally endorsed, evidence-based guidelines Covering:- assessment, diagnosis, management.

Rotterdam PCOS diagnostic criteria now endorsed globally.

1. Menstrual disturbances, ovulatory dysfunction (< 21 or > 35 days)
2. Hyperandrogenism (clinically or biochemically)
3. Polycystic ovaries on ultrasound

+ Exclusion of thyroid disease (TSH), Hyperprolactinemia (prolactin) and NCCAH (17OHP)
Assessment of Hyperandrogenism

- Clinical hyperandrogenism
  - Hirsutism
  - Acne
  - Androgenic alopecia (female pattern baldness)

The Ferriman-Gallwey scale for hirsutism
(≥4-6 indicates hirsutism)

- Biochemical hyperandrogenism
  - Bioavailable testosterone, calculated free testosterone or free androgen index
Diagnosis of Polycystic Ovarian Morphology

- Use transvaginal ultrasound
  Follicle number per ovary ≥ 18 and/or ovarian volume ≥ 10ml if using **new technology**
  Follicle number per ovary ≥ 12 and/or ovarian volume ≥ 10ml if using **old technology**

- Transabdominal ultrasound
  Ovarian volume ≥ 10ml

Old technology

New technology
Problems with the management of PCOS

Despite the high prevalence of PCOS and substantial research, the underlying cause of PCOS is unknown and there is **no cure**.

No drug approved for the treatment of PCOS, most drugs used in an off-label fashion.

Current treatments treat the symptoms of PCOS **not** the cause.
Androgens and PCOS

Androgen excess is a key diagnostic trait of PCOS.

Hyperandrogenism - cause or consequence of PCOS?

Women exposed to excess androgen due to congenital adrenal hyperplasia or female-to-male transgenders display polycystic ovaries.

Due to ethical and logistical constraints, difficult to prove in humans.

Animals models → investigate underlying mechanisms involved in PCOS pathogenesis.
Development of PCOS mouse model by exposure to androgen excess

Optimised PCOS mouse model

- DHT implant
- ~3 wks of age
- Control (blank) or DHT (dihydrotestosterone) implants
- 13 wks
- Fully developed PCOS phenotype

Human PCOS traits found in mouse model

**Endocrine features:**
Hyperandrogenism

**Reproductive features:**
Irregular cycles/acyclicity
Oligo-/anovulation
Polycystic ovaries
Antral follicle arrest
\( \downarrow \) follicle health

**Metabolic features:**
Obesity
Adipocyte hypertrophy

Androgen excess replicates a breadth of PCOS features in our PCOS mouse model.

Body fat (g):
- Control 4.4 ± 0.1
- DHT 5.1 ± 0.3 *
Is there a role for androgens in PCOS?

- PCOS mouse
  - DHT
  - Closely replicates features of humans PCOS

- Androgen receptor knockout mouse (ARKO)
  - Completely androgen resistant mouse model
  - Can not respond to androgens via the AR
Are intra- or extra-ovarian mechanisms involved?

- Androgen excess can induce a full range of PCOS traits.
- Does androgen excess initiate the development of PCOS via intra- or extra-ovarian AR-mediated mechanisms?

DHT-induced PCOS mouse + Global and cell specific androgen receptor knockout models

Induce PCOS by androgen excess (postnatal DHT treatment)

- Global loss of AR signalling
- Brain specific loss of AR signalling
- Granulosa cell specific loss of AR signalling

Assess the development of PCOS traits

PCOS not observed = AR signalling required
WT + Blank

WT + DHT

NeurARKO + DHT

ARKO + DHT

GCARKO + DHT

= corpus luteum

= arrested follicle
Caldwell, et al. PNAS 2017

<table>
<thead>
<tr>
<th>PCOS traits assessed</th>
<th>Development of clinical PCOS traits?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irregular cycles/acyclicity</td>
<td>✓</td>
</tr>
<tr>
<td>Oligo- or anovulation</td>
<td>✗</td>
</tr>
<tr>
<td>Multi-cystic ovarian appearance</td>
<td>✓</td>
</tr>
<tr>
<td>▲ Unhealthy large antral follicles</td>
<td>✗</td>
</tr>
<tr>
<td>Granulosa Cell Layer Thickness</td>
<td>✓</td>
</tr>
<tr>
<td>▼ Granulosa Cell Layer Thickness</td>
<td>✗</td>
</tr>
<tr>
<td>▲ Theca Cell Layer Thickness</td>
<td>✓</td>
</tr>
<tr>
<td>▲ body weight</td>
<td>✗</td>
</tr>
<tr>
<td>▲ body fat</td>
<td>✓</td>
</tr>
<tr>
<td>Adipocyte Hypertrophy</td>
<td>✗</td>
</tr>
<tr>
<td>▼ Adiponectin</td>
<td>✓</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>✓</td>
</tr>
</tbody>
</table>

Active AR ▲ Global loss of AR ▲ Neuronal loss of AR ▲ Granulosa cell loss of AR ▲ + DHT = androgen excess

AR signalling within the brain but not the ovary is a major mediator in the development of PCOS.
Findings pinpoint the brain as a prime target site for androgen actions in the pathogenesis of PCOS.
Summary of PCOS and its possible origins

PCOS is the most common endocrine disorder of women in their reproductive years.

PCOS is a complex, heterogeneous disorder with reproductive, endocrine, metabolic and psychological features.

Rotterdam PCOS diagnostic criteria now endorsed globally.

1. Menstrual disturbances, ovulatory dysfunction (< 21 or > 35 days)
2. Hyperandrogenism (clinically or biochemically)
3. Polycystic ovaries on ultrasound

Current treatments treat the symptoms of PCOS not the cause.

New evidence supports the brain as key site involved in the pathogenesis of PCOS.
Video: https://youtu.be/NhyYZCBq5A8
Projects in Reproductive Biology

Oocyte and Ovarian Biology Research Unit

We perform basic research on ovarian and oocyte (egg) function for translation into the clinic to improve the success of fertility treatments and IVF in humans.

Research Projects

Biomarkers for fertility and IVF
Dr Angelique Riepsamen
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Improving egg quality
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Improving egg quality
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Oncofertility
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**TABLE 20-6**

**Actions of Estrogen and Progesterone**

**Estrogen**

**Effects on Sex-Specific Tissues**

Is essential for egg maturation and release

Stimulates growth and maintenance of entire female reproductive tract

Stimulates granulosa cell proliferation, which leads to follicle maturation

Thins the cervical mucus to permit sperm penetration

Enhances transport of sperm to the oviduct by stimulating upward contractions of the uterus and oviduct

Stimulates growth of the endometrium and myometrium

Induces synthesis of endometrial progesterone receptors

Triggers onset of parturition by increasing uterine responsiveness to oxytocin during late gestation through a twofold effect: by inducing synthesis of myometrial oxytocin receptors and by increasing myometrial gap junctions so that the uterus can contract as a coordinated unit in response to oxytocin
Other Reproductive Effects
Promotes development of secondary sexual characteristics
Controls GnRH and gonadotropin secretion
  Low levels inhibit secretion
  High levels responsible for triggering LH surge
Stimulates duct development in the breasts during gestation
Inhibits milk-secreting actions of prolactin during gestation

Nonreproductive Effects
Promotes fat deposition
Increases bone density
Closes the epiphyseal plates
Improves blood cholesterol profile by increasing HDL and decreasing LDL
Promotes vasodilation by increasing nitric oxide production in arterioles (cardioprotective)

Progesterone
Prepares a suitable environment for nourishment of a developing embryo/fetus
Promotes formation of a thick mucus plug in cervical canal
Inhibits hypothalamic GnRH and gonadotropin secretion
Stimulates alveolar development in the breasts during gestation
Inhibits milk-secreting actions of prolactin during gestation
Inhibits uterine contractions during gestation
FSH and LH throughout the female life course