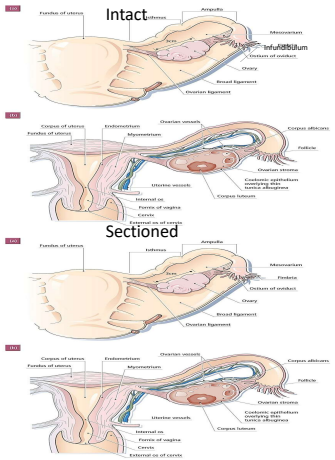
Medicine
School of Women's & Children's Health
Discipline of Obstetrics & Gynaecology

Endocrine control of female reproductive function

Kirsty Walters, PhD
Fertility Research Centre,
School of Women's & Children's Health,
University of New South Wales,
Sydney, Australia

1

Female reproductive organs in human



Ovary
Ovarian bursa
Oviduct
Uterus
Cervix
Vagina

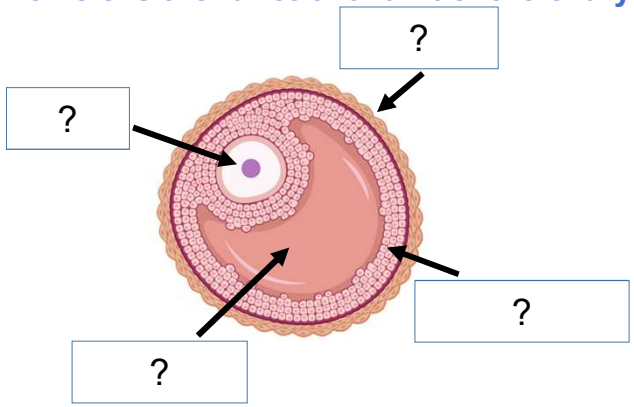
Ovary contains:
- stromal matrix (connective tissue, nerves, lymphatic and blood vessels)
- smooth muscle fibres
- follicles
- corpora lutea
- corpora albicans
- surface epithelium, overlaying tunica albuginea

Essential Reproduction
Fig. 8.1

2

2

The follicle is the functional unit of the ovary



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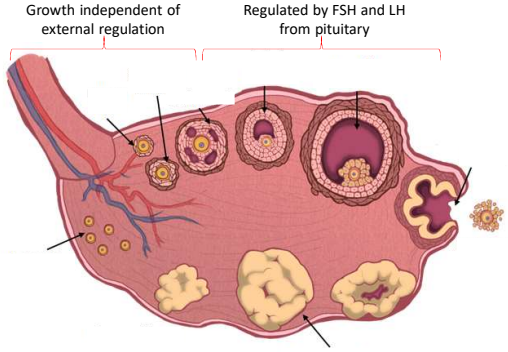
The follicle is the functional unit of the ovary

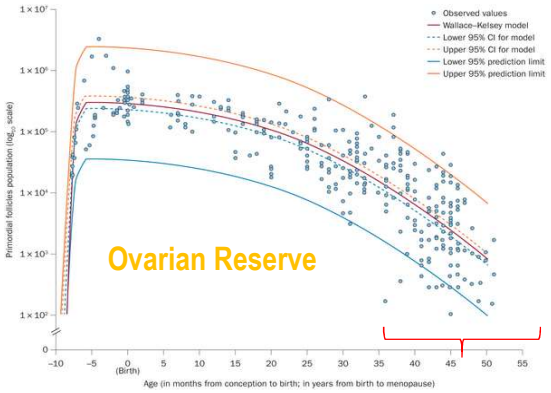


FOLLICULOGENESIS (growth and development of the follicle) accompanies and supports OOGENESIS (growth and maturation of the oocyte)

4

4

Primordial follicle pool and lifetime fertility

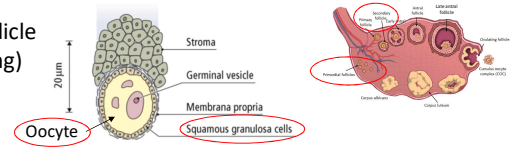


Wallace & Kelsey (2010) Human ovarian reserve from conception to the menopause *Plos ONE* 5(1):e8772.

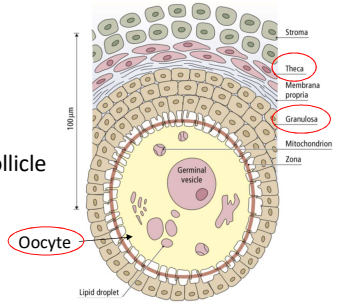
5

Cells in ovarian follicles

Primordial follicle (Non-growing)



Preantral follicle



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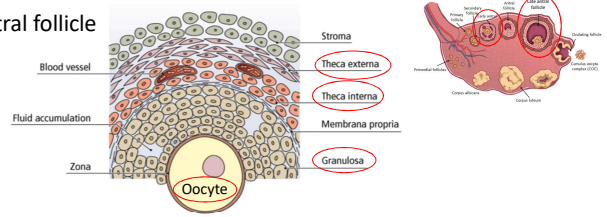
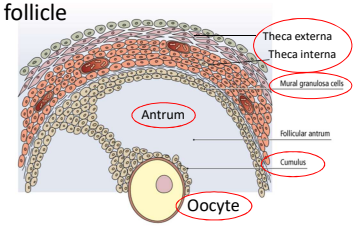
Cells in ovarian follicles

Early antral follicle



Late antral follicle

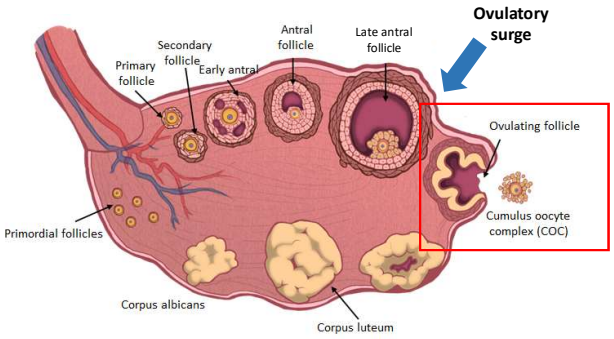


Essential Reproduction 7th Edition 2013

7

Preovulatory follicle & ovulation

What hormone is involved?



Corpus luteum / corpus albicans

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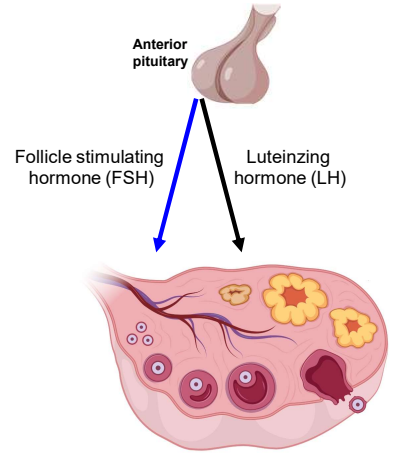
8

Key regulators of follicle development



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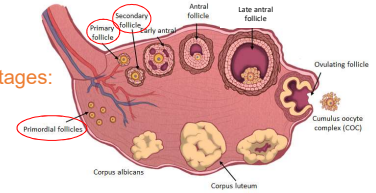
9

Regulators of follicle growth and recruitment

Primordial to preantral stages:



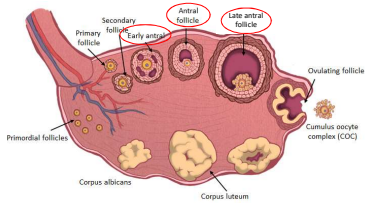
- Gonadotropin INDEPENDENT i.e. no exogenous factors
- Intraovarian/paracrine growth factors and cytokines important
- Balance of stimulatory (activation/recruitment) and inhibitory (quiescence/apoptosis) factors
  - Anti Mullerian Hormone (AMH) produced by granulosa cells of larger follicles *inhibits* primordial follicle recruitment ($Amh^{-/-}$)
  - LIF and Kit-ligand *promote* primordial follicle growth
- See Box 8.1 Essential Reproduction, 2013; McLaughlin & McIver (2009) *Reproduction* 137:1-11 (Review)

10

10

Regulators of follicle growth and recruitment

Early antral and beyond:

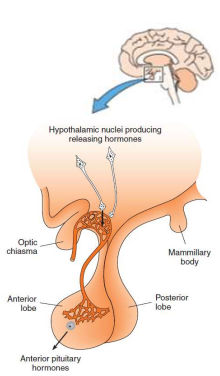


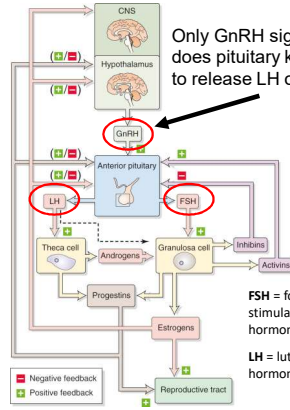
- Gonadotropin DEPENDENT i.e. FSH and LH
- Granulosa and theca cells acquire FSHR and LHR
- Inhibin-Activin-Follistatin axis (TGFβ Family)
  - –ve and +ve feedback to anterior pituitary of FSH synthesis/secretion

11

11

Hypothalamic-pituitary-gonadal axis





Ovary

Only GnRH signal, so how does pituitary know whether to release LH or FSH?

FSH = follicle stimulating hormone

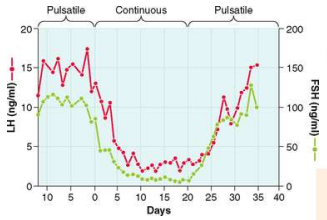
LH = luteinising hormone

[Ch 55, Boron and Boulpaep, Medical Physiology (2017) 3rd Ed]

12

12

Relationship between GnRH pulse rate and production of FSH or LH



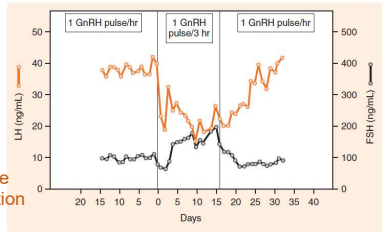
Belchetz PE, et al (1978) Science 202:631–633.

GnRH must be released in pulses for FSH/LH secretion

GnRH pulse rate produces the change in FSH/LH release

Fast pulse rate – increase in LH release

Slow pulse rate – increase in FSH secretion



[Ch 5, Endocrine & Reproductive Physiology (2013) 4th Ed]

13

13

Activin, follistatin and inhibin in the ovary

- **Activin** and **inhibin** are produced by granulosa cells and to act via autocrine/paracrine mechanisms to regulate folliculogenesis and luteinisation.
- 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.
- **Inhibin:** -ve
  - *Suppresses* FSH (although FSH stimulates inhibin secretion = negative feedback)
  - Reduced by GnRH and enhanced by IGF-1
- **Follistatin:** -ve
  - *Inhibits* FSH release
  - Binding and bio-neutralisation of activin

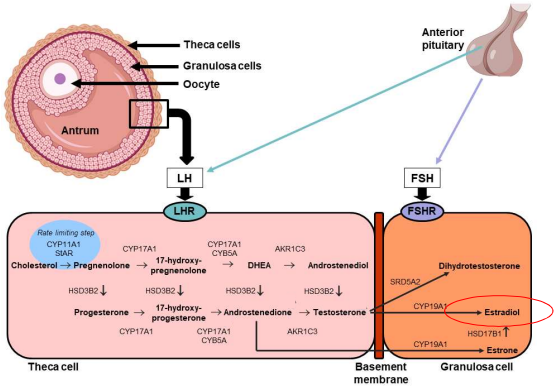
14

14

Steroid hormone production in the ovary

How do FSH and LH regulate follicle development?

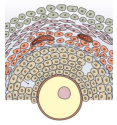
Regulating the two cell, two-gonadotropin theory of ovarian steroidogenesis



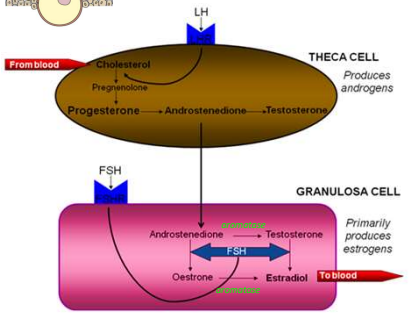
15

15

2-cell 2-gonadotropin hypothesis



Early antral follicles



Roles of androgens:
1. Substrates for conversion to estrogens
2. Synergize with FSH to stimulate GC proliferation and ↑ follicle size
3. Stimulate aromatase (CYP19A1) activity thus promoting estrogen synthesis

↑ androgens from theca fuels massive increase in estrogen which further stimulates GC proliferation

Positive feedback loop = E2 surge

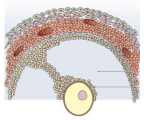
GCs acutely FSH and estrogen responsive, therefore rapid growth

Adapted from Johnson, M. H. (2007). Essential Reproduction, *Sixth edn*

16

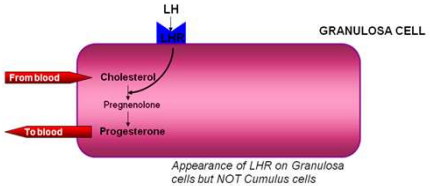
16

2-cell 2-gonadotropin hypothesis



Late antral or preovulatory follicles

Only 1 cell required for P4 synthesis.



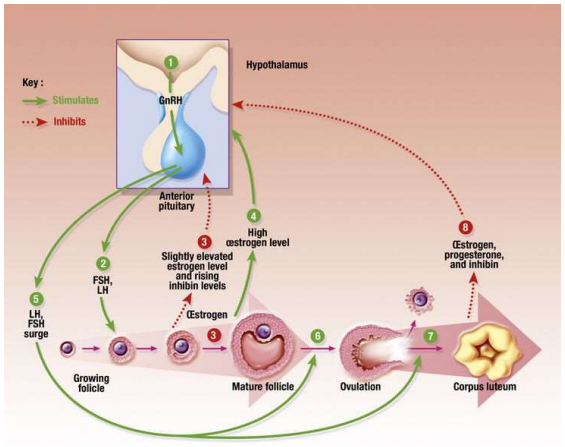
Appearance of LHR on Granulosa cells but NOT Cumulus cells

↑ 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 → increased production of progesterone = luteinisation

Progesterone + Progesterone Receptor = ovulation

Adapted from Johnson, M. H. (2007). Essential Reproduction, *Sixth edn* (Massachusetts, Blackwell Publishing

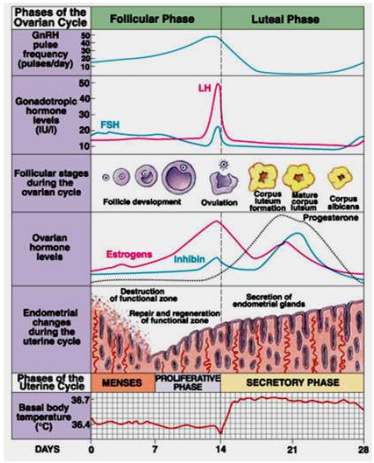
17



18

18

Hormonal profile during the menstrual cycle



- 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

19

When do the different follicle types arise during the menstrual cycle?

Table 8.2 Human follicular development

Morphology	Day of menstrual cycle	Diameter (mm)*	FSH/LH receptors present?	Oestrogen in peripheral blood (pmol/l)
Preantral	Throughout	<0.5	–	NA
Very early antral	Throughout	<2	–	NA
Early antral	1–6	2–7	+	<20
Expanding antral†	6–10	7–10	+	100–200
Expanded antral	10–12	10–20	+	200–400
Preovulatory	13–14	20–25	+	800+‡

*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.
‡10^3–10^4 higher oestrogen concentrations within the follicular fluid itself.
NA, not applicable.

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20

20

Comparison of follicular development across different species

Table 8.1 Duration of phases of follicular development in the non-pregnant animal

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

*Also includes very early antral development (see Table 8.2).
NK, not known.

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21

21

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

22

22

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

23

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.

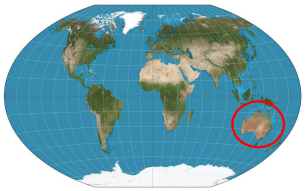
24

24

Polycystic ovary syndrome (PCOS)

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

Worldwide prevalence of 10%



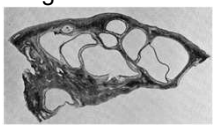
Present in 12-18% of women of reproductive age in Australia

A lifelong condition that impacts systems across the body.

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



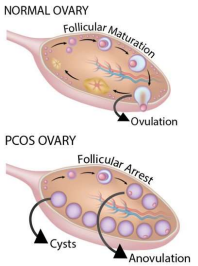
25

Clinical features of PCOS

Endocrine traits:
- Hyperandrogenism
  - Acne
  - Hirsutism
- LH hypersecretion

Metabolic traits:

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



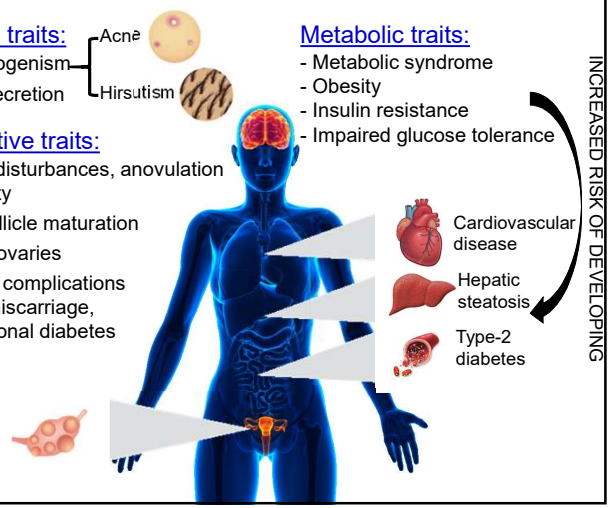
26

Clinical features of PCOS

Endocrine traits:
- Hyperandrogenism
  - Acne
  - Hirsutism
- LH hypersecretion

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

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



27

How do you diagnose PCOS?

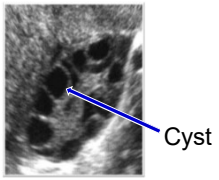
Rotterdam, AE-PCOS and NIH diagnostic criteria's.

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

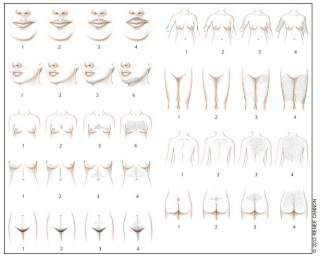
+ Exclusion of thyroid disease (TSH), hyperprolactinemia (prolactin) and non-classical congenital adrenal hyperplasia (NCCAH)



28

Assessment of Hyperandrogenism

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



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

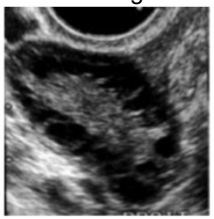
- Biochemical hyperandrogenism
  - Bioavailable testosterone, calculated free testosterone or free androgen index

29

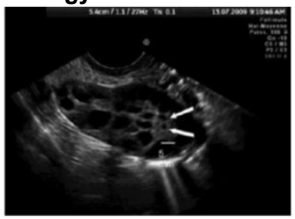
29

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**



Old technology



New technology

- Transabdominal ultrasound
  - Ovarian volume ≥ 10ml

30

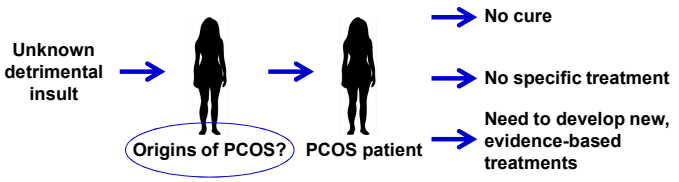
30

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.



31

31

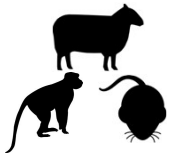
Androgens and PCOS

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.



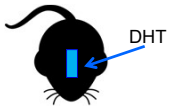
Animals models → investigate underlying mechanisms involved in PCOS pathogenesis.

32

32

Development of PCOS mouse model by exposure to androgen excess

Optimised PCOS mouse model



~3 wks of age — 13 wks — 16 wks

Control (blank) or DHT (dihydrotestosterone) implants

Fully developed PCOS phenotype

Caldwell, et al. Endocrinology 2014

33

Human PCOS traits found in mouse model

Endocrine features:

Hyperandrogenism

Reproductive features:

Irregular cycles/acyclicity

Oligo-/anovulation

Polycystic ovaries

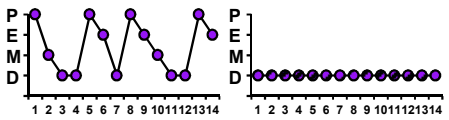
Antral follicle arrest

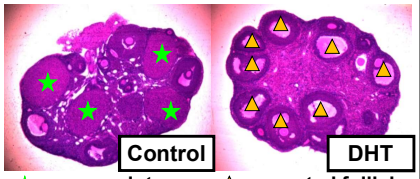
↓ follicle health

Metabolic features:

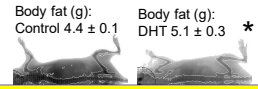
Obesity

Adipocyte hypertrophy





★= corpus luteum △= arrested follicle



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

34

Is there a role for androgens in PCOS?

PCOS mouse

+

Androgen receptor knockout mouse (ARKO)

↓

Closely replicates features of humans PCOS

↓

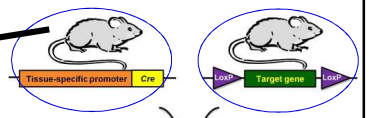
Completely androgen resistant mouse model

Can not respond to androgens via the AR

35

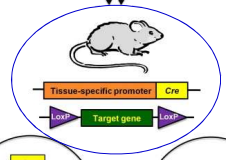
Development of ARKO mice by Cre/LoxP technology

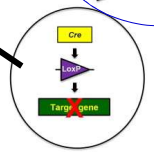
Can be global (whole body deletion) or tissue or cell specific depending on where the promoter is expressed.



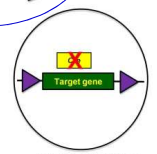
X

Androgen receptor knockout mouse (ARKO)





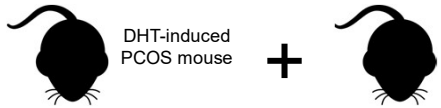
Target cell recombination



All other cell types

36

Are intra- or extra-ovarian mechanisms involved?

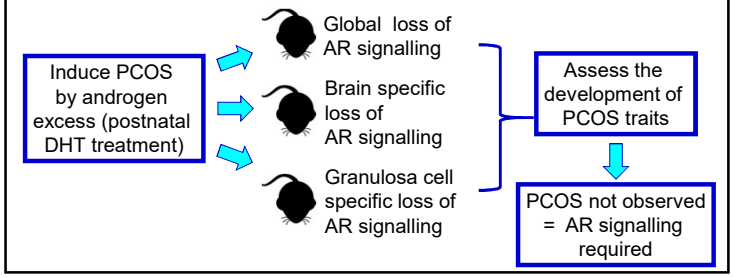


DHT-induced PCOS mouse

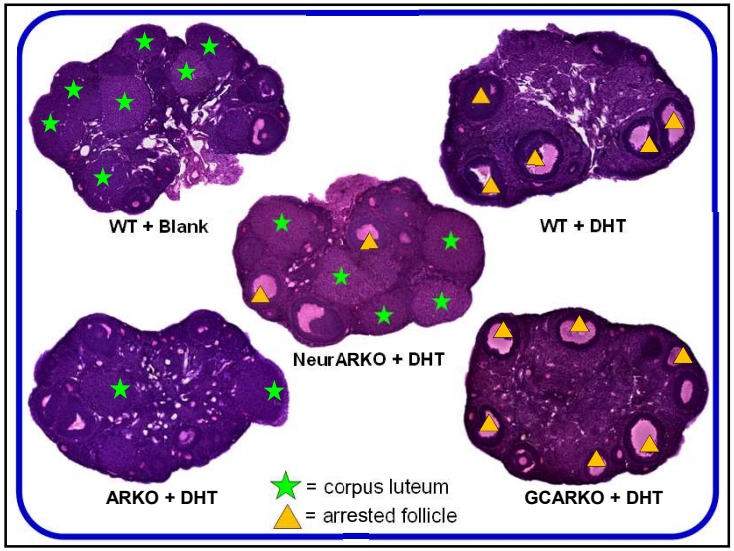
+

Global and cell specific androgen receptor knockout models

- 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?



37



38

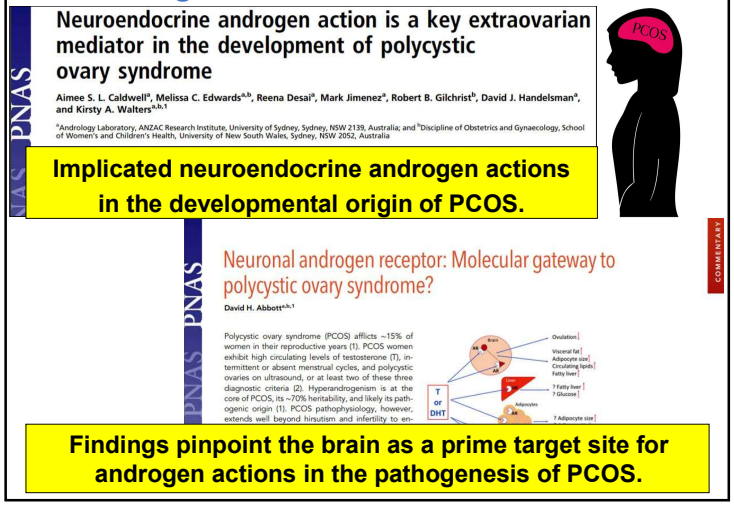
Caldwell, et al. PNAS 2017

PCOS traits assessed	Active AR	Global loss of AR	Neuronal loss of AR	Granulosa cell loss of AR
	+ DHT = androgen excess			
	Development of clinical PCOS traits?			
Irregular cycles/acyclicity	✓	✗	✓	Partial
Oligo- or anovulation	✓	✗	✗	✓
Multi-cystic ovarian appearance	✓	✗	Partial	✓
↑ Unhealthy large antral follicles	✓	✗	✗	✓
↓ Granulosa Cell Layer Thickness	✓	✗	✓	✗
↑ Theca Cell Layer Thickness	✓	✗	✓	✓
↑ body weight	✓	✗	✗	✓
↑ body fat	✓	✗	✗	✓
Adipocyte Hypertrophy	✓	✗	Partial	✓
↓ Adiponectin	✓	✗	✓	✓
Dyslipidemia	✓	✗	✗	✓

AR signalling within the brain but not the ovary is a major mediator in the development of PCOS.

39

Significant advance in the field

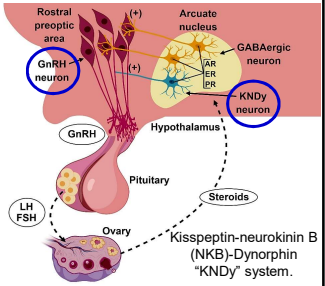


40

First clinical study targeting the neuroendocrine pathophysiology of PCOS

Neurokinin B Receptor Antagonism in Women With Polycystic Ovary Syndrome: A Randomized, Placebo-Controlled Trial

Jyothis T. George, Rahul Kakkar, Jayne Marshall, Martin L. Scott, Richard D. Finkelman, Tony W. Ho, Johannes Veldhuis, Karolina Skorupskaite, Richard A. Anderson, Stuart McIntosh, and Lorraine Webber



Patients:
Women with PCOS, exhibiting following criteria:
- polycystic ovaries
- hyperandrogenism
- amenorrhea/oligomenorrhea

Treatment:
Neurokinin B antagonist AZD4901 (neurokinin-3 receptor antagonist (NK-3)) at a dose of 20, 40 or 80mg/day or placebo for 28 days (12-14 patients/group).

Results: ↓ LH pulse frequency and LH and testosterone concentrations.

Implications: Potential new approach for the treatment of PCOS.

Can targeting AR-driven neuroendocrine pathways be a viable option to treat all PCOS traits?

George, et al. J Clin Endocinol Metab 2016

41

Neurokinin-3 receptor antagonism ameliorates PCOS metabolic traits

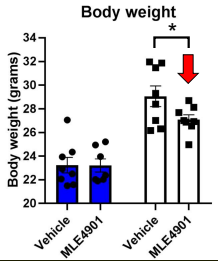
DHT-induced PCOS mouse

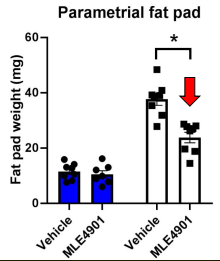
+

Treatment with NK3 receptor antagonist (MLE4901) for 4 weeks

Assess the development of PCOS traits

Professor Richard Anderson





Support targeting neuroendocrine pathways in the development of novel treatments for PCOS.

42

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.

UNSW PCOS video: https://www.youtube.com/watch?v=NhyYZCBq5A8

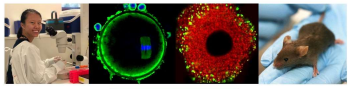
43

UNSW Women's & Children's Health — Fertility & Research Centre

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



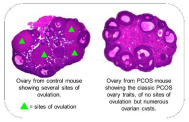
Research Projects

Biomarkers for fertility and IVF
Measuring reproductive potential by using non-invasive markers of egg quality. Development of diagnostics for use in IVF.

Improving egg quality
Investigating the effects novel factors that improve egg quality by altering its metabolism or the function of its surrounding cells

Polycystic Ovary Syndrome
Identifying target mechanisms involved in the development of PCOS. Test the efficacy of new treatments for PCOS in mouse models.

Oncofertility
Regulating biological pathways to protect ovaries during chemotherapy. Developing state-of-the-art techniques to preserve female fertility.



Be part of a team and gain hands-on experience in:
Cell culture
Microscopy
Molecular biology
Immunohistochemistry
Mass spectrometry
Protein and DNA work
Oocyte micro-manipulation

Dr Angelique Riepsamen
a.riepsamen@unsw.edu.au

A/Prof Kirsty Walters, PhD
Fertility Research Centre,
School of Women's & Children's Health,
UNSW,Sydney, Australia
Email: k.walters@unsw.edu.au

Biomarkers for fertility and IVF
Dr Angelique Riepsamen
a.riepsamen@unsw.edu.au

Polycystic ovary syndrome (PCOS)
Dr Kirsty Walters
k.walters@unsw.edu.au

Improving egg quality
Dr Dulama Richani
d.richani@unsw.edu.au

Oncofertility
Dr Michael Bertoldo
Michael.bertoldo@unsw.edu.au

44

44