Reproduction – ovarian function

Rudolf Cardinal, 8 Nov 98.

- This is a complex topic!
- The ovary makes (1) ova; (2) oestrogens; (3) progestagens.
- While the testis releases sperm continuously, ova (sing. ovum, L. egg) are released episodically, at *ovulation.*
- **The period prior to ovulation is characterized by *oestrogen* dominance. The period following ovulation is characterized by *progestagen* dominance.** The whole is called the **ovarian cycle.**
- The term "menstrual cycle" refers to the cyclicity of the body and behaviour of the adult female, and is only applicable to species that menstruate, i.e. higher primates. It is a consequence of the underlying ovarian cycle. In species that do not menstruate, we refer to the "oestrous cycle".
- The cycle reflects the fact that the female genital tract must act at different times to transport gametes to the site of fertilization (oestrogenic phase) and to provide the site of implantation of the fertilized egg and its subsequent development (progestagenic phase).

The ovary

- The ovary consists of **stromal tissue** containing **primordial follicles** (homologous to tubules in the testis) and also **interstitial glands** (homologous to Leydig cells). Little is known about the function of the interstitial glands and I will not mention them again.
- As in the male, gamete production comprises *mitosis*, *meiosis* and *oocyte maturation*.
- However, in the female the primordial germ cells that entered the embryonic gonad continued their development and proliferated mitotically. They are known as *oogonia* during this process, and they stop once they have entered their first meiotic division. They are now *primary oocytes.*
- As they enter meiosis, the oocytes become surrounded by ovarian mesenchymal cells to form *primordial follicles.* Follicular cells secrete a basement membrane, the **membrana propria,** around the outside of the follicle.
- **Oocytes are not replaced.**
- From puberty, a few primordial follicles recommence growth every day, so a continuous trickle of developing follicles is formed.

Follicular development

We first consider the development of a single follicle.

- **The preantral phase** (fig 4.3). The follicle first grows rapidly, from 20 μm to 200–400 μm depending on the species. Most of this growth occurs in the primary oocyte, which grows to 60–120 μm. This is a time of protein synthesis – it's part of the "packaging" phase of gametogenesis.
- The surrounding **granulosa cells** divide to become several layers thick, and secrete a glycoprotein that forms a layer called the **zona pellucida** between themselves and the oocyte. (There are gap junctions between the granulosa cells, and between the granulosa cells and the oocyte via cytoplasmic processes. This network is important for oocyte nutrition, since the granulosa layer is *avascular* – indeed, *acellular*.) Cells of the ovarian stroma condense on the membrana propria to form the **theca,** which is vascularized. **FSH and LH receptors develop.**
- We now have an advanced **preantral follicle.**
- The process thus far is independent of any external control.

- **The antral phase** (fig 4.4). In the absence of FSH and LH, follicles undergo *atresia*. The granulosa shows reduced synthetic activity, the oocyte dies, the follicle is invaded by leukocytes and macrophages and becomes fibrous scar tissue.
- Adequate tonic FSH and LH converts the preantral follicles to **antral follicles** (a.k.a. Graafian follicles). Both the granulosa and theca proliferate further. The theca divides into two layers: the **theca interna,** glandular and highly vascular, surrounded by the **theca externa,** a fibrous capsule. Fluid starts to appear between the granulosa cells, creating **follicular fluid** within the **follicular antrum.**
- The oocyte is surrounded by a mass of granulosa cells, the **cumulus oophorus,** and this is suspended in the follicular fluid and connected only by a thin stalk to the rest of the granulosa.

- *The follicle makes androgens and oestrogens.* The principal androgens are androstenedione and testosterone; the principle oestrogen is oestradiol 17β. The largest follicles release their steroids into the circulation, culminating in an **oestrogen surge.**
- Only the theca interna has LH receptors. LH stimulates these cells to make androgens from cholesterol. The theca does not make large quantities of oestrogens. [*Similar to Leydig cells.*] The granulosa cells receive these androgens and aromatize them to oestrogens. This process is stimulated by FSH. Only the granulosa has FSH receptors. [*Similar to Sertoli cells.*]
- Androgens stimulate aromatase activity, so increase oestrogen production. Oestrogen binds to receptors on granulosa cells, stimulating them to proliferate and to synthesize more oestrogens. This is a system of **positive feedback.**
- The combination of oestrogen and FSH causes *LH receptors to develop on the granulosa cells.*

- **The preovulatory phase.** The antral follicle will die unless a *surge of LH* coincides with the appearance of LH receptors on the outer layer of granulosa cells. The LH surge has two effects over the next few *hours*.
  1. Terminal growth changes occur in the follicle cells and the oocyte. The **oocyte** resumes meiosis. The first meiotic division results in half the chromosomes, but nearly all the cytoplasm, going into one cell – the *secondary oocyte.* The remaining chromosomes are discarded as the *first polar body*. Then meiosis arrests again. At the same time, *cytoplasmic maturation* occurs.[1]
  2. The **follicle** itself matures. A rapid expansion of the follicular fluid volume makes the follicle grow to **25 mm** or more in the human. There is a transient rise in androgens and oestrogens, but then they decline to very low levels. **The granulosa cells no longer converts androgen→oestrogen, but instead synthesizes progesterone. LH also stimulates this progesterone synthesis.** In addition, the cells lose their capacity to bind oestrogen and FSH.

- **Ovulation.** The follicle bulges from the ovarian surface. At one point (the *stigma*) the epithelial wall thins, balloons outwards and ruptures. Follicular fluid flows out onto the surface of the ovary, carrying the oocyte and surrounding cumulus. In man, the ovarian surface is directly exposed to the peritoneal cavity. The *fimbria* of the *oviduct* (Fallopian tube) bear cilia and sweep the egg mass into the *ostium* of the tube.
- The post-ovulatory follicle collapses and a clot forms in it.
- Enzyme activity is responsible for follicular rupture, not an increase in hydrostatic pressure.

- **The corpus luteum.** The follicle's core becomes fibrotic and the granulosa cells (plus some theca) become vascularized and **luteinized**[2]. It is now a *corpus luteum*, and secretes **progestagens.** The principle progestagen is progesterone. A few other hormones are secreted, varying with species (e.g. 17α-hydroxyprogesterone in primates, some oestradiol 17β in man, probably from the remaining theca cells, but these are less important).
- The endocrine support of the CL shows considerable species variation. In man, LH is luteotrophic and prolactin may be. Oestrogen is luteolytic in man.

- **Luteolysis.** *There is an important species difference.*
- **In most mammals,** the uterus produces prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), a *luteolytic factor* that causes the corpus luteum to regress.
- **In primates,** the corpus luteum regresses on its own unless rescued [see later].

In man the length of the preantral phase is unknown (but is 14 days in mice); the antral phase takes 8–12 days; the preovulatory phase lasts 37 h; the luteal phase lasts 12–15 days.

The number of follicles that ovulate depends on how many were rescued at the late preantral and late antral stages. It is characteristic of a species (ranging from one to several hundred). In man, about 15–20 follicles start the process of antrum formation, but usually only one is sufficiently primed with LH receptors for the LH surge to induce preovulatory changes.[3]

Overall, <0.1% of follicles complete the process to become a corpus luteum.

[1] These effects are stimulated by LH, but the oocyte does not have LH receptors, so the effect must be through follicular cells. The mechanism is unknown.
[2] Lutein is a carotenoid pigment that is yellow/orange.
[3] Administration of extra FSH (for antral growth) and extra LH (for preovulatory growth) can be used to induce *superovulation.*

The ovarian cycle and its control

- One **ovarian cycle** is the time between successive ovulations. In man, it is 24–32 days long. (The **menstrual cycle** begins on the first day of menstruation, so ovulation occurs in the middle of the menstrual cycle, on day ~12.)
- The oestrogen-dominated period prior to ovulation is called the **follicular phase,** since the oestrogens are made by follicles. In man, it lasts 10–14 days.
- The progestagen-dominated period after ovulation is called the **luteal phase,** as progesterone comes from the corpus luteum. In man, it lasts 12–15 days.
- There is considerable species variation in the organization of the ovarian cycle, and I will only talk about the human.

- As you know, lactotrophs in the anterior pituitary secrete prolactin.
- Gonadotrophs in the anterior pituitary secrete the glycoprotein *gonadotrophic hormones*, **LH and FSH**. Synthesis and secretion depend on **gonadotrophin hormone releasing hormone (GnRH)** from the hypothalamus.
- GnRH secretion is *pulsatile* (~1/hour) and this is critical for its effect (continued receptor occupancy causes receptor downregulation).
- GnRH has a **self-priming** effect. The secretory response to a second pulse of GnRH is larger than the first
- LH and FSH secretion may be regulated by changing the *amplitude* and *frequency* of the pulses, or the response of the gonadotrophs.

- **Oestradiol** normally *suppresses* LH/FSH secretion (negative feedback). However, if oestradiol levels increase greatly (e.g. 200–400% more than in the early follicular phase) and remain high for ~48 hours, then LH/FSH secretion is *enhanced* (positive feedback).
- **Progesterone,** at high concentrations, *enhances the negative feedback effects of oestradiol* to keep LH/FSH very low. It also *prevents the positive feedback effects of oestradiol.*
- Oestradiol and progesterone can act at the pituitary, but also at the hypothalamus. The positive feedback effect of oestradiol occurs because it increases the number of pituitary GnRH receptors.
- **Inhibin** is a protein hormone that influences *FSH* (but not LH?) secretion. Complicated and unclear. Main effect may be to reduce FSH. It acts only at the pituitary.

So, let's look at the cycle again – refer back to the events occurring at the follicle.

- **Follicular phase.** At the start of the menstrual cycle, luteal levels of oestrogen and progesterone fall, so negative feedback is relaxed and FSH/LH levels rise. (At this time, the antral phase of follicular growth is occurring and FSH/LH prevents follicular atresia.)
- Follicular growth causes the production of oestrogens and inhibin. Oestrogen, androgen, LH and inhibin levels increase; FSH levels fall.
- In the second half of this phase there is an oestradiol surge, which initiates positive feedback. This triggers a surge of LH/FSH, which in turn triggers the preovulatory phase. Oestrogen levels then fall precipitously and progesterone levels start to rise (see above). Lacking a continuing positive feedback stimulus, LH/FSH levels drop.
- *The length of the follicular phase is regulated by the rate of maturation of the principal preantral follicle – the "ovarian" or "pelvic clock".*

- **Luteal phase.** Progesterone (from the CL) rises. Oestrogens and androgens are still being made in significant quantities, but the presence of progesterone prevents any positive feedback effect, and enhanced the negative feedback effects of oestrogens, so LH and FSH levels reach their lowest point.
- When luteolysis occurs, both oestrogens and progesterone decline again and LH/FSH levels rise once more.

SUMMARY

Effects of hormones on gonadotrophin release

- progestagens, inhibin and low oestrogens → ↓gonadotrophins
- high oestrogens → ↑↑gonadotrophins
- progesterone enhances oestrogen negative feedback and blocks oestrogen positive feedback

Mechanism

(other factors – light [diurnal/seasonal], touch, olfaction, social factors)
↓
hypothalamus — inhibition: *progesterone (↓frequency), oestrogens (↓amplitude)*
↓ GnRH
pituitary — inhibition: *inhibin, progesterone, low oestrogens*
promotion: *high oestrogens (1. induce GnRH receptors, 2. enhance self-priming effect of GnRH)*
progesterone blocks this effect of oestrogens
↓
LH, FSH

The menstrual cycle

Follicular phase

a) *preantral*
oocyte grows
granulosa proliferates and secretes ZP
theca develops
FSH+LH receptors develop
Hormone-independent.

Oestrogen ↓. Progesterone ↓, relaxes –ve feedback. FSH ↑, LH ↑.

b) *antral*
tonic FSH (and LH) prevents atresia
theca: proliferates (interna, externa); LH stimulates cholesterol→androgens (androstenedione, testosterone)
granulosa: proliferates.
- secretes follicular fluid, forming antrum
- cumulus oophorus forms
- secretes oestrogens
- FSH stimulates aromatization: androgens → oestrogens
- oestrogen receptors (→ more proliferation)
- oestrogen + FSH → LH receptors develop

Inhibin ↓ causes FSH ↓. Then oestrogen ↑↑ causes LH ↑↑ (and FSH ↑↑).

c) *preovulatory*
LH surge (with LH receptors on granulosa + theca) prevents atresia.
Oocyte: LH → via follicle somehow → to second metaphase (polar body)
Theca: proliferates then regresses
Granulosa: LH → don't aromatize
make progesterone
stimulate progesterone synthesis
lose oestrogen + FSH receptors.

Oestrogen ↓↓. Progesterone ↑. Together cause low LH/FSH.

d) *ovulation*
? prostaglandins → proteases/collagenases → stigma bursts, etc.

Luteal phase

e) *corpus luteum*
Granulosa (now vascularized; luteinizes) and theca make progesterone.
In humans, LH (and prolactin?) is luteotrophic, oestrogen is luteolytic.
Progesterone supports uterus (uterotrophic).

f) *luteolysis*
corpus luteum → corpus albicans
Primates: low LH insufficiently luteotrophic.
Other mammals: $PGF_{2\alpha}$ from uterus is luteolytic.
Steroid support for uterus lost. *Menstruation.*

Other effects of oestrogens and progesterone – a quick overview only

This should all make functional sense now.
Remember – follicular phase = oestrogens, luteal phase = progesterone.
You should know the uterine anatomy and layers.

Oviducts	oestrogens:	↑ activity of cilia, ↑ epithelial secretion
	progesterone:	↓ ciliary activity, ↓ secretion
Uterus	**oestrogens:**	proliferation of endometrium ↑blood supply to endometrium ↑RNA/protein synthesis **induce progesterone$_R$**
	progesterone:	"secretory phase" ↑secretion of uterine glands further thickening of endometrium (only when oestrogen-primed)
	withdrawal of progesterone:	sloughing of endometrium (spiral arteries constrict to reduce blood loss) and menses
Cervix + vagina	oestrogens:	cervix and vagina become secretory, mucus less viscous (*facilitate sperm penetration*)
	progesterone:	very viscous mucus (*form a plug to avoid abortion*)
Mammary glands	oestrogen and progesterone stimulate growth (look up details / we'll cover it later)	
Brain	effects on sexual behaviour, appetite (↑ by P), other behavioural changes body temperature (1°C increase, 2–3 days after ovulation)	

We will look at some of the other effects when we cover pregnancy and lactation.

Menopause

- Irregular menstrual cycles, first seen during puberty, reappear in women in their early forties onwards and mark the onset of the **menopause.** This period of change may last 10 years or more and is called the **climacteric.**
- It results from a decline in the number of ovarian follicles and a reduction in their responsiveness to LH/FSH.
- In addition, the responsiveness of the pituitary and hypothalamus to oestrogens falls.
- As the follicles fail, **oestrogen secretion declines,** though androgen levels tend to rise.
- **Ovulation ceases.**
- As there is no negative feedback, LH and FSH levels *increase* dramatically.

- **Oestrogen withdrawal** causes
  atrophy of reproductive organs
  vasomotor changes (hot flushes, night sweats)
  increased bone catabolism (anti-parathyroid effect of oestrogens lost) ⟶ osteoporosis
  behavioural changes