

Lecture 30: Female Reproductive Endocrinology

Most species have a distinct breeding season - reproduction shuts down for both sexes during parts of the year in which resources are limited. Breeding is timed so that young are growing fastest when resources are most abundant.

Within the breeding season, the endocrine control of reproduction in males and females is very different - males are continuously fertile, but females ovulate cyclically.

Control of ovulation is complex. Could start the description at several places - arbitrarily, I'll begin with ovary and follicles, and work up from there.

The ovary and ovulation: follicles, corpora lutea

(**Overhead:** Fig. 2.3 Nalbandov - structure of mammalian ovary).

(**Overhead:** Fig. 2.5 Nalbandov - structure of bird's L ovary - R ovary not functional)

Even before a female is born, her ovaries hold ova (eggs, female gametes). In an immature female, each egg is enclosed in a ***primary follicle*** (small, no membrane). Most eggs in an adult female are also in primary follicles. At any time, a small subset develop into ***secondary follicles*** (larger, surrounded by membrane). In each estrous cycle, large secondary follicles are recruited to become ***Graafian follicles***.

Mature, Graafian follicles have a fluid filled cavity (the ***antrum***). The egg itself projects into this space, surrounded by ***granulosa cells***, which primarily secrete estrogen. The projection into the antrum is called the ***cumulus oophorus***. A 'ripe' follicle moves up to the surface of the ovary so that it bulges out, with the cumulus oophorus on the side away from the ovarian wall. A site (the ***stigma***) develops on the outer surface, where the tissue changes so that the follicle can rupture. At ovulation, the follicle splits and the egg shoots out with the antral fluid.

Many secondary and Graafian follicles regress without ovulating (called ***atresia***). This is not well understood, but it is normal.

After ovulating, the follicle fills with blood and lymph. The blood clot is resorbed as ***luteinization*** occurs, and is replaced by granulosa cells that form a ***corpus luteum (CL)***, 'dark body' plural = ***corpora lutea***). These cells primarily secrete progesterone.

After a period (usually a few days to a few weeks, but longer in some species), the CL regresses, progesterone secretion drops, and it converts to a non-functional ***corpus albicans*** ('white body') made mostly of connective tissue - eventually becomes barely detectable scar on surface of ovary.

Endocrine control of ovarian cycles:

Follicular phase: prior to ovulation, cells in the ***follicle*** primarily secrete ***estrogen***.

Luteal phase: after ovulation, cells in the ***corpus luteum*** primarily secrete ***progesterone***, but also secrete some estrogen.

Some shorthand - most of the estrogen is estradiol - E_2
Progesterone - P_4

Noted before that there are many follicles in ovary. After an ovulation, the CL's from that ovulation secrete P_4 . But other pre-ovulatory follicle are growing, and they secrete E_2 .

Result - the ***ratio of E_2/P_4*** varies through time.

- $E_2:P_4$ increases as follicle grows
- $E_2:P_4$ drops abruptly at ovulation
- $E_2:P_4$ increases as CL's regress

Variation in $E_2:P_4$ ratio is important in understanding how endocrine feedback loops control ovulation.

Higher levels controlling folliculogenesis and ovulation. ***Hypothalamus*** controls ***anterior pituitary*** (AP), which controls ovary.

The AP is a gland distinct from the brain. The posterior pituitary (PP) is actually an extension of the brain - so PP secretions are neurohormones. AP hormones are glandular hormones (not neurohormones). The hypothalamus (in the brain) secretes hormones into the ***hypothalamic-hypophyseal portal veins***.

(**Overhead:** Fig 3. Perry: relationships of AP and PP to brain)

(**Overhead:** Fig 4. Perry: hypophyseal portal veins to AP, neurons to PP).

(Diagram of loops: GnRH, ***gonadotropins*** (FSH and LH), ovarian steroids (estrogen and progesterone)).

(**Overhead:** Fig 9.31: Eckert ; the endocrine loops controlling ovulation)

(**Overhead:** 9.32 Eckert: two primate menstrual cycles: menstrual vs estrus)

Sequence of events:

1. Hypothalamus secretes Gonadotropin Releasing Hormone (GnRH).
2. GnRH stimulates secretion of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) by the AP. (always a + effect).
3. FSH stimulates growth of pre-ovulatory follicles(s).
4. As follicles grow, they secrete estrogen in increasing amounts. Two endocrine steps here -

- LH stimulates ovary (thecal cells, outside follicle) to secrete testosterone.
FSH stimulates conversion of androgen to estradiol (granulosa cells, in follicle).
5. LH and FSH feedback negatively on the secretion of GnRH by the hypothalamus. This helps to terminate the stimulation of follicles to grow.
 6. Estrogen and progesterone normally feedback negatively on the hypothalamus and AP. But as E₂:P₄ ratio increases, the feedback becomes positive.
 7. Note that the follicle is growing with constant level of FSH. It is an increase in *sensitivity* of ovary to FSH that causes follicular growth, initially.
 8. As E₂ increases (b/c ovary is growing) E₂:P₄ ratio increases and feedback of estrogen on hypothalamus and AP switches to *positive* feedback.
 9. Shift to positive feedback causes *estrogen surge*.
 10. Estrogen surge, with positive feedback, causes ovulatory pulse of LH.
 11. LH pulse causes ovulation.
 12. Follicle becomes CL and secretes much more P₄ than previously (but also secretes estrogens).
 13. Drop in E₂ causes LH to return to baseline. If non-conceptive, FSH also drops to baseline. Feedback of steroids on AP and hypothalamus switches back to negative. *Inhibin*, an ovarian peptide hormone, also contributes to negative feedback.
 14. CL collapses to become (nonsecretory) corpus albicantia after a period that varies among species. Prostaglandins, especially PGF_{2α}, contribute to regression of CL (*luteolysis*). PGF_{2α} may have multiple sources, but uterine endometrium is main source.

IF PREGNANT:

15. Progesterone maintains uterine wall in condition for implantation of zygote. Zygote implants.
16. After implantation, placenta (chorion) begins secreting *chorionic gonadotropin* (CG). CG keeps the CL from regressing. Implantation may also inhibit production of prostaglandins.
17. Continued presence of CL maintains P₄ secretion, which maintains uterine lining.
18. The placenta takes over the secretion of P₄, and the CL becomes nonfunctional corpus albicantia. There is a great deal of variation among species when (if) this occurs. Some, like horses, ovulate again in midgestation to produce another persistent CL as a source of P₄.

AN aside: Human pregnancy tests use an enzymatic reaction catalyzed by presence of CG, which is completely diagnostic. But continually elevated P₄ is also pretty clear indication of pregnancy (or pseudopregnancy) in field research. CG is a peptide (structure varies among species, so highly specific antibody can be hard to obtain), so RIA of P₄ (steroid) is easier in practice.

Three refinements:

A. Time scale of hormone fluctuations:

1. The secretion of GnRH by hypothalamus, and FSH and LH by AP is **pulsatile** \Rightarrow released in short bursts. The reason for this is not understood, but it may have to do with avoiding negative short-short loop feedback of gonadotropins on hypothalamus.

(**Overheads:** Compare Fig 1 Serra, p192, with Greenwald et al. Fig 47.9, p 675 - sampling 1x/day vs 1x/6 min).

One example of how pulsatility of LH secretion relates to function - summation rather than pulses during ovulatory LH pulse in rats.

(**Overheads:** Greenwald et al Fig 46.5 - rat 4-day cycle
Fig 46.6 - pulses of LH
Fig 46.7 summation of pulses during LH pulse)

2. Steroids are secreted more continuously \Rightarrow called **tonic** secretion. Secretion itself is pulsatile, but amplitude of pulses is low, so circulating levels do not show as much short-term (hourly) variation as peptide hormones.
3. Steroids fluctuate in a circadian rhythm - high in the AM and low in the PM. Circadian variation in estrogen and progesterone secretion is not great, but this is important for other steroids (androgens and corticosteroids), which vary by factor of 2x-7x in a typical day.
4. Obviously, this creates headaches in measuring a representative hormone level with a single sample. One solution - use a time-pooled sample, in urine or feces. Here the short-term fluctuations are averaged out over the period in which the urine or feces was pooled.

B. Biological activity of hormones. Hormones secreted into the blood vary in their level of activity - the same concentration of hormone can have different effect in different context.

1. Hormones generally have negative feedback on hormone **receptor densities**. For example, if circulating cortisol levels remain high for a long time (days, weeks), the density of cortisol receptors will decrease in target tissues.
2. **Steroid binding globulins.** Much of the steroids in circulation are inactivated by binding to proteins. This serves to increase the duration over which hormones have an effect (bound hormone is released as free hormone is cleared). A consequence is that variation in hormone *or in binding globulin* can alter level of effect.

Consequence:

1. If circulating level of a hormone differs (between individuals, times), effect of hormone probably differs.
2. But if circulating level of hormone does not differ (between individuals, times), it is still possible that effect of hormone differs.

Two solutions: Can assay hormone and binding globulin levels in blood. Can assay steroid hormones in saliva - bound hormone does not pass through filtration (too big a molecule), so saliva concentration parallels active concentration in blood).

C. *Interspecific variation.* There is more than one way to accomplish many endocrine processes. The general pattern above applies to most birds and mammals, but the details can differ among species. Mention one here.

Ovulation is *spontaneous* in some species (ovulation occurs whether or not mating occurs). Ovulation is *induced* by mating in other species.

(**Overhead:** Fig. 5.2 Nalbandov)

(**Overheads:** Greenwald et al, 4 figs.)

Fig 45.3: push-pull perfusion in mating rabbits

Fig 45.1 schematic of reflex ovulation

Fig 45.2 GnRH release due to mating

Fig 45.7 response of LH and FSH to GnRH

After LH surge, the process is similar to spontaneous ovulation

Induced ovulators tend to be solitary, species - e.g most mustelids, most cat species Why? Benefit: ready to mate when encounter a male.

Cost: pseudopregnancy - whether or not a zygote implants, the uterine lining is maintained and the endocrine events of pregnancy (high P₄) run their normal course.

Hormonal effects on female reproductive behavior

1. *Mating* has two components:

Proceptivity - female becomes sexually attractive to males

Receptivity - female is willing to mate

Both components are mainly stimulated by estrogen. Estrogen alone can cause induce mating behavior, but this requires pharmacological doses (higher than normal physiological range).

Pretreatment with progesterone allows elevated E₂ within physiological range to induce mating.

If P4 is given simultaneously with E2, then it has a negative effect on inducing estrous behavior

(**Overhead:** Fig 47.5 Greenwald et al, p. 666 - data from sheep on P4 pretreatment).

2. **Parental** behavior and care of young is stimulated by *prolactin* (from anterior pituitary) and *progesterone*.

Example - Florida Scrub Jay. Cooperative breeders - live in groups that include helpers - mainly adult offspring from previous years who have not dispersed. Helpers typically don't breed (we'll discuss this at length in later lectures) but do help to raise the offspring produced by dominant male and female.

(**Overhead:** Schoech et al, Fig 2. & Fig. 3)

Prolactin levels rise throughout breeding season for all individuals. Superimposed on this are several patterns:

- Higher in females than in males (different scales on y-axis in Figs 2 & 3)
- Higher in breeders than nonbreeders
- Variation among years

(**Overhead:** Schoech et al Fig 4)

Consistent with control of parental behavior by prolactin levels, breeders (with higher Prl) feed chicks more than nonbreeders.

But females do not feed more than males, as would be predicted by a 1:1 relationship between Prl and parental care.

(**Overhead:** Schoech et al Fig 5)

Overall, \uparrow Prl \rightarrow \uparrow feeding rate
Breeders seem to feed at a high rate no matter what their Prl level.
Helpers feed more if their Prl is high.

All of the patterns are consistent with conclusion that \uparrow Prl \rightarrow \uparrow in parental care (feeding of chicks), up to a threshold of about 40 ng/ml. Above that, further increase in Prl does not increase parental effort. **Thresholds** are common in endocrine processes \Rightarrow above a certain hormone concentration, *all receptors for that hormone are occupied*. Cannot increase response further, unless the density of receptors increases.

(**Overhead:** Schoech et al Fig 5)

More chicks \rightarrow higher Prl \rightarrow higher parental effort. This suggests an increase in begging or other stimulation by chicks may increase Prl to meet the higher parental effort that a large clutch requires.

3. Lactation is stimulated by *prolactin*,
Milk let-down is stimulated by *oxytocin* (from posterior pituitary).

Pheromonal effects on reproduction.

Substances in urine, feces, other secretions can have dramatic effects on reproduction in individuals exposed to the substances. Vomeronasal organ (closely related to smell) is highly sensitive to pheromones, but normal sense of smell is also involved.

Lee-Boot effect. Mice housed in all-female groups stop cycling. (Evolutionarily, this saves wasted effort of coming into estrus when there is little chance of establishing pregnancy).

Whitten effect Females that are housed together tend to ovulate synchronously.

Whitten housed female mice in groups of 10-30, then housed each female alone with one male. Mice have an estrous cycle of 5 days, so by chance would expect 1/5 of the mice to mate on each of the next 5 days.

	Observed	Expected by chance	O-E
Day 1:	43 mated	58	-15
Day 2	44	58	-14
Day 3	146	58	+88
Day 4	42	58	-16
Day 5	14	58	-44

In mice, it turns out that exposure to male pheromones terminates suppression of cycles that occurs in all female groups, which leads to synchronization with the experimental design above.

This effect occurs in humans, as shown by studies of women living in dormitories. In humans and other primates, the synchronization occurs in absence of males - pheromone involved is from other females.

(Overhead: McClintock Table 1 & Fig 1)

Why synchronize estrous? Predator dilution. Common cause of death for juveniles of many species is predation. If all young are born at once, prey swamp the predator's ability to eat them all, and a higher proportion live through to age at which they are less vulnerable.

Bruce effect

Pregnant female mouse exposed to a strange male will abort or resorb embryos, come into estrus again and mate with the new male. Benefit to the male is obvious - he doesn't wait to begin producing own offspring. For female, this is 'best-of-a-bad-situation' solution. The male is likely to kill offspring other than his own (seen in many species, including some without Bruce effect). Given that the offspring are not likely to escape infanticide, best solution is to bypass the energetic costs of the remaining period of gestation (gestation and lactation are energetically costly - up to 3x basal metabolic rate of nonpregnant individuals), and establish a new pregnancy.