Functional physiology of the female genital organs of the domestic animals

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Generative Organs - Ovaries

- The ovary is the primary female reproductive organ and has two important functions:

- **producing the female reproductive cell** (the egg or ovum)

- and **producing the hormones estrogen and progesterone**.

- The ovarian follicles are the key structures that produce both the gametes and the ovarian hormones during their growth in sequential reproductive cycle in most domestic animals. The growth of the follicles is rhythmic and continuous during the entire year in animals like cow but shows a seasonal influence in animals such as mares, sheep, goats, buffalo and camel.

- The sequential growth of the ovarian follicles starts to some extent before birth, is halted at birth and becomes cyclical after attainment of puberty.

- The activation of follicle growth is complex and involves many environmental, nutritional, hormonal and other cues.

- The process of **oogenesis** (formation of ovum-the female gamete) occurs in coordination with the other cells of the ovary that enclose the ovum producing cells in the structure called **follicle**. The follicle cells interact with the primary oocyte resulting in their sequential growth via a process known as **folliculogenesis.** Both oogenesis and folliculogenesis occur in coordination after birth but are discussed separately after the embryonic development.

Embryonic development of ovary

- The first appearance of the gonad is essentially the same in the two sexes, and consists in a thickening of the mesothelial layer of the peritoneum. The thick plate of epithelium extends deeply, pushing before it the mesoderm and forming a distinct projection. This is termed the **gonadal ridge**. The gonadal ridge, in turn, develops into a gonad. This is a testis in the male and an ovary in the female.

- At first, the mesonephros and gonadal ridge are continuous, but as the embryo grows the gonadal ridge gradually becomes separated off from the mesonephros. However, some cells of mesonephric origin join the gonadal ridge. Furthermore, the gonadal ridge still remains connected to the remnant of that body by a fold of peritoneum, namely the **mesorchium or mesovarium**. About the seventh week the distinction of sex in the gonadal ridge begins to be perceptible.

- The **ovary** is thus formed mainly from the **genital ridge** and partly from the **mesonephros**. Later the mass is differentiated into a central part, the medulla of ovary, covered by a surface layer, the germinal epithelium. Between the cells of the germinal epithelium a number of larger cells, the immature ova, are found. The immature ova, in turn, are carried into the stroma beneath by bud-like ingrowths (*genital cords of the germinal epithelium*). The surface germinal epithelium ultimately forms the permanent epithelial covering of this organ. Furthermore, it soon loses its connection with the central mass. Instead, the **tunica albuginea** of the ovaries develops between them.

- The **ovary** is first apparent as a thickening of the coelomic epithelium on the medial aspect of the mesonephros, around day 22 in sheep, and day 30 in cattle.

- The **ovigerous cords** are loose fetal epithelial structures, also called **germ cell nests or cysts**.

- Evidence of the beginning of ovigerous cord formation was found on day 60. By day 75 of gestation, the ovigerous cords were more extensive and mesonephric-derived cell streams were detectable. By day 90 (and still present at day 105), both ovigerous cords and cell streams/rete tubules were definitive structures of the developing ovaries. Ovaries appeared to develop in "lobular" segments around the periphery of the ovary. Some lobes appeared to be at slightly different developmental stages, as assessed by the extent or definition of ovigerous cord formation.

- During early embryonic development, cells from the dorsal endoderm of the yolk sac migrate along the hindgut to the gonadal ridge. These **primordial germ cells** (PGCs) multiply by mitosis and once they have reached the gonadal ridge they are called oogonia (diploid stem cells of the ovary).

- Once oogonia enter this area they attempt to associate with the other somatic cells, derived from both the peritoneum and mesonephros. Development proceeds and the oogonia become fully surrounded by a layer of connective tissue cells (pre-granulosa cells) in an irregular manner. It has been hypothesized that the ovarian surface epithelial cells penetrate into the ovary to form the granulosa cells In this way, the structures formed are known as **primordial follicles**.

- Newer research shows that before 70 days of gestation the gonadal ridge/ovarian primordium is formed by proliferation of **Gonadal Ridge Epithelial like (GREL)** cells at the surface epithelium of the mesonephros. Primordial germ cells (PGCs) migrate into the ovarian primordium. After 70 days, stroma from the underlying mesonephros begins to penetrate the primordium, partitioning the developing ovary into irregularly-shaped **ovigerous cords** composed of **GREL cells and PGCs/oogonia**. The cords are always separated from the stroma by a basal lamina. Around 130 days of gestation the stroma expands laterally below the outermost layers of GREL cells forming a sub-epithelial basal lamina and establishing an epithelial-stromal interface. It is at this stage that a mature surface epithelium develops from the GREL cells on the surface of the ovary primordium. Expansion of the stroma continues to partition the ovigerous cords into smaller groups of cells eventually forming follicles containing an oogonium/oocyte surrounded by GREL cells, which become granulosa cells, all enclosed by a basal lamina.

- Irrespective of whether the ovarian surface epithelial cells penetrate into the ovary to form the granulosa cells of follicles,

or

  Ovarian surface epithelial cells and granulosa cells have a common precursor, the GREL cell

It is for certain that the oogonia are diploid cells, they divide mitotically and produce the diploid primary oocytes which then undergo meiosis and are arrested at prophase and then enclosed by the granulosa and other somatic cells to form the primordial follicles.

Oogenesis is the origin and development of the ovum. Oogenesis, which begins in fetal life, is not completed until animals are sexually mature.

During development of the embryo, cells from the inner cell mass undergo differentiation to become specialized stem cells that form various tissues of the fetus.
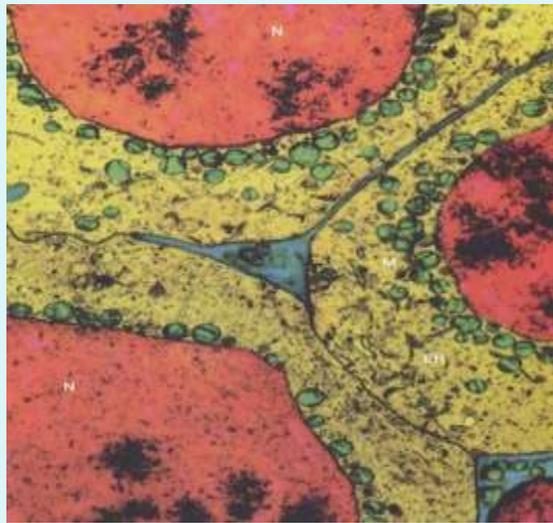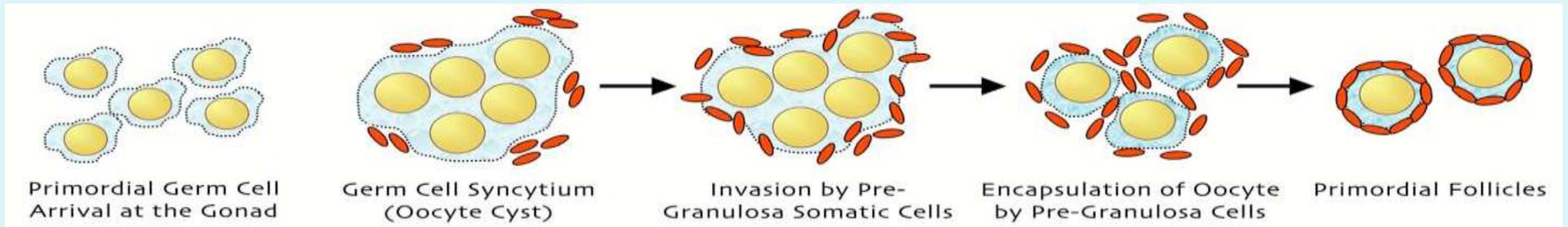
Some stem cells become **primordial germ cells (PGC)**, which are precursors of **oogonia** in the female and spermatogonia in the male. **PGCs** migrate from the embryonic yolk sac to the **genital ridge** and populate the developing gonads -- **the bilateral ovaries** of the female and the bilateral testes of the male.

PGCs in the bovine female fetus differentiate into oogonia during the first trimester of gestation. Oogonia divide mitotically well into the second trimester of gestation, generating a peak of approximately **2.7 million germ cells** at around **Day 110** of gestation.

In cattle, the first primordial germ cells were identified in an 18 day old embryo. The primordial germ cells loose their motility after reaching the developing ovaries. They are now called **oogonia** and these cells show a high frequency of mitotic division and produce diploid **primary oocytes**.

During the second trimester, oogonia form **egg nests** in the ovarian cortex and enter the **first prophase of meiosis** where they become **arrested** in the **diplotene stage**. Somatic germinal epithelial cells from the surface of the ovary invade the egg nests and form a single flattened layer of epithelial cells around each oocyte to form the primordial follicle. **The primordial follicle** comprises the arrested oocyte surrounded by the single layer of epithelial cells.

Germ Cells Division and Follicle Formation

Primordial Germ Cell Arrival at the Gonad

Germ Cell Syncytium (Oocyte Cyst)

Invasion by Pre-Granulosa Somatic Cells

Encapsulation of Oocyte by Pre-Granulosa Cells
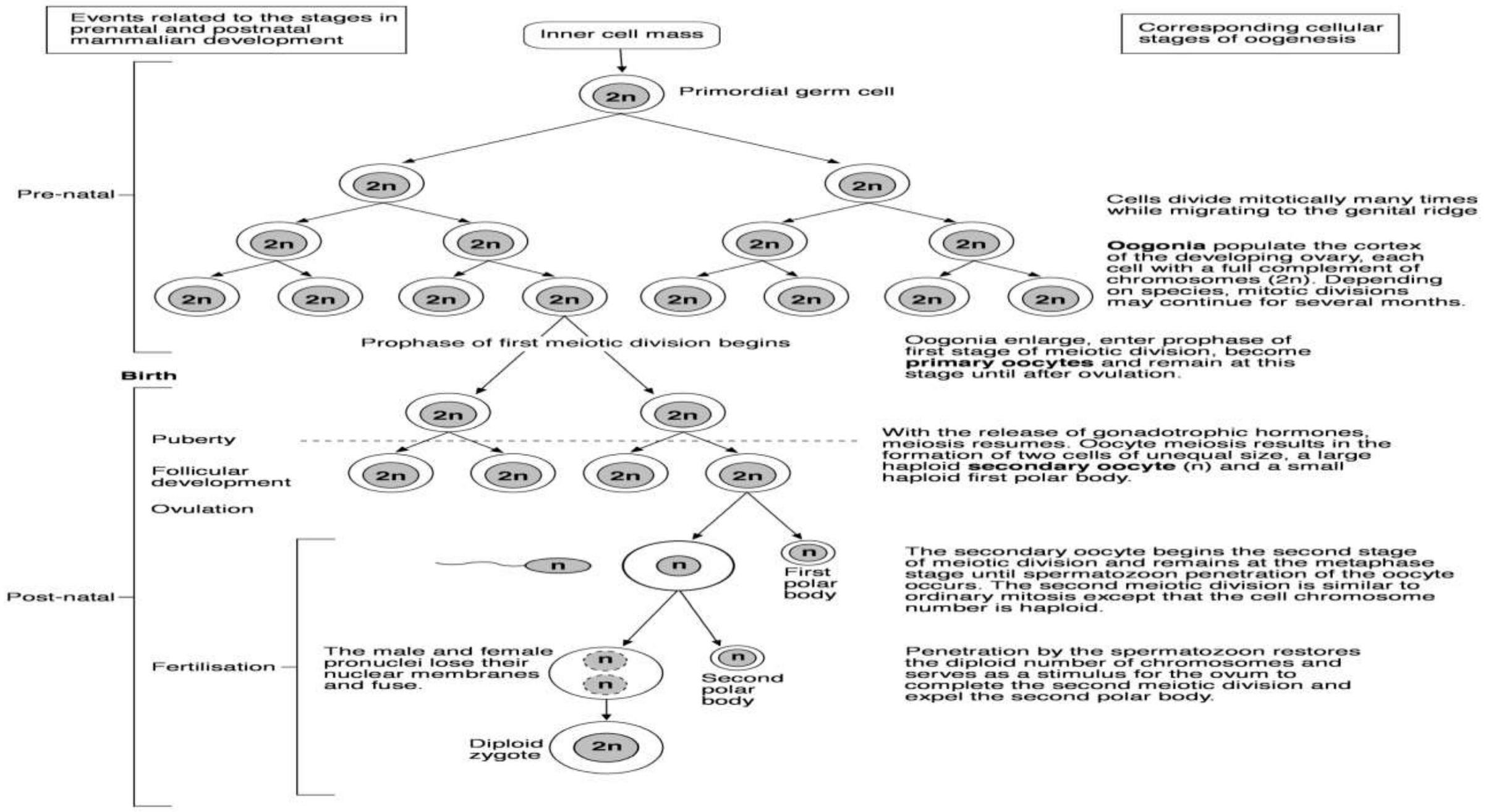
Primordial Follicles

from Makabe and van Blerkom, 2006

- The highest number of oogonia were present at Day 50 of gestation in the pig and Day 110 in cattle.
- The number of oogonia then decreases till birth. The PGCs undergo cortical proliferation and divide by mitosis to yield **oogonia** (2n). The oogonia divide by mitosis (**oocytogenesis**) to yield **primary oocytes** (2n). The mitotic division of oogonia (2n) leads to **finite number of primary oocytes** for the reproductive cycle.
- The primary oocytes undergo meiosis-1 (**ootidogenesis**) which is however halted at prophase-I and resumed at puberty and completed at ovulation. The **primary oocytes (2n)** become enclosed in a single layer of pre-granulosa cells, surrounded by a thin basement membrane and called **primordial follicles.**
- The development of primary oocytes from PGCs all occur prenatally with primary oocytes blocked at prophase I.
- The growth of oocytes is resumed after puberty and the meiotic block is removed by the action of luteinizing hormone surge. **The primary oocyte** completes its first meiotic division producing a **secondary oocyte** and the first polar body is released

Maximum number of female germ cells reached in fetal ovaries during gestation in different species and the number of germ cells in the ovaries at the time of birth or nearly after

Species	Maximum number of germ cells (Day of gestation)	Number of germ cells close after birth (Day after birth)
Heifer	2,700,000 (110)	68,000 (13 days after birth)
Female Piglet	1,100,000 (50)	500,000 (at birth)
Buffalo Heifer	23,540 (210)	20,000 (at birth)
Rat	75,000 (18)	27,000 (2 days after birth)
Human female	6,800,000 (150)	2,000,000 (at birth)

Events related to the stages in prenatal and postnatal mammalian development

Corresponding cellular stages of oogenesis

Inner cell mass

2n — Primordial germ cell

Pre-natal

Cells divide mitotically many times while migrating to the genital ridge

Oogonia populate the cortex of the developing ovary, each cell with a full complement of chromosomes (2n). Depending on species, mitotic divisions may continue for several months.

Prophase of first meiotic division begins

Oogonia enlarge, enter prophase of first stage of meiotic division, become **primary oocytes** and remain at this stage until after ovulation.

Birth

Puberty

With the release of gonadotrophic hormones, meiosis resumes. Oocyte meiosis results in the formation of two cells of unequal size, a large haploid **secondary oocyte** (n) and a small haploid first polar body.

Follicular development

Ovulation

First polar body

The secondary oocyte begins the second stage of meiotic division and remains at the metaphase stage until spermatozoon penetration of the oocyte occurs. The second meiotic division is similar to ordinary mitosis except that the cell chromosome number is haploid.

Post-natal

Fertilisation

The male and female pronuclei lose their nuclear membranes and fuse.

Second polar body

Penetration by the spermatozoon restores the diploid number of chromosomes and serves as a stimulus for the ovum to complete the second meiotic division and expel the second polar body.

Diploid zygote — 2n

Figure 2.3 Oogenesis, which begins in foetal life, is not completed until animals are sexually mature. Oocytes, gametes produced by female animals, provide the maternal genetic material and nourishment for the developing zygote.
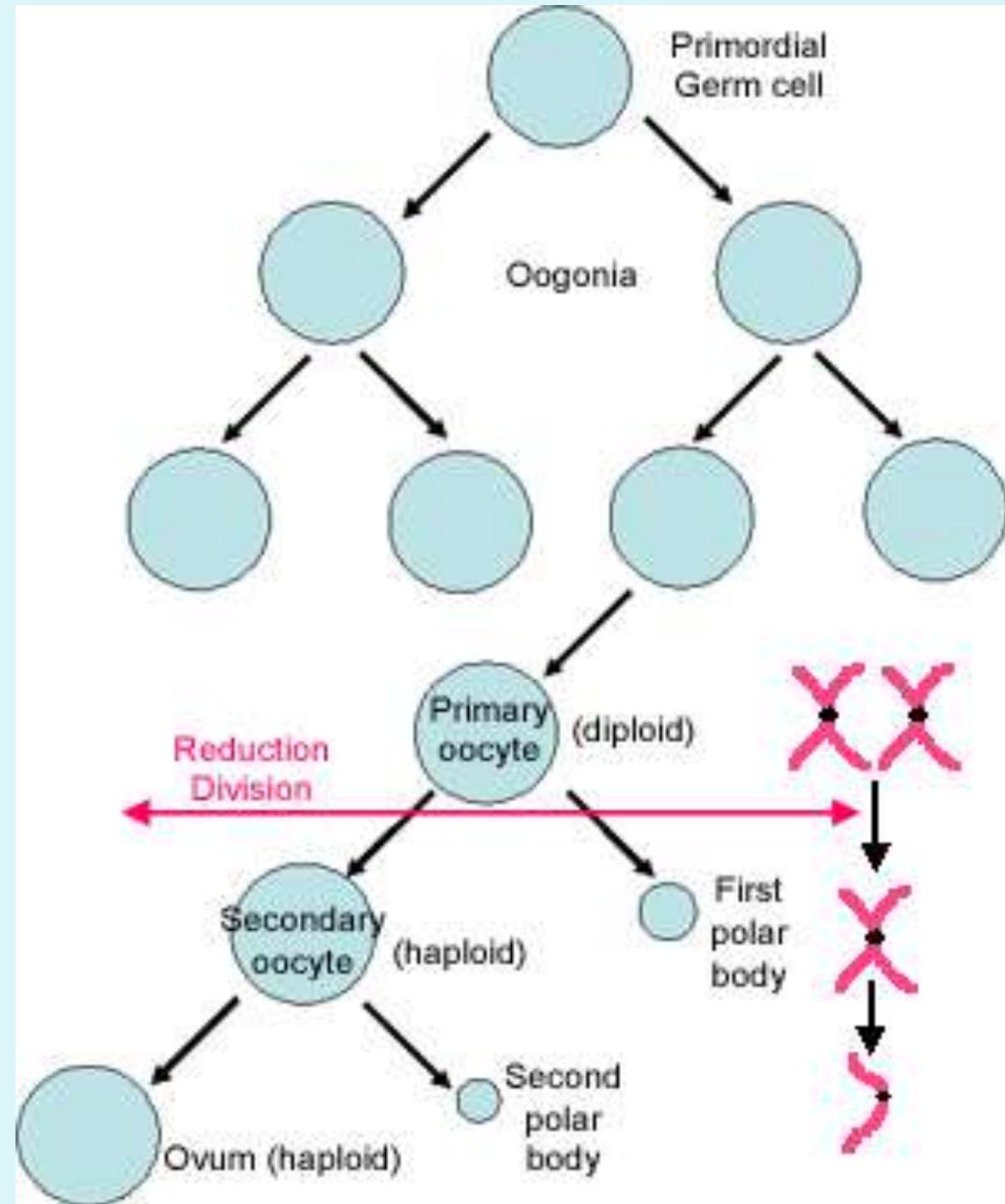
Once the **primordial follicle** is formed, the **follicular epithelial cells** that are in direct contact with the oocyte interact through localized cellular signals to regulate oocyte maturation. Follicle growth is regulated by signals between the oocyte and surrounding epithelial cells and between the epithelial cells and other somatic cells in the ovarian cortex and by signals emanating from systemic circulation.

Some primordial follicles that populate the fetal ovary begin to grow during the second trimester of gestation, but most remain in an arrested state. Growth is characterized first by enlargement of the flattened epithelial cells to become cuboidal-shaped granulosa cells. Next, the arrested oocyte increases in size and surrounding granulosa cells divide to form multiple layers. Ultimately a fluid-filled central antrum forms and partially surrounds a cluster of cumulus granulosa cells surrounding the oocyte.

Gamete production

Rhythmic growth and development of the female gamete (oocyte) occurs within the functional unit (follicle) of the ovary during the estrous cycle to release the oocyte at ovulation and cells of the ovulated follicle transform themselves into a temporary endocrine gland the corpus luteum (CL). The uterus, ovary, pituitary, hypothalamus and environment coordinate to maintain the rhythmic estrous cycle, follicle growth and release of oocyte in different patterns in different domestic animal species.

The first meiotic division is completed 4 h before ovulation and a secondary oocyte is formed with a polar body (1st polar body) in most domestic animals except the bitch in which the first meiotic division is completed after ovulation and primary oocytes are ovulated.

In bitches the eggs **are ovulated** as **primary oocytes** and are not capable of being fertilized until about 60 h after **ovulation** when they undergo the first meiotic division to become **secondary oocytes**.

The transition of the primary oocyte to a secondary oocyte reduces the chromosome numbers to half (1n).

SECOND MEIOTIC DIVISION STARTS

corpus albicans

FIRST MEIOTIC DIVISION COMPLETED

if no fertilisation

corpus luteum

FSH, LH SECRETION AT SEXUAL MATURITY

At birth (no further development until sexual maturity)

Graafian follicle containing secondary oocyte

Early primary follicle

Primary follicle

Secondary follicle

Primordial follicles containing primary oocytes

OVULATION

Oogenesis

Migration to germinal ridge
Mitotic divisions

Primordial Germ Cells

Oogonia

Follicle Cells

Initiation of Meiosis
Interphase
DNA synthesis
Meiotic prophase begins

Primary Oocyte

Prophase of Meiosis
leptotene
zygotene
pachytene
diplotene
dictyotene
Meiotic Arrest

Gonadotropin Independent

Growth

Gonadotropin Dependent

zona pellucida formation
mRNA production
protein synthesis
cortical granule formation

nuclear maturation
cytoplasmic maturation
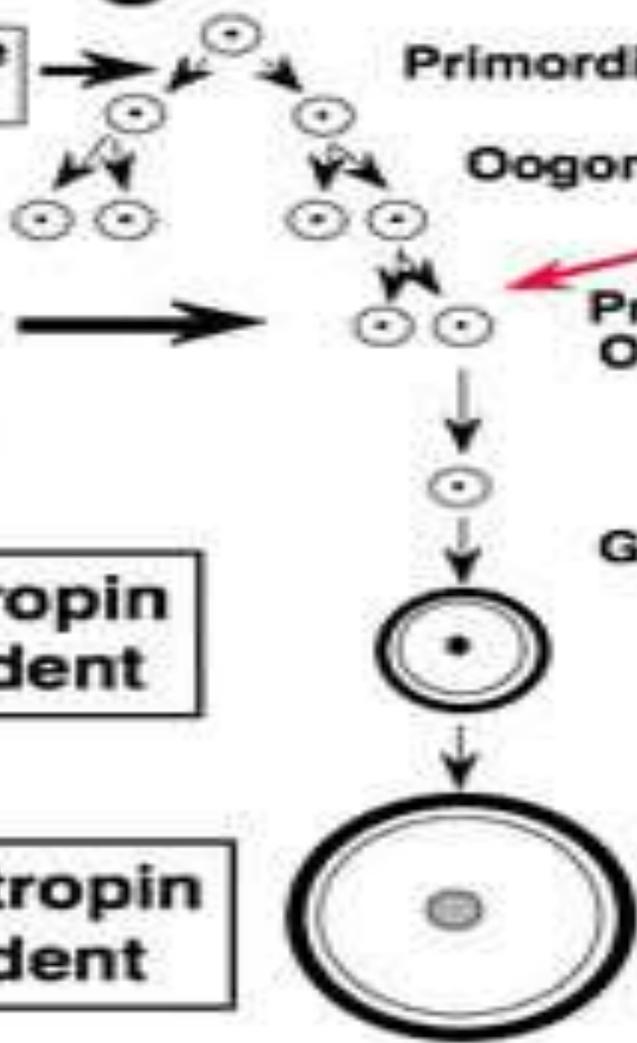
Folliculogenesis

Follicle The follicle is the basic functional unit of the ovary containing the oocyte which is surrounded by specialized epithelial cells (granulosa and theca cells) which perform endocrine function. Usually a follicle contains one oocyte however, polyovular follicles containing several oocytes may develop in carnivores, sows and ewes.

The primordial follicles sequentially undergo growth and proliferation to form the **primary follicles (preantral)** once they leave the resting follicle pool. The primary follicles then grow into **secondary** and then **preovulatroy follicles (mature follicles)** or degenerate.

Primary follicles The cells surrounding the **primary oocyte** proliferate by mitosis, acquire the receptors for FSH hormone and are known as **granulosa cells**. A glycoprotein layer known as **zona pellucida** develop at the interphase between the granulosa cells and the oocyte. With the growth of follicle under the influence of FSH, stromal cells surrounding it differentiate into spindle shaped cells called theca cells which are highly vascularized.

The growth of the primordial follicles is resumed at **puberty** subsequent to which the follicles grow in a cyclic fashion that is repeated at defined intervals regularly throughout the year in **non seasonal** breeding species.

Although the **follicular growth** is **resumed** at **puberty** however in cattle, some workers first observed primordial, primary, and secondary follicles at Days 90, 140, and 210, respectively, of the 280-day gestation period.

- Follicular growth can be classified into three phases according to their developmental stage and gonadotropin dependence :

- (1) follicular growth through primordial, primary, and secondary stages (gonadotropin-independent phase),

- (2) transition from preantral to early antral stage (gonadotropin-responsive phase), and

- (3) continual growth beyond the early antral stage (gonadotropin-dependent phase), which includes follicle recruitment, selection, and ovulation.

Puberty is the age at which gonads become active and start producing the female gamete oocyte

Gonadotropin-independent phase

Primordial follicle activated ⟶ Primary and Sec follicle

Gonadotropin-dependent phase

- Preantral ⟶ Antral follicles

Gonadotropin-dependent phase

- Early antral ⟶ Follicle recruitment, selection, and ovulation-Follicular waves

- Ovaries of cattle contain two different pools of follicles, the **non-growing** pool and the **growing** pool. The non-growing pool contains the primordial follicles, whereas the growing pool contains the **primary, secondary and tertiary follicles.** Entry of primordial follicles into the growth phase occurs throughout the reproductive life. The **primordial follicles** continuously leave the **arrested pool** and undergo the primordial to primary follicle transition.

Pool of resting primordial follicles

Recruitment

Primary → Secondary → Large preantral → Small antral → Large antral → OVULATION

non - growing pool

(primordial follicles, 35 – 100 µm)
Size of the pool depends on: oogonia multiplication, time of
meiosis initiation, loss of germ cells by apoptosis

Recruitment
Growth factors ?
Gonadotropins ?

gonadotropin-
dependent

growing pool
(primary, secondary and tertiary follicles)

antral follicles
(antrum formation: ∅ ~ 0.3 mm)

∅ ~ 2 mm

**ovulatory
follicles**

obligatory
gonadotropin-
dependent
(follicular waves)

- The growth of follicles from the primordial to ovulatory follicles is complex and requires a couple of days to months.

- The sequence of events involves the growth of primordial follicles into primary, secondary, preantral and then antral follicles (follicles with a central fluid filled cavity-the antrum). This transformation involves the proliferation of granulosa cells and reorganization of cells forming the follicle.

- During this transition the oocytes increase in size and the surrounding squamous pre-granulosa cells become cuboidal and proliferate to form a layer of cuboidal cells around the growing oocyte. The follicle is now called the **primary follicle**. The mechanisms responsible for the initiation of follicular growth during this phase are poorly understood although some molecules such as growth factors and gonadotropins have been discussed.

Secondary follicle

As the follicle becomes larger, small liquid filled areas appear between the granulosa cells. Eventually a liquid filled cavity the **antrum** develops between the granulosa cells. The liquid between the cavity is called liquor folliculi. As the antrum becomes larger and larger, the oocyte is pushed off to one side of the follicle.

The oocyte is surrounded by a layer of follicular cells (**corona radiata**) and rests on a little hillock of granulosa cells (**cumulus oöphorus**). The theca layer differentiates into a **theca interna** and **theca externa**. The theca interna is active in steroid synthesis. In response to **LH** they synthesize **androgens** which diffuse to granulosa cells and are converted to estrogens by the **aromatase enzyme**.

The theca externa resembles connective tissue and blends into the rest of the ovarian stroma.

(a) Stages of Folliculogenesis

① Primordial follicle
- Oocyte nucleus
- Oocyte
- Ovary cortex cells
- Granulosa cells

② Primary follicle
- Granulosa cells
- Oocyte
- Zona pellucida
- Oocyte nucleus

③ Secondary follicle
- Granulosa cells
- Oocyte
- Oocyte nucleus
- Zona pellucida
- Theca cells

④ Tertiary follicle
- Antrum
- Cortex
- Oocyte
- Theca cells
- Granulosa cells
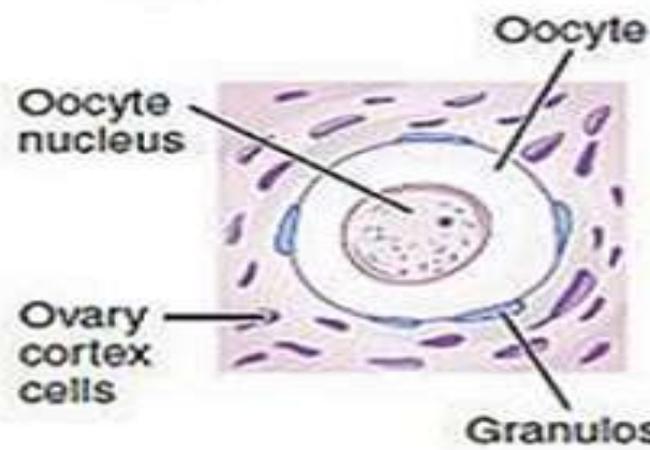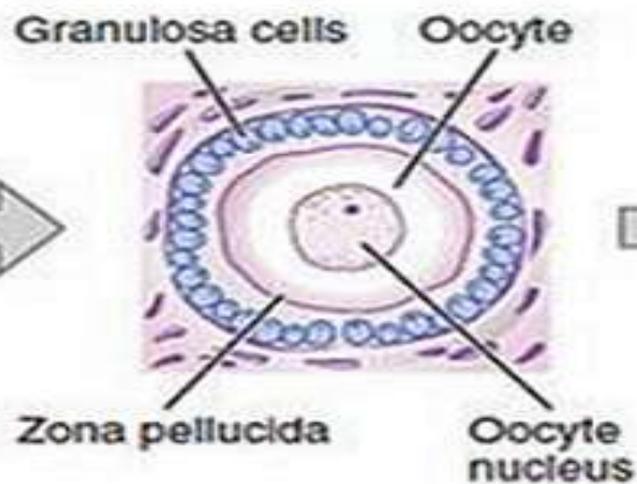
⑤ Ovulating follicle
- Oocyte
- Granulosa cells
- Ovary
- Ruptured follicle
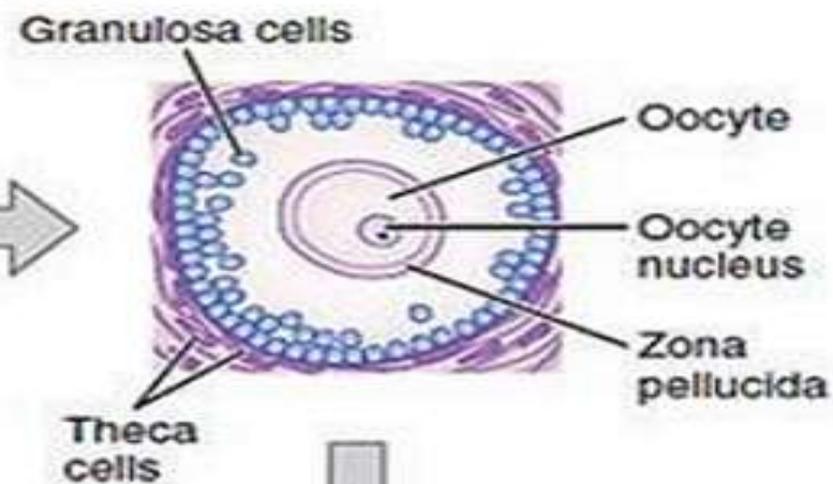
⑥ Corpus luteum
- Ovary
- Corpus luteum
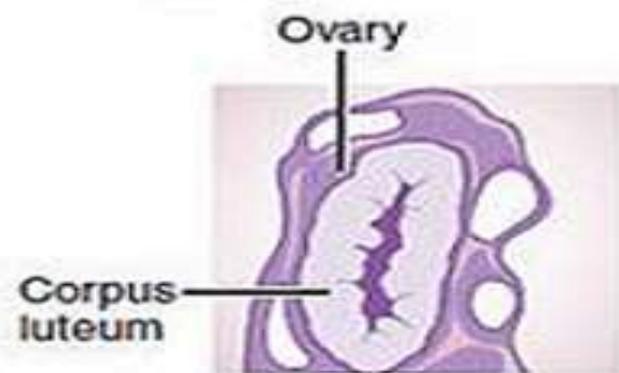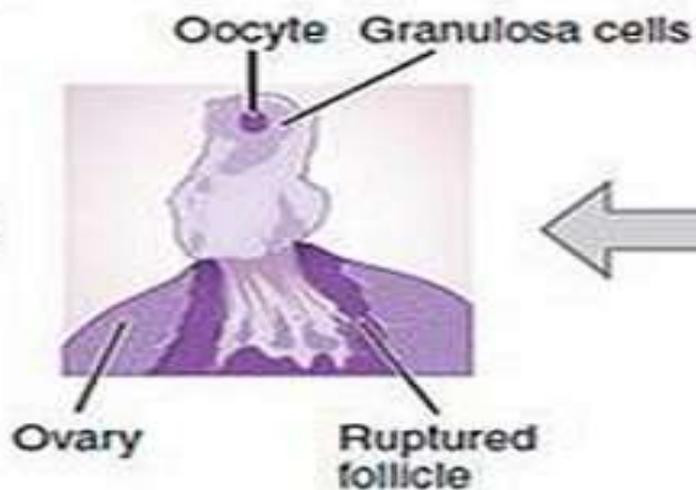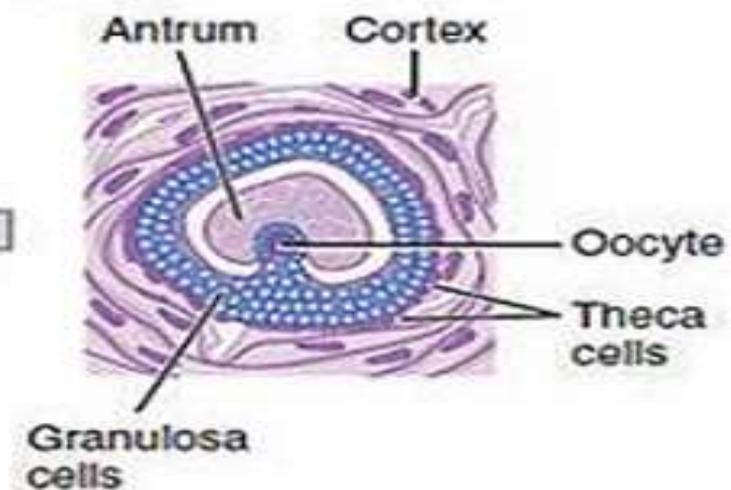
It is now known that the **antral (tertiary) follicles** are selected to grow and this growth occurs in a **wave like fashion** in most domestic mammalian species. The follicular growth waves lead to growth in the size and fluid secretions within the follicle, expression of estrus, and ovulation with release of mature oocyte.

Steroids & Growth Factors → FSH Growth Factors → FSH Growth Factors → FSH & LH Growth Factors

Primordial   Primary   Preantral   Antral   Graafian

?   10 days   10 days   40 days

80 to 100 days
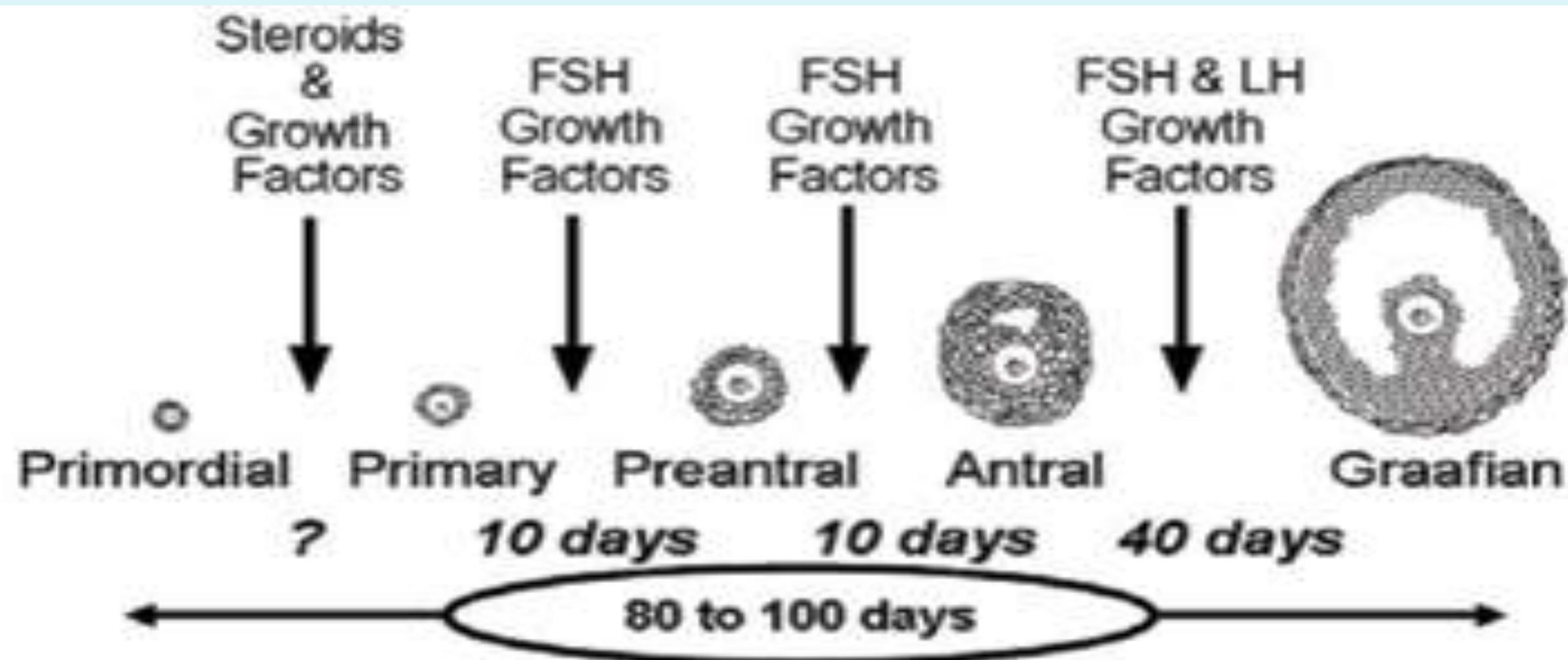
Figure 2 - Bovine folliculogenesis with approximate timeline for each stage and examples of hormones and factors that regulate transition from one stage to the next
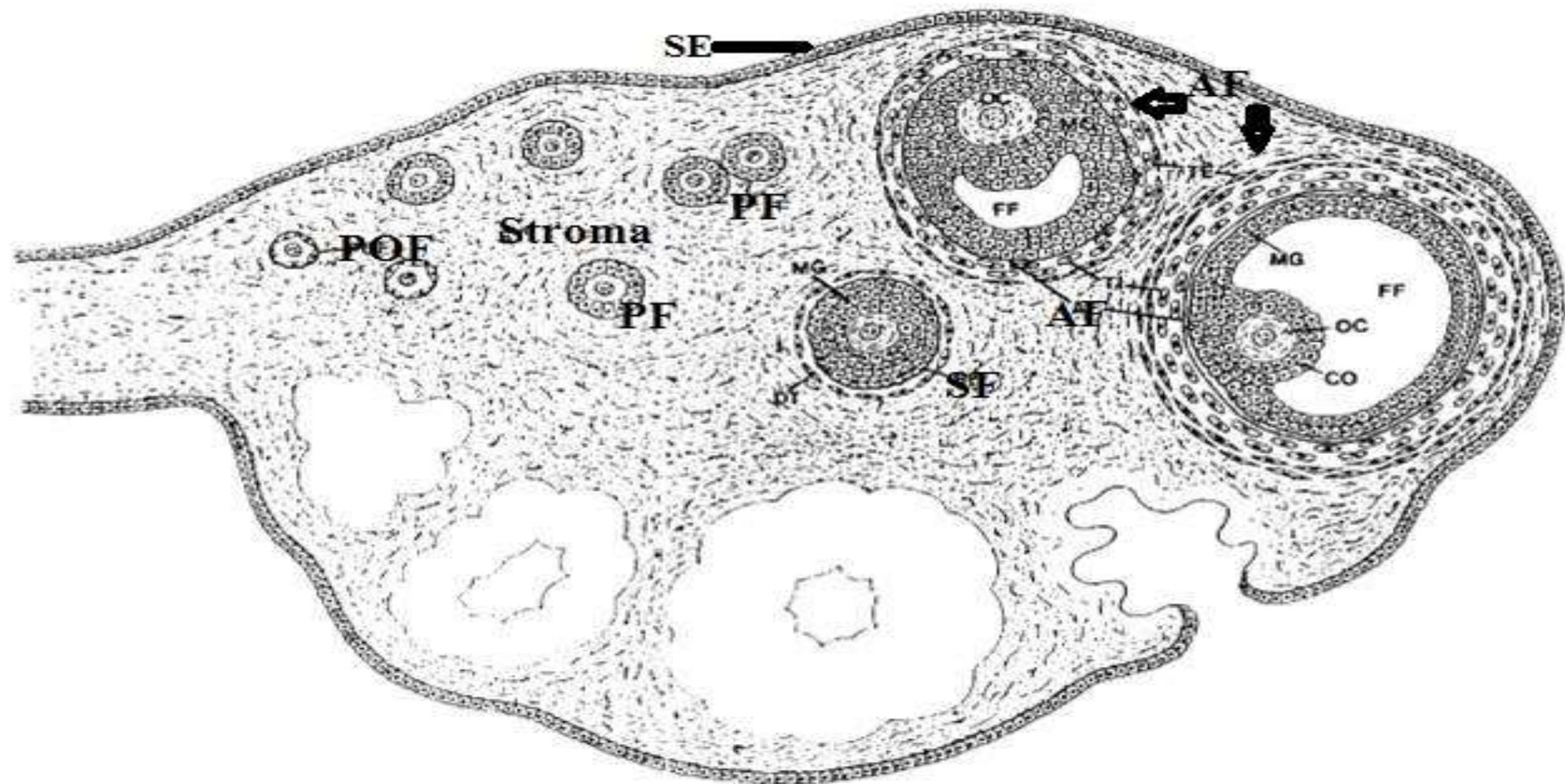
Fig. 2.12. Stages of follicle development in the bovine ovary. SE, surface epithelium; POF, primordial follicle; PF, primary follicle; SF, secondary follicle; AF, antral follicle; MG, membrana granulosa; DT, developing theca; OC, oocyte; FF, follicular fluid; TI, theca interna; TE, theca externa; CO, cumulus oophorus; ST, stromal tissue. (From Van den Hurk *et al.*, 2000.)

PRIMARY FOLLICLE

Basement Laminae
Granulosa Cells

GROWING FOLLICLE

Basement Laminae
Granulosa Cells
Zona Pellucida
Fully Grown Oocyte

GRANULOSA CELLS
Pseudostratified
(bitch, rabbit,...)
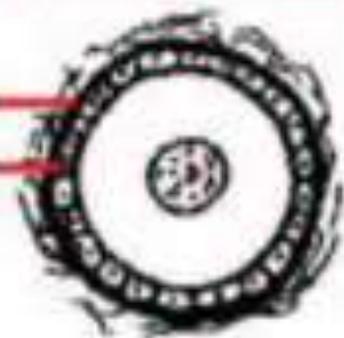
GROWING FOLLICLE

Basement Laminae
Granulosa Cells
Zona Pellucida
Fully Grown Oocyte

GRAAFIAN (TERTIARY) FOLLICLE
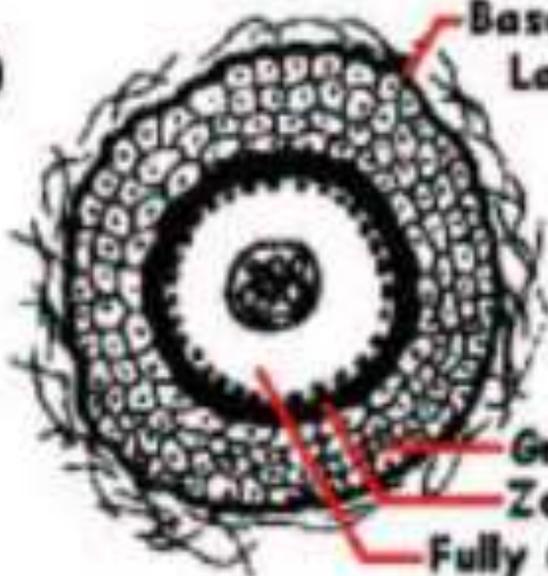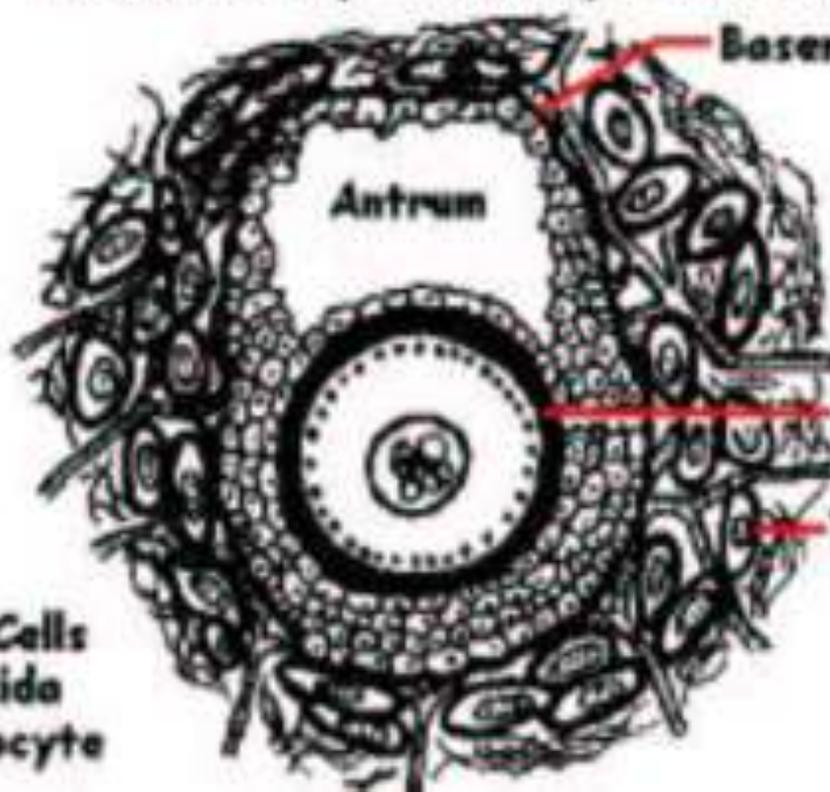
Basement Laminae
Antrum
Blood Vessel
Zona Pellucida
Theca Cells

1.6% Atresic

6.6% Atresic

40.5% Atresic

30.0% Atresic

67.4% Atresic

0.13–0.28 mm

0.29–0.67 mm

0.68–1.52 mm

1.53–3.67 mm

3.68–8.57 mm

Takes 15 days to reach next class

Takes 12 days to reach next class

Takes 3.3 days to reach next class

Takes 3.5 days to reach next class

Takes 7–8 days to reach 8.56 mm size class

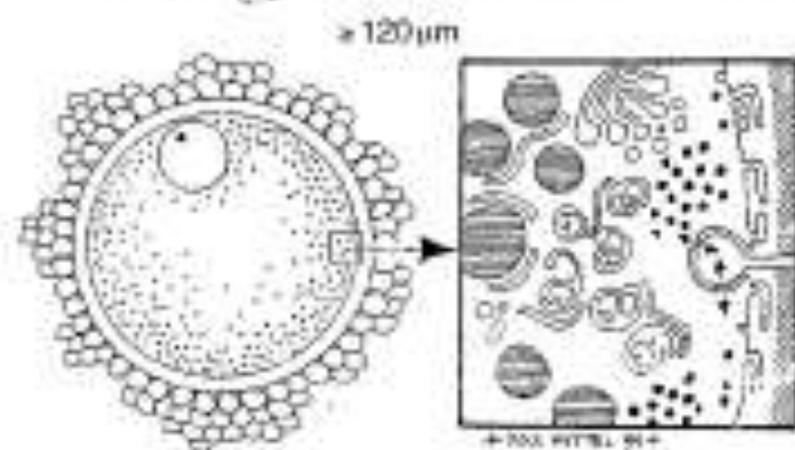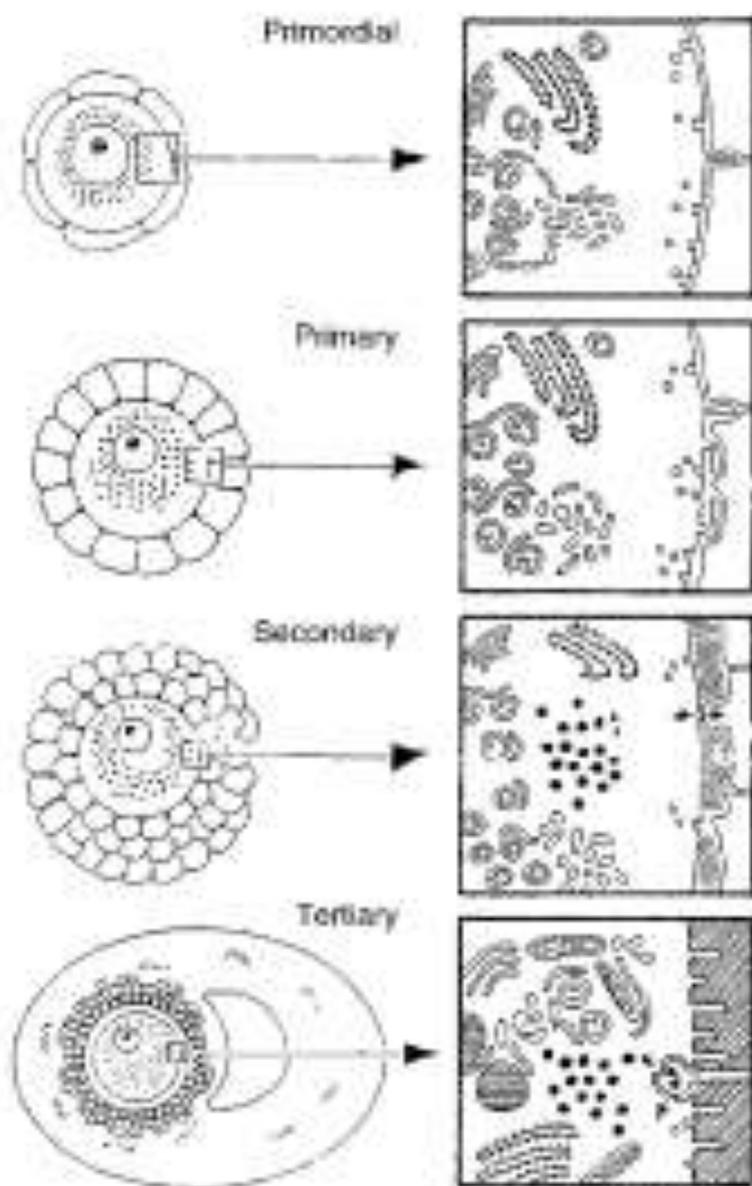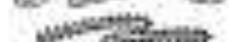120 per ovary

60 per ovary

30 per ovary

25 per ovary

5 per ovary
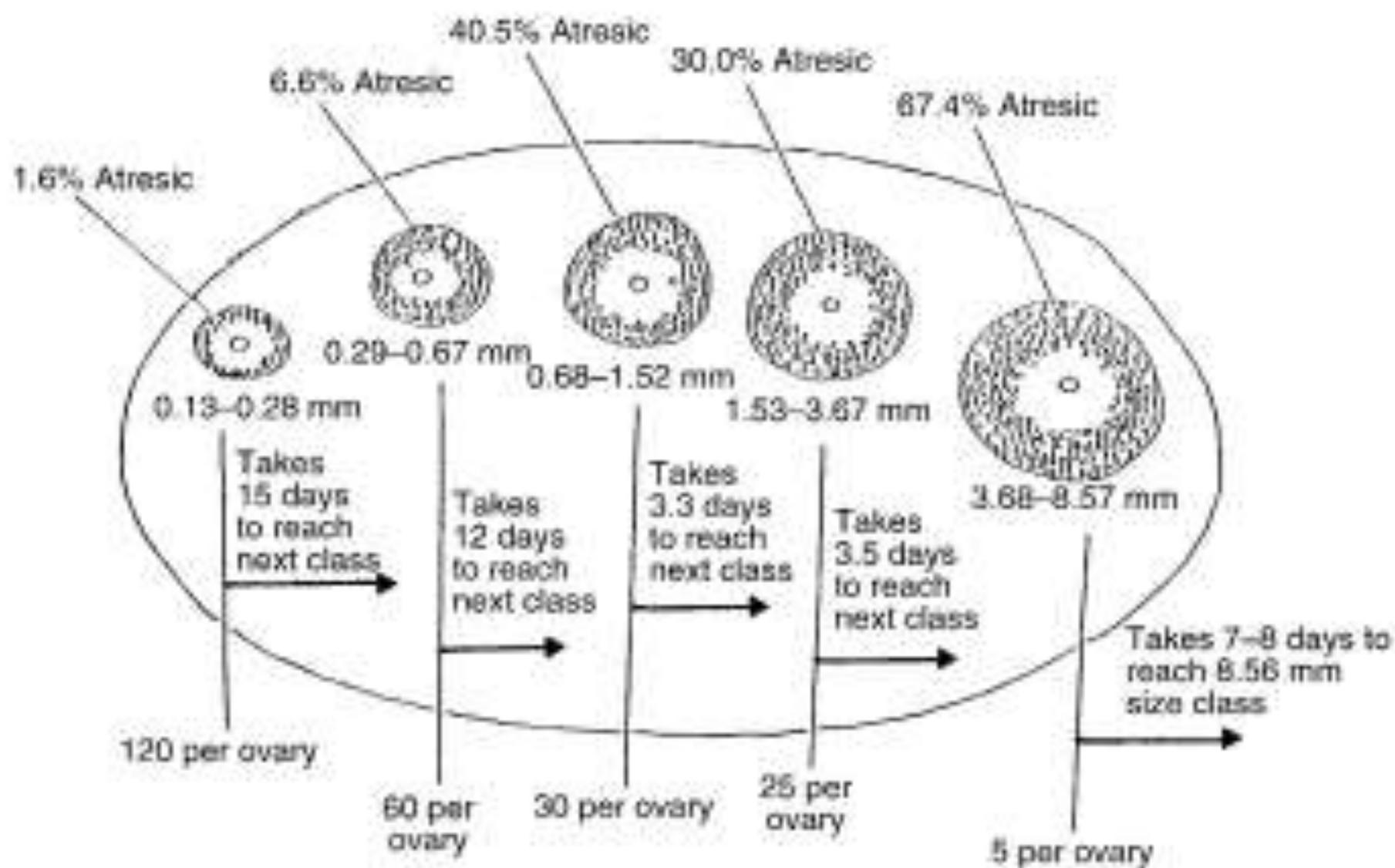
Fig. 2.13. Follicle size categories in the bovine ovary (based on data from Lussier et al., 1987).

Follicular stage

Primordial

Primary

Secondary

Tertiary

90–100 μm

≥ 120 μm
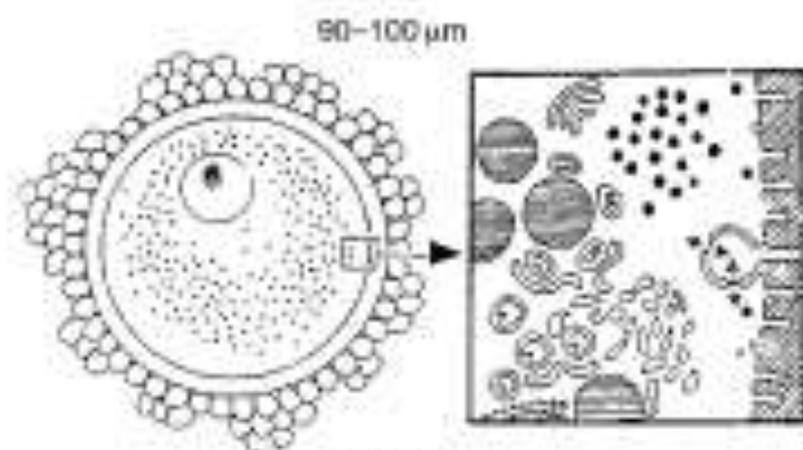
Mitochondria

Rough endoplasmic reticulum

Coated pits

Smooth endoplasmic reticulum

Golgi complex

Cortical granules

Gap junctions

Zona pellucida

Lipid droplets

- In most domestic animals the later stages of follicle development occurs in a **wave-like pattern** during estrous cycles (**cattle, sheep, goats, horses and buffalo**) or periods of reproductive activity (**llamas and camels**). A follicle wave is the organized development of a cohort of gonadotrophin-dependent follicles all of which initially increase in size, but most of which subsequently regress and die by atresia (subordinate follicles). The number of remaining (dominant) follicles is specific to the species and is indicative of litter size. Follicle waves develop during both luteal and follicular phases and it is the dominant follicle(s) of the last follicular wave that ovulates. However, there are cases where dominant follicles from the last two follicle waves can ovulate (**sheep and goats**). There are exceptions to the organized wave-like pattern of follicle growth where follicle development is apparently **continuous (pigs and chickens)**. In these animals many follicles develop to intermediate diameters and at specific times follicles that are destined to ovulate are selected from this pool and continue growing to ovulation

- Follicular growth in camels depends on **mating**
- **No** luteal phase in **non-mated camels**
- In **mated** non-pregnant camels **luteal phase is short** 6-9 days

- Follicular recruitment 2-4 days, growth 10-12 days and dominance at 6mm

- Follicles ovulate at 9-10 mm
- **No follicle above 2-2.5 cm ovulates**

Differences among species in follicle diameter, oocyte diameter and number of granulosa cells

Species	Follicular diameter (µm)			Oocyte diameter (µm)			Mean number of granulosa cells		
	PL	PR	S	PL	PR	S	PL	PR	S
Cattle	36	49	88	28	32	44	7	15	62
Buffalo	35	42	53	25	27	29	4-8	8-20	-
Sheep	41	75	129	35	52	73	16	128	637
Goat	20	24	44	16	17	25	6	11	31
Cat	28	41	75	23	30	41	7	13	46
Dog	28	43	102	22	28	48	6	15	62
Human	35	42	77	32	32	48	13	52	360
Pig	34	40	85	26	27	39	5	8	50

PL: primordial follicle, *PR*: primary follicle, *S*: secondary follicle

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The sequential growth and conversion of the antral follicles to preovulatory follicles and their ovulation is a part of their growth in waves known as **follicular waves** that will be discussed separately.

Follicular atresia

Majority of the secondary follicles degenerate by a process called atresia. The zona pellucida, oocyte and follicular cells degenerate and are reabsorbed. The basal lamina of granulosa cells become hyalinized and is then called glassy membrane. The theca interna cells mix back with the stroma. In the queen, bitch and rodents the theca interna cells may persist as interstitial endocrine cells.

- In mammalian ovaries, more than 90% of follicles undergo a degenerative process known as atresia.
- Two kinds of degenerate (attretic) ovarian follicles are recognized in swine: hemorrhagic and **milky.** Cell degradation system, which is responsible for the selective degradation of most short-lived proteins during follicular atresia probably results in the milky appearance of the follicles in the sow.
- In female camels the fate of dominant follicle in the absence of mating is atresia. During this process some follicles become **hemorrhagic.** Some follicles will continue to grow into large follicles of **greater than 3-4 cm** but will never ovulate and ultimately regress by atresia. The fate of follicle atresia in camel is thus to some extent dependent on whether the camel was mated or not.

Ovarian hormones

| Hormone	Production and Regulation	Action
Oestrogen	GnRH from the hypothalamus causes FSH secretion from the pituitary gland which stimulates the developing *follicles* in the ovary to secrete this hormone. The granulosa cells of the follicle secrete estrogen	**Increases uterine contractions and is responsible for onset of behavioral estrus. It increases vaginal mucus secretion. Higher estradiol causes positive feedback for LH surge. Estrogens promote mammary development**
Progesterone	This is produced by the corpus luteum after ovulation, and also the placenta during pregnancy.	**Progesterone is known as the *hormone of pregnancy*. It converts the endometrium to its secretory stage to prepare for implantation, reduces the immune response within the uterus to allow for acceptance of the conceptus, and decreases contractility of the smooth muscle.**
Inhibin	GnRH from the Hypothalamus causes FSH secretion from the Pituitary Gland which stimulates the Granulosa cells.	**Inhibits FSH secretion.**
Relaxin	This is produced by the Corpus Luteum towards the end of pregnancy as a result of fetal ACTH production.	**Relaxes the cervix and pelvic ligaments in preparation for parturition.**
Oxytocin	Oxytocin is mainly produced by the Hypothalamus and secreted by the Pituitary Gland, however in some species including primates and ruminants it is produced by the Corpus Luteum.	**In addition to allowing Milk let-down and promoting uterine contractions, oxytocin works along with oestrogen to induce endometrial production of PGF-2alpha resulting in regression of the Corpus Luteum.**

Ovulation

Ovulation is the release of mature ovum from the ovarian follicle. The key event in the process of ovulation is the pre-ovulatory surge in luteinizing hormone (LH). This surge in LH is required to initiate the processes of ovulation. Ovulation involves rupture of the follicle wall and release of the oocyte. The follicle bulges out at the surface of the ovary in most domestic animals except the mare. A small soft oval area on the follicle the *macula pellucida* (**stigma**) appears and ruptures with release of follicular fluid and the ovum. In ruminants the oocytes have lost their corona at the time of ovulation. The fimbriae of the oviduct picks up the ovum. Ovulation leaves a hollow cavity at the ovarian surface (ovulation fossa). The oocyte is 100-150µm at ovulation.

- Ovulation is either spontaneous (in response to gonadotropins and ovarian steroids) after endogenous surge of LH or induced (in which ovulation occurs in response to LH surge induced after coitus).

- The domestic animals **cattle, buffalo, sheep, goat, mare, sow and bitch** are spontaneous ovulator species. **Rats, mice, monkeys and humans** are also spontaneous ovulators.

- Animals like **cat, camel, rabbit, mink and ferrets** are induced ovulator species that will ovulate only in response to a mating.

Because of follicle development, ovulation and CL formation changes do occur in the bovine ovary in the shape which is shown
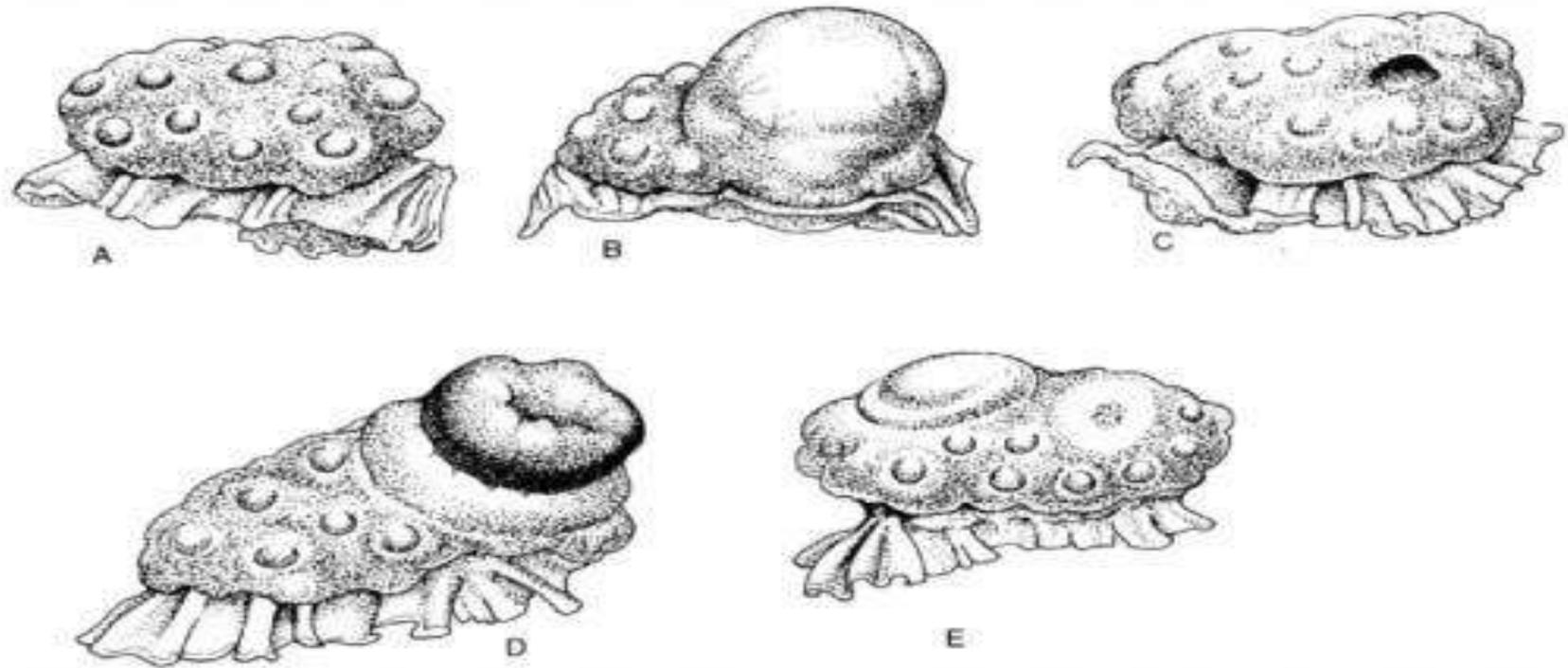
A. Small Secondary Follicles
B. Mature Follicle Pre-Rupture
C. Recently Ruptured Follicle
D. Mature Corpus Luteum
E. Regressing Corpus Luteum
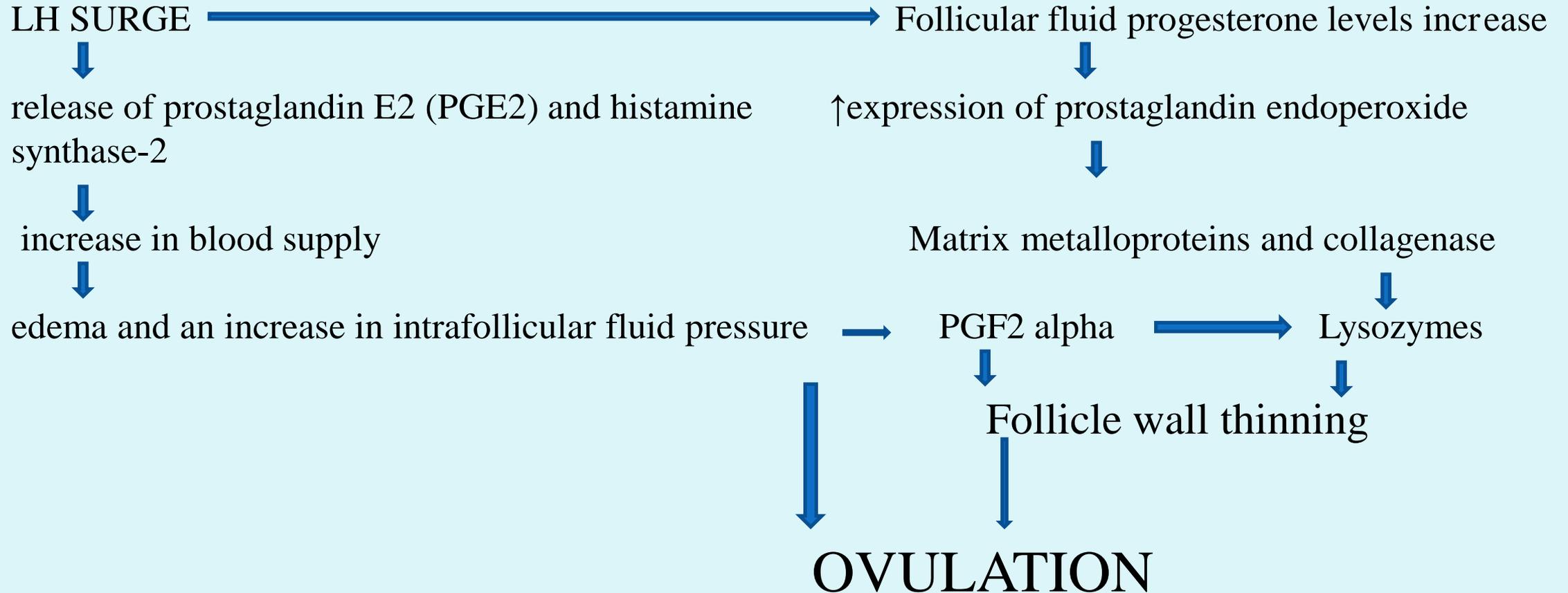
Corpus luteum in regression

Stigma

Tertiary follicle

Bovine ovary with a Graafian follicle ready to rupture.

Stigma of Graafian Follicle

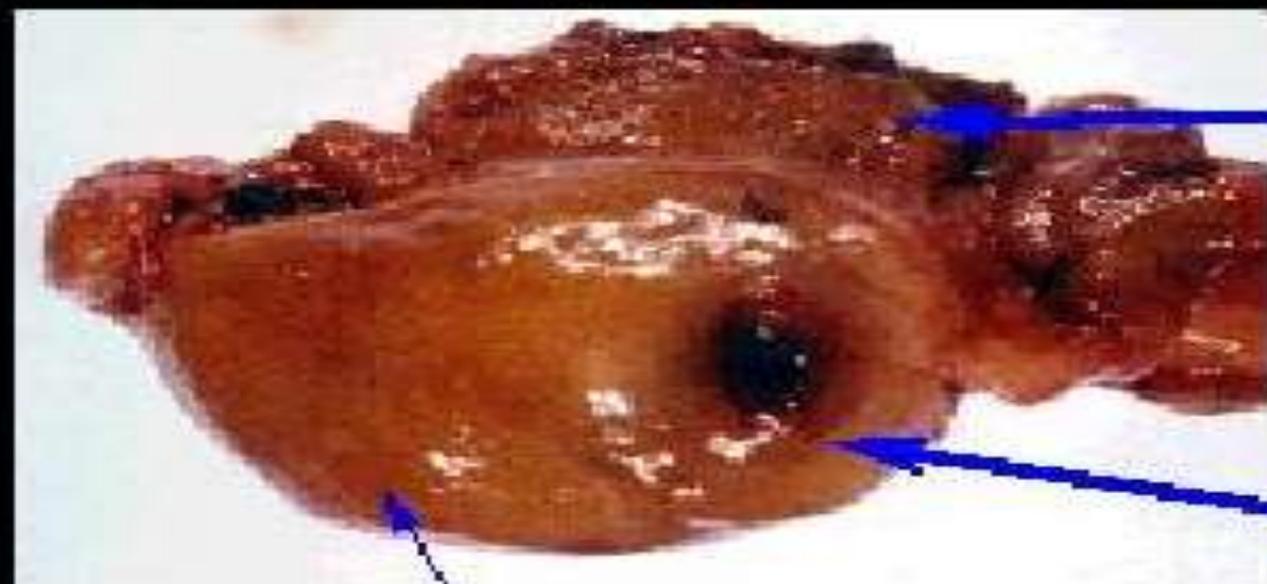# **Ovulation** The mechanisms involved in ovulation are shown below

LH SURGE ⟶ Follicular fluid progesterone levels increase

release of prostaglandin E2 (PGE2) and histamine synthase-2

↑expression of prostaglandin endoperoxide

increase in blood supply

Matrix metalloproteins and collagenase

edema and an increase in intrafollicular fluid pressure ⟶ PGF2 alpha ⟶ Lysozymes

Follicle wall thinning

OVULATION

Table 1 The diameter of the mature follicle and characteristic weight in different mammalian species.

Follicle (mm)	Weight (kg)	Species	Ref.
0.42	0.03	Mouse	Griffin et al. (2006)
0.55	0.2–0.25	Albino rat	Sangha and Guraya (1989)
0.64	0.2	Hamster	Griffin et al. (2006)
2.8	2.7	Rabbit	Osteen and Mills (1980)
4	3	Cat	Izumi et al. (2012)
6	9–10	Beagle (dog)	Reynaud et al. (2009)
6	23	Sheep	Aurich (2011)
7.5	35	Serrana goat	Simões et al. (2006)
8	150	Gilt	Chiou et al. (2004)
7–12	48–84	Alpaca	Bravo et al. (1991)
7–12	130–200	Llama	Bravo et al. (1991)
16	250–800	Water buffalo	Taneja et al. (1996)
20	700	Cow	Evans (2003)
20	2700	Elephant	Lueders et al. (2011)
23	60	Human	Evans (2003)
20–25	800	Summatran rhinoceros	Hermes et al. (2007)
27–38	300–550	Camel dromedarius	Manjunatha et al. (2012)
30–34	1800	White rhinoceros	Hermes et al. (2007)
50	1000	Black rhinoceros	Hermes et al. (2007)
55	450	Horse	Aurich (2011)
120	1900	Indian rhinoceros	Hermes et al. (2007)

Ovulation leads to formation of **ovulation depression (OVD)** on the ovary due to rupture of follicle wall and release of follicular fluid. Subsequently blood fills in the cavity. This is early **corpus haemorrhagicum**

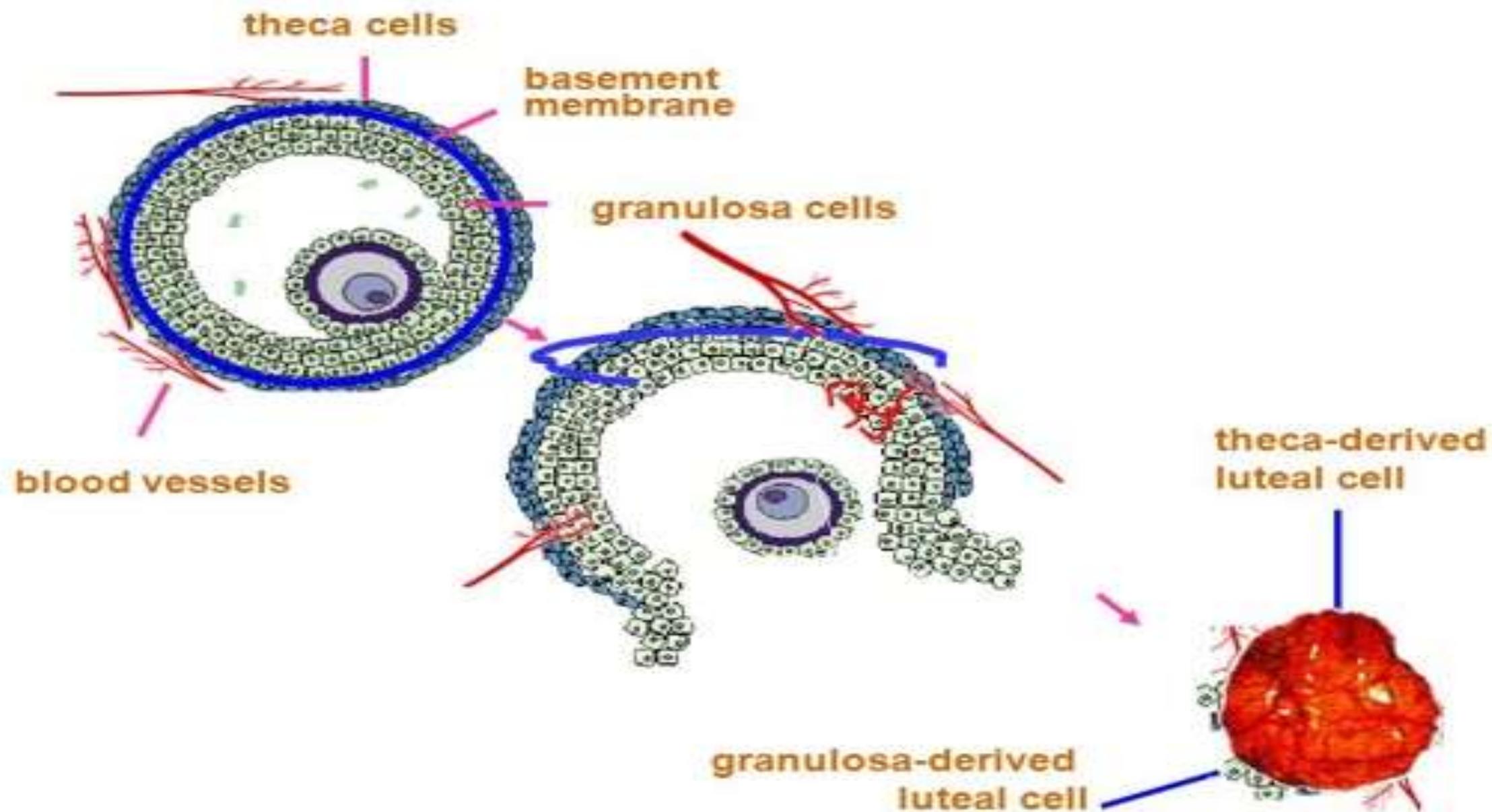(OVD)

# Bovine Corpus Hemorrhagicum

Mesovarium

Corpus Hemorrhagicum

Ovary

Ovulation and Corpus Luteum Formation

theca cells

basement membrane

granulosa cells

blood vessels

theca-derived luteal cell

granulosa-derived luteal cell

Corpus luteum

- Corpus luteum is the structure formed by tissue remodeling of the ovulated follicular cells and serves as an temporary endocrine gland on the ovary. The word corpus luteum is a latin word meaning **yellow body**. The corpus luteum is, however, actually yellow only in the cow and in all other domestic species it is red. The yellow coloration of the corpus luteum is due to the pigment, **lutein**. The loss of fluid from the follicular cavity causes the follicle to collapse into many folds. As a result, some **granulosa** and **thecal** layers are pushed into the apex of the ruptured follicle. The protrusion of tissue and ruptured blood vessels forms a structure known as the **corpus haemorrhagicum**. Following this, the **theca interna** and **granulosa** cells differentiate into large and small **luteal cells**. Available evidence suggests that LH has an important role in establishing a fully functional CL in the cow but is not required to maintain its function. The arterial blood flow to the ovary varies in proportion to the luteal activity. The blood flow to the ovary is highest during luteal phase, decreases with luteal (CL) regression.
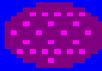
Corpus luteum

Above view of the surface of the ovary
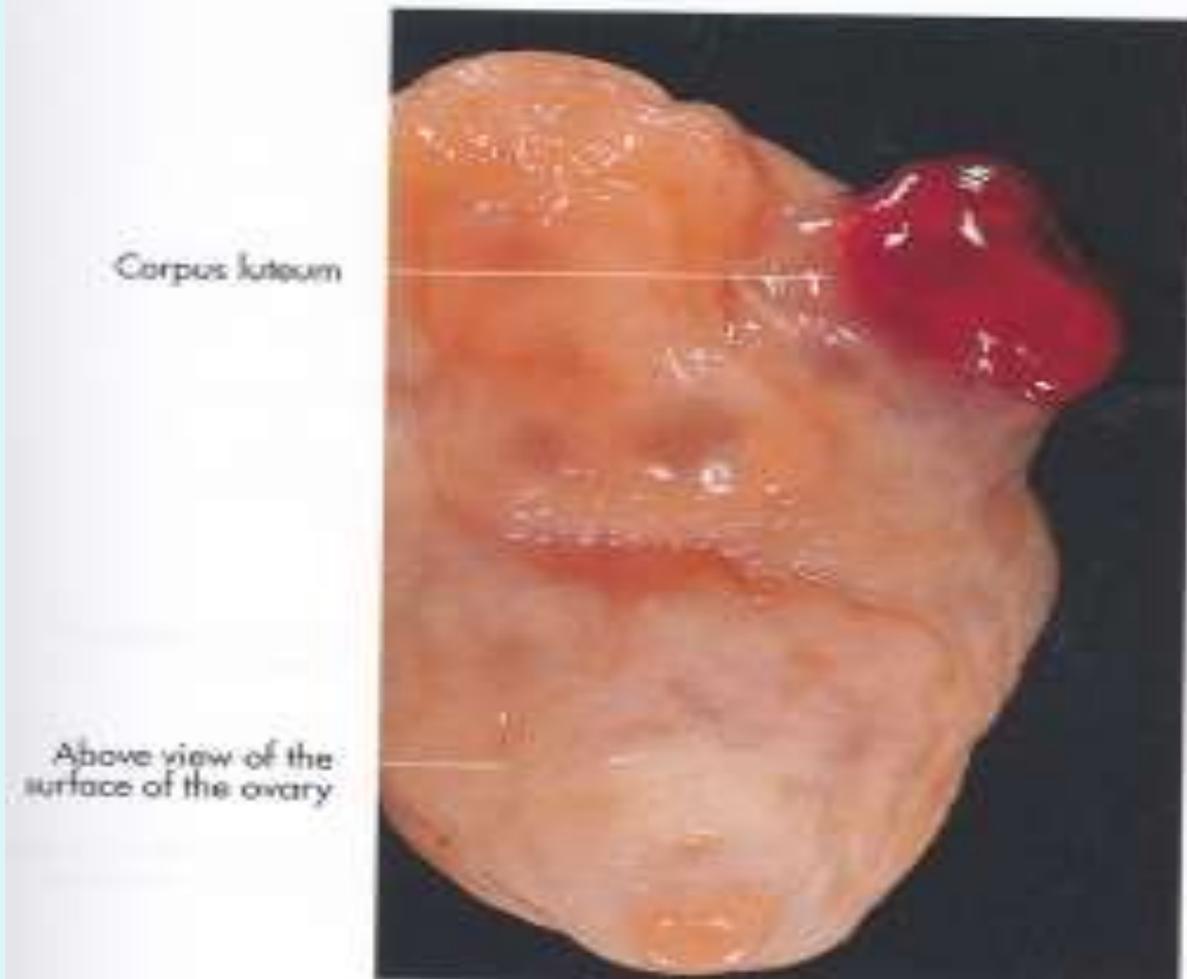
Bovine Corpus luteum

2 cm.
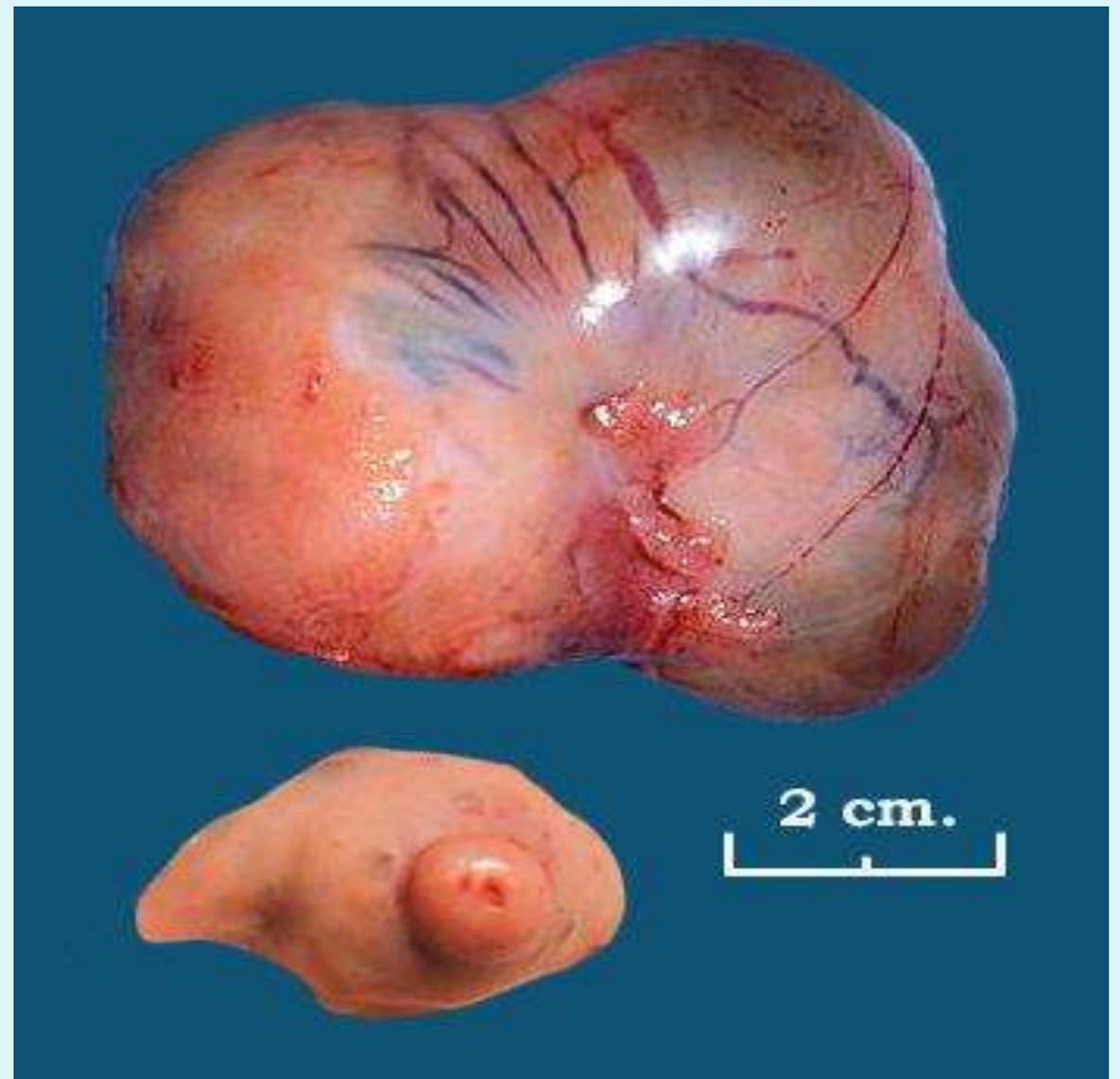
Comparison of bovine and equine ovaries and the corpus luteum which is embedded inside in the mare ovary

- **Small luteal cells** (<20μm)
- Small luteal cells are formed from remodeled **follicular theca cells**. These cells proliferate during luteinization. Small luteal cells contain many lipid droplets within their cytoplasm, which is an important source of cholesterol esters for progesterone synthesis. The small luteal cells are responsive to LH.
- **Large luteal cells** (20-40μm)
- Large luteal cells are formed from **follicular granulosa cells** that have undergone hypertrophy. These large luteal cells are the endocrine cells of the corpus luteum, producing large amounts of the hormone progesterone.

Corpus Luteum

- **Large luteal cells**
  - (Granulosa cells)
  - PGF receptors
  - 85 % of progesterone
  - Not LH responsive
- **Small Luteal cells**
  - (Thecal cells)
  - No PGF receptors
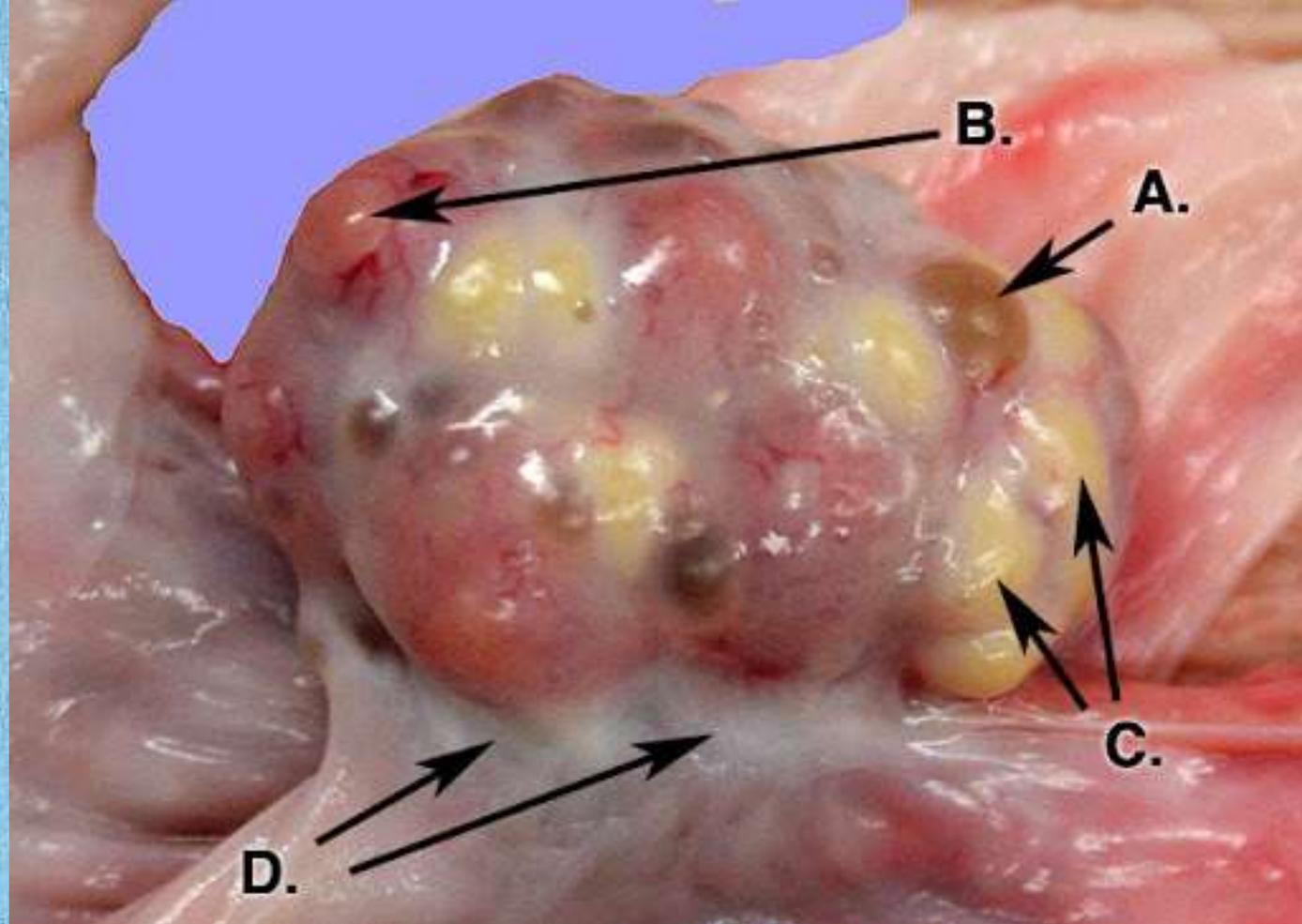 - 15% of progesterone
  - LH responsive
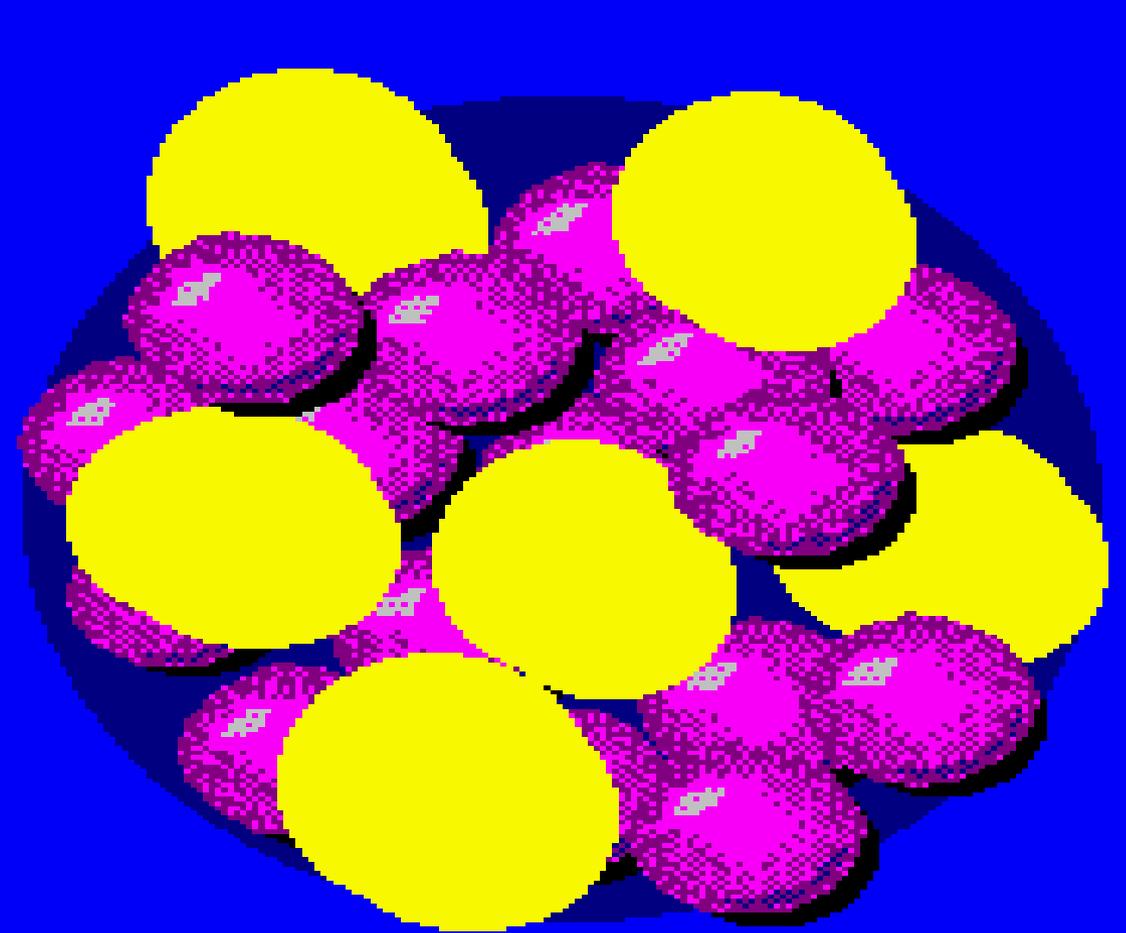
In the non-pregnant animal, corpora lutea are transient structures. **Cyclic corpora lutea** undergo proliferation and vascularization directly after ovulation. In the cow the CL **increases** rapidly between **Days 3 and 12** and remain constant until Day 16 when the regression of the corpora lutea begins under the influence of PGF2 alpha and CL degenerate into a connective tissue scar, the **corpus albicans** (since it is white in color). If the ovum is fertilized and pregnancy is established the corpus luteum remains fully developed and active throughout for at least part of the pregnancy in some animals (ex: mare, sheep) and throughout gestation in a few domestic animals (cow, goat, sow). This CL of pregnancy is called **corpus luteum verum** whereas when the corpus luteum regresses in the absence of pregnancy it is known as **corpus luteum spurium** (False yellow body). In pregnant cattle the CL increases in size for 2-3 months of gestation and then regresses gradually with fast regression and decrease in progesterone near parturition.

The **buffalo CL** is more embedded in the ovarian stroma and less projecting on the ovarian surface

The equine CL formed after an ovulation is termed a **primary CL**. However, during diestrus or early pregnancy **secondary CL** are also formed. **Accessory corpora lutea** may result from luteinization of anovulatory follicles in estrous mares (hemorrhagic anovulatory follicle, HAF), and especially **during early pregnancy from day 35 of gestation** onwards. The primary CL is maintained and continues to produce progesterone along with supplemental CL (combined secondary and accessory CL) that forms after day 35 until the **placenta becomes the principal source of progesterone** starting **by day 50 to 70 of gestation**. By day 90 to 100 the placenta produces sufficient progesterone and other progestins to maintain pregnancy without any ovarian support.

Old Cl ('C. albicans') from the previous estrous cycle

Lu.

Cm

B.

A.

C.

D.
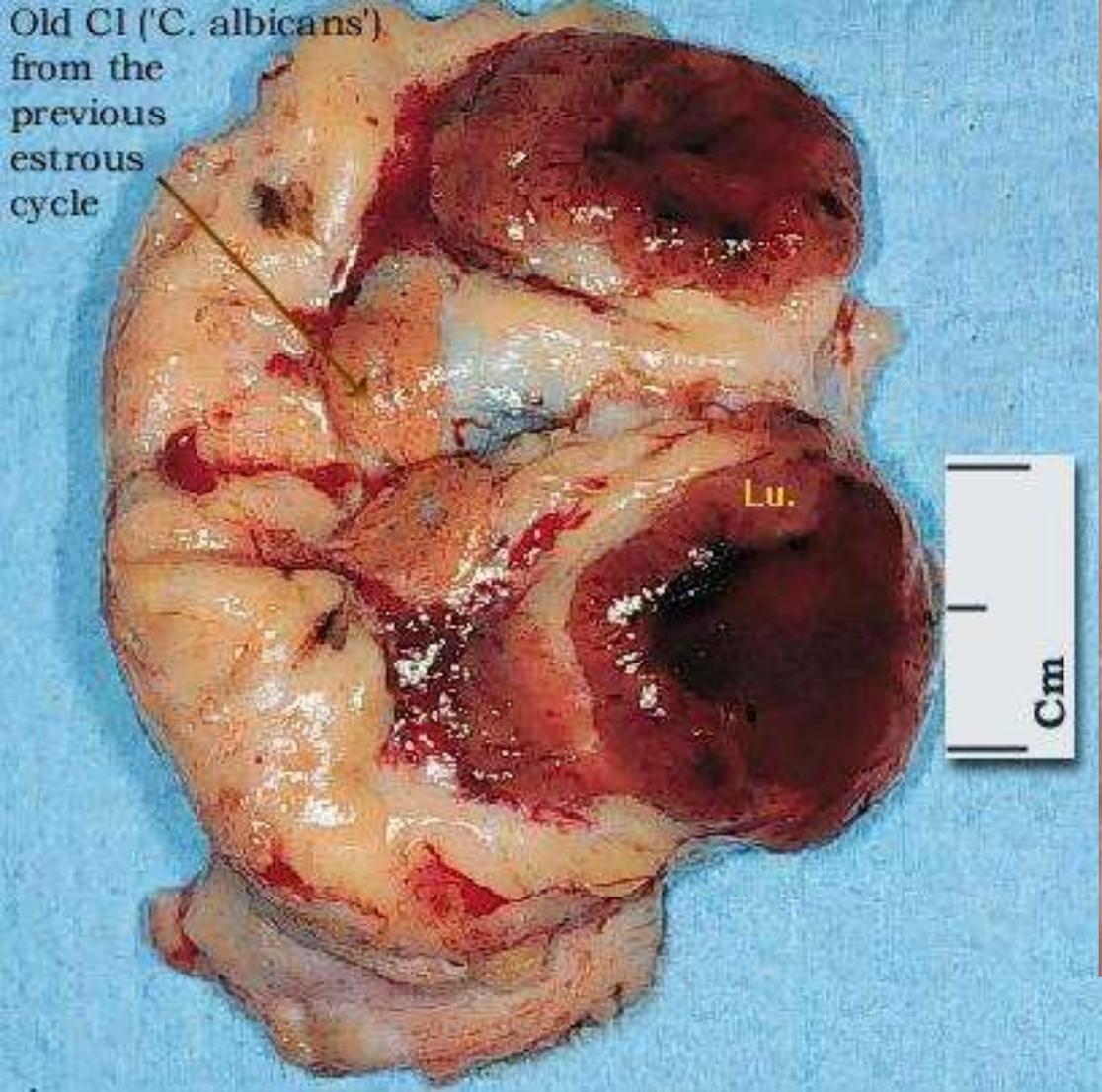
Corpora lutea (Hemorrhagic) on a mare ovary (opened)

Corpora lutea on a sow ovary B, Corpus albicans C, Follicles A

Luteal progesterone is essential for maintaining pregnancy in the **mare before Day 50** of gestation. **By Day 100 of gestation, the placenta** is capable of maintaining pregnancy without luteal support. **The primary, secondary and accessory corpora lutea** of the mare regress starting on Day 90 and complete regression by Day 180 of gestation.

Progestins: The equine placenta appears not to synthesize progesterone. However, it secretes copious quantities of progestins (5-alpha-pregnanes), which serve the same function for maintenance of pregnancy. Toward the end of gestation, blood levels of these progestins are typically 100 times the maximal level of progesterone

Ovulation can occur in a mare on the twenty-third day of pregnancy. Ovarian activity reaches its peak during the second and third month of pregnancy, when gonadotrophic hormone is abundant in the blood.
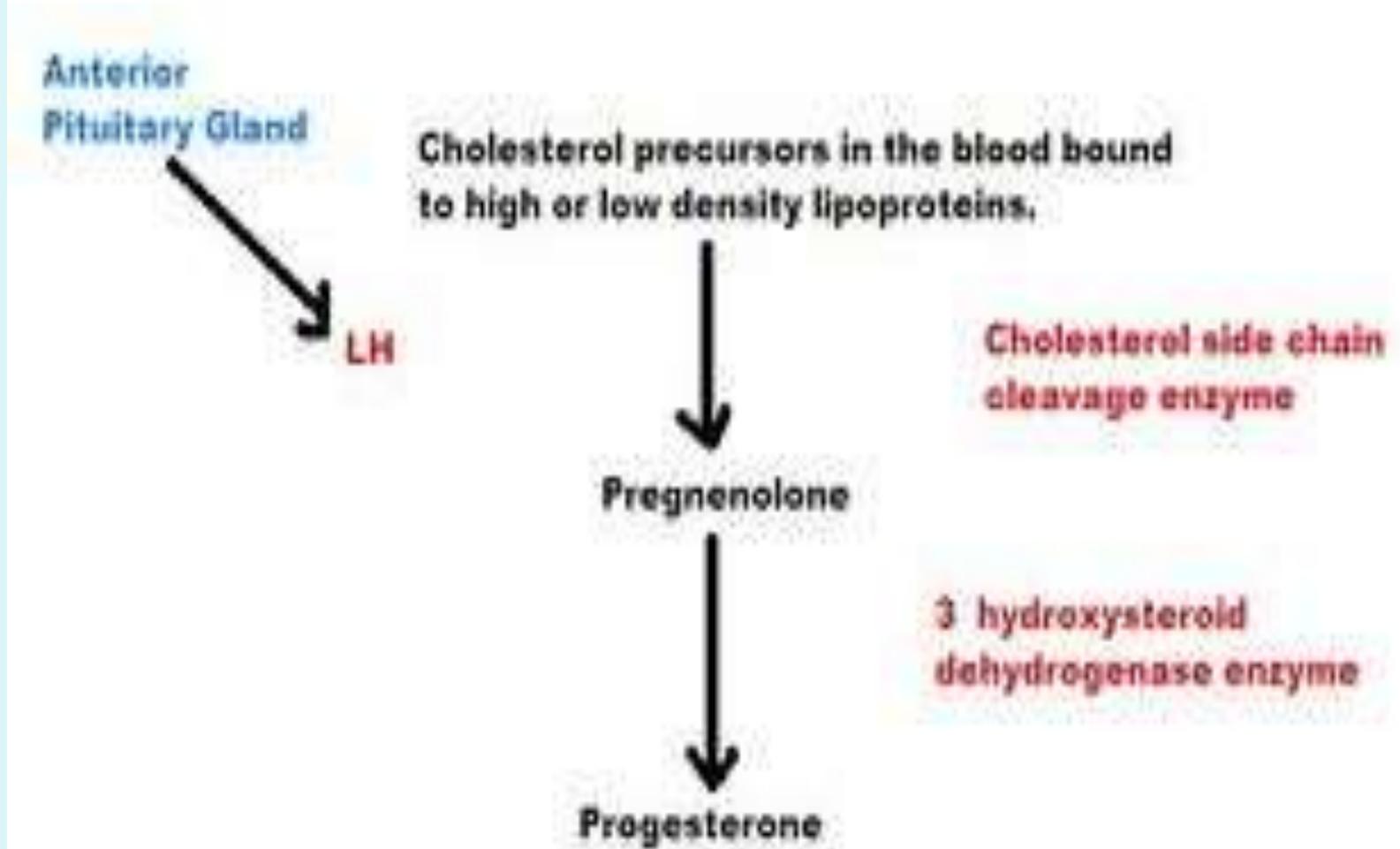
The **sheep placenta** produces progesterone **by Day 50** and maintains the pregnancy

- The primary source of progesterone in the **female dromedary camel** is the corpus luteum, however in the absence of mating ovulation does not occur therefore, in the absence of mating and ovulation, progesterone plasma level remain very low throughout the follicular wave (<1 ng/mL). However, after mating, progesterone concentrations start to rise reaching 3 ng/mL by day 8-9 after mating and increases throughout gestation.

- In mated but non-pregnant camels the CL is short lived and persists only for 6-11 days. Thus the **luteal phase** is difficult to be defined in camels and **is short lived**.

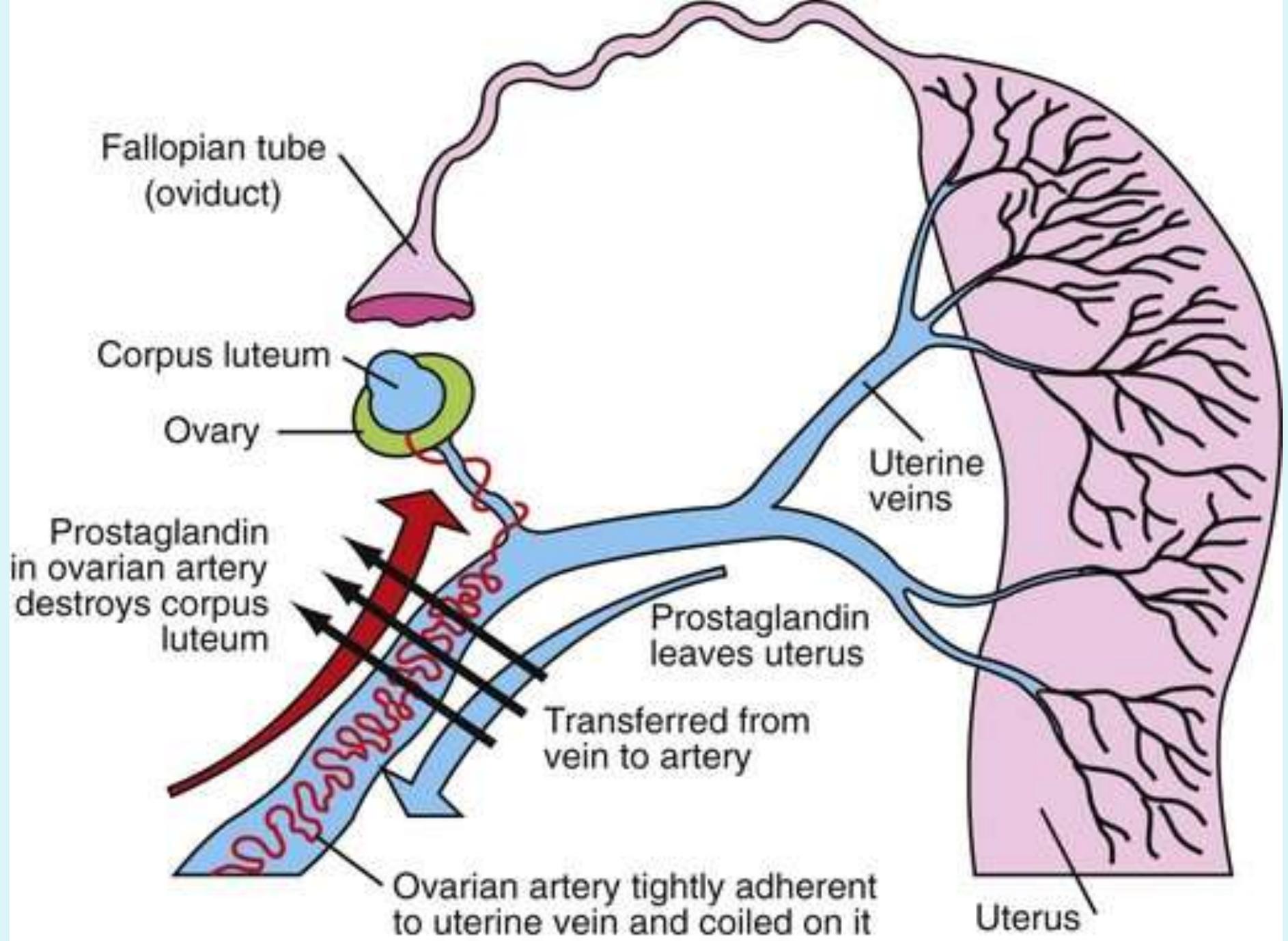# Control of Progesterone Biosynthesis in the Corpus Luteum

Progesterone production **begins** about after about **24 h of ovulation** in cattle and is maximum between Day 6 and Day 18-20 post estrus. The period of growth of CL is rapid between day 3 and 12 of the estrous cycle and remains relatively constant until Day 16 when regression begins. The diameter of the **mature CL** is **larger than that of a mature graafian follicle** except in the mare, in which it is smaller. Progestagens are necessary for maintaining pregnancy in domestic farm animals. In sheep and mare progesterone is produced by the placenta after a certain time of gestation (beyond Day 50 in sheep and beyond Day 90 in mare) and thus removal of CL would not terminate pregnancy in these species. Progesterone is secreted by the CL as **granules**. In aged animals the functions of the CL decline producing less progesterone.

Progesterone Biosynthesis

Anterior Pituitary Gland

Cholesterol precursors in the blood bound to high or low density lipoproteins.

LH

Cholesterol side chain cleavage enzyme

Pregnenolone

3 hydroxysteroid dehydrogenase enzyme

Progesterone

The Luteolytic Mechanism

The CL regresses in the absence of an established pregnancy. This occurs due to the release of prostaglandin from the uterine endometrium in many species except in **primates** where it originates from the CL itself. **PGF2α** from the uterus is transported to the ipsilateral ovary through a vascular **counter-current exchange** mechanism. This involves two closely associated blood vessels in which blood from one vessel flows in the opposite direction to that of the adjacent vessel. Low molecular weight substances in high concentrations in one vessel cross over into the adjacent vessel, where they are low in concentration. The ovarian artery lies in close association with the utero-ovarian vein. By counter-current exchange, PGF2α is transferred across the wall of the uterine vein into the ovarian artery by passive diffusion. This ensures a high proportion of PGF2α produced by the uterine glands will be transported directly to the ovary and corpus luteum without dilution in the systemic circulation. This is an important mechanism, because much PGF2α is denatured during its passage through the circulatory system. **In mares PG is known to reach the ovary by the systemic route.**

Fallopian tube (oviduct)

Corpus luteum

Ovary

Prostaglandin in ovarian artery destroys corpus luteum

Uterine veins

Prostaglandin leaves uterus

Transferred from vein to artery

Ovarian artery tightly adherent to uterine vein and coiled on it

Uterus

During diestrus progesterone increases phospholipid stores and prostaglandin synthase-2 (PTGS-2) in uterine epithelium. Arachidonic acid (fatty acids present in the phospholipid layer of cell membranes) generated by phospholipase A-2 is converted by PTGS-2 to substrates for synthesis of prostaglandin F2 alpha (PGF). Exposure of uterus to progesterone for 10-12 days results in down regulation of progesterone receptors and up regulation of oxytocin (OXT-R) and estrogen (ESR-1) receptors. The interaction of neurohypophyseal and ovarian oxytocin with oxytocin receptors in the endometrium evokes the secretion of luteolytic pulses of uterine PGF. Intra and extra cellular calcium may be required for oxytocin to stimulate PGF2 secretion from uterus in the bovine species. The uterine luteolysin appears to travel from the uterus to the ovary bearing the CL via counter current exchange mechanism in cattle, sheep, goats and pigs and **systemic route** in horses. The combined effects of estrogen, progesterone and oxytocin result in luteolysis in mares and the CL of mares are responsive to luteolytic effects of PG after Day 5 post ovulation. The PG causes severe vasoconstriction and the blood supply to the CL is reduced dramatically thus causing rapid cellular death.

The **porcine** CL are unique among domestic animals since a single injection of PGF2a does not induce luteolysis before day 12 of the estrous cycle (Guthrie & Polge 1976), although PGF2a receptors (PTGFR) are already abundant on the surface of porcine luteal cells during the early luteal phase (Gadsby et al. 1990, 1993). On the other hand, repeated administration of PGF2a on day 5 of the estrous cycle does promote luteolysis in pigs (Estill et al. 1993). Despite many studies, the molecular mechanism of luteolytic sensitivity (LS) acquisition in porcine CL still remains poorly understood. Estrogens are luteotropic in sows.

- The luteolytic mechanisms of camel prostaglandins are unique. The PG released from the **left uterine horn** can lyse the **CL on either the right or left ovary** but the PG from right uterine horn can only lyse the CL over the right ovary.

- The pregnancy is exclusively established in the left uterine horn and an extra fetal membrane termed the **epidermal membrane** is present in camels.

Canine reproductive physiology exhibits several unusual features. Among the most interesting of these are the **lack of an acute luteolytic mechanism**, coinciding with the apparent luteal independency of a uterine luteolysin in absence of pregnancy, **contrasting with the acute prepartum luteolysis observed in pregnant animals**. These features indicate the existence of mechanisms different from those in other species for regulating the extended luteal regression observed in non-pregnant dogs, and the actively regulated termination of luteal function observed prepartum as a prerequisite for parturition. Nevertheless, the supply of progesterone (P4) depends on corpora lutea (CL) as its primary source in both conditions, resulting in **P4 levels that are similar in pregnant and non-pregnant bitches** during almost the entire luteal life span prior to the prepartum luteolysis.

Unlike in livestock, in which lutein cells can be divided into small- (theca interna-derived), and large- (granulosa cell derived) lutein cells, no such distinction can be made in dogs. Although, as in other species, likewise, canine lutein cells develop from both of these cell populations, only one type of steroidogenic cell can be identified in dogs There is no uterine luteolysin in non-pregnant dogs and hysterectomy does not affect CL function. It appears thus, that in lacking an active luteolytic principle, the CL life span of non-pregnant dogs is regulated by some intrinsic regulatory mechanisms. At the regulatory level, the canine CL also presents some species-specific peculiarities compared with other domestic animals. Both LH and prolactin (PRL) have luteotropic roles in the canine CL. However, PRL appears to be the predominant factor and appears to be required for CL maintenance starting around day 25 after ovulation. Studies suggest that - as in the non-pregnant bitch - also in the pregnant bitch luteal production of prostaglandins is associated with luteal support rather than luteolysis. **In dogs** the **CL** is relatively **resistant** to the lytic effects of **prostaglandins during** the **first** 2 to 4 weeks of **pregnancy**.

FUNCTIONS OF THE UTERUS

- **Sperm Transport**: Estrogen from the ovaries stimulates myometrial contractions to assist in sperm transport toward oviducts when female is in estrus. During transportation the sperm is also capacitated to get it ready for fertilization.

- **Regulation of Corpus Luteum (CL)** Uterine glands secretes prostaglandin F2α, which functions to destroys the CL to regulate the estrous cycle.

- **Embryo Development & Placental attachment**: The uterus provides early developing embryo the proper nutrition via its abundant endometrial vasculature that secrete histotrophes which are an admixture of sugars, vitamins, amino acids etc. Eventually, placental attachment or placental invasion occurs and functions to provide an interface between the maternal and fetal circulations allowing for nutrient and gas exchange necessary for fetal growth and development

- **Parturition and Post-Partum Involution**: Increased myometrial contractions assist in expulsion of the fetus during parturition. After parturition, the uterus goes through a process called involution, which allows the uterus to return to its original size and prepare itself for the next pregnancy.

The Uterus Communicates with the ovary about presence of embryo – determines the life of the *corpus luteum* via a process called ***maternal recognition of pregnancy***

Uterus is the site of semen deposition during artificial insemination: mare, cat, sheep, goat, dog

Endometrial glands and uterine fluid

- These **tubular glands** are straight at the time of **estrus**, they then undergo remodeling, become more coiled and secrete histotrophes under the increasing progesterone concentrations.

- The volume and composition of uterine fluid show variation during the estrous cycle. In sheep the volume of the fluid in the uterus exceeds that of the oviduct during estrus whereas during the luteal phase the reverse is true.

- Uterine fluid accumulations are **undesirable during estrus** in cattle and mares and hampers fertility.

Uterine proteins

- The uterus actively **secretes** many proteins and some proteins **transudate** into the uterine lumen from blood. These proteins have complex functions of embryonic growth, implantation and maternal recognition of pregnancy. A uterine protein named **blastokinin** (uteroglobulin) influences the formation of blastocysts from morulae (Hafez and Hafez, 2000). A large number of endometrial proteins are secreted during early pregnancy in cattle (Forde, 2014) and other domestic animal species which help in embryonic implantation.

Uterine contractions

Uterine contractions are predominant during estrus and at parturition. During estrus myometrial contractions originate in the posterior part of the reproductive tract and predominantly toward the oviduct, whereas during parturition uterine contractions originate in the anterior part of the uterus and direct the fetus towards the birth canal. The uterine contractions are low during luteal phase. Thus uterine contractions increase under the influence of estrogen and decrease under progesterone influence

Uterotubal Junction

- The point at which the uterine tube connects to the uterus is conventionally called the uterotubal junction; it is closed until three to five days post-ovulation. In some species, such as the cow, the uterotubal junction (UTJ) controls the movement of the embryo from the oviduct to the uterus by estradiol regulation. High concentrations of estradiol are believed to cause a blockage in the UTJ, which is reversed/opened when estradiol levels decrease, thus allowing the movement of the embryo into the uterus. In other species, there is no obvious blockage at the UTJ

- **Pigs** have a constriction at the UTJ which is believed to be the mechanism for blockage of polyspermy in the pig. This constriction acts as a major barrier for sperm transport and limits the number of sperm released into the ampulla region of the uterine tube.

- **Descent** of the **equine** conceptus into the **uterine** lumen occurs at day 5 to 6 after ovulation but is only possible when the **embryo** secretes **prostaglandin E2**. **Unfertilized ova** never enter the **mare uterus** and they are lost in the oviduct. Although maintenance of **equine** pregnancy probably involves secretion of a conceptus derived anti-luteolytic factor, which has been termed the **endometrial prostaglandin synthesis inhibitor**. PGE_2 is also considered to play a luteoprotective role in mares. The presence of the conceptus was shown to block the induction of COX-2 expression in equine endometrium at Day 15, suggesting an important mechanism by which it may suppress uterine $PGF_{2\alpha}$ release and prevent luteolysis during early pregnancy.

Functions of CERVIX

- The cervix performs many functions including prevention of entry of microbes in the uterus as it remains tightly closed except during estrus and at parturition.

- The cervix also secretes cervical mucus which helps in sperm transport. The sperm enters the cervical mucus and travels in it faster

- The crypts of the cervix serve as pockets for formation of sperm reservoir. From which sperm are released and travel towards the uterus. It has also been proposed that some of the sperms are trapped in the cervical crypts and this prevents polyspermy.
- Mucus and anatomy of cervix act as a sperm filter in some species
- The cervix prevents large numbers of sperm from reaching oviduct in cow and ewe.
- The cervix safeguards the fetus during pregnancy by forming the cervical seal.

Cervical mucus

The uterine cervix has many **Nabothian glands** that secrete mucus. The cervical mucus consists of macromolecules of mucin of epithelial origin which is composed of 25% amino acids and 75% carbohydrates including glycoproteins (such as lipoproteins, albumin), carbohydrates (such as galactose, glucosamine and sialic acid) and enzymes (glucuronidase, amylase, phosphorylase, esterase and phosphatase). Cervical mucus has many rheological properties (elasticity, viscocity and stickiness). The estrual cervical mucus shows fern pattern on drying on a glass slide. This appears due to the high chloride content of the mucus. The cervical secretions form a mucus plug during pregnancy preventing the entry of external material including microbes entering the uterus.

The spaces between the micelles of the cervical mucus are enlarged at estrus (2-5 micron) that permit passage of sperm and diffusion of soluble substances. In many species the sperms leave the seminal plasma (that is lost after mating to the exterior) and travel through the cervical mucus.

The cervical mucus is stringy and thin at estrus and thick and viscous during luteal phase

The cervical mucus flows towards vagina as the cilia beat towards vagina.

Cervical softening at parturition

- During the course of gestation there is substantial increase in the mass of cervix and the secretions are sticky and tightly close the opening.

- The cervical softening (ripening) at parturition is a complex process that involves many hormones and relaxin.

- Coupled with that there is increased synthesis of proteoglycans and hyaluronate, appearance of a new proteoglycan and breakdown and reorganization of the collagen network. The cervical mucus plug liquefies at parturition and provide lubrication for passage of the fetus.

Functions of the vagina

The vagina has multiple functions. It is the copulatory organ in which the semen is deposited during natural mating in many species. The vagina distends at copulation and at parturition to allow mating and delivery of the fetus.

- The rugae vulgaris and rhomboid shaped arrangement of the musculature allow distension of the vagina at mating and at parturition.
- During mating the contractions in the vagina are activated by the fluid secreted into the vagina during pre-coital stimulation.
- The vagina is the major site for sperm antigen-antibody reaction.
- The vaginal fluid is composed of transudate through the vaginal wall and mixed with cervical and endometrial fluids.
- During estrus the vascularity of the vaginal wall is increased.
- The vagina secretes some specific odor substances (volatile fatty acids- the pheromones) that attracts the male.

Functions of oviduct

- The uterine or fallopian tube (salpinx) is the **site of fertilization.** There are three sections of the uterine tube: infundibulum, ampulla, and isthmus. The primary function of the uterine tube is the **transportation** of the freshly **ovulated oocyte** and **sperm** to the **site of fertilization**, followed by the **transport** of the **fertilized egg** to the **uterus.** The infundibulum contains finger-like projections called **fimbriae** which increase the surface area of the infundibulum.

- Once the oocyte is ovulated and collected by the infundibulum, it is transported to the **ampulla** of the uterine tube.
- An undefined area where the ampulla continues with the isthmus (also conventionally called the **ampullary-isthmic junction**) is the site of fertilization.
- The isthmus of the uterine tube is smaller in diameter and transports the fertilized oocyte to the uterus for subsequent embryonic development.
- Limited embryonic development occurs in the isthmus of the uterine tube, depending on species

- The ciliated cells of the oviductal mucosa have slender motile cilia (**kino-cilia**) that extend into the lumen.
- The cilia beat toward the uterus.
- The constant beating of cilia and the oviductal contractions keep the oocyte in constant rotation that helps in fertilization and prevent oviductal implantation.
- The **non-ciliated** cells are **secretary** and produce secretary granules during the follicular phase of estrus.
- The oviductal fluid has several functions including sperm capacitation, sperm hyper-activation, fertilization and early pre-implantation development.
- The oviductal fluid is composed of **transudate** from serum and secretary products from oviductal epithelium.

- Oviductal contractions facilitate **mixing of oviductal contents** and **denude the ova** and delay the **transport of ova**.

- The pattern and amplitude of contractions vary in different segments of oviduct. In the **isthmus** the contractions peristaltic and antiperistaltic contractions are segmental, vigorous and continuous.

- In the **ampulla** strong peristaltic waves occur in a segmental fashion toward the mid-portion of the oviduct.

THANKS