Reproductive Organs Basics– Differences between Species

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Embryonic Development

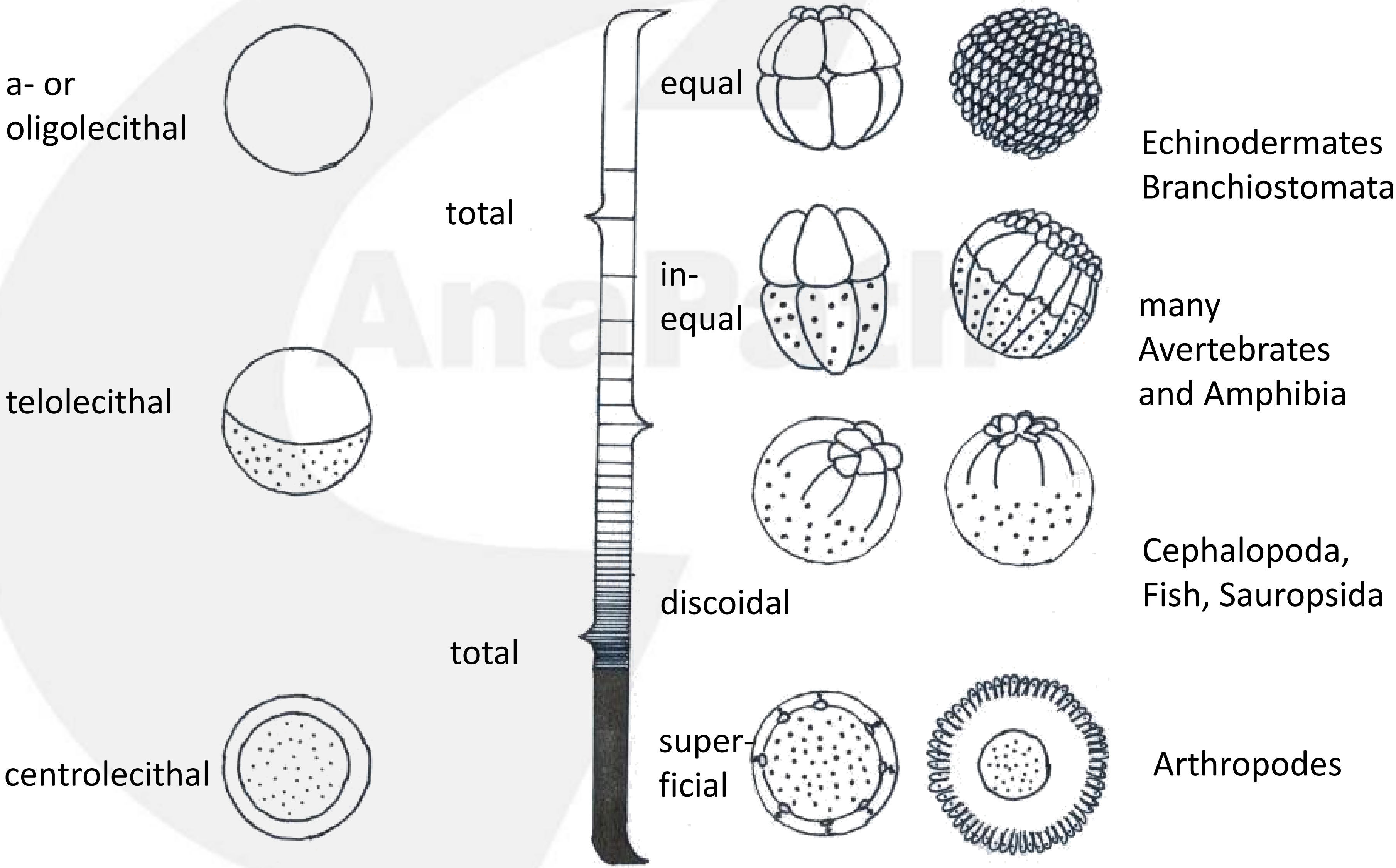
Type of Eggs (Oocytes)

- Oocytes with nucleus and cytoplasm, complete apparatus for synthesis
- Special is the deposition of larger amounts if reserve substances = yolk
- Rule: as more intense the brood care as less eggs and as more yolk (except mammals with alimentation by materna organism)

Cleavage: Cygote to Blastula

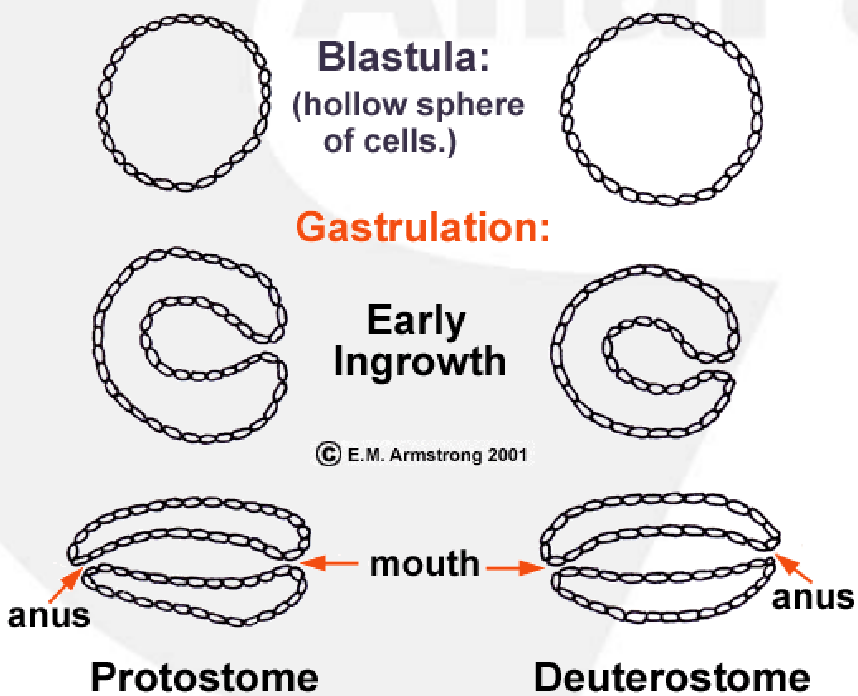
- Cell division without gowth
- No differentiation at beginning
- aegual/in-aequal cleavage (depending on distribution of yolk), discoidal, superficial
- holoblastic (complete) cleavage: bilateral (nematodes, chordates) , radial (porifers, echinodermates), rotational (mammals) , and spiral (turbellaria, annelides, molluscs)
- Morula
- Blastula – hollow sphere of cells (cells of wall more or less of same size) filled with fluid (exceptions are blastulas of e.g. coelenterates with compact sterroblastula)

Relationship: Oocyte and Cleavage



Blastopore

- Opening into the archenteron
- Primitive gut
- In protostome development, the blastopore, becomes the animal's mouth (nematodes, plathelminths, molluscs etc).
- In deuterostome development, the blastopore becomes the animal's anus (echinodermates, chordates).



http://waynesword.palomar.edu/images/gastru1.gif

Cleavage: Gastrula

- Reorganization of single-layered blastula into three-layered structure.
- Ectoderm gives rise to epidermis, neural crest, nervous system.
- Mesoderm gives rise to somites, which form muscle; cartilage of ribs/vertebrae; dermis, notochord, blood/vessels, bone, connective tissue.
- Endoderm gives rise to epithelium of digestive system and respiratory system, liver and pancreas.
- Following gastrulation, cells in the body are organized into sheets of connected cells (e.g. epithelia), or mesh of isolated cells (i.e. mesenchyme).

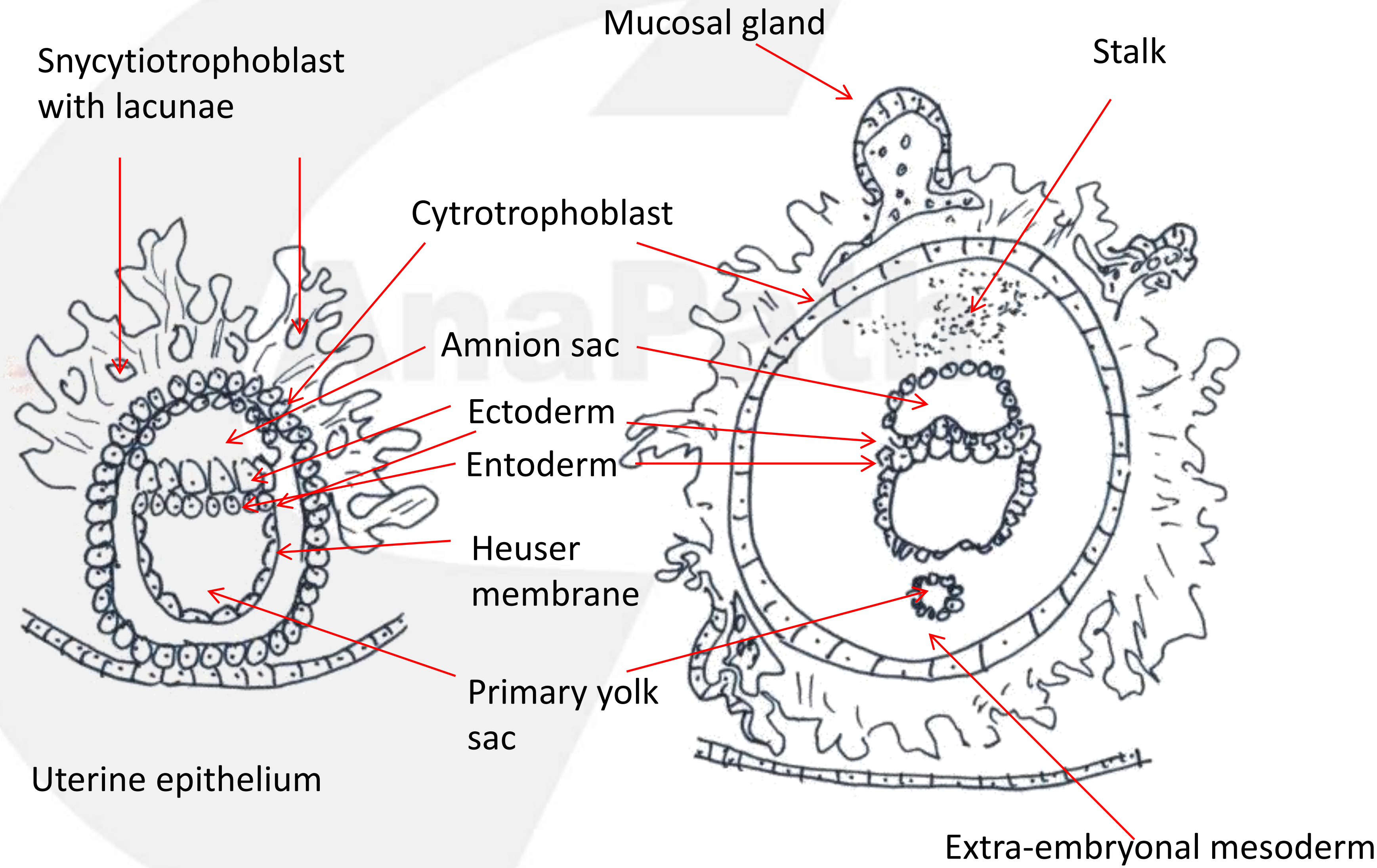
Organ development

- **Histological differentiation**
- **Early differentiation into bluestems with subsequent morphological differentiation of organs**
- **Induction principles:**
  e.g. neural tube induced by blastopore
  e.g. diverticulum of neural tube causes lens development

Mammals: Blastocyst

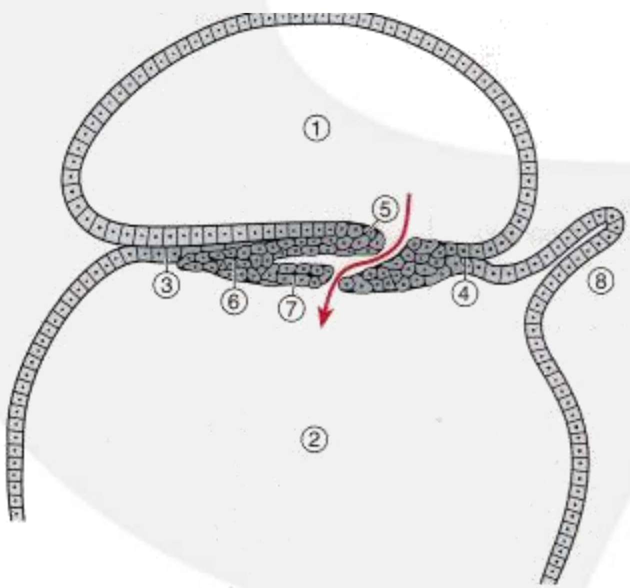
- Human blastocyst comprises 70-100 cells
- Possesses inner cell mass (embryoblast) that subsequently forms the embryo, and outer cell layer (trophoblast)
- Blastocoele is a fluid-filled cavity
- Trophoblast forms inner cell layer (cytoptrophoblast) and outer spongious layer (syncytiotrophoblast)
- In embryoblast fomation of entoderm/ectoderm whereby entoderm forms roof of blastocoel
- Cleft formation between ectoderm/cytotrophoblast: amnion sac
- Approximately 2 days after nidation (human) primary yolk sac below entoderm lined by Heuser membran
- Membrane replaced by entoderm cells and formation of secondary yolk sac

Mammals: Blastocyst



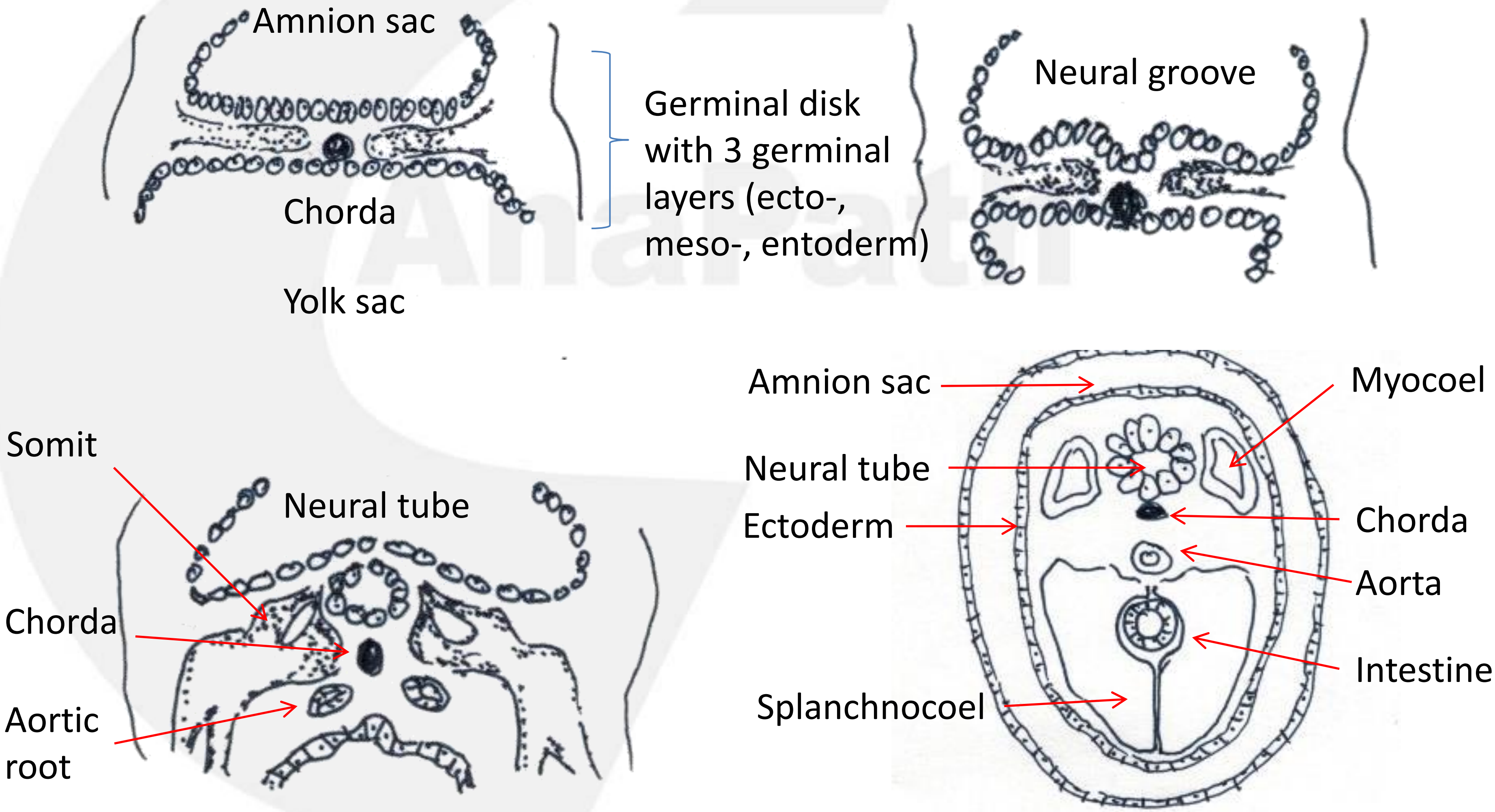
Germinal disk

- Ectoderm and entoderm form floor of amnion sac.
- Formation of primitive streak determines longitudinal axis of organism.
- Primitive pit growth forms head/chorda structures that causes connection between yolk sac and amnion cavity (Canalis neurentericus)
- Differentiation of neural material above chorda.



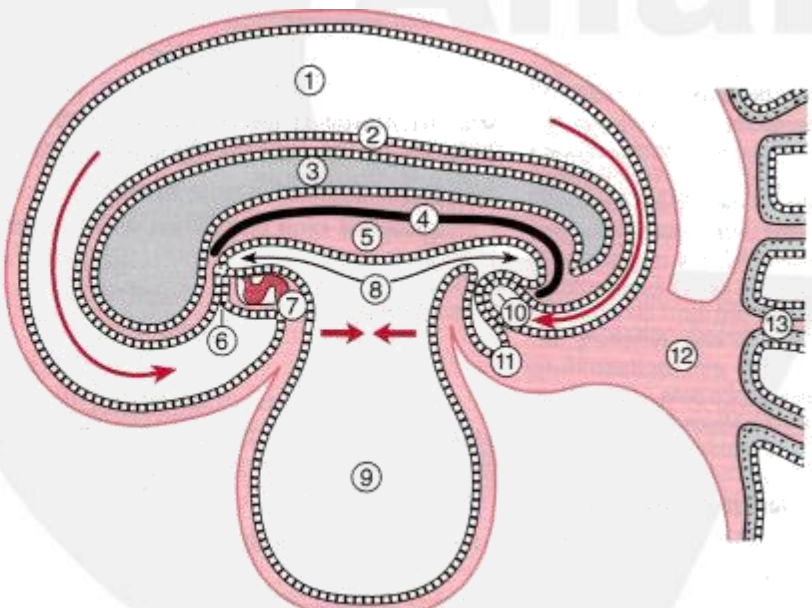
1 – amnion sac
2 – yolk sac
3 – prechordal plate
4 – cloace membrane
5 – primitive pit
6 – prechordal mesoderm
7 – chorda mesoderm
8 - allantois

Development of Chorda and Neural Tube



Chorion

- **Extra-embryonal cleft formation (called 'Chorion cavity') surrounding germinal disk, amnion cavity, yolk sac) formed by trophoblast cell layers (now called 'Chorion') and a mesenchymal layer**
- **Amnion cavity narrows upper part of yolk sac and ingrowing allantois forming umbilical cord**



1 – amnion sac
2 – ectoderm
3 – neural tube
4 – chorda
5 – mesoderm
6 – stomatodeum with oropharyngeal membrane
7 – heart anlage
8 – intestinal tube
9 – yolk sac
10- proctodeum
11 – allantois
12 – stalk
13 – placenta

Placenta – Development and Species Differences

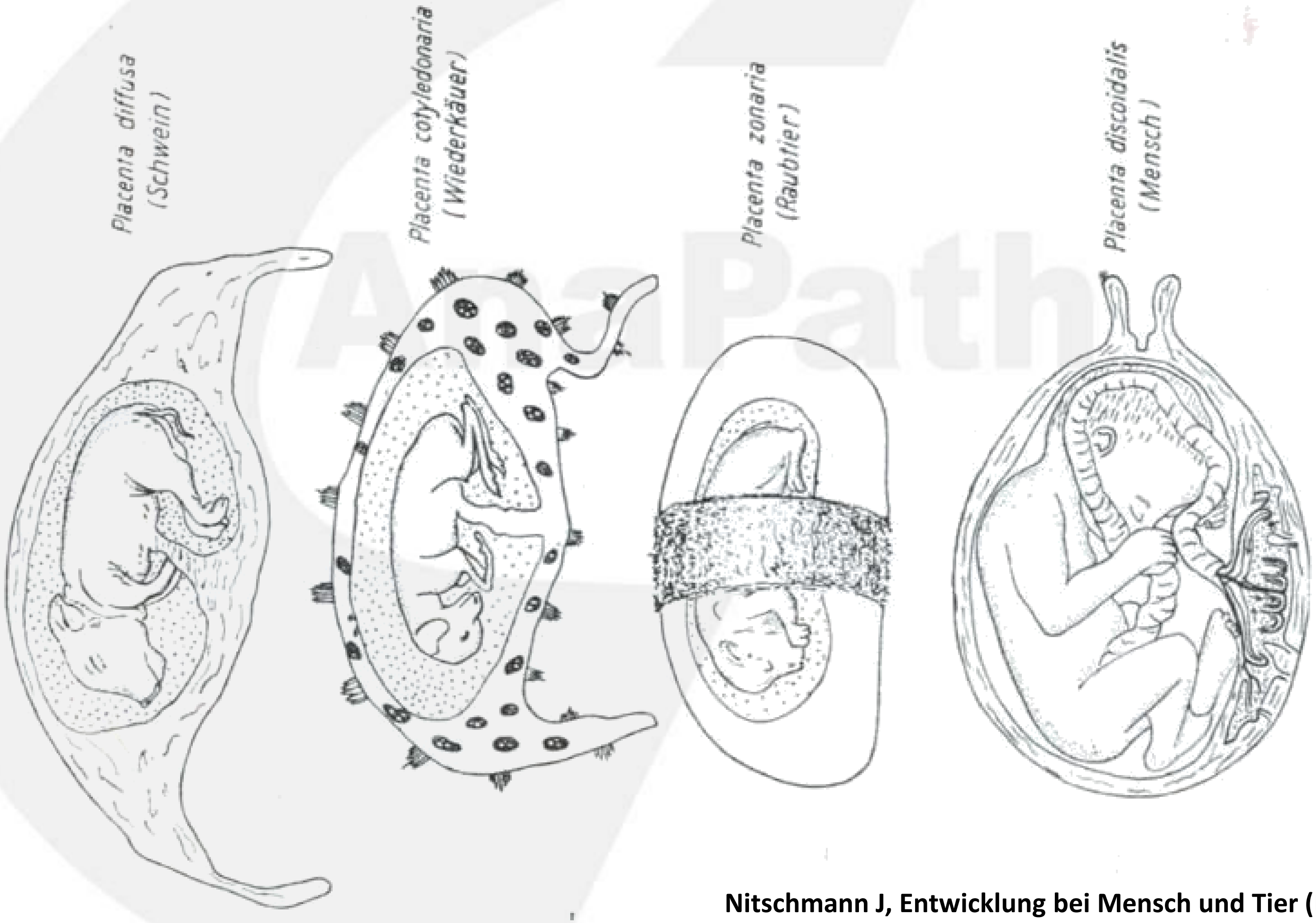
- **Placenta discoidalis**
- **In human, loss of zona pelucida afer 5.5 to 6 days**
- **Syncytiotrophoblast enters uterine mucosal layer (proteolysis), growths in and is embedded in mucosa after 11 to 12 days**
- **Trophoblast develops into chorion under formation of massive syncytial wall with lacunae**
- **Connection of syncytium with endothelium of maternal blood vessels (utero-placentar circulation)**
- **Chorionic villi formed by trophoblast that ingrowth further that connect in week 4 to intraembryonic circulation**
- **In month 3, formation of disks by chorionic villi**

Placenta

Placenta – Forms

- **Placenta diffusa: Complete chorionic surface connects to uterine mucosa, e.g. pig**
- **Placenta cotelydonaria: islets of chorionic placentomes, e.g. cattle, lemures etc.**
- **Placenta discoidalis / bidiscoidalis: monkeys, primates**
- **Placenta zonaria: mixed form with belt-like formation of connection, e.g. carnivores**

Placenta – Forms



Nitschmann J, Entwicklung bei Mensch und Tier (Embryologie)
Akademie-Verlag, Berlin, 1986

Placenta – Histological Types

- **Epitheliochorial type: uterine epithelium and the chorion are in contact, and there is no erosion of the epithelium. (e.g. pig, lemurs)**
- **Syndesmochorial type: fetal epithelium in contact with maternal submucosa, e.g. ruminants**
- **Endotheliochorial type: maternal vessels endothelium in contact with the chorion of the fetal membranes, e.g. carnivores, primates**

Placenta

Decidua: endometrium during pregnancy that forms maternal part of the placenta

- **Discoidal placenta**
- **Hemochorial placentation (invasion of maternal tissue by the trophoblast cause disappearance of maternal blood vessels)**
- **Direct contact with maternal blood**

2 structures:

1. **Choriovitellinae placenta:**

- **Trophoblasts adhered to basement membrane**
- **Asscociated with decidua capsularis**
- **Degenerates and disappears by day 14 of gestation**

Placenta

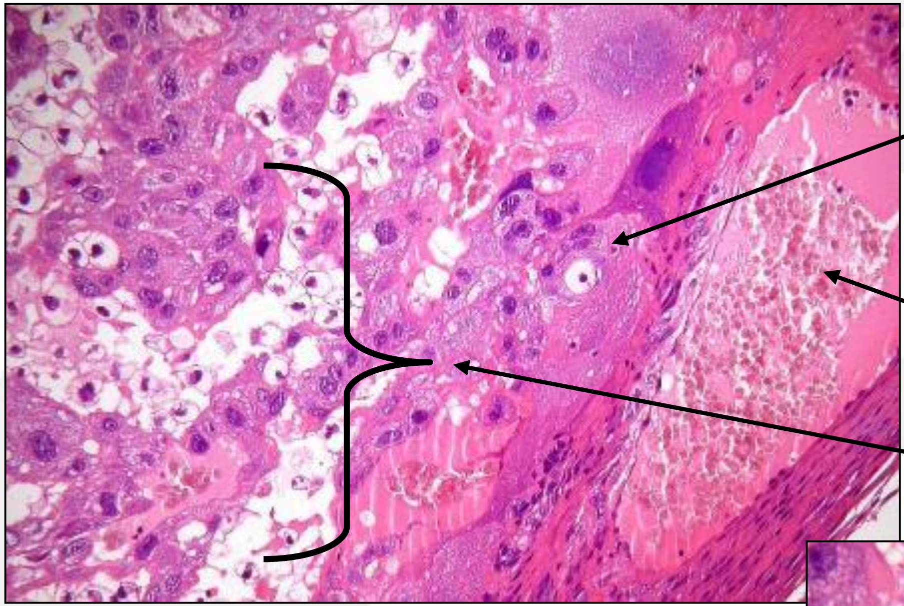
2.Chorioallantoic placenta:

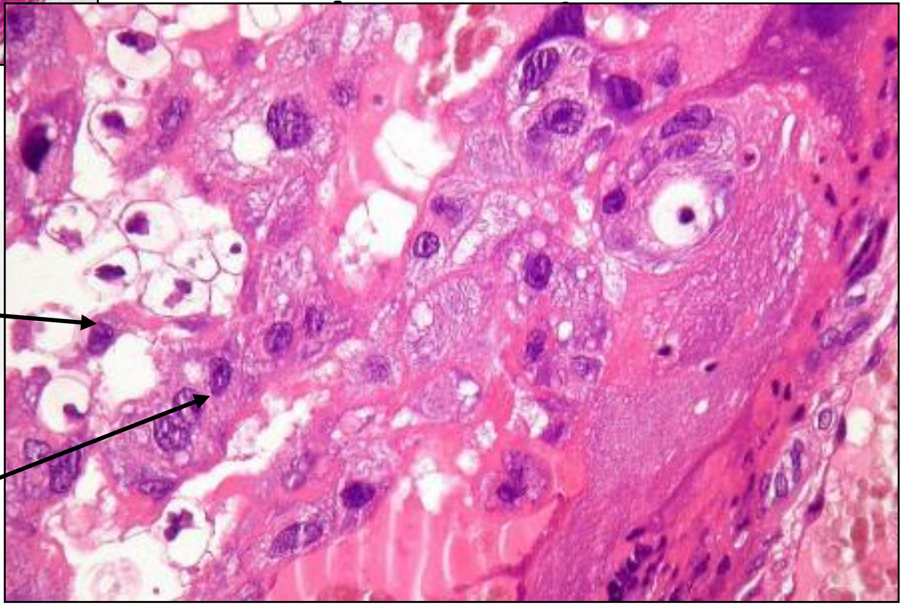
- **Develops before degeneration of choriovitellinae placenta in mesometrial uterine region**
- **Two zones:**

  a) Junctional zone adjacent to decidua basalis consisting of outer giant cells (trophoblasts) and maternal vascular channels (trophospongium with highly packed basophilic spongioblast cells)
  In decidua basalis, maternal blood spaces lined by cytotrophoblast and syncitiotrophoblast.

  Trophospongium contains necrotic areas.

Placenta


Trophoblasts
Maternal vascular spaces

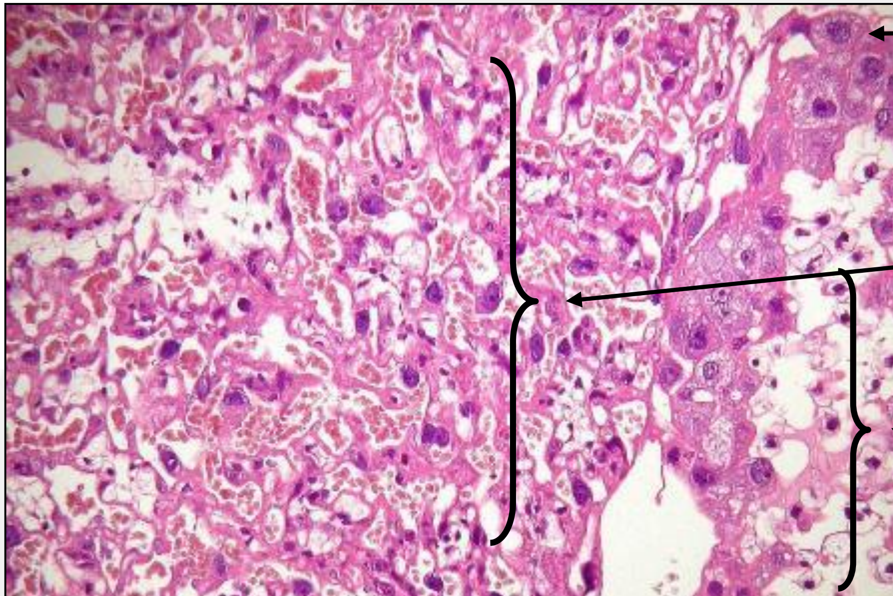

Cytotrophoblasts
Syncytiotrophoblasts

Placenta

b) Labyrinth zone with trophoblasts, maternal vas-
cular spaces and fetal vessels (fetus site)
Strands from the trophospongiosum extend into
the labyrinth.
Embryonic capillaries lined by the endothelial cells.

Reicherts membrane at embryonic site

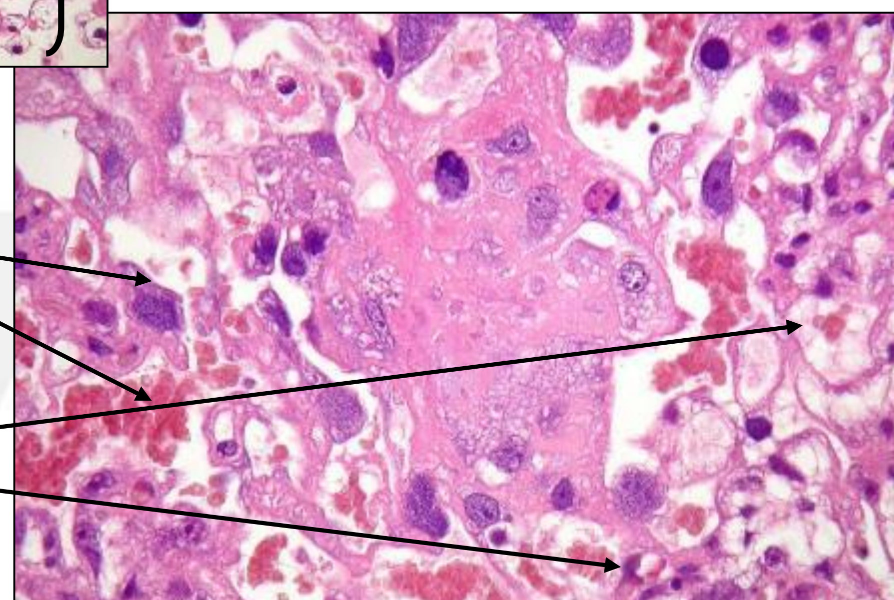
Placenta



Trophoblasts

Labyrinth zone

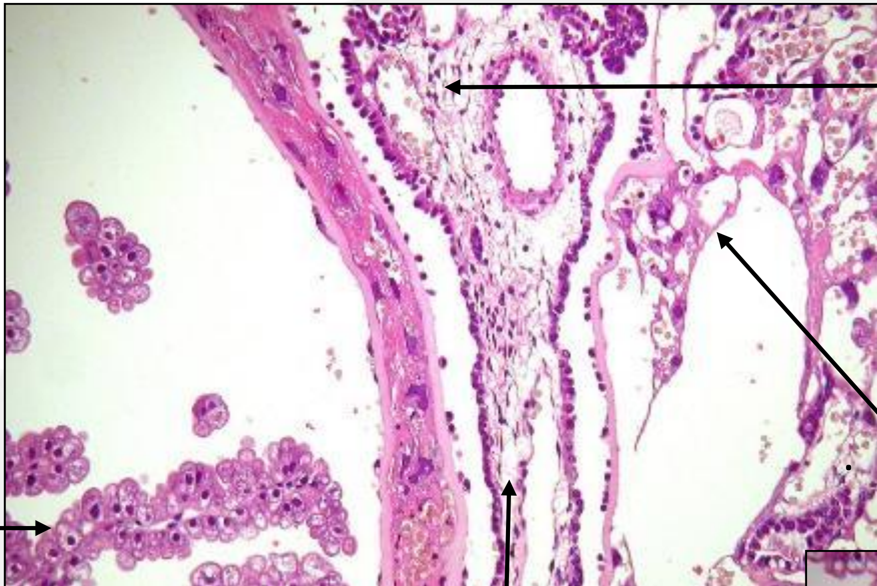
Trophospongium



Maternal blood spaces

Embryonal vessles

Placenta

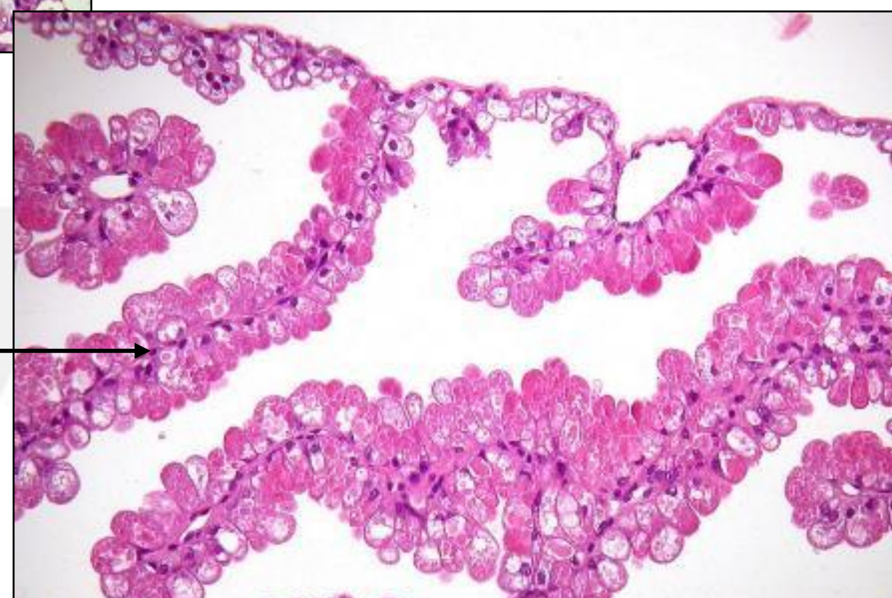


Decidua capsularis (reflex-um): decidua of the endo-metrium covering implan-ted ovum

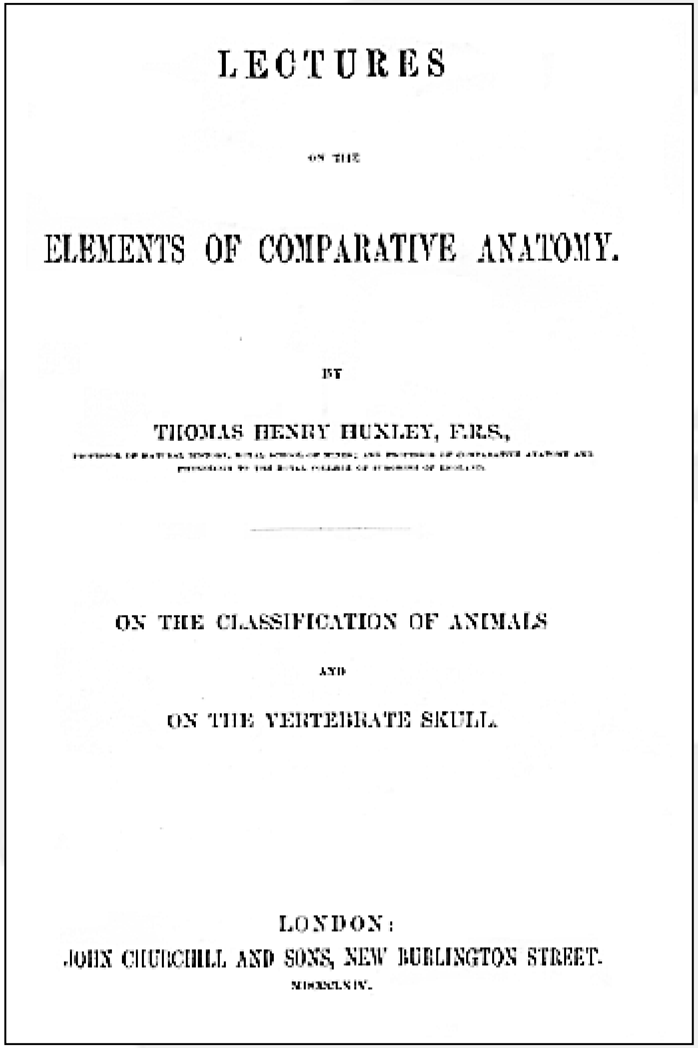
Labyrinth

Reicherts membrane

Yolk sac membrane (Vitelline membrane)



Placenta: Thomas Henry Huxley, 1864



LECTURES

ON THE

ELEMENTS OF COMPARATIVE ANATOMY.

BY

THOMAS HENRY HUXLEY, F.R.S.,

ON THE CLASSIFICATION OF ANIMALS

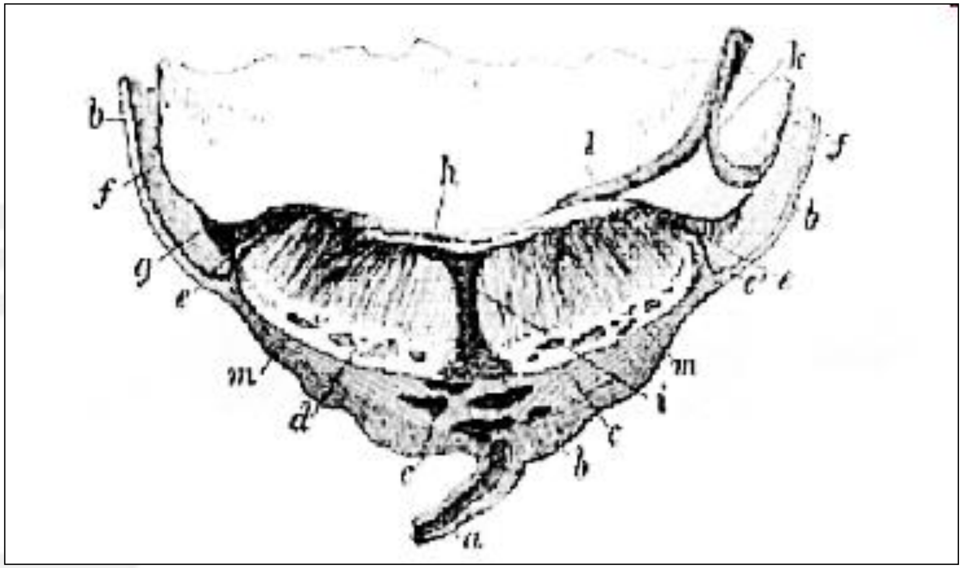
AND

ON THE VERTEBRATE SKULL.

LONDON:

JOHN CHURCHILL AND SONS, NEW BURLINGTON STREET.

MDCCCLXIV.



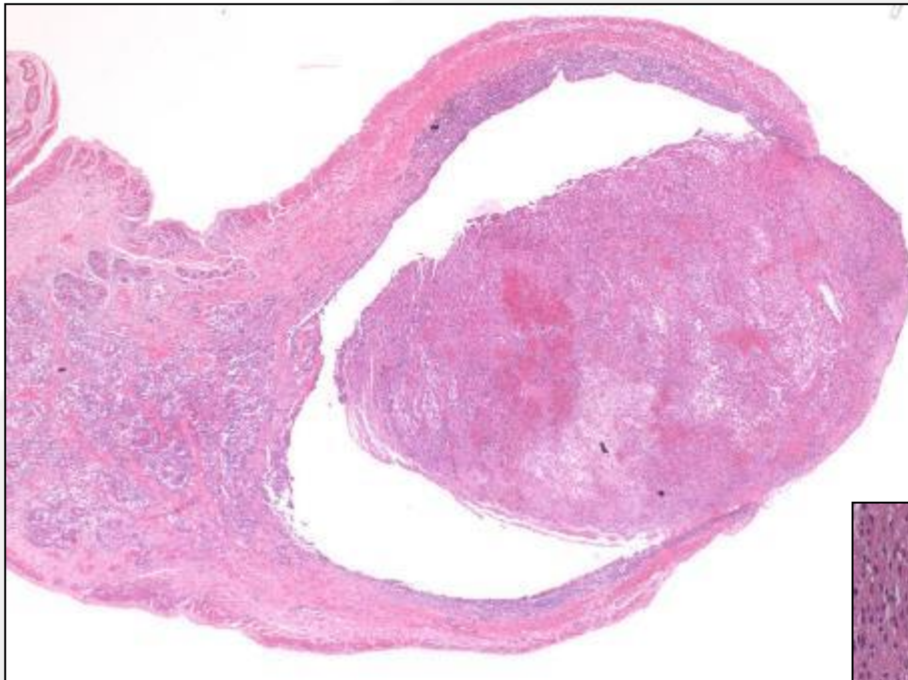
Deciduale Reaction (Deciduoma)

- **Uterine lining (endometrium) during pregnancy that**
- **Forms the maternal part of the placenta**
- **Formed under the influence of progesterone**
- **Characteristic cells**

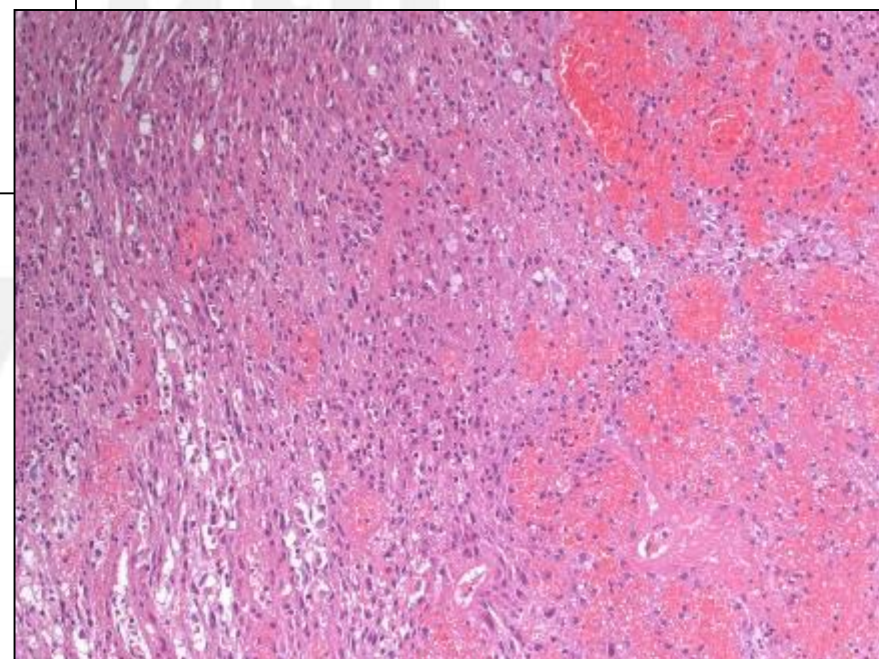
- **High progesterone levels may trigger decidual reaction in rodents**

Tawfik OW, Sagrillo C, Johnson DC, Dey SK. Decidualization in the rat: role of leukotrienes and prostaglandins. Prostaglandins Leukot Med. 29: 221-7 (1987)
Miller MM, O'Morchoe CC. Decidual reaction induced by prostaglandin F2α in the mature oophorectomized rat. Cell Tiss Res, 225: 189-199 (1982)

Deciduale Reaction (Deciduoma)

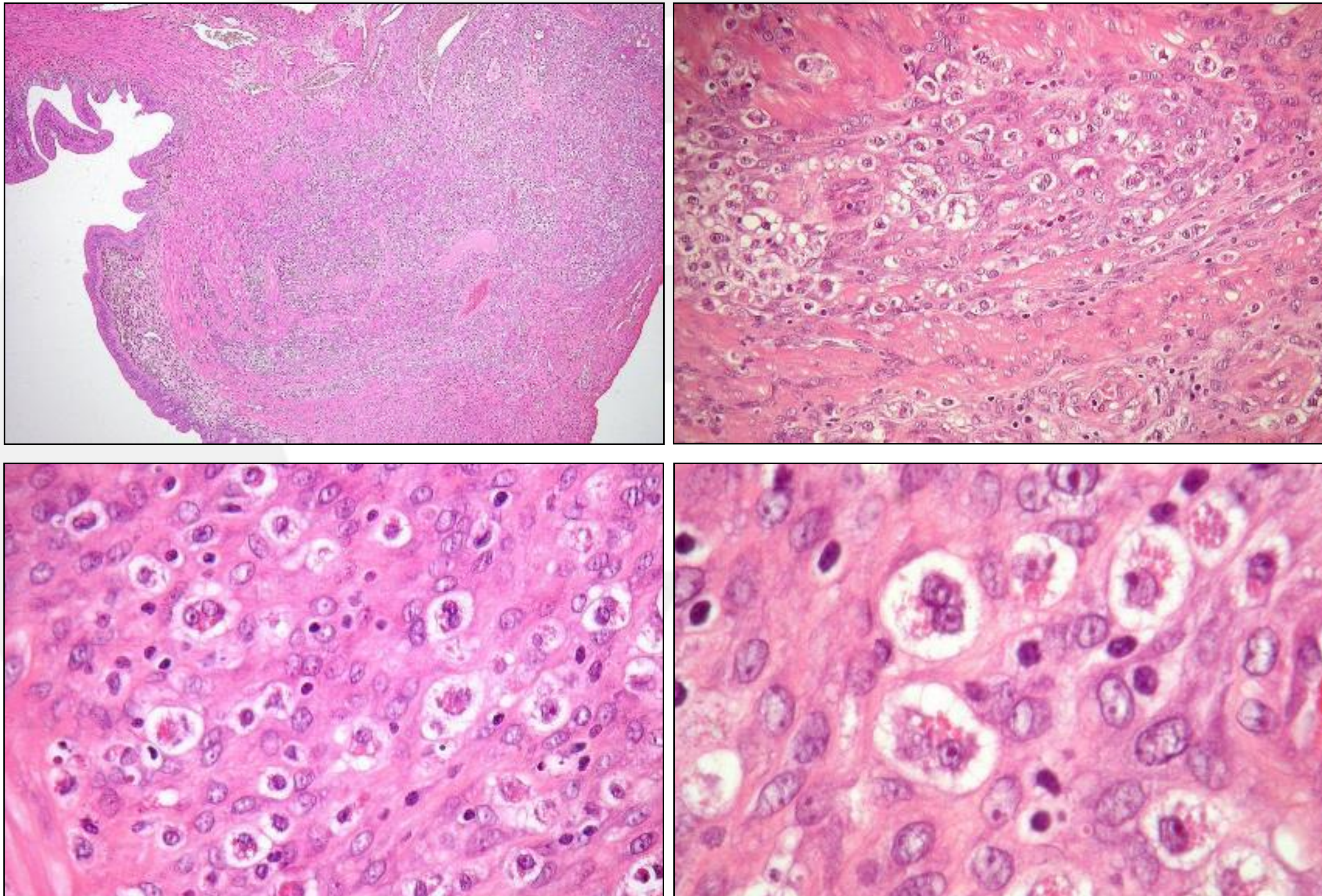


Most prominent at anti-mesometrial site.



Cellular and vascular changes in endometrium at the time of implantation by hormones.

Deciduoma (mesometrial site): Granulated Metrial Gland Cells



Sex Determination

Sex Determination

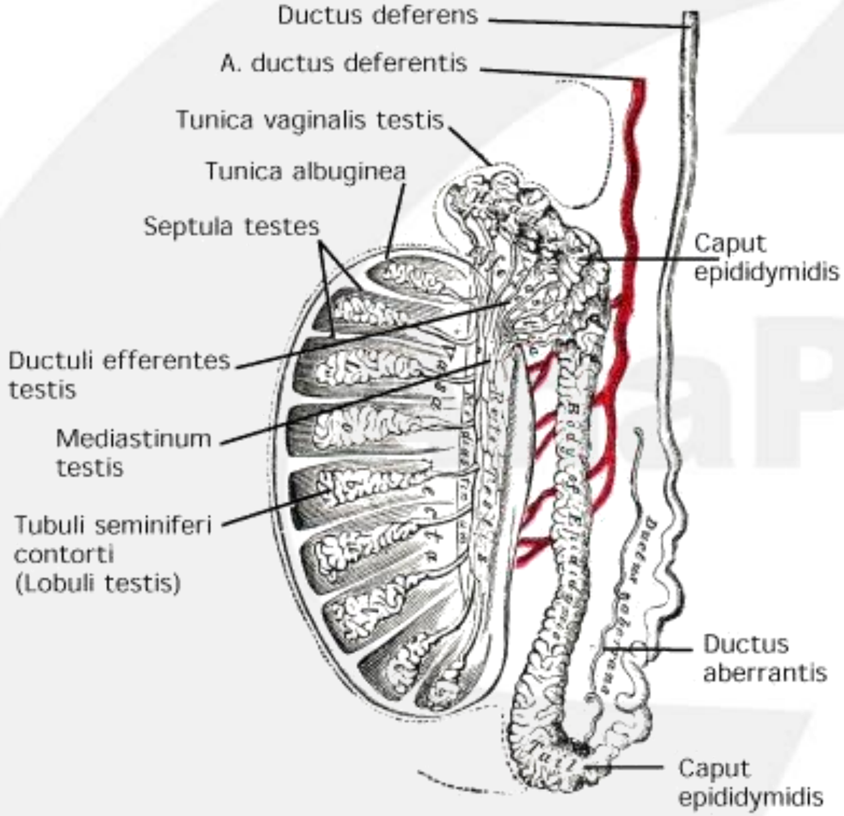
- Definitive decision by steroid hormones
- Y-chromosome bears all information to from male phenotype
- At the beginning, indifferent gonads and two genital tubes, i.e. Müllerian and Wolffian ducts
- Wollfian duct (mesonephros) regresses in females but forms vas deferens in males
- Müllerian duct forms oviduct, uterus, and vagina
- Y chromosome induces development of testes in primitive gonads
- Leydig cells: secretion of testosteron with subsequent regression of Müllerian duct

Gross Anatomy - Basics

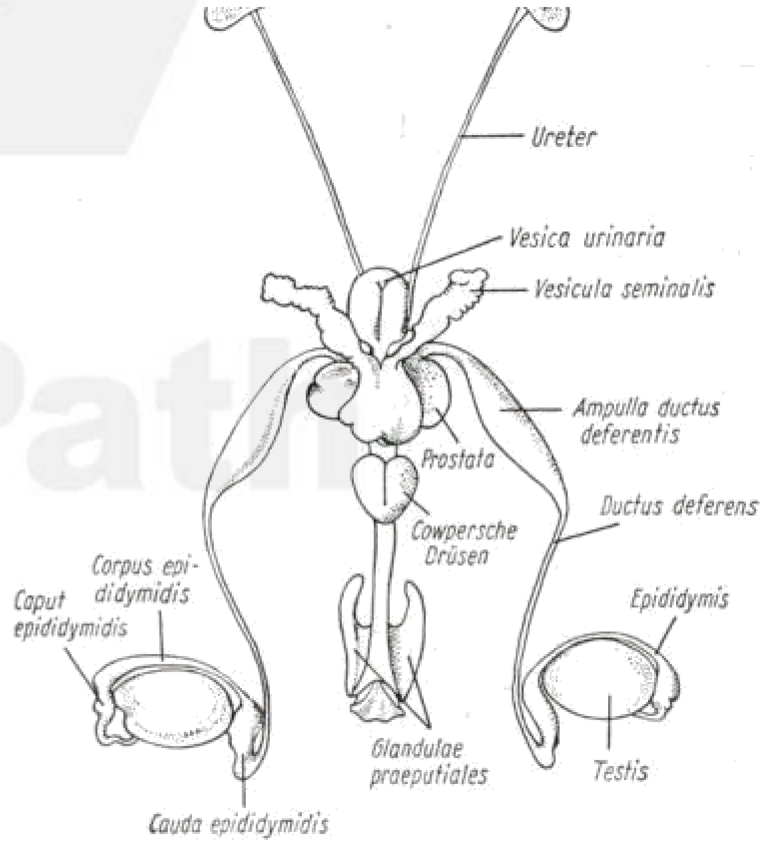
Male Sex Organs – General

- Monotremata similarly to reptiles where conducting parts open into urogenital sinus that is divided into separate sperm and urine ducts but entering a common cloace
- Testes are located species specific
- When descending from the original intra-peritoneal position than formation of scrotum where the testes are fixed by a ligament
- Descensus is partially only temporary (in Monotremata, some Insectivora, Proboscida etc. the testes are always intra-abdominal)
- Testes are lined by a fibrous capsule (Tunica albuginea) covered by peritoneum
- From Tunica albuginea separate septa that divide the testes in lobules which contains tubuli seminiferi
- Spermatic tubules enters the rete testis which connects via ductuli efferentes to the epididymides
- From epididymides, a vas deferens enters the urogenital sinus after the starting point of the urethra

Male Sex Organs - General



http://www.urologielehrbuch.de/01/Gray_Hoden_Aufbau.jpg



Kämpfe L, Kittel R, Klapperstück J, Leitfaden der Anatomie der Wirbeltiere. Gustav Fischer, Jena, 1980

Male Sex Organs - General

Accessory organs:

- **Seminal vesicles as adenxa of vas deferens**
- **rat: lobulated, approx. 2.5x0.5 cm**
- **mouse/hamster: lobulated, approx. 1.5x0.5 cm**
- **dog: NOT PRESENT**
- **pig: compact, approx. 5.0-7.0 cm**
- **rabbit: lobulated, approx. 3.5x1.0 cm**

- glands derived from the urogenital sinus:

a) prostate that secretes near the entry of vas deferens

- **rat: lobulated, round, approx. diameter 2.5 cm (several parts)**
- **mouse/hamster: lobulated, round, approx. diameter 1.0 to 1.5 cm**
- **dog: round, approx. diameter 2.0-3.0 cm**
- **pig: small, similar**
- **rabbit: round, approx. diameter 3.0 cm**

Male Sex Organs - General

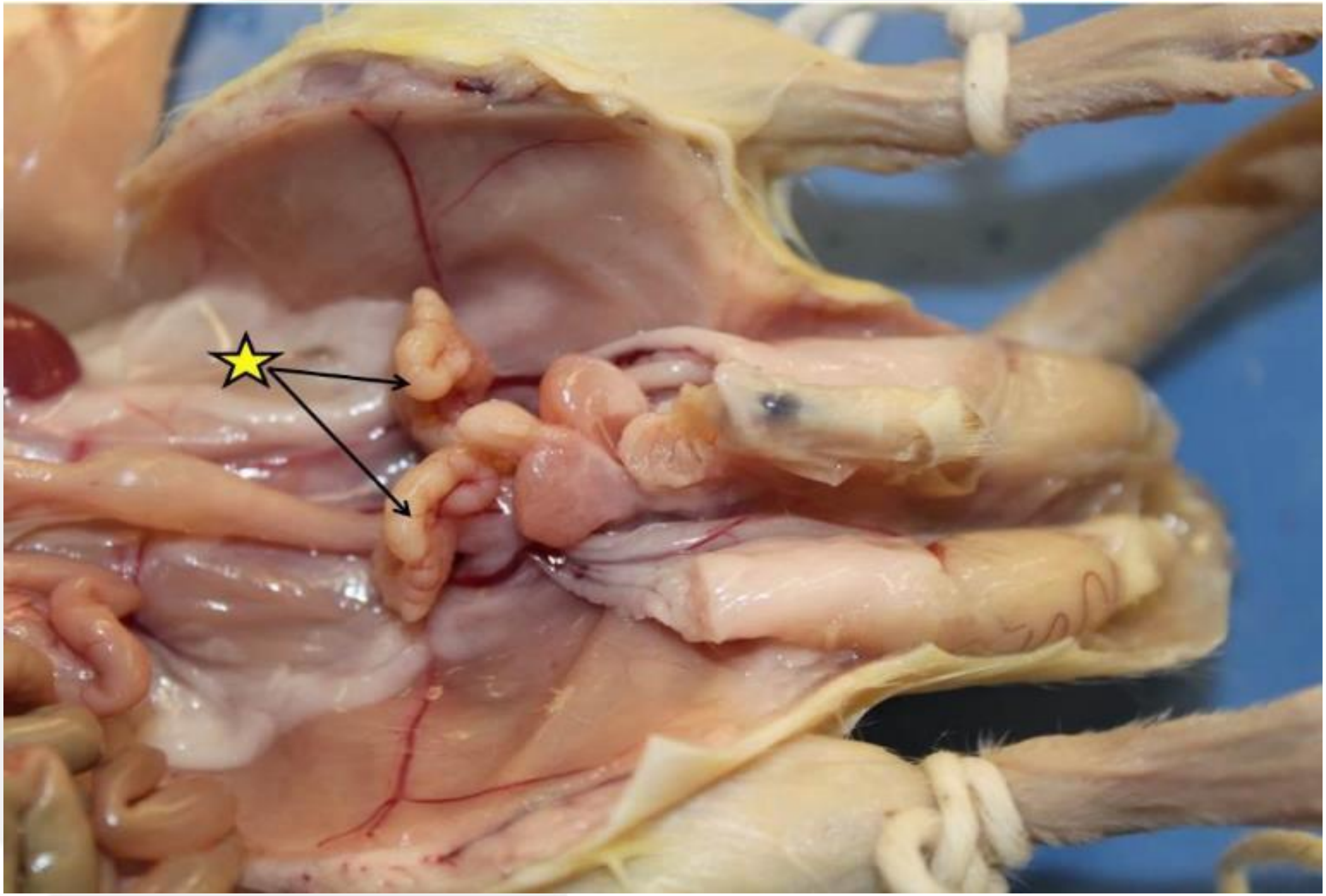
b) Cowper's glands (bulbourethral glands) that enters caudally

- rat: extremely small (approx. 0.3 cm)
- mouse/hamster: larger in mice, 0.5-1.0 cm
- dog: very small (approx. 0.5 cm)
- pig: extremely large, up to 500 ml secretion
- rabbit: very small (approx. 0.5 cm)

Penis:

- Cavernous type
- Fibroelastic type (horse)
- Glans penis
- Bone (penis bone: carnivores; glans penis bone: e.g. cat)

Male Sex Organs – Main Differences in Species

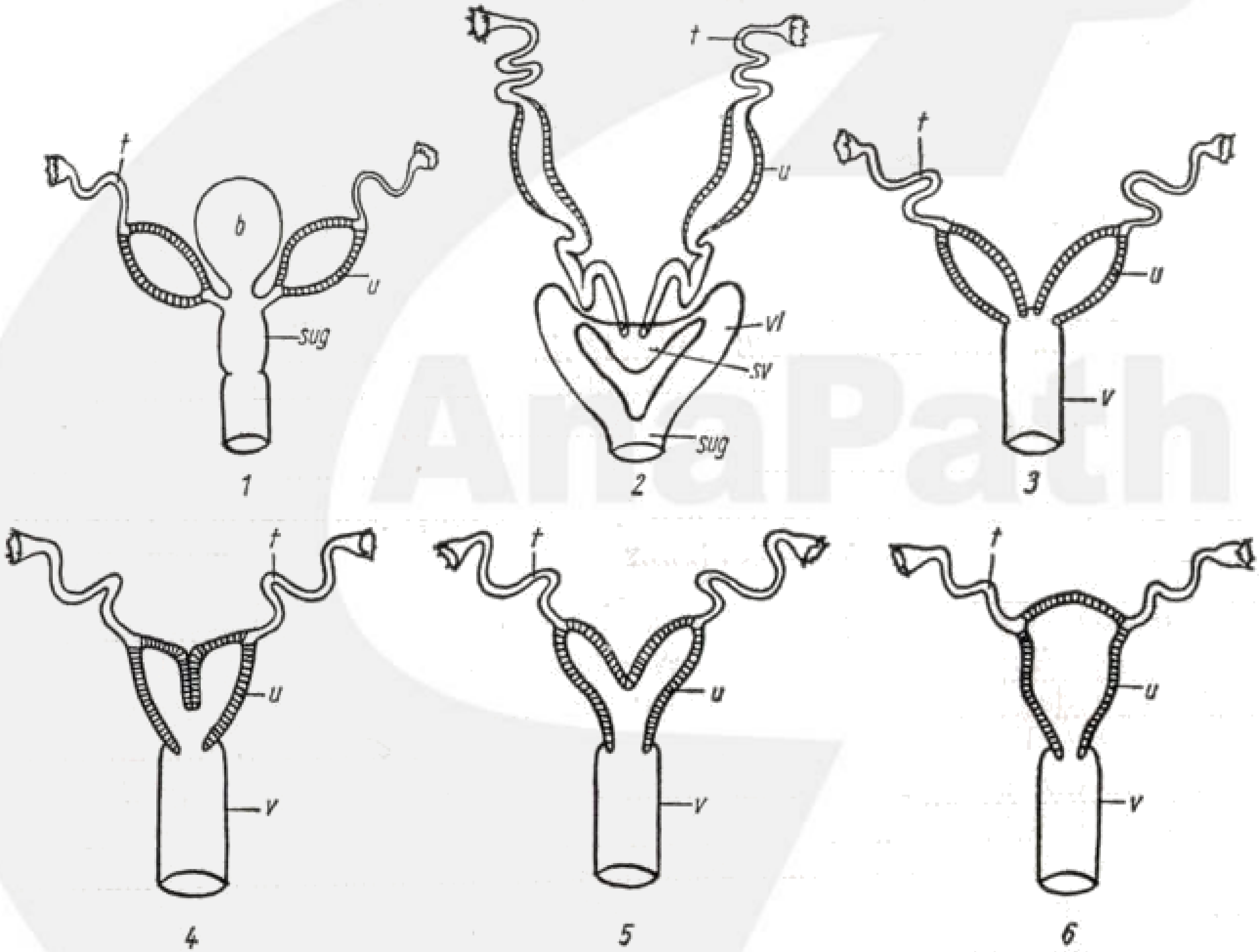


http://classconnection.s3.amazonaws.com/642/flashcards/400642/jpg/19_seminal_vesicle1326567329296.jpg

Female Sex Organs – General

- Ovaries very uniform in mammals with exception of Monotremata (due to size of ooctyes and richness in yolk, similar to reptiles)
- Müller’s duct forms in general 3 parts: oviduct (tuba uterina) with infundibulum and fimbrae), uterus, vagina,
- Müller’s duct including vagina double present and separated in Monotremata
- Fusion of vaginae laterales in Marsupials forms unpaired vaginal sinus
- Only one vagina in all other mammals but:
  - both uteri enters one vagina (uterus duplex) in Rodentia, Elephas
  - both uteri are caudally unified but cranial there are 2 horns (uterus bicornis) (Insectivora, Perissodactyla, Carnivora, some Ruminantia)
  - complete fusion of uteri (uterus simplex) (Simiae, Hominidae)

Female Sex Organs – General



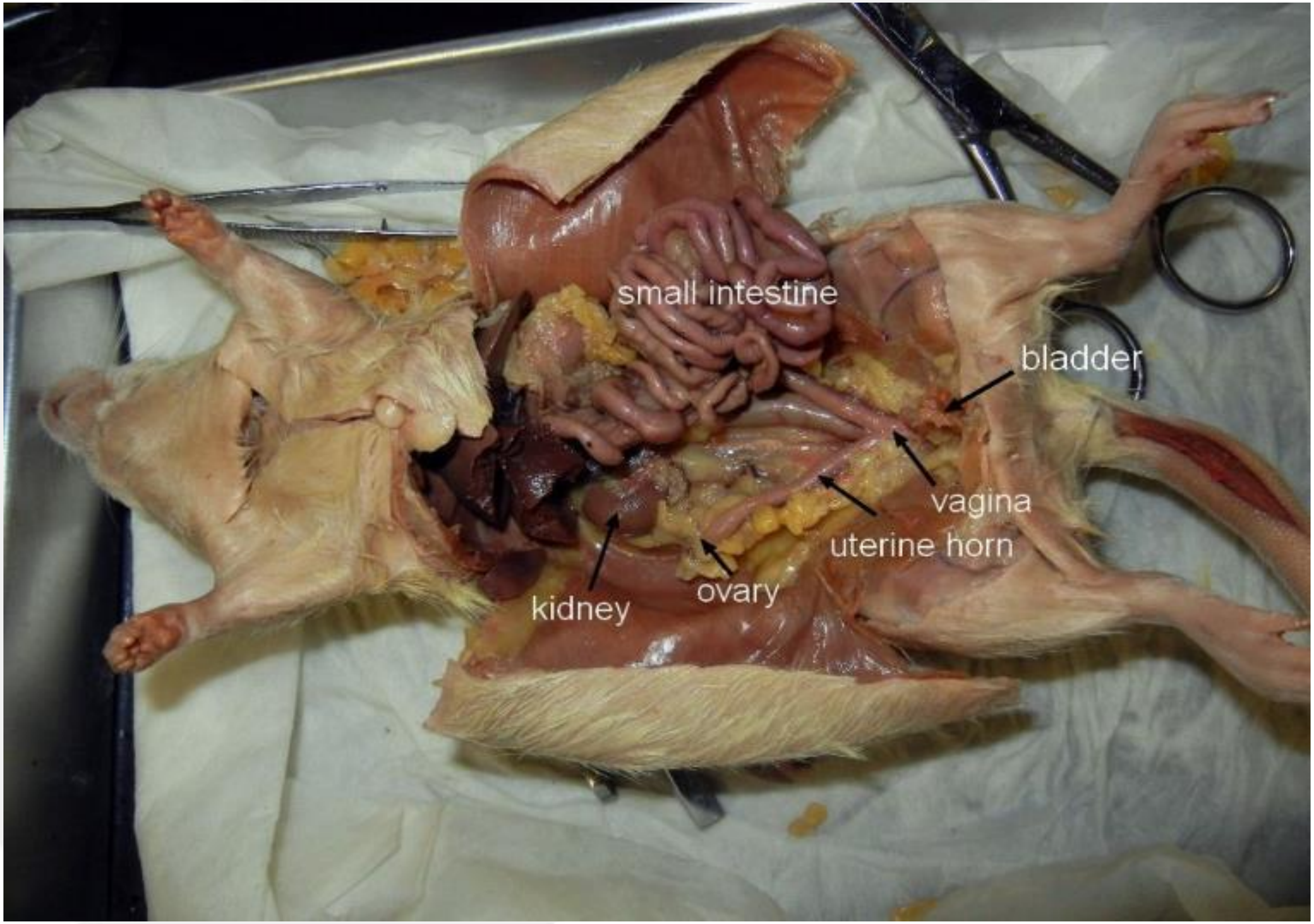
1 – Monotremates
2 – Marsupials
3 – Uterus duplex
4 – Uterus bipartitus
5 – Uterus bicornis
6 – Uterus simpex
sug – Urogenital sinus
su – Vaginal sinus
t – Tuba uterina
V – Vagina
Vl – Vagina lateralis

Kämpfe L, Kittel R, Klapperstück J, Leitfaden der Anatomie der Wirbeltiere. Gustav Fischer, Jena, 1980

Female Sex Organs – General

- **External organs are:**
  - Vestibulum vaginae that develops from the short urogenital sinus
  - Clitoris that includes all elements of a penis including cavernous body, glans and preputium (including clitoral glands analogue to preputial glands)

Female Sex Organs – Rat



http://www.biologycorner.com/resources/rat_urogenital_labeled.jpg

Maturation

Major Differences in Strains: Maturity Males

Historical Control Data in 4 Weeks Studies																
	Categorie ALL				**Categorie 1**				**Categorie 2**				**Categorie 3**			
	COBE		Others		COBE		Others		COBE		Others		COBE		Others	
No. of Studies	81		50		46		31		29		13		4		2	
No. of Pathologists	18		16		14		12		11		6		4		2	
Organ/Finding	COBE		Others		COBE		Others		COBE		Others		COBE		Others	
Testes	**315 M**	-	**189 M**	-	**179 M**	-	**122 M**	-	**118 M**	-	**44 M**	-	**12 M**	-	**8 M**	-
Immature	9.56	-	19.75	-	15.04	-	24.66	-	2.62	-	15.28	-	0	-	0	-

Historical Control Data in 13 Weeks Studies																
	Categorie ALL				**Categorie 1**				**Categorie 2**				**Categorie 3**			
	COBE		Others		COBE		Others		COBE		Others		COBE		Others	
No. of Studies	35		20		11		3		18		12		3		3	
No. of Pathologists	14		10		9		3		11		7		3		2	
Organ/Finding	COBE		Others		COBE		Others		COBE		Others		COBE		Others	
Testes	**187 M**	-	**93 M**	-	**63 M**	-	**13 M**	-	**95 M**	-	**66 M**	-	**17 M**	-	**7 M**	-
Immature	1.72	-	7.46	-	0	-	25	-	2.45	-	5.56	-	0	-	0	-

C1=6-8 months, C2=9-11 months, C3=12-14 months, H=Harlan, O=Others

Weber K: Scientific Round Table: Overcoming Challenges When Changing Animal Models – a CRO Perspective, ACT, Palm Springs, November 1-4, 2009

Issues – Dog: 28-Day Studies

ID	Type (28 days)	Age/Delivery (months)	Ø Age(month)	Accli. days	Pretest days	Body Weight: Pretest (kg) M	F	Total Months
1	continuous infusion	7 - 8.5	7.75	91	6	7.8-10.0	7.3-9.2	12
2	continuous infusion	11 - 13	12.5	28		8.9-10.6	7.9-10.1	15
3	4-weeks continuous infusion	7 - 8	7.5	35		8.0-10.0	7.1-9.5	10
4	continuous infusion	6 - 8	7	30	7	6.5 - 9.1	6.7 - 8.9	9
5	continuous infusion	5.5 - 7.5	6.5	26	7	7.4 - 10.2	6.2 - 9.9	9
6	continuous infusion	8.5 - 9.5	9	41	6	6.8 - 10.8	6.8 - 10.8	12
54	oral	4 - 6	5	21	6	6 - 8	6 - 8	7
55	inhalation	7 - 8	7.5	6	7	6 - 9	6 - 9	9
57	oral	6 - 8	7	16	7	6 - 9	6 - 9	9
59	oral	5 - 7	6	18	7	6 - 9	6 - 9	8

Weber K: Scientific Round Table: Overcoming Challenges When Changing Animal Models – a CRO Perspective, ACT, Palm Springs, November 1-4, 2009

Testes

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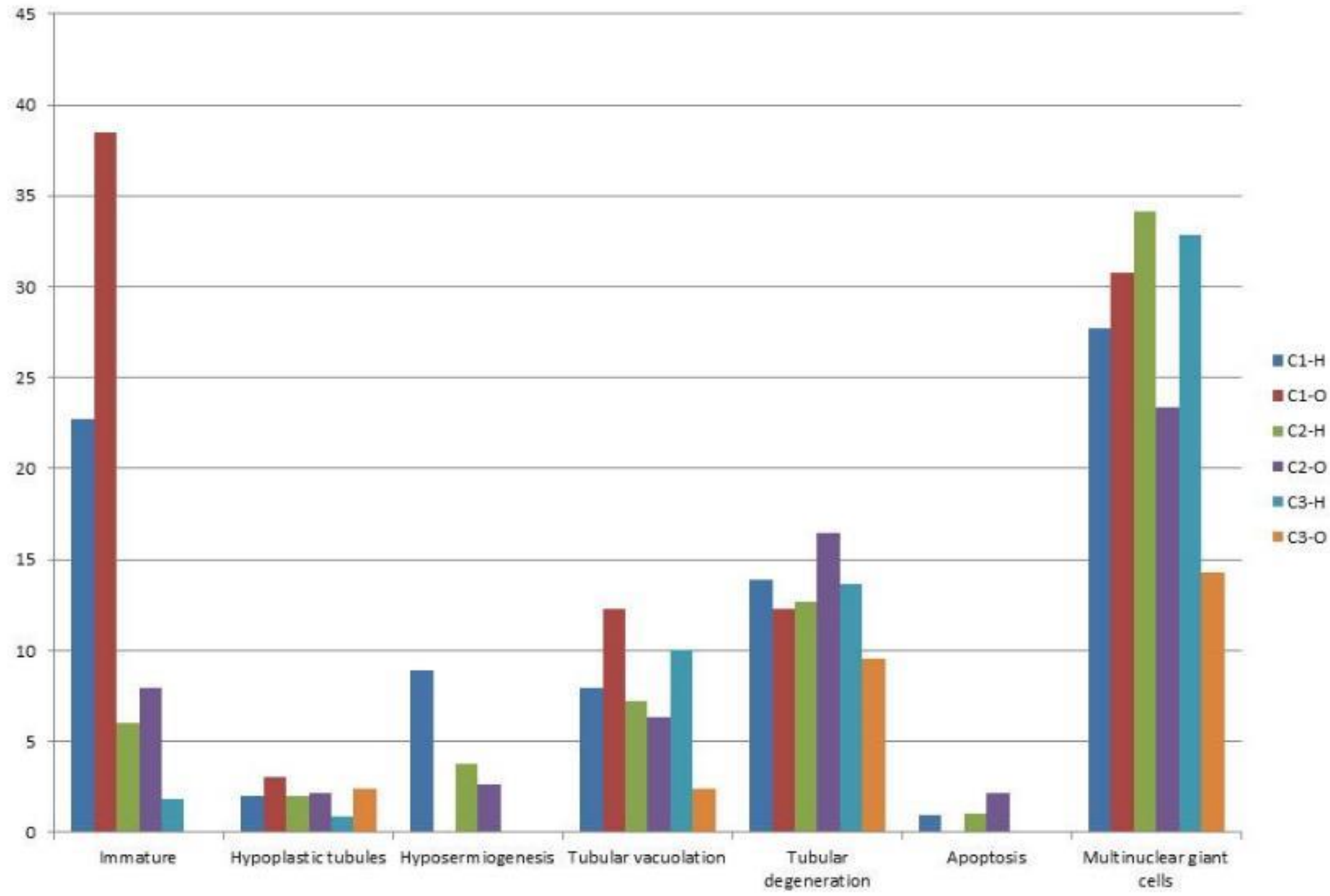
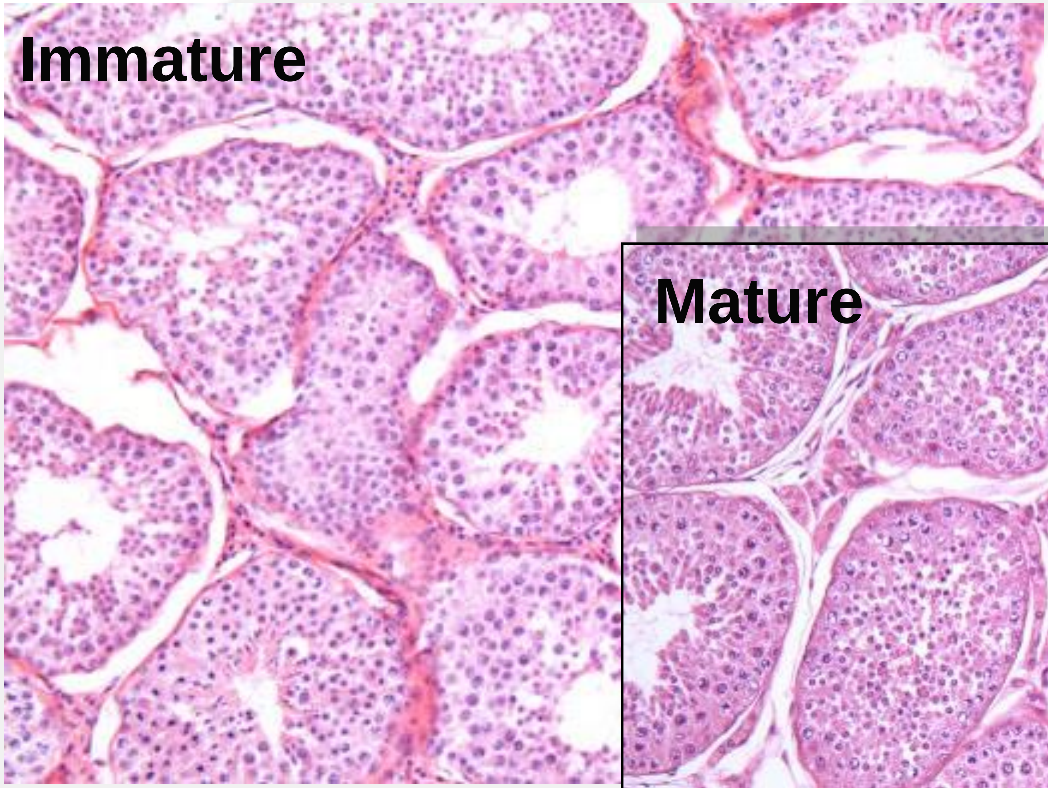
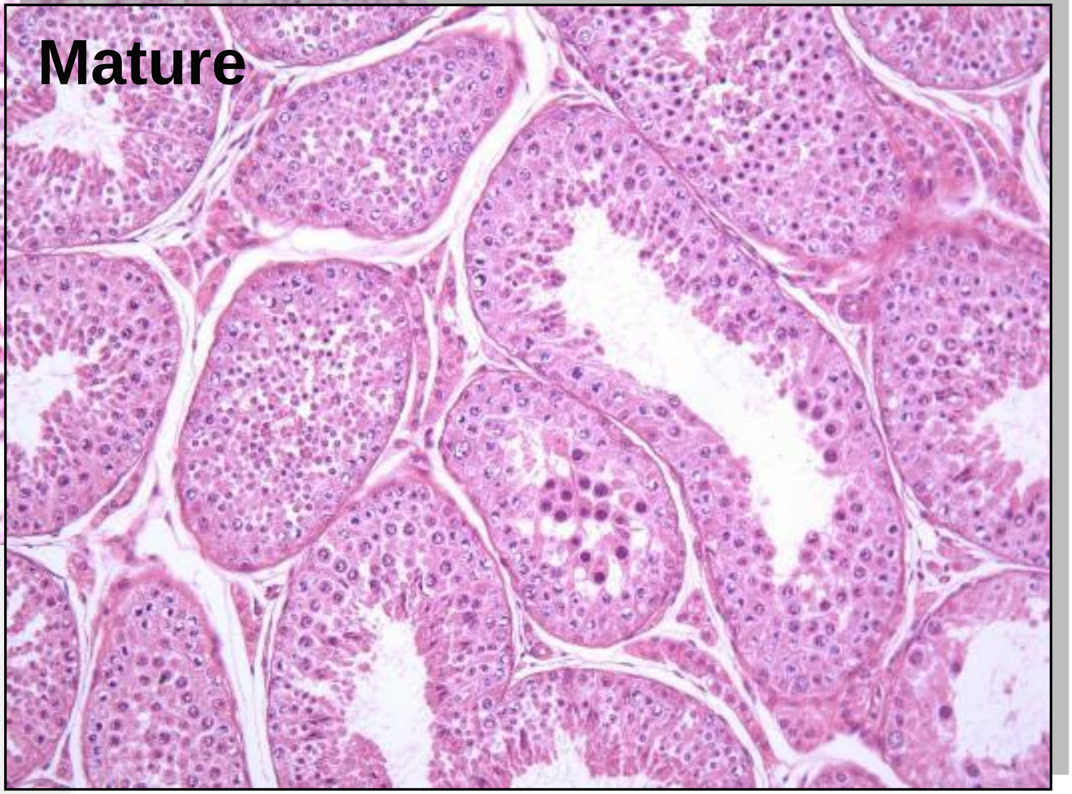


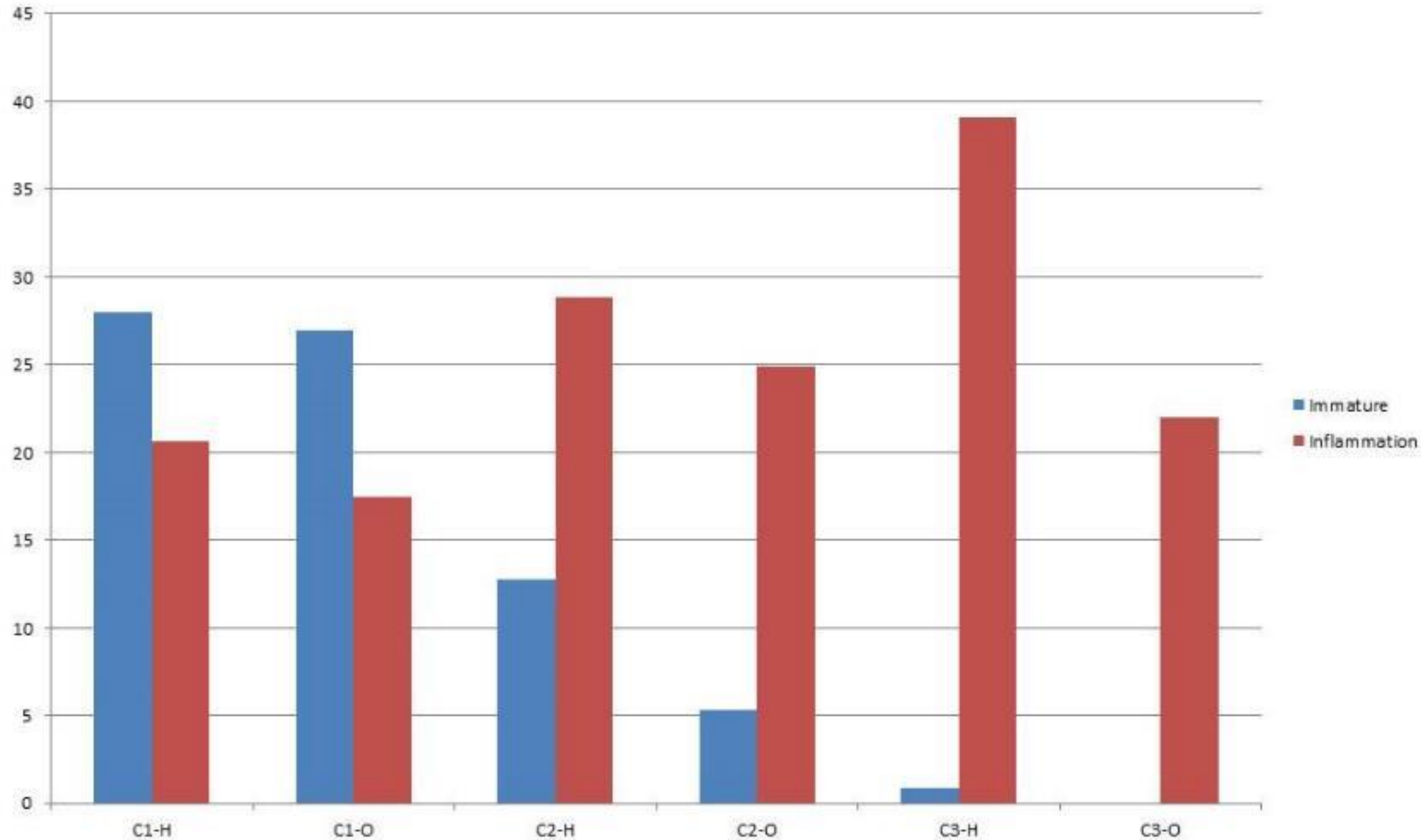
Testes – Dog: 28-Day Studies



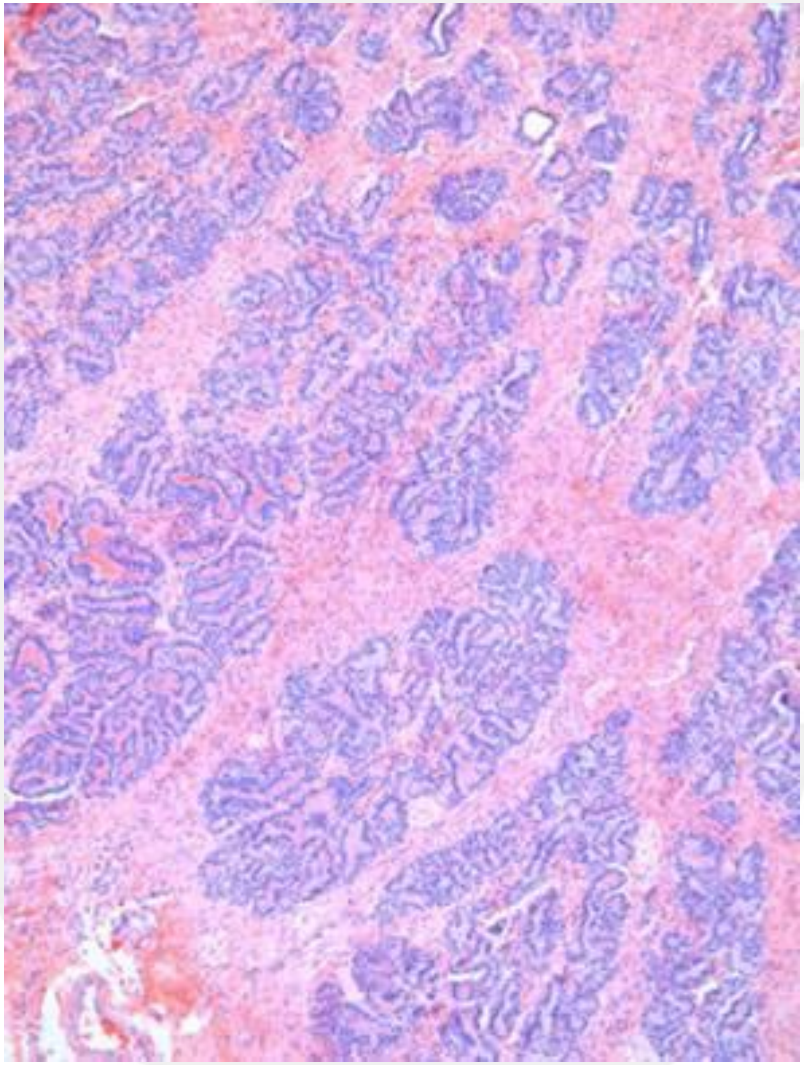


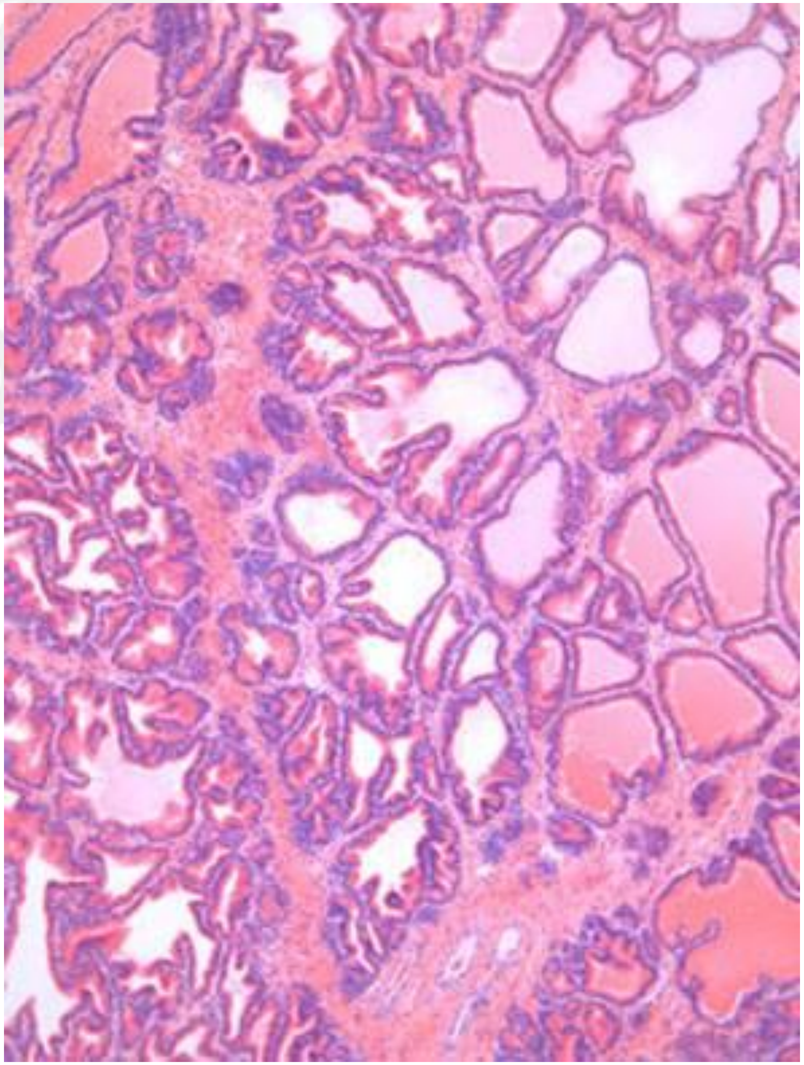
Prostate

C1=6-8 months, C2=9-11 months, C3=12-14 months, H=Harlan, O=Others



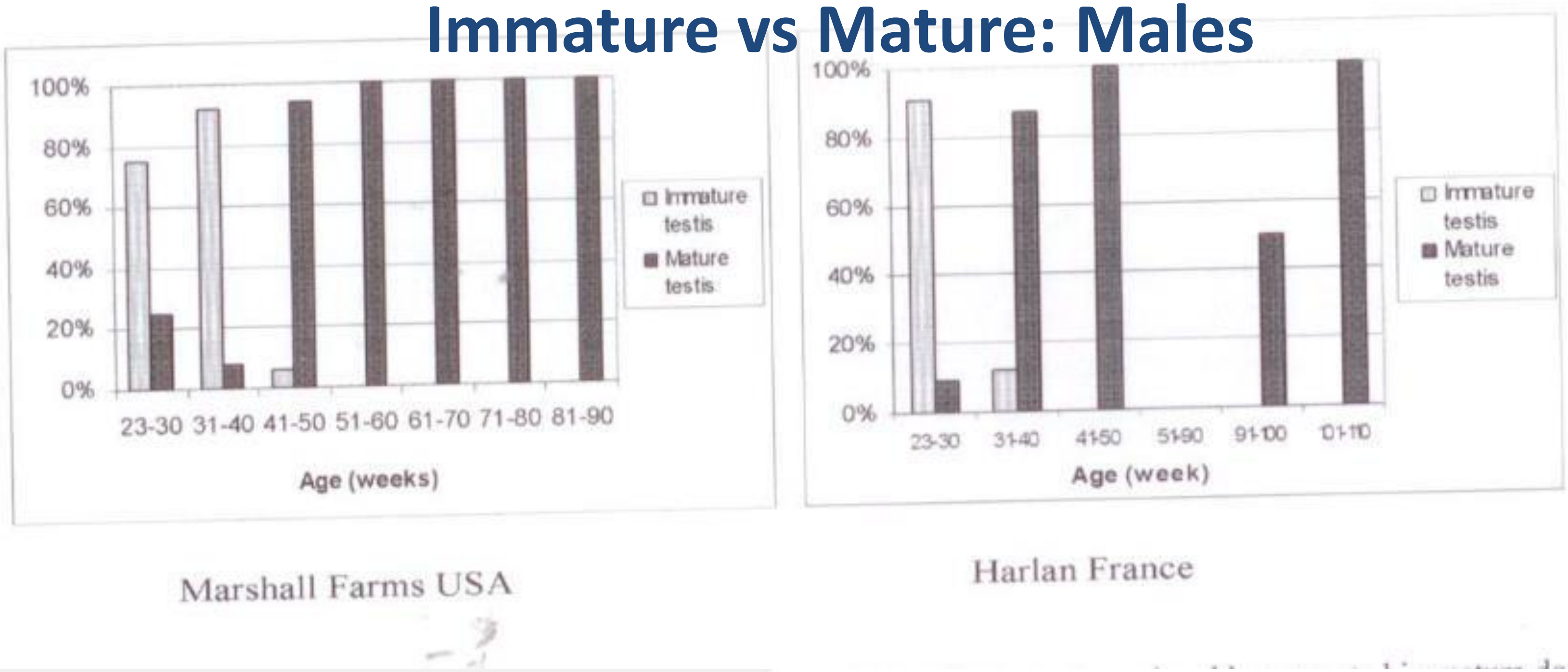
Immature vs Mature: Males





Reported Differences on Maturity in Males

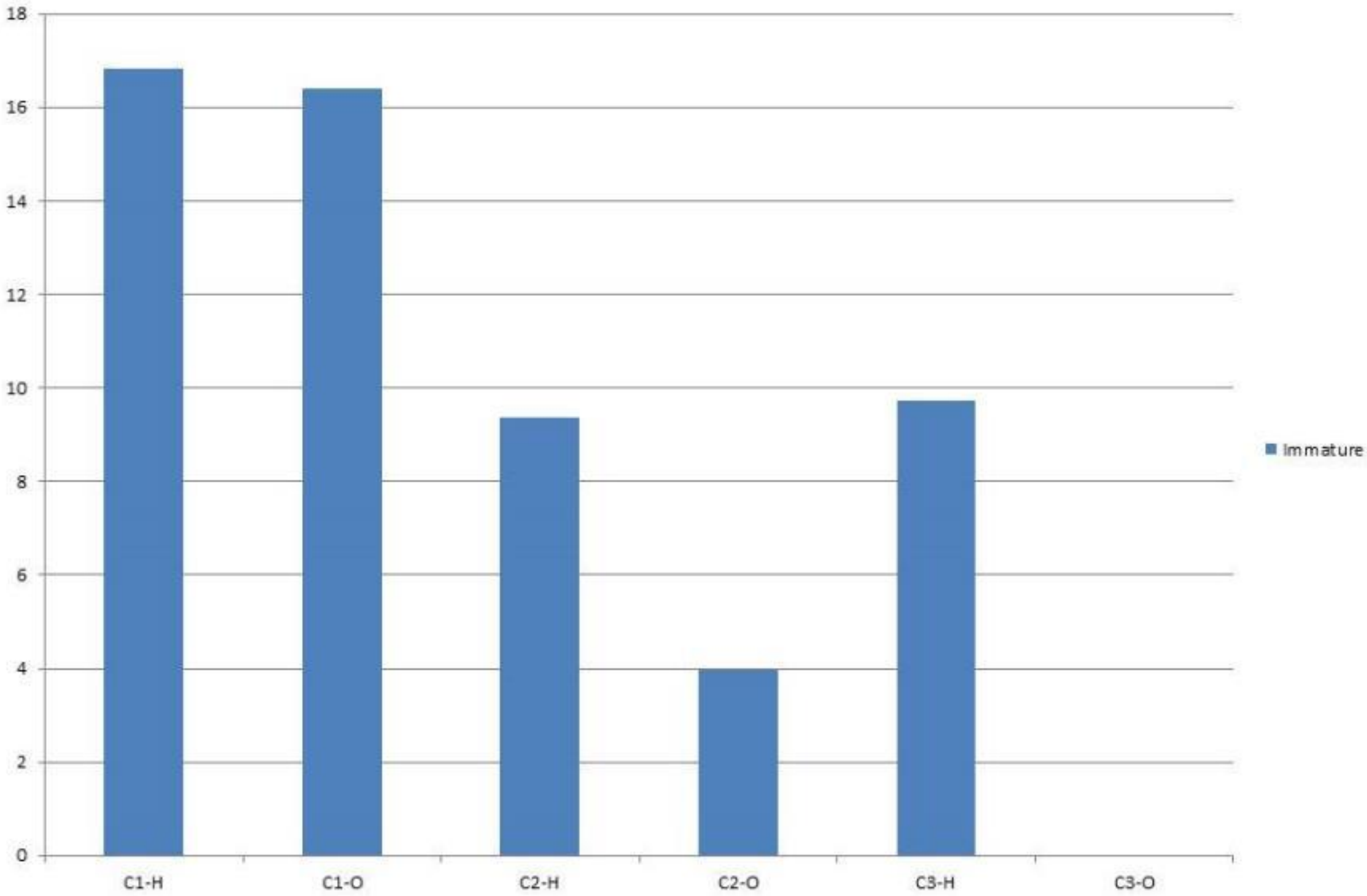
Immature vs Mature: Males



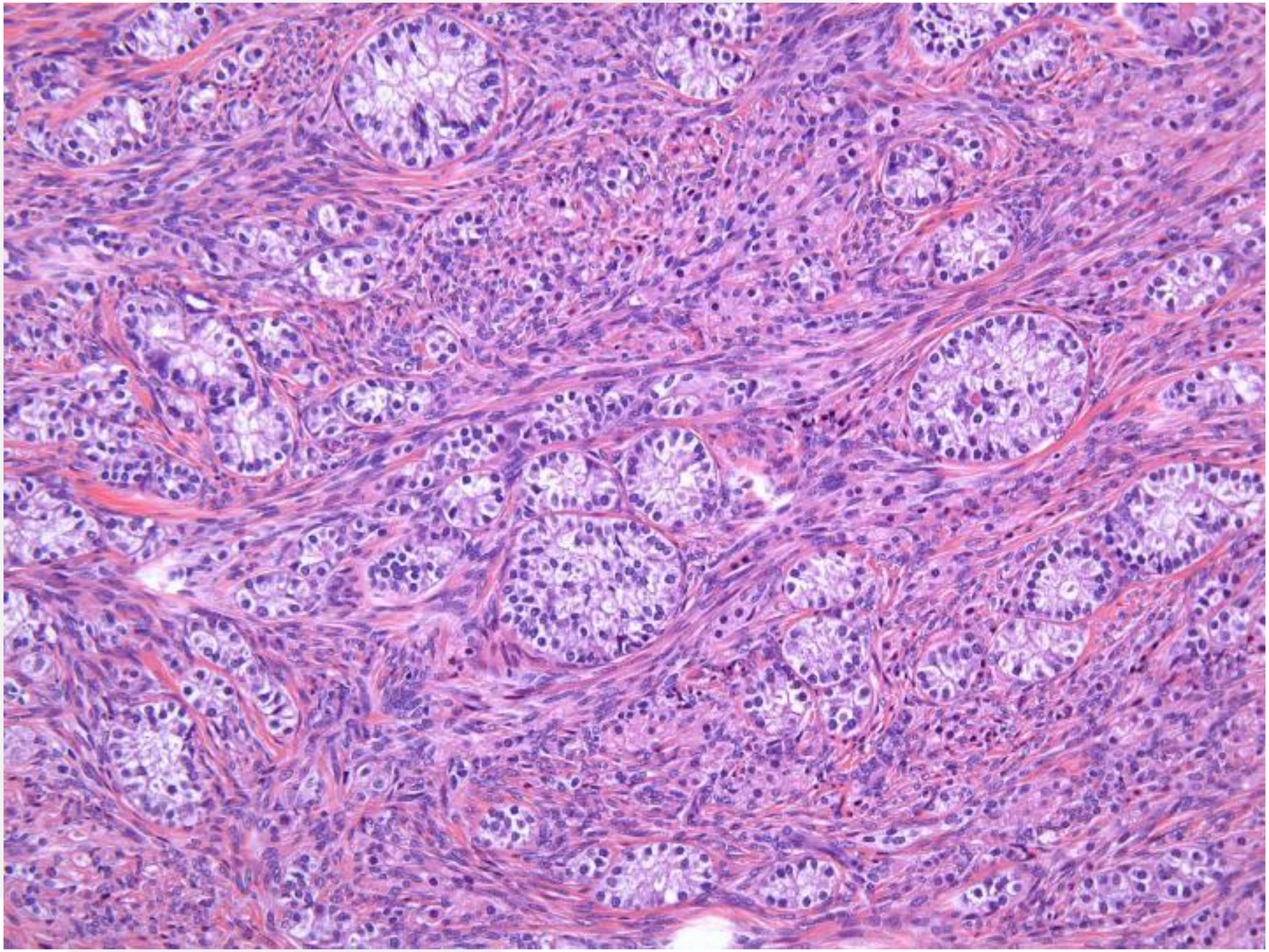
Dorso L, et al. (2008): Toxicol Pathol. 36: 917-925

Ovaries

C1=6-8 months, C2=9-11 months, C3=12-14 months, H=Harlan, O=Others



Immature: Females



Pitfall: Maturity

Table 3 Historical Control Data confirm the Variability of Immature and Mature Animals throughout Study Groups

STUDY / FINDING	CONTROL	GROUP 2	GROUP 3	GROUP 4	GROUP 5	ORIGIN
1 (13 W)	N=6	N=4	N=4	N=6		CHINA
Immature	5	3	4	6		
Mature	1	1	0	0		
2 (13 K)	N=6	N=4	N=4	N=6		CHINA
Immature	5	1	4	5		
Mature	1	3	0	1		
3 (4 W)	N=5	N=3	N=3	N=5		CHINA
Immature	5	3	3	5		
Mature	0	0	0	0		
4 (52 W)	N=6	N=4	N=4	N=6		CHINA
Immature	5	3	3	5		
Mature	1	1	1	1		
5 (28 D)	N=5	N=3	N=3	N=5	N=5	CHINA

STUDY / FINDING	CONTROL	GROUP 2	GROUP 3	GROUP 4	GROUP 5	ORIGIN
						TOTAL
TOTAL	N=109	N=75	N=75	N=109	N=5	N=373
Immature	97 (88.99)	63 (84.00)	64 (85.33)	91 (83.49)	5 (100.00)	320 (85.79)
Mature	12 (11.01)	12 (16.00)	11 (14.67)	18 (16.51)	0 (0)	53 (14.21)

Pitfall: Maturity - Males

Animal No.	Testes	Epididymides	Prostate	Seminal Vesicles
11	Incomplete mature. Only some tubules produce sperm.	Full with sperm, little detritus.	Start of dilation of acini.	Large. Colloid production.
12	Immature. However, one mature tubule.	Immature. Empty tubules.	Start of dilation of acini.	No colloid.
13	Immature. Sertoli's cells only.	Immature. Empty tubules.	Partial dilation of acini.	Large. Colloid production.
14	Mature.	Little sperm, much detritus.	Full dilation of acini.	Large. Colloid production.
15	Mature	Unilateral sperm. On contralateral site mainly mucus.	Partial dilation of acini.	Large.

Pitfall: Maturity - Females

Animal No.	Ovaries
21	Already cycled (corpus luteum)
22	Not cycled before, only secondary follicles.
23	Not cycled before (no c.lutea). Prior to first cycle (enlarged secondary follicles).
24	Not cycled before, only secondary follicles.
25	Not cycled before (no c.lutea). Prior to first cycle (Graafian follicle).
26	Not cycled before, only secondary follicles.
27	Not cycled before, only secondary follicles.

Pitfall: Maturity

Discussion

Despite of the evidence of increased organ weights and the statement of a higher proportion of the animals in the high dose males, it is deemed that the portion if mature animals reflects a normal variability and distribution of the ratio mature/immature animal under the conditions of a 28-day study.

Group 1 males at an age of 2.99-3.38 years were all immature. Recovery and main test animals are evaluated together due the fact that only 2 study weeks make the difference. Although immature, the testes showed different appearance in that the most immature animal showed Sertoli's cells only associated with the appearance of single scattered spermatogonia (no. 1) whereas other animals made progression ranging from testes with single round spermatids (nos. 2, 3, 6) associated with an increased mitotic index (nos. 4, 5). In most animals, the epididymal tubules were empty (nos. 1-3, 4, 5) or some detritus (assumed to represent remnants of pre-sperm cells) (nos. 2, 6). In the prostate gland, the acini were still fully collapsed (nos. 1, 6) or started dilation (nos. 2-5). Seminal vesicles were small and did not contain colloid (Plates 1-3)....

…..Historical control data also confirm an uneven distribution of mature and immature animals within short term studies (table 3)….

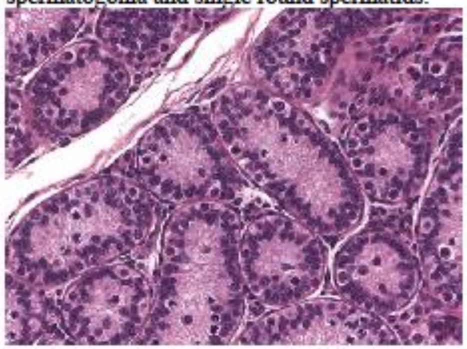
Furthermore, mature cynomolgus monkeys in toxicity studies were described at ages from 5–8 years old and body weights ranging from 4.0 to 7.0 kg, whereas immature/prepubertal animals were those of an approximate age of 3 years at body weights ranging from 2.5–3.5 kg (Dreef HC, vanEsch E, DeRijk EPCT (2007): Spermatogenesis in the Cynomolgus Monkey (Macaca fascicularis): A Practical Guide for Routine Morphological Staging. Toxicol Pathol, 35:395-404).

In addition, the ovaries from all females of this study were re-evaluated. One control animal and two high dose animals only showed corpora lutea, and hence were known to be under cycle before. In most other animals, there were only secondary follicles, and tertiary or Graafian follicles as an indicator for entering a first cycle were rarely observed.

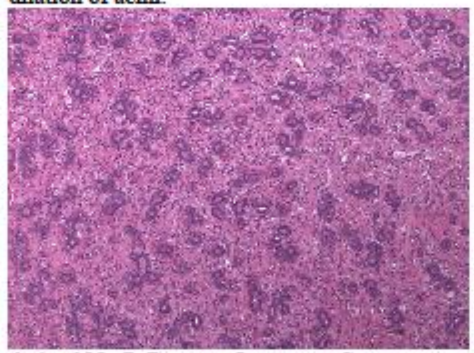
Furthermore, the epiphyseal growth plate from all males (except no. 18: no visible on section) were re-evaluated (Plates 18-21). No differences were noted between controls and test item-treated animals.

Pitfall: Maturity - Males

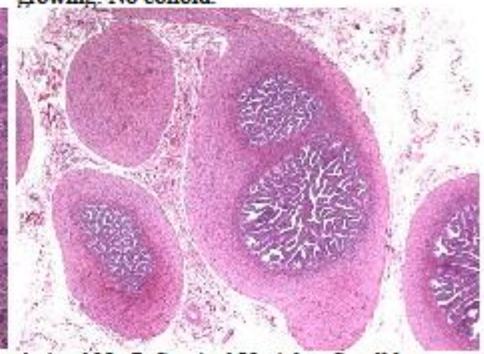
Animal No.3: Testes. Immature. Some spermatogonia and single round spermatids.



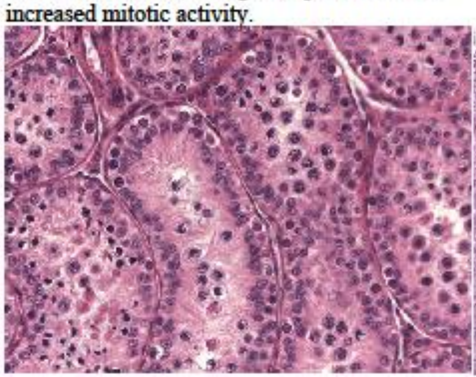
Animal No.3: Prostate. Immature. Starts dilation of acini.



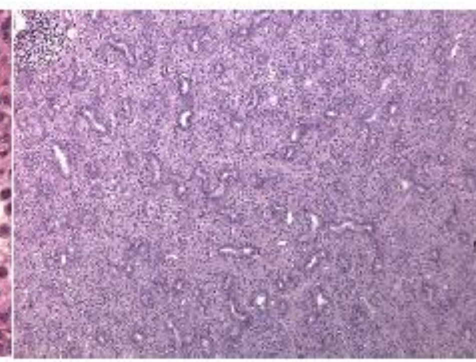
Animal No.3: Seminal Vesicles. Small but growing. No colloid.



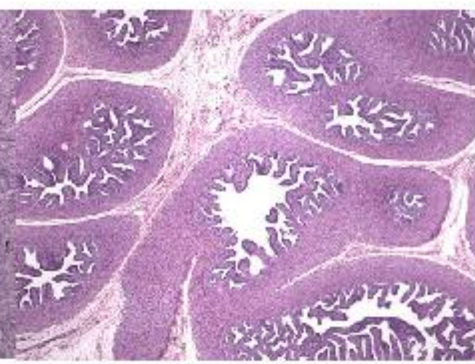
Animal No.7: Testes. Immature. Some spermatogonia, single round spermatids, transformation into elongated spermatids and increased mitotic activity.



Animal No.7: Prostate. Immature. Starts dilation of acini.



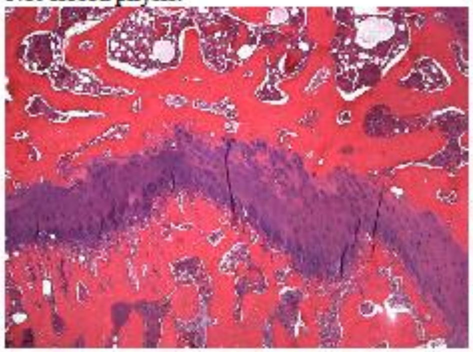
Animal No.7: Seminal Vesicles. Small but growing. No colloid.



Pitfall: Maturity - Females

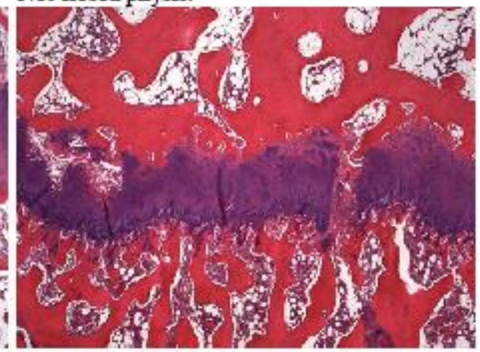
Plate 18

Animal No.1: Femur, epiphyseal growth plate. Not closed physis.

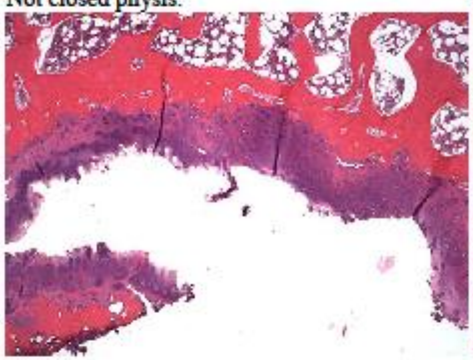


Animal No.2: Femur, epiphyseal growth plate. Not closed physis.

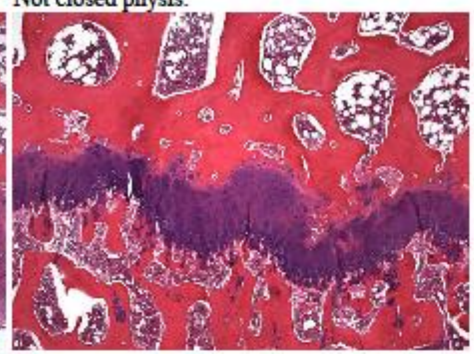
Animal No.3: Femur, epiphyseal growth plate. Not closed physis.



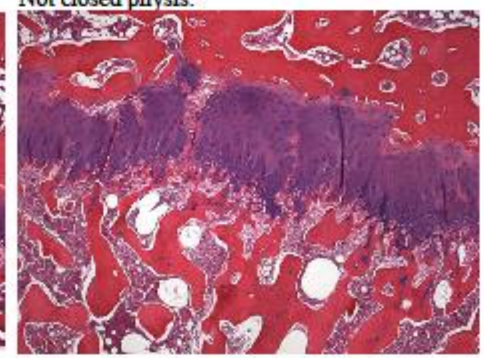
Animal No.4: Femur, epiphyseal growth plate. Not closed physis.



Animal No.5: Femur, epiphyseal growth plate. Not closed physis.

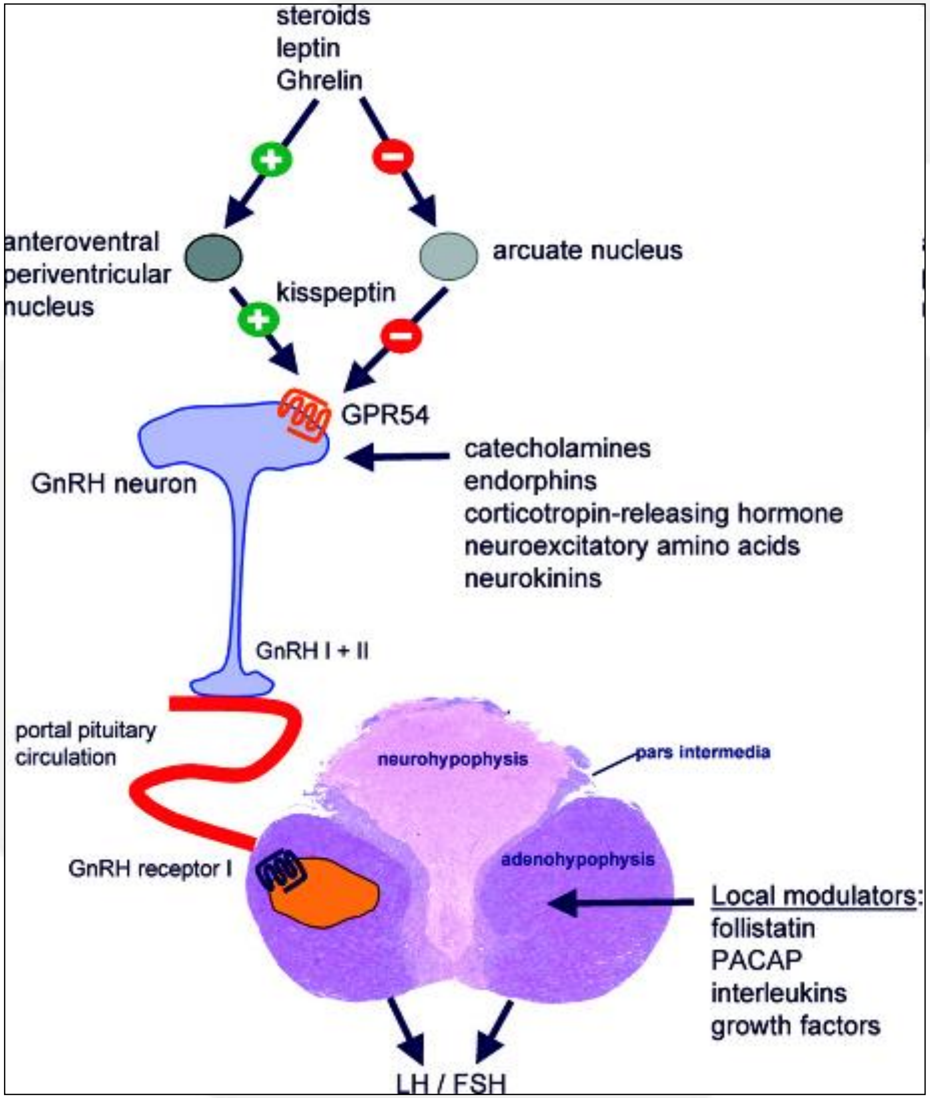


Animal No.6: Femur, epiphyseal growth plate. Not closed physis.



Physiology

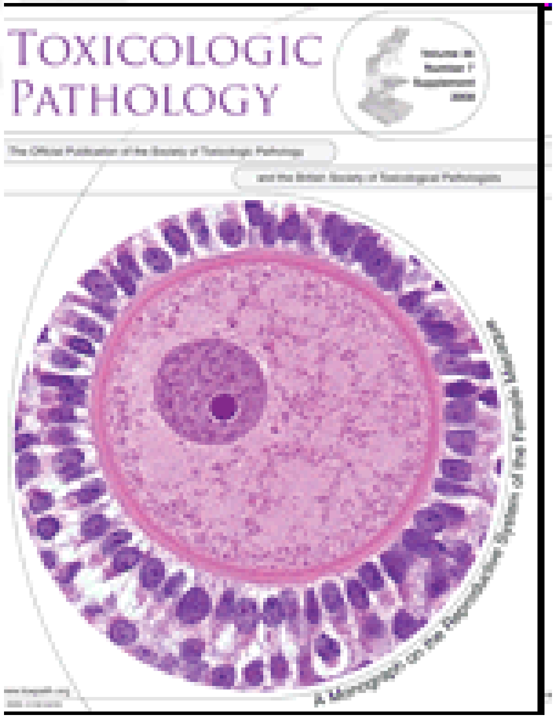
Endocrine Regulation - General



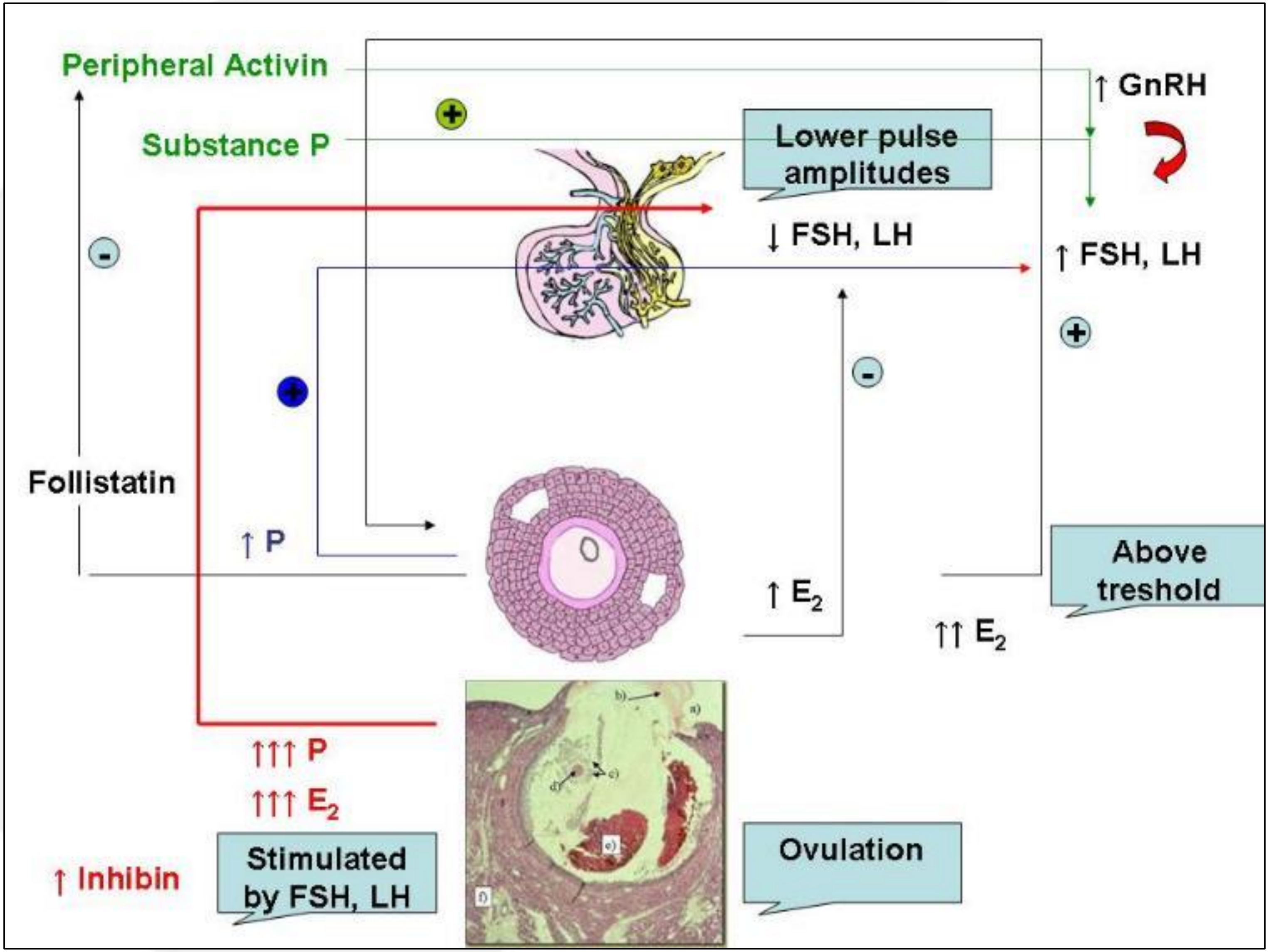
- preproGnRH along axons portal blood
- GnRH is released pulsatile
- increased or decreased frequency of GnRH pulses reduces or abolishes gonadotropin secretion (disturbed cycle)
- Kisspeptin-expressing HT neurons
- is sensit3
- ive to steroid levels mediating negative feedback regulation of gona dotropins
- no sex steroids affects GnRH secretion by direct action on GnRH neurons
- Kisspeptin neurons in n. arcuate are direct targets of sex steroids in all species

Female Monkeys

Buse et al., A Monograph on Female Reproductive Pathophysiology in Macaques, Toxicol Pathol. 36, suppl. (2008)



Endocrine Regulation - Female



Cycling Monkeys

- Cynomolgus: Monitoring by daily vaginal smears for menstruation
- May be combined with frequent sampling of steroid and peptide hormone analysis (not in general design in toxicity studies)
- Marmosets: No external sign of cycle (monitoring by regular progesterone)
- Cynomolgus and marmosets do not exhibit seasonal variations in ovarian activity, but in rhesus monkey there is a pronounced annual rhytm
- Cynomolgus: No cycle synchronization

Main Cycle Differences: Primates vs. Rodents

- **Life span of corpus luteum in primates approx. 2 weeks**
- **If pregnant: c. luteum with extended duration of function and delayed luteal regression (time for implantation and luteal-placement shift)**
- **Unlinke in rodents, prolactin does play a major role during the luteal phase**
- **Luteolysis in primates does not involve a uterine signal**

Main Differences: Cynomolgus vs. Human

Summarized by VanEsch et al. *Toxicol Pathol. 36, suppl. (2008)*

• Sexual maturity:	2.5-4 y	vs.	10-18 y
• Menopause:	20-25 y	vs.	50 y
• Cycle:	28-32 d	vs.	28-30 d
• Implantation:	9-15 d	vs.	6-13 d
• Gestation:	134-184 d	vs.	259-294 d

Ovarian Cycle

- Day 1 of menstrual bleeding is designated as the day 1
- Entire duration is 28 to 32 days in cynomolgus monkey
- Follicular phase: 12 to 14 days
- Periovulatory interval is approximately 3 days
- Luteal phase: 14 to 16 days
- Determination by daily vaginal smears possible

Ovarian Cycle: Endocrine Profile 1

- **Follicular phase:**
  - predominant E_2 increases, LH/FSH and P low
  - rise of E_2 is by end phase along with increase FSH

- **Periovulatory phase:**
  - rise of E_2 (D12), followed by LH (D12.5), FSH (D13), and later P (permitting oocyte maturation, preventing atreasia)

Ovarian Cycle: Endocrine Profile 2

- **Luteal phase:**
  - gonadotropins at levels comparable to follicular phase
  - P clearly elevated during the midluteal phase (peak D22)

- **Ovulation: E_2 decreases**
  - contraction and release of oocyte by local prostaglandins, plasminogen activator, leucotrienes, angio tensins, catecholamines, vasoactive growth factors

Ovarian Cycle Monitoring

Bleeding pattern:

- No menstrual bleeding
- Slight menstrual bleeding
- Heavy menstrual bleeding
- Very heavy (i.e., visible) menstrual bleeding

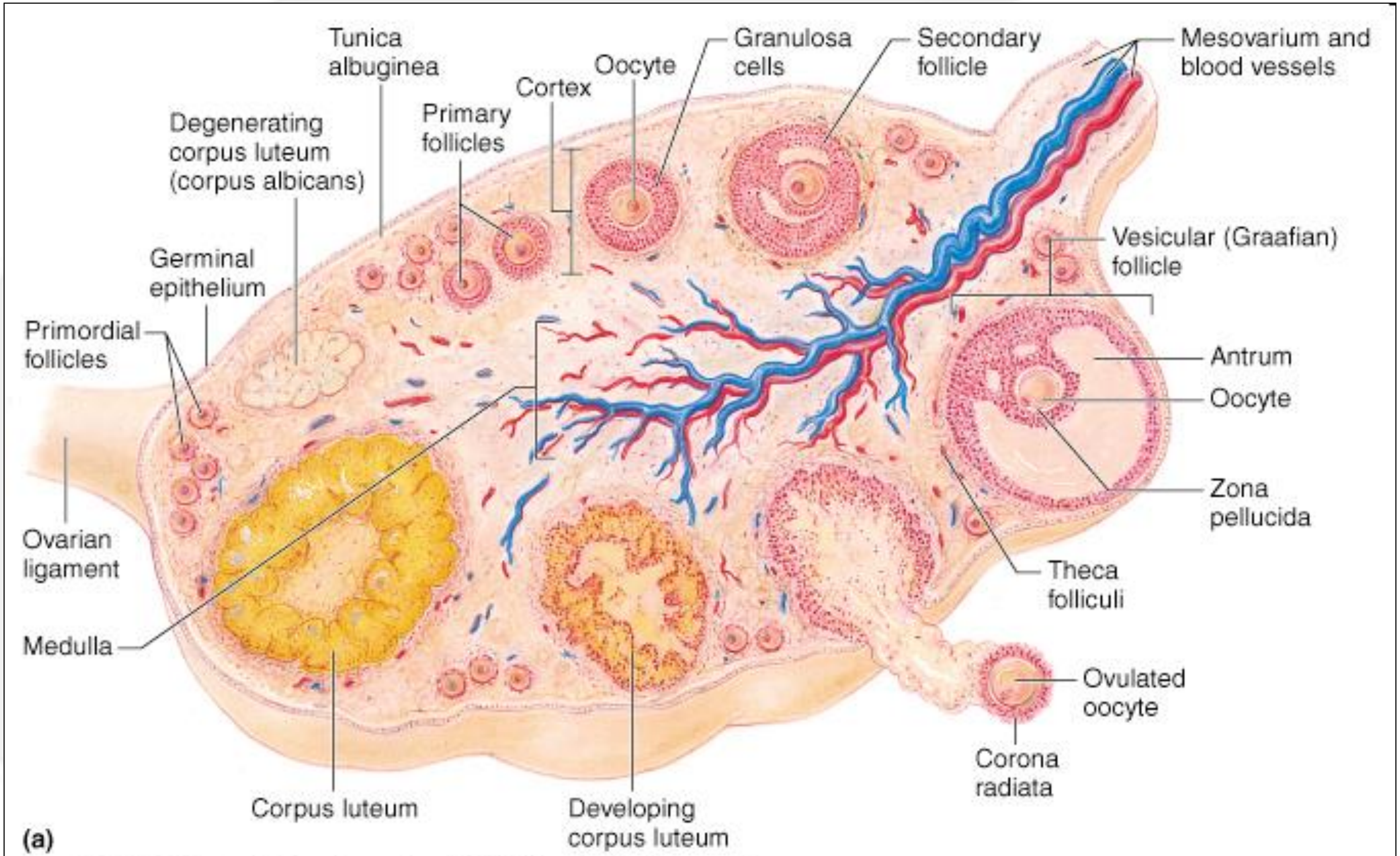
Hormone profile by blood sampling (about 2 mL):

- Days 1, 4, 7, 10, 11, 12, 13, 14, 15, 16, 18, 20, 22, 24, and 27 of menstrual cycle

Menarche and Menopause

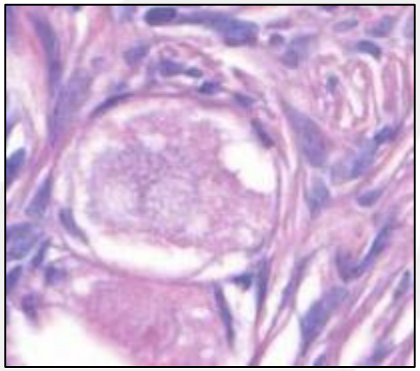
- Approx. 20 w following birth, LH/FSH levels peaks in circulation followed by a decline toward pre-pubertal levels in Cynomolgus
- Onset of puberty triggered by initiation of pulsatile GnRH release
- Juvenile/prepubertal ovary: follicle development to pre-antral and early antral follicles but no preovulatory stage
- Menarche at 2-3 y
- Menopause: increased gonadotropins, reduced E2, only slightly reduced P, increased GnRH secretion and primary follicles decreases

Normal Ovarian Structures

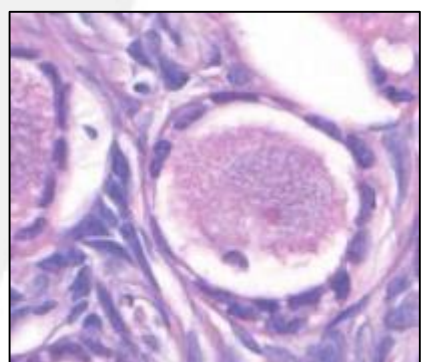


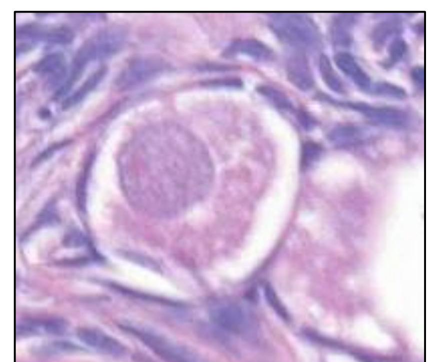
Primoridal and Primary Follicle

Category 1: Oocytes surrounded by flattened granulosa epithelial cells (classical primordial follicle):



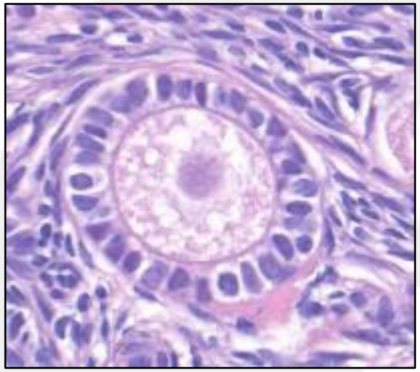
Category 2: Oocytes surrounded by both flattened and cuboidal cells (intermediate, primordial, activated primary, or early primary follicle):



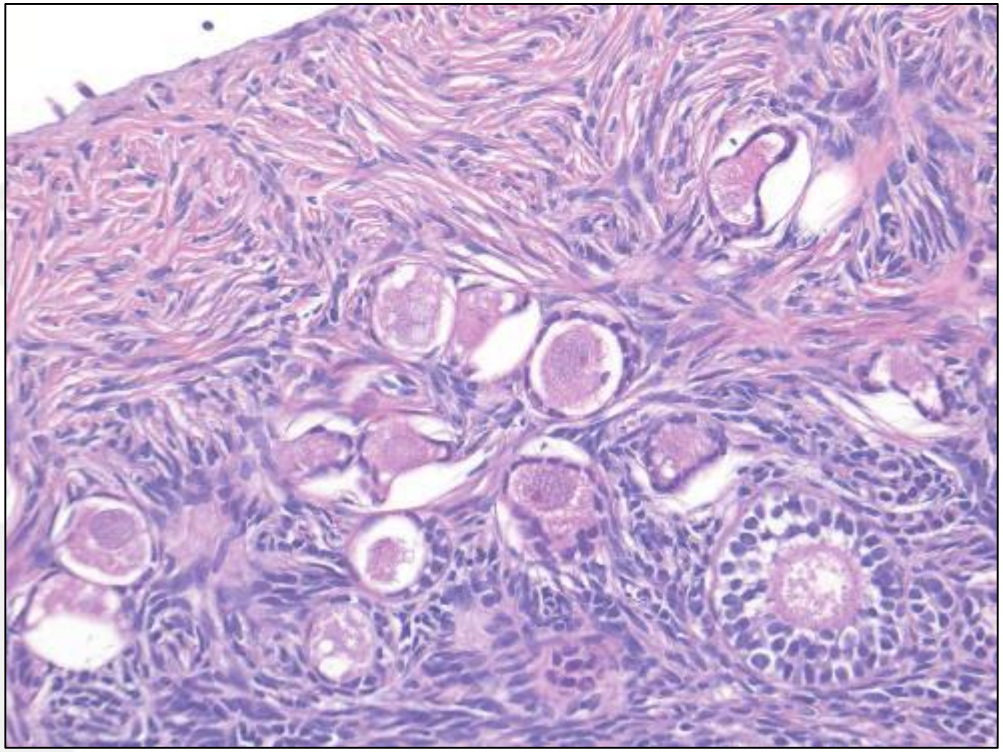


Primorodial and Primary Follicle

Category 3: Oocytes with a single layer of surrounding cuboidal cells (classical primary follicle):

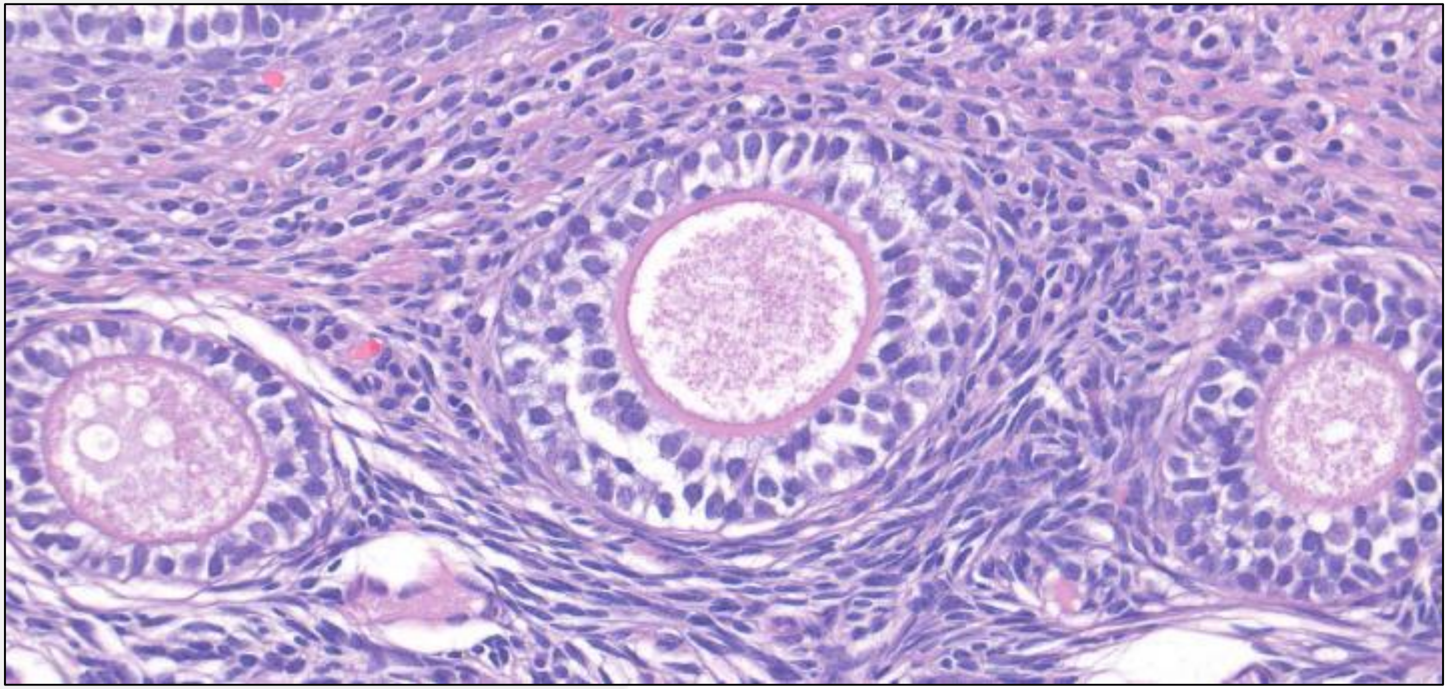


Primordial follicle represents dormant oocyte stage (dictyotene prophase) persisting over years



Secondary (Pre-antral) Follicle

- More than one layer of granulosa epithelial cells
- Distinct glycoprotein layer (pellucid zone or oolemma)separating it from the oocyte



Secondary (Pre-antral) Follicle: Receptors

- **Progesterone receptors expressed by almost all granulosa cells, internal and external theca cells (equally present in young and mature females)**

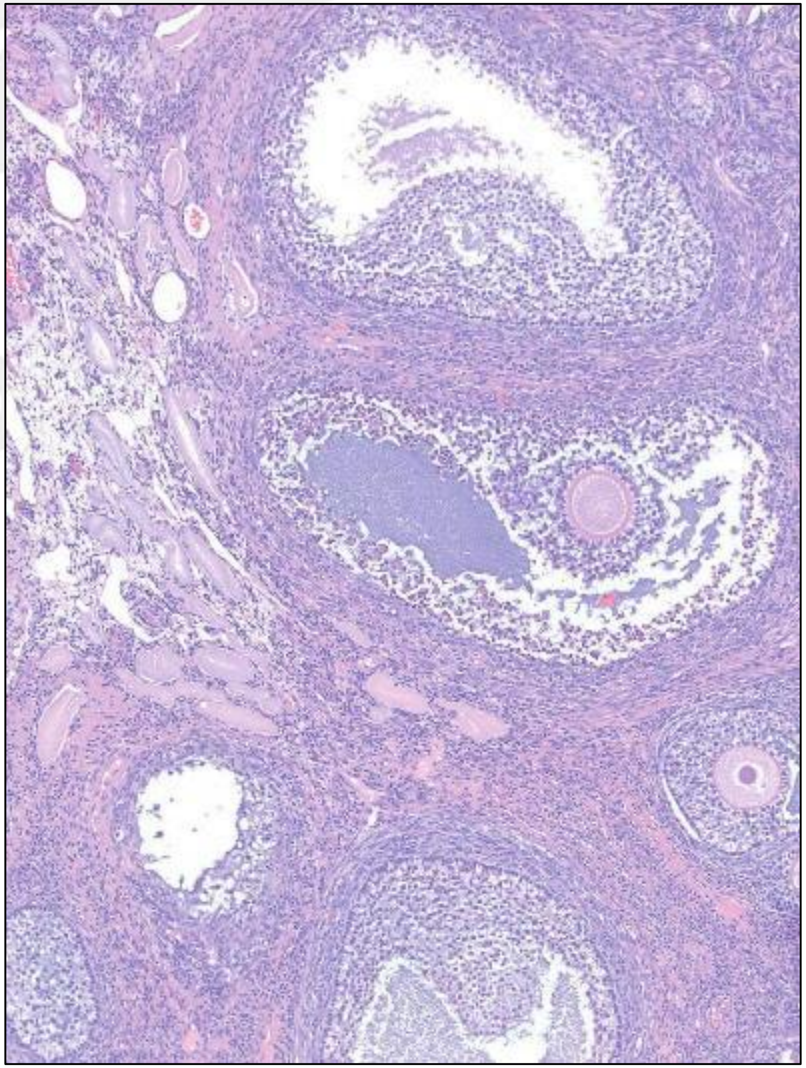
- **Estrogen receptors expressed by a few granulosa cells slowly increasing in number with the size folliclesand in advanced secondary follicles in 10% ofgranulosa cells**

- **Estrogen receptors expressed by some internal theca cells inpreantral follicles**

- **Androgen receptors not expressed by secondary follicles**

Tertiary Follicle

- With antrum lined by granulosa cells
- Follicular liquor (mostly by granulosa cells)
- Follicle growth attributes to granulosa cell proliferation
- Expansion of antral cavity
- Oocyte reaches a diameter of about 120 µm



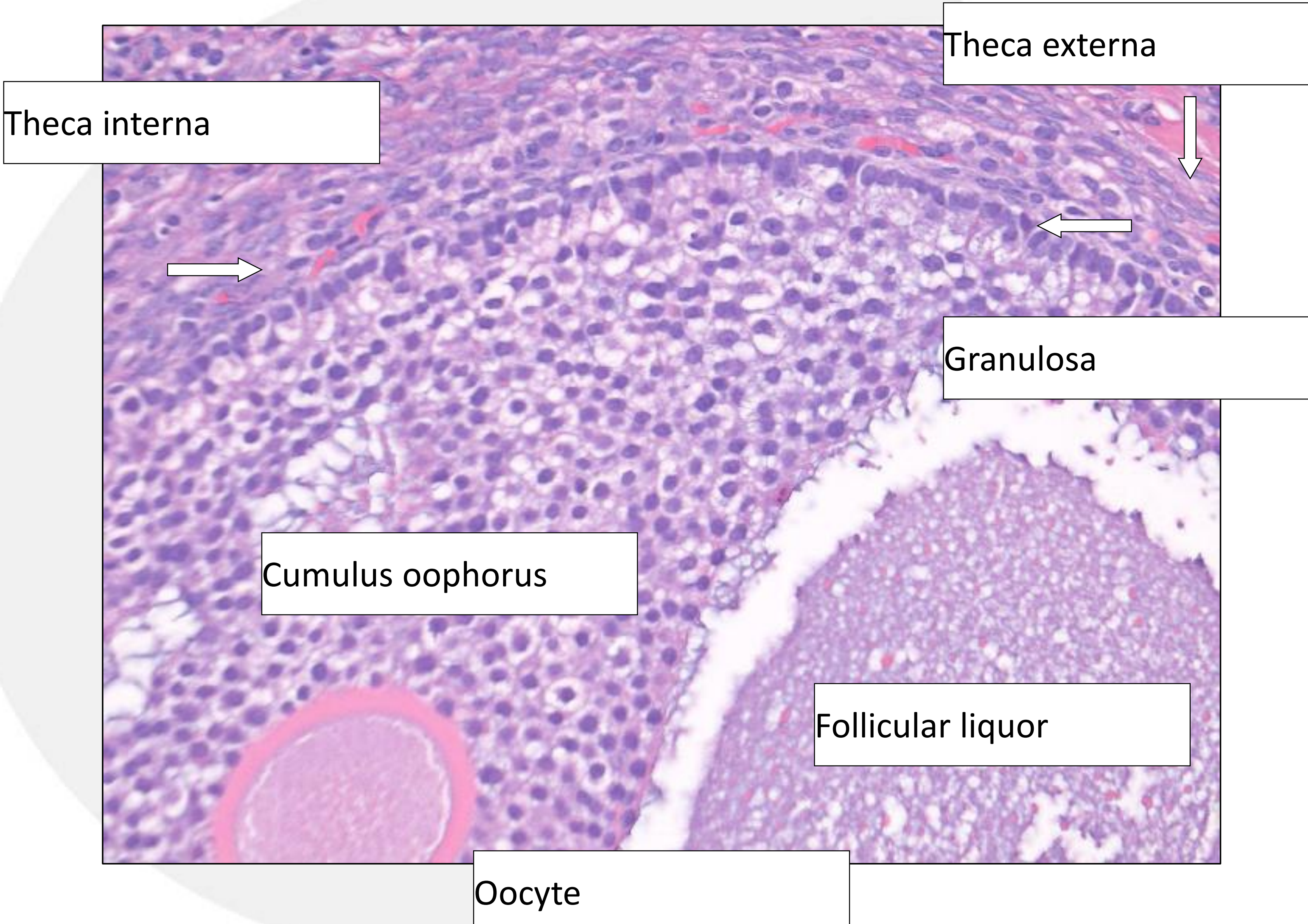
Tertiary Follicle: Receptors

- P receptors expressed by 50% of the granulosa cells (persist throughout luteal phase.
- Relatively high expressed in theca cells

- E_2 receptor expression increases with follicle growth, (almost 100% of granulosa cells in advanced tertiarys)
- Quick decline when transformationof granulosa cells into luteal epithelial cells

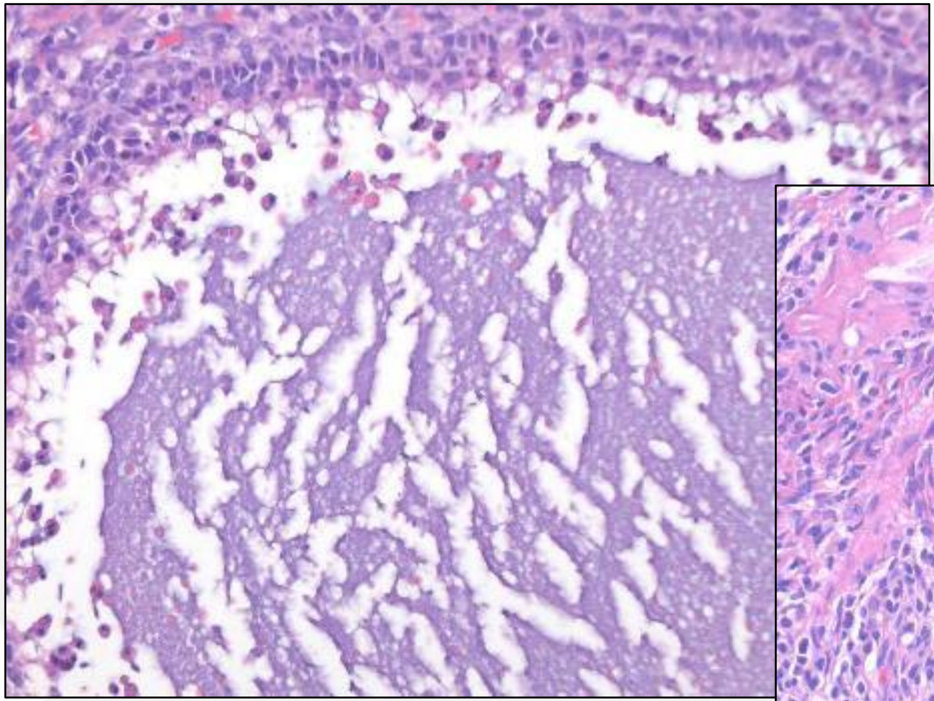
- Androgen receptor expression by over 80% of granulosa cells and by almost 100% of external theca cells.
- Absent in internal theca

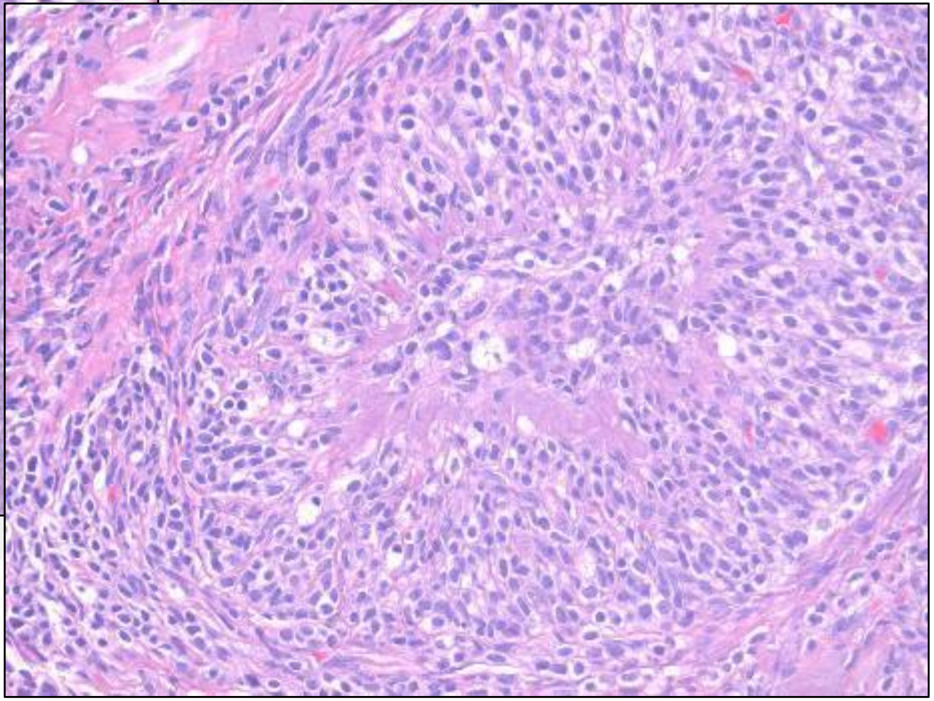
Tertiary Follicle



Tertiary Follicle: Atresia

- Majority of tertiary follicles undergo degeneration (atresia)
- Atresia may be seen also in secondary follicles





Twins

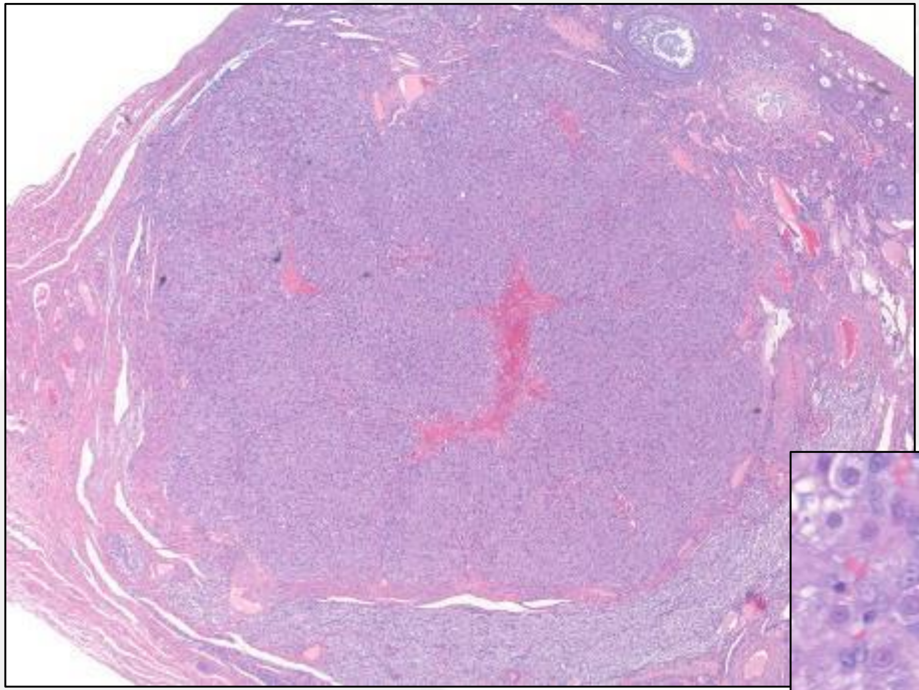
- **Very rare (Cynomolgus gives birth normally to one offspring)**

Corpus Luteum

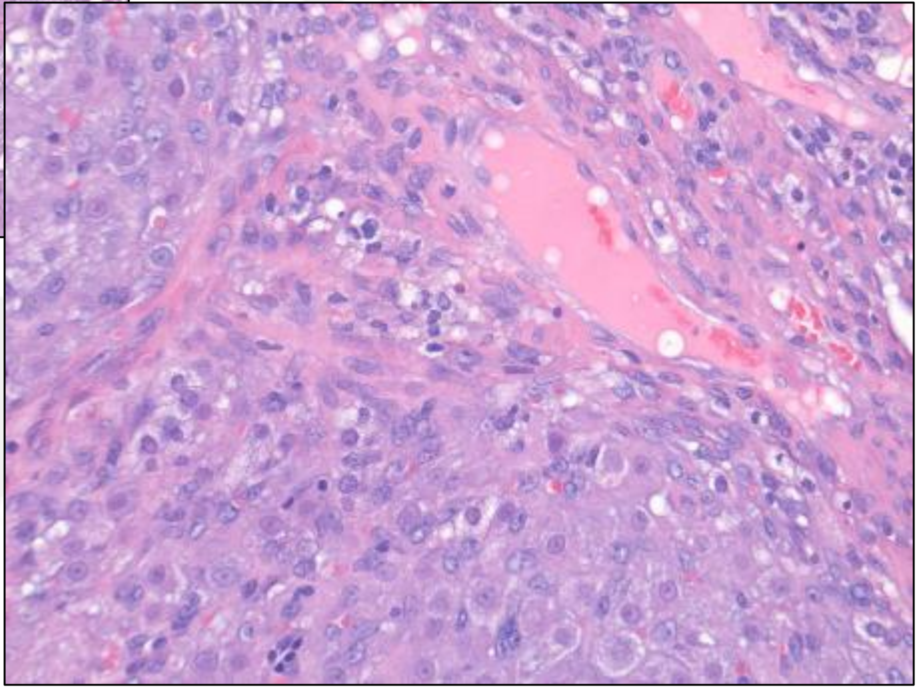
- Prior to ovulation, follicular metabolism switches
- From estrogen to progesterone synthesis
- Granulosa epithelial cells transform into large granulosa luteal cells
- Luteal cell growth by steroid genesis
- Formation of C.lutea

- If no fertilization, C. lutea involute (atrophy) and disappears within weeks

Corpus Luteum

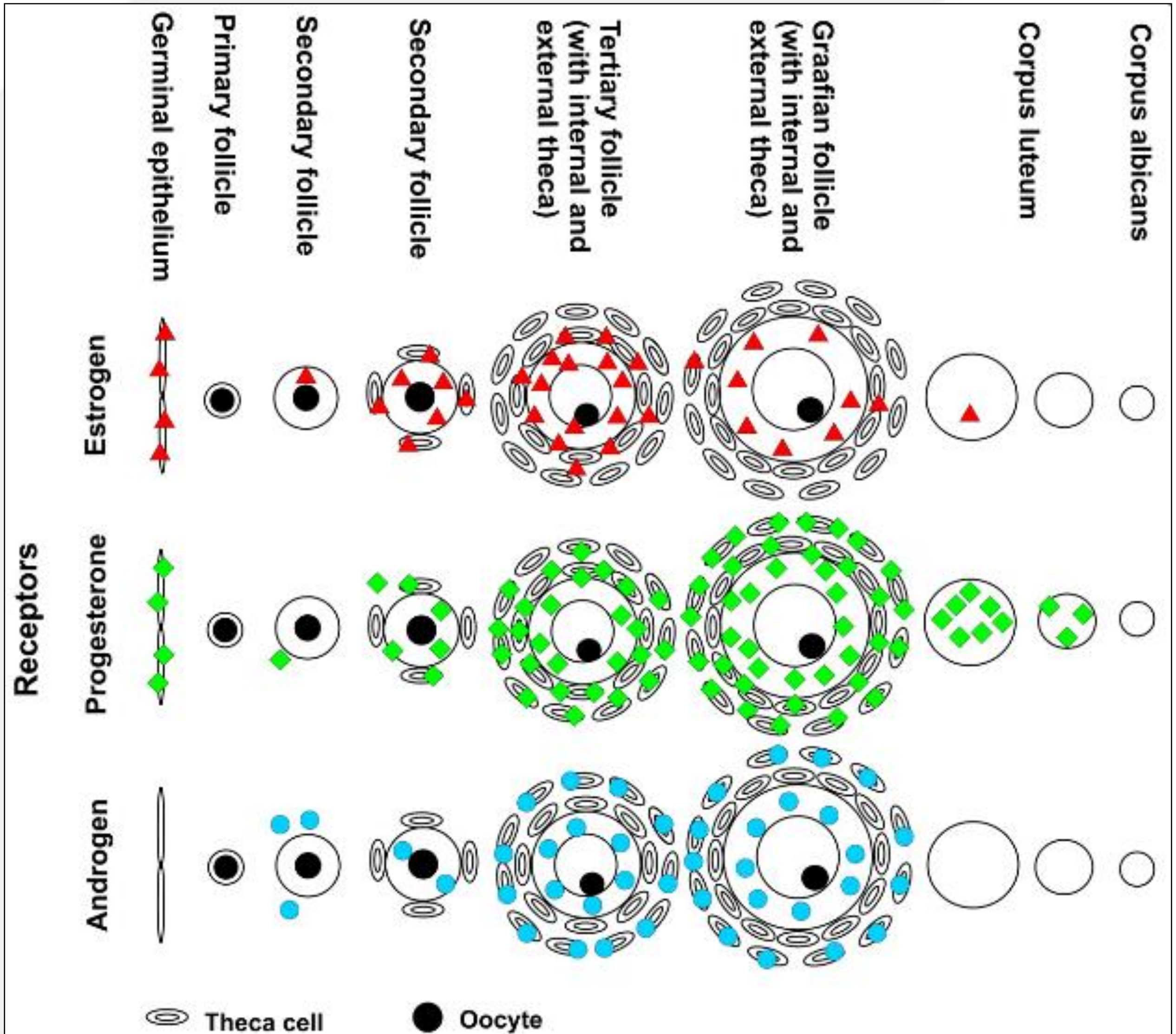


C.Lutea may be very large



Fine vacuolated luteal cells

Receptor Distribution During Follicle Development (Cynomolgus)



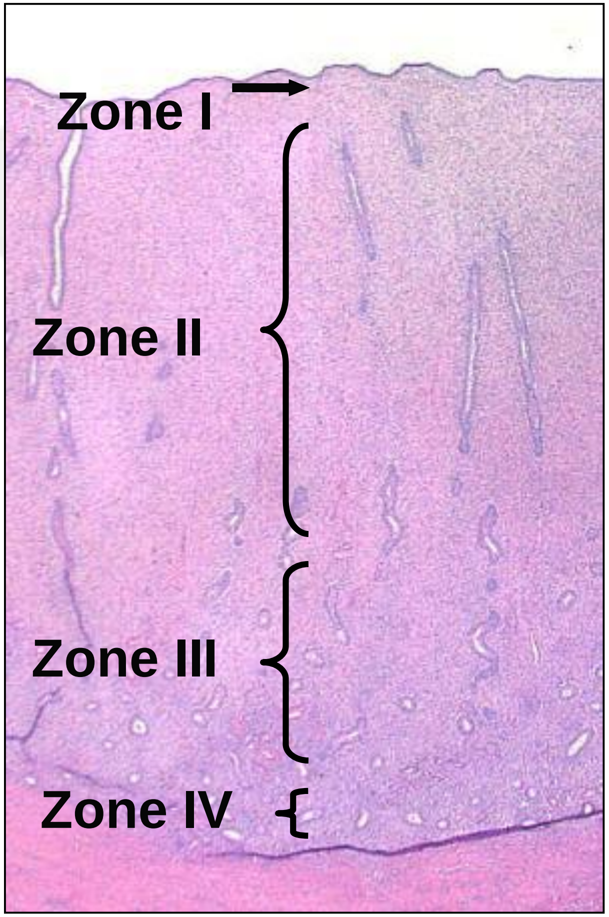
Buse et al, Toxicol Pathol. 36, suppl. (2008)

Endometrium

- Endometrial surface and glands lined by single layer secretory type epithelium
- Surface epithelium: less cyclic variation than glandular epithelium
- Glandular epithelium consists of a different cell types, varying over the length of glands and during cycle phases

Endometrium – Zonation

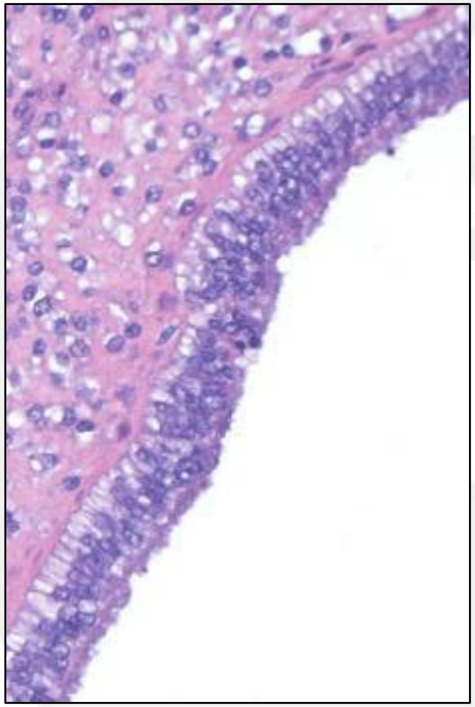
- 3 functional zones: compacta, spongiosa, basalis
- Zona functionalis = compacta and upper spongiosa (shed during menstruation)
  - More affected by fluctuations in circulating ovarian hormones than zona basalis
- Zona basalis: renewal after menstruation (escape from shedding)



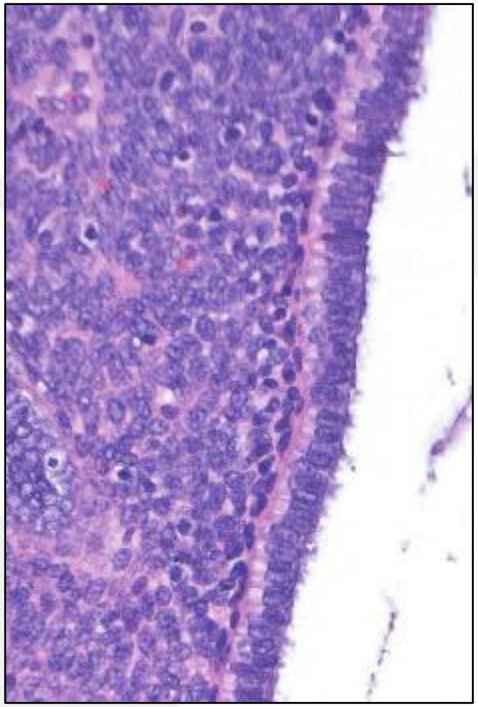
Zona I : superficial epithelium
Zone II : thick, with glands
Zone III: bodies of glands
Zone IV: basal, blind end of glands

Endometrium – Morphology varies under E/P

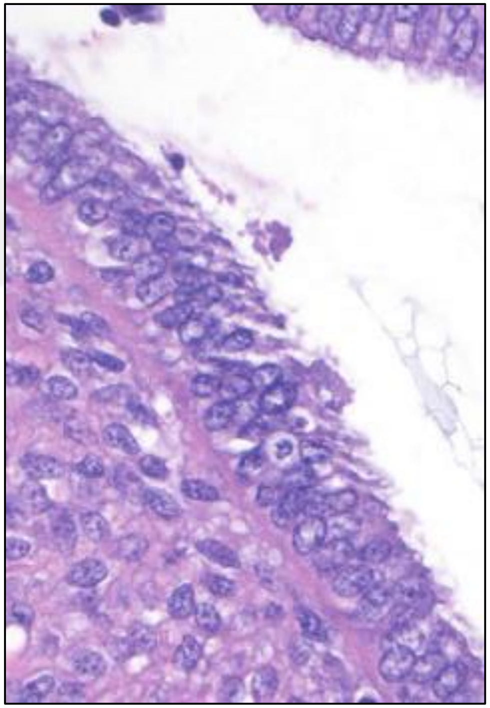
- **Secretory cells**



- **Ciliated cells**
- **increase under E**



- **Clear cells**
- **increase under E**



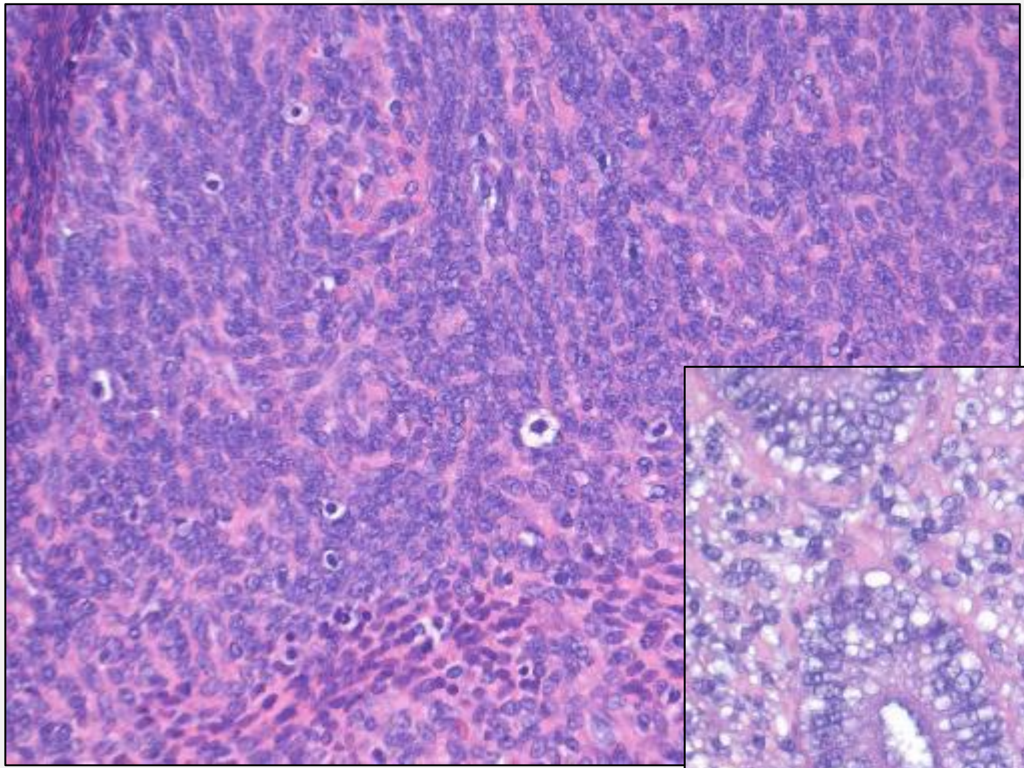
Endometrium – Stroma

- Endometrial stroma surrounds and supports glands
- Mainly formed by endometrial stromal cells and blood vessels
- So-called "endometrial lymphocytes" are a unique type of LGL
- Endometrial lymphocytes mainly during luteal phase
- Round, often with clear cytoplasm with a centrally located round, kidney-shaped, or more segmented nucleus and eosinophilic cytoplasmic granules
- Once mistaken for infiltrating leukocytes and erroneously named 'endometrial granulocytes'

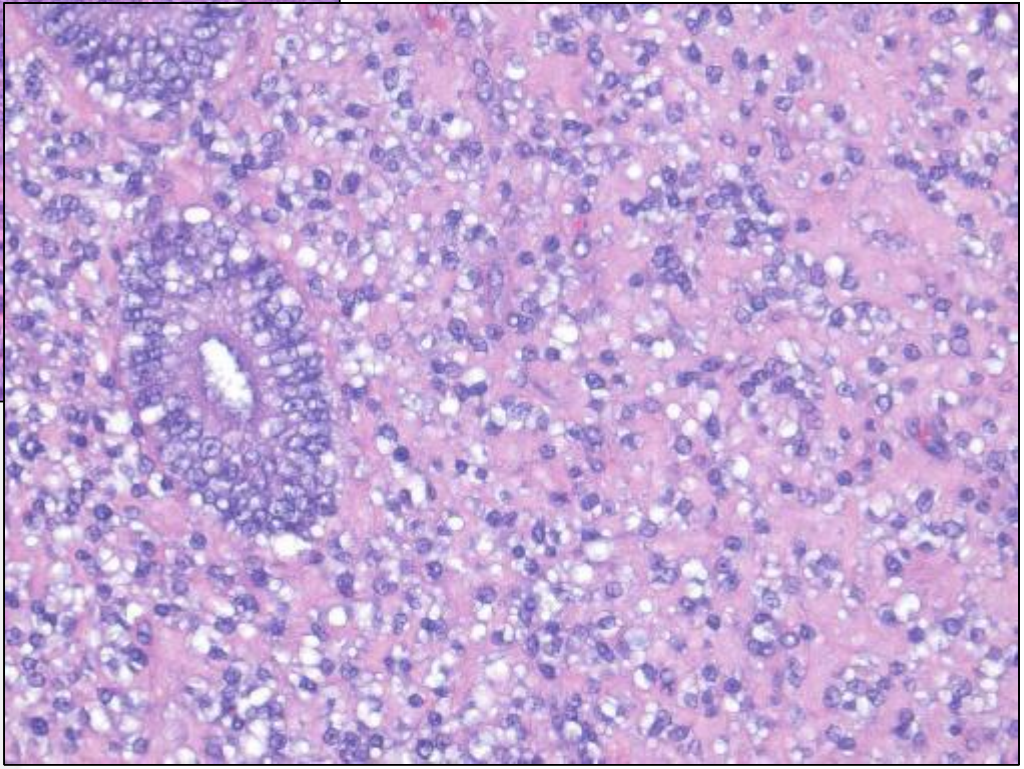
Endometrium – Decidualization

- Typical morphological change under progesterone dominance during luteal phase
- Stromal cell nlarges and becomes ‘decidualized’
- Decidualization: process in which stromal cells trans-form to large, polyhedral, cytoplasm-rich cells storing alarge amount of glycogen
- Decidual changes in the stroma localized around spiral arteries and underneath superficial epithelium
- At higher progesterone levels in the whole stroma, functionalis and even part of the basalis
- Can be so prominent that glands become constricted, causing obstruction/dilation of lower parts of the glands

Endometrium – Stroma



Silent stroma with en-dometrial lymphocyte

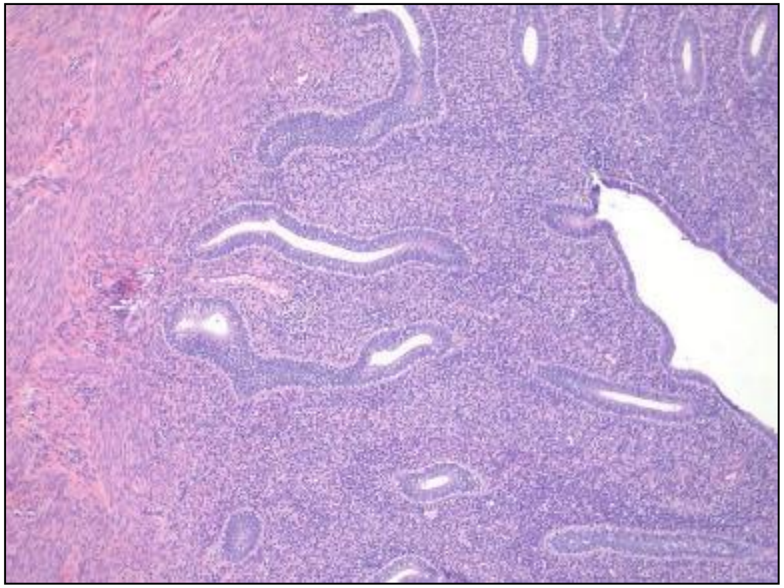


Decidualized stroma

Endometrium – Cycle Morphology – Mid Follicular

Mid-follicular phase (estradiol levels increase) is characterized by:

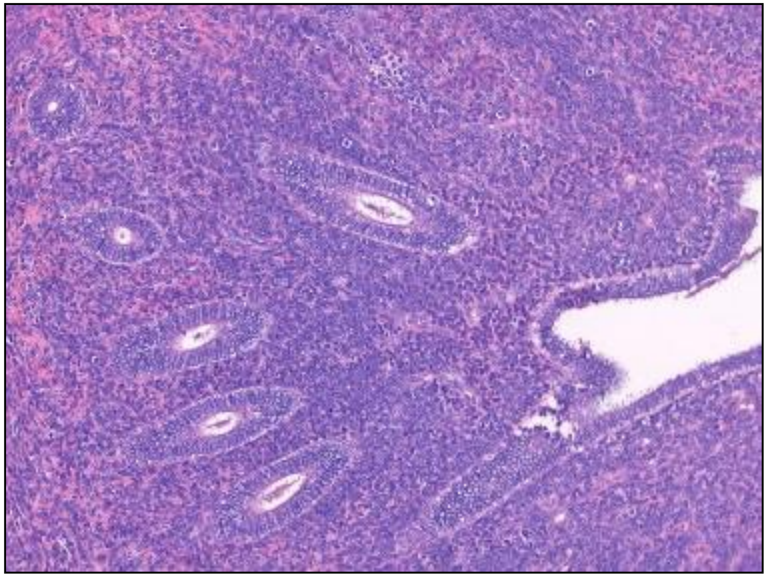
- High endometrium with straight, occasionally tortuous tubular glands within edematous stroma
- Stroma in zona basalis is less compact
- Demarcation between functionalis and basalis less clear
- Increase in height of endometrium by stromal edema
- Basalis is similar to early follicular phase



Endometrium – Cycle Morphology: Early Follicular

Early follicular phase is characterized by:

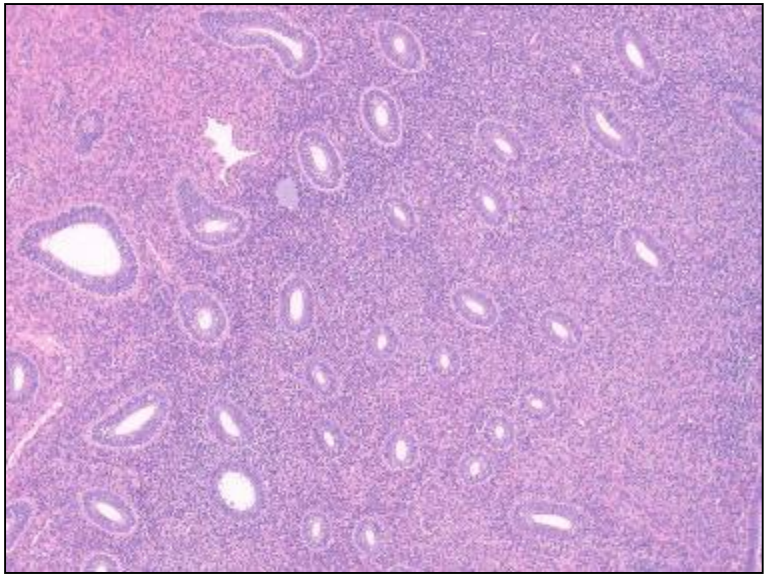
- **low, inactive endometrium**
- **sparse, narrow, and straight tubular glands within loose stroma**
- **zona basalis easily recognized due to more compact stroma**



Endometrium – Cycle Morphology: Late Follicular

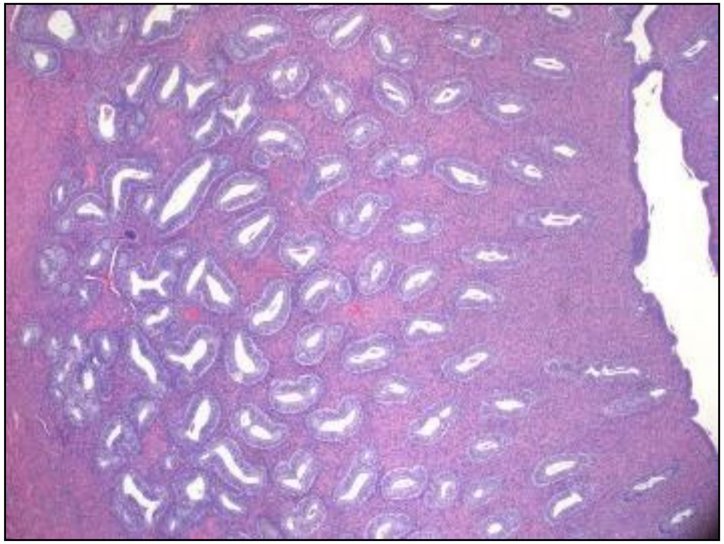
Late follicular phase is characterized by:

- **Glands of functionalis with maximum of nuclear pseudo-stratification**
- **PAS-positive perinuclear cytoplasmic vacuoles indicated secretory activity**
- **Stromal edema diminishes but is persistent until ovulation**



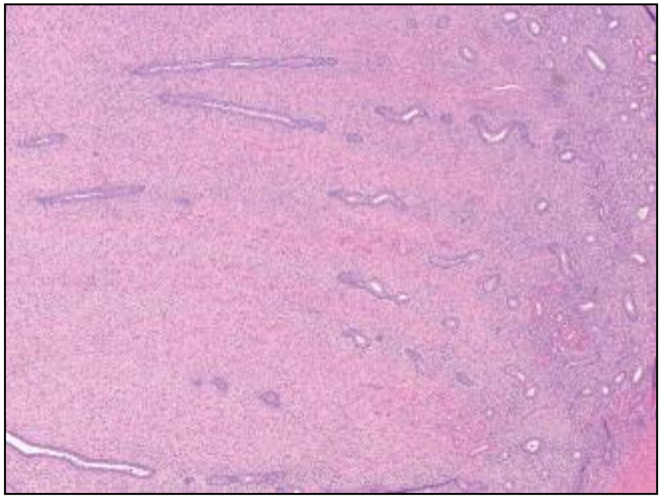
Endometrium – Cycle Morphology: Early Luteal Phase

- Early luteal phase mean serum P:E2 ratio is 42:1
- Morphological basis for implantation
- Portions of glands in functionalis are tortuous
- Glands lined by a columnar epithelium of medium height
- Characteristic presence of subnuclear vacuolation in glands of functionalis glycogen accumulation)
- Surface epithelial cells differ from epithelial cells lining the glands in that they are high columnar
- Loose stroma of functionalis
- Glands with increased sacculation
- Spiral arteries starts growing



Endometrium – Cycle Morphology: Mid Luteal Phase

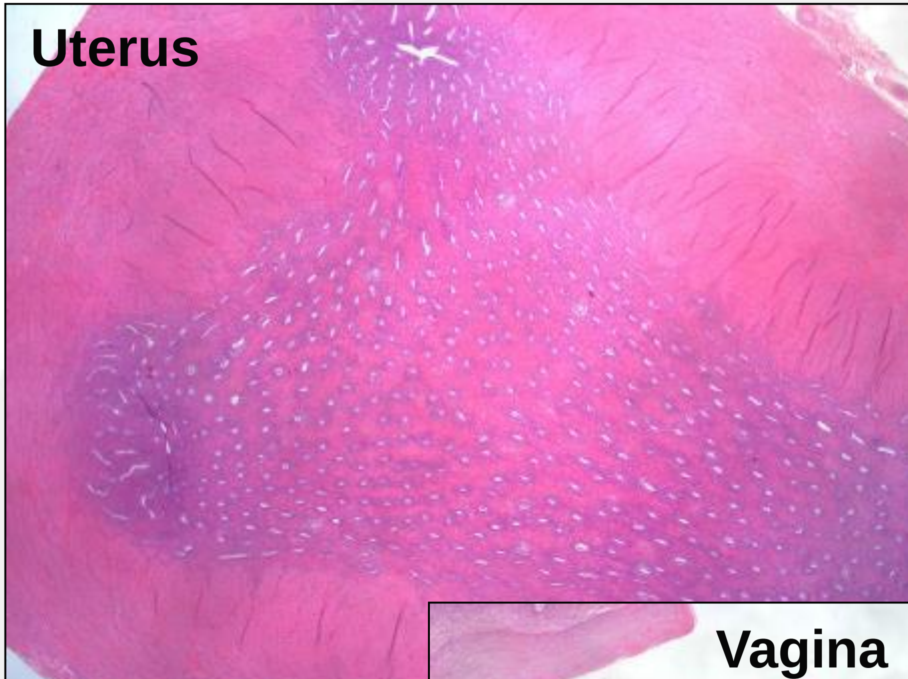
- **Mitotic activity is completely absent in surface and glandular epithelium in functionalis.**
- **Epithelium PAS-positive, homogeneous to granular secretory material within the gland lumina**
- **Hallmark is significant proliferative activity in deepest part of the glands in the zona basalis**
- **Epithelia in this zone are high columnar with nuclear pseudostratification and mitotic figures**
- **Significant amounts of glycogen may be in these cells**
- **Stroma of basalis is dense**
- **Spiral arteries are most prominent at the basalis–functionalis junction**



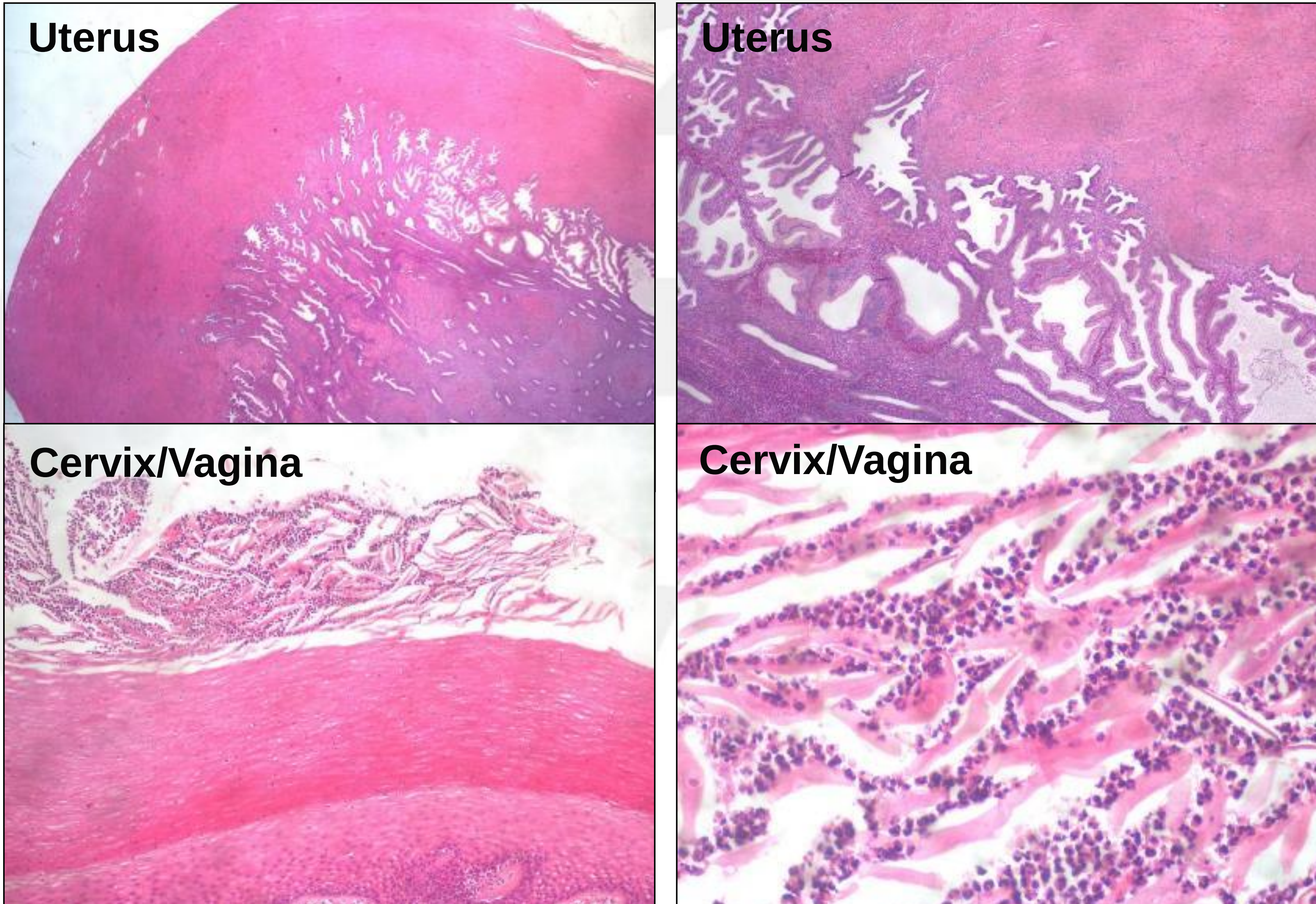
Endometrium – Cycle Morphology: Late Luteal Phase

- **Spiral arteries fully developed**
- **Stromal cells adjacent to spirals pseudodecidualized**
- **Glands in zona functionalis less tortuous with large amounts of homogeneous, PAS-positive material**
- **Epithelial cell lining glands are columnar to cuboidal**
- **Surface epithelium is low columnar with small cytoplasmic protrusions on luminal surface**
- **In area underneath surface epithelium, the stroma can be edematous**
- **Hemorrhages and fibrin leakage**
- **Numerous endometrial lymphocytes**
- **Mitotic activity absent in glands and stroma**
- **Spiral arteries prominent**

Classical Cycle Morphology: Proliferation



Classical Cycle Morphology: Secretion



Endometrium – Cycle Morphology: Regeneration

- **Estradiol levels low, hence regeneration mediated by other factors**
- **First signs in remaining upper parts of glands of the functionalis (starts already during late menstruation phase**
- **Proliferation and migration from remaining portion of glands in zona functionalis and upper basalis**
- **Epithelium starts to proliferate from stumps of remaining glands**
- **Newly formed surface epithelium is flat**

Cervix – Histology

- **Stratified squamous epithelium overlying a stroma composed of dense connective tissue and smoothmuscle**
- **In contrast to vaginal mucosa, the cervical squamous epithelium lacks prominent rete and often has less superficial keratin**
- **Squamous mucosa is divided into three layers:**
  - **germinal basal/parabasal zone**
  - **stratum spinosum of intermediate cells**
  - **superficial zone of mature keratinocytes.**

Cervix – Histology

(A) prepubertal

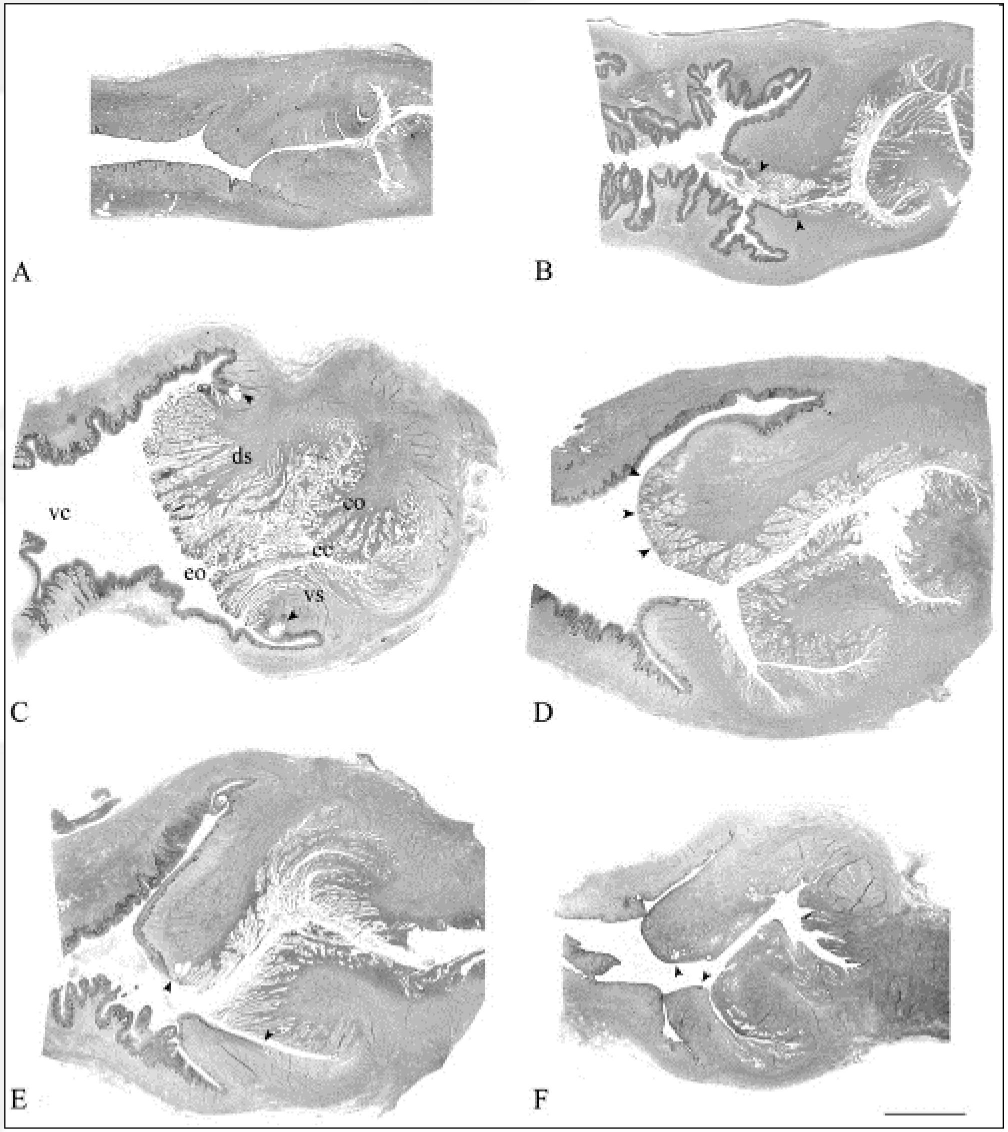
(B) early pubertal

(C) premenopausal

(D) premenopausal

(E) premenopausal

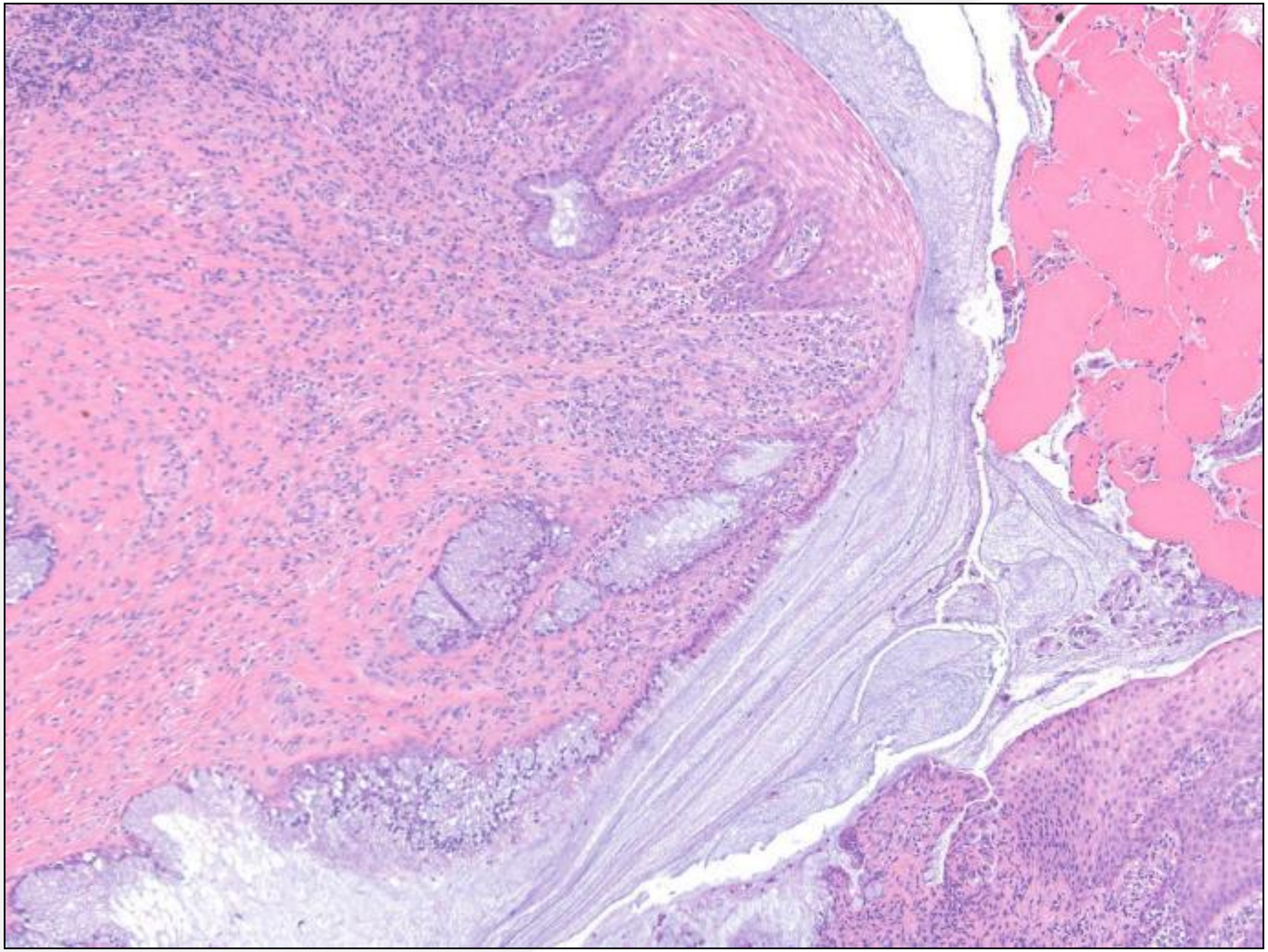
(F) ovariectomized



Cervix – Development

- **Prior to puberty, squamous and glandular epithelia are atrophic, cervical shelves and colliculi are rudimentary, and squamocolumnar junction (SCJ) is in distinct (where squamous epithelium abruptly shifts to tall columnar glandular epithelium)**
- **Estrogen exposure (about 2-3 y of age) induces marked increase in vaginal and cervical squamous maturation and keratinization and moderate increase in endocervical gland hypertrophy**
- **Prior to ovulation, SCJ remains within the endocervical canal**
- **With complete menstrual cycle activity, the glandular cervix becomes enlarged shifting the original SCJ to the exocervix (called ectopy)**

Cervix – Squamocolumbar junction (SCJ)

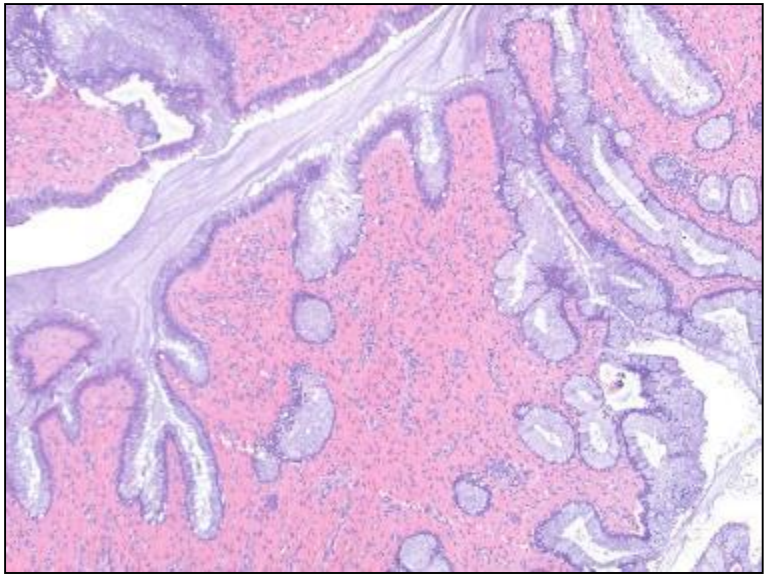


Cervix – T-Zone

- **T-zone forms a second "functional" SCJ**
- **expands with increasing age**
- **Composed of squamous epithelium lacking superficial maturation and the abundant intracytoplasmic glycogen seen in normal maturing keratinocytes of the spinosum**
- **Squamous metaplasia occur in response to factors, including the lower pH of the vagina, hormones, local infection, inflammation, and microtrauma**
- **Squamous metaplasia also common in basal portion of endocervical glands cycling macaques (estrogen is a key factor)**

Cervix – Endocervix

- Mucosa composed of simple columnar epithelial cells lining main canal and colliculi and form infolded glandular structures
- In cycling animals, cells have basal nuclei and clear to pale eosinophilic vacuolated cytoplasm
- Primary roles of secretory and ciliated columnar cells is to secrete and distribute mucus
- Secretory activity peaks in luteal phase (cells may reach about 50 µm in height



Cervix – Endocrine Regulation: Estrogen & Progesteron

- Ovarian hormones induce keratinization in vagina and adjacent exocervix
- Cells are particularly sensitive to estrogen (basal cell proliferation, maturation, desquamation)
- SCJ increases in thickness under estrogen (but not keratinized, characteristic: accumulation of intracytoplasmic glycogen in cells of spinosum)
- Loss of estrogen causes diffuse atrophy of squamousepithelium
- Estrogens and progestogens induce marked hypertrophy and mucus secretion of endocervical glands
- Estradiol induces a more profuse, watery, alkaline mucus
- Progesterone induces more viscous, acidic mucus

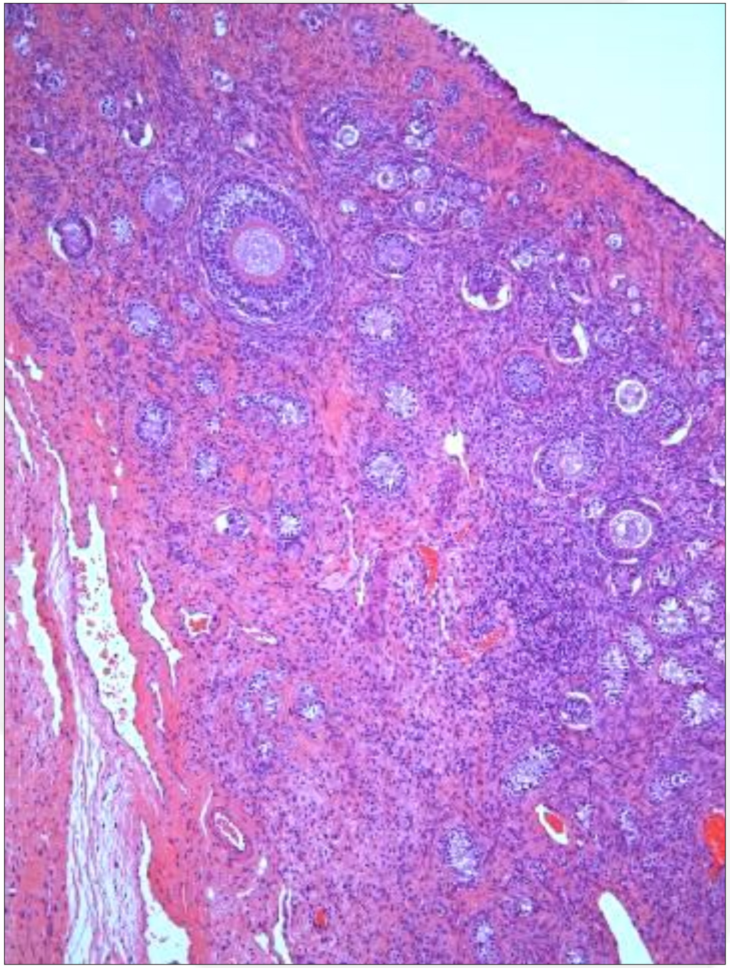
Female Dogs

Estrus cycle

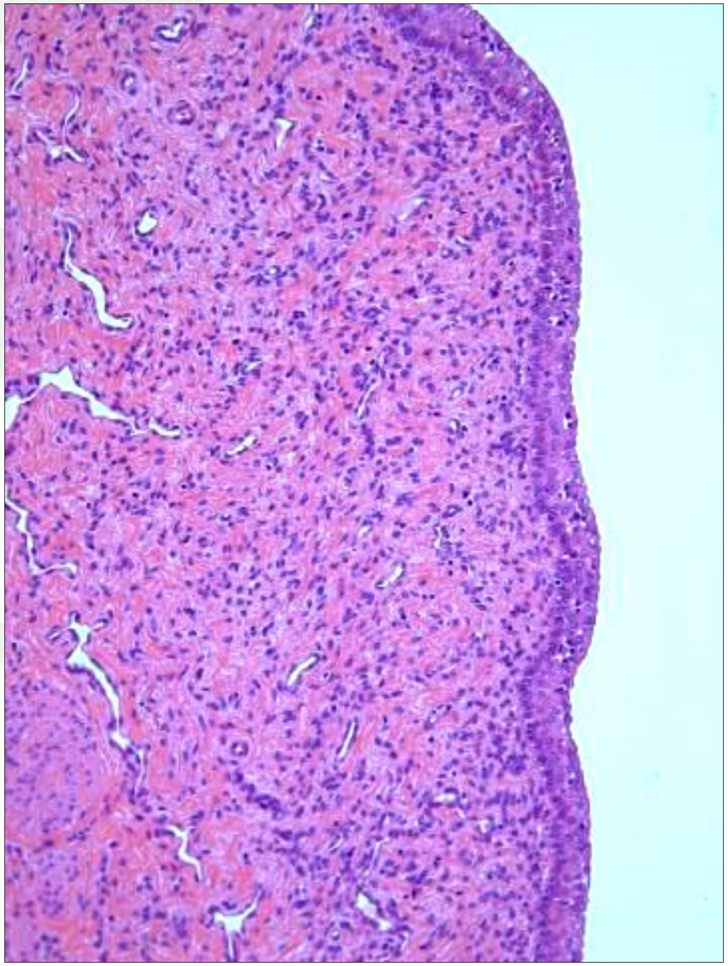
- **Anestrus: 4-5 months**
- **Proestrus: 9-11 days**
- **Estrus: 9-11 days**
- **Metestrus: 2-3 months**

Immaturity: Ovary, Vagina

Ovary:
small follicles, no corpora lutea



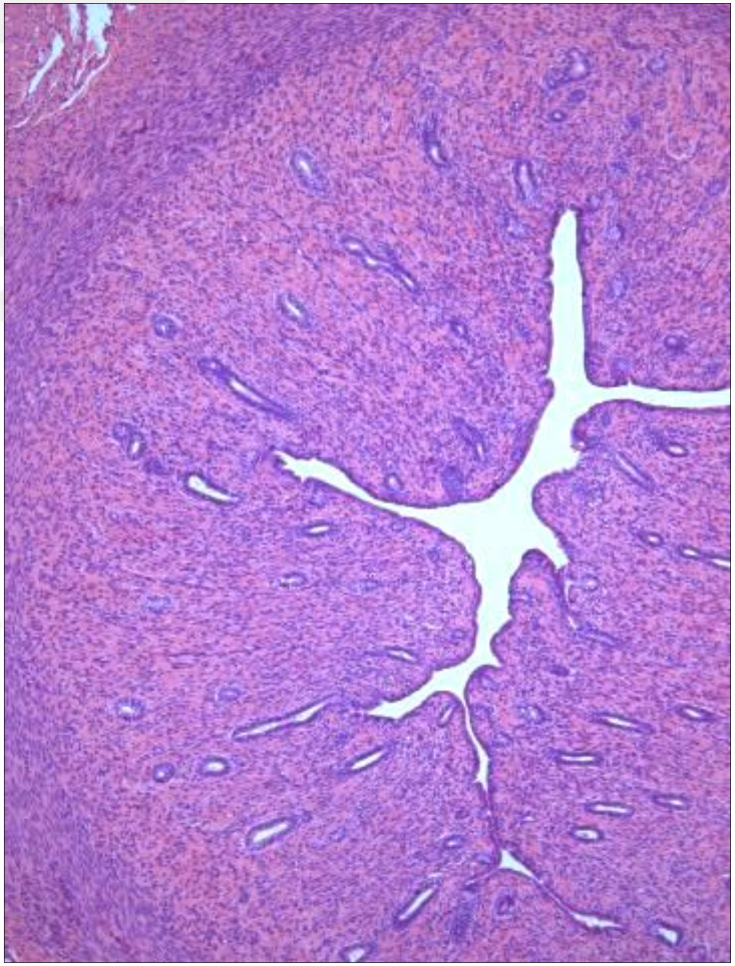
Vagina:
Thin epithelium



Immaturity: Uterus

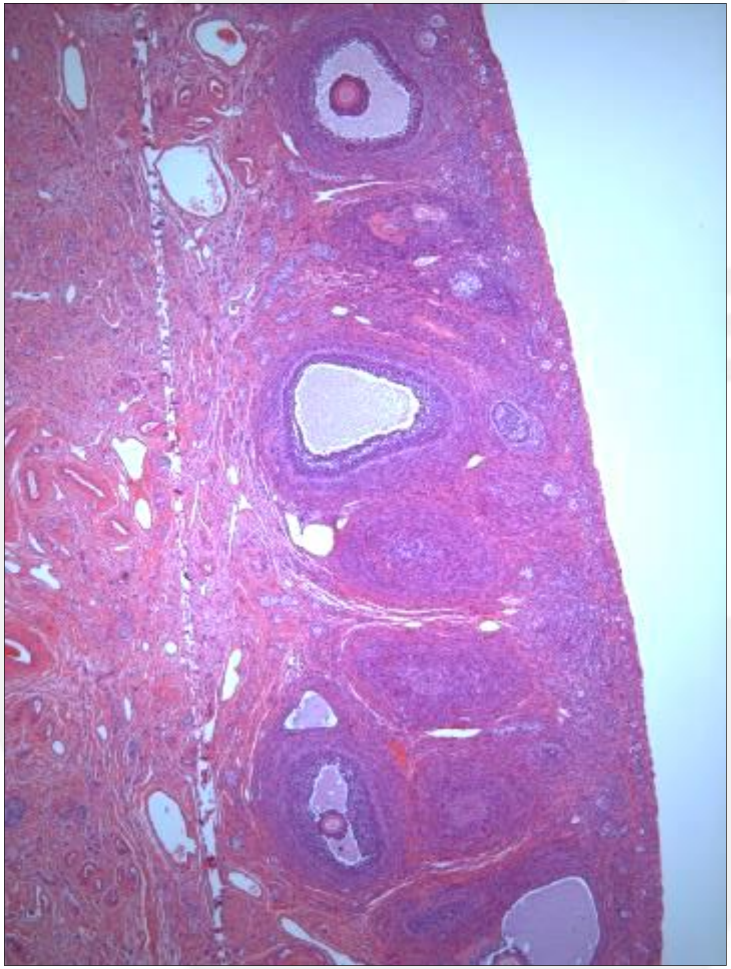
Small diameter

Few glands only

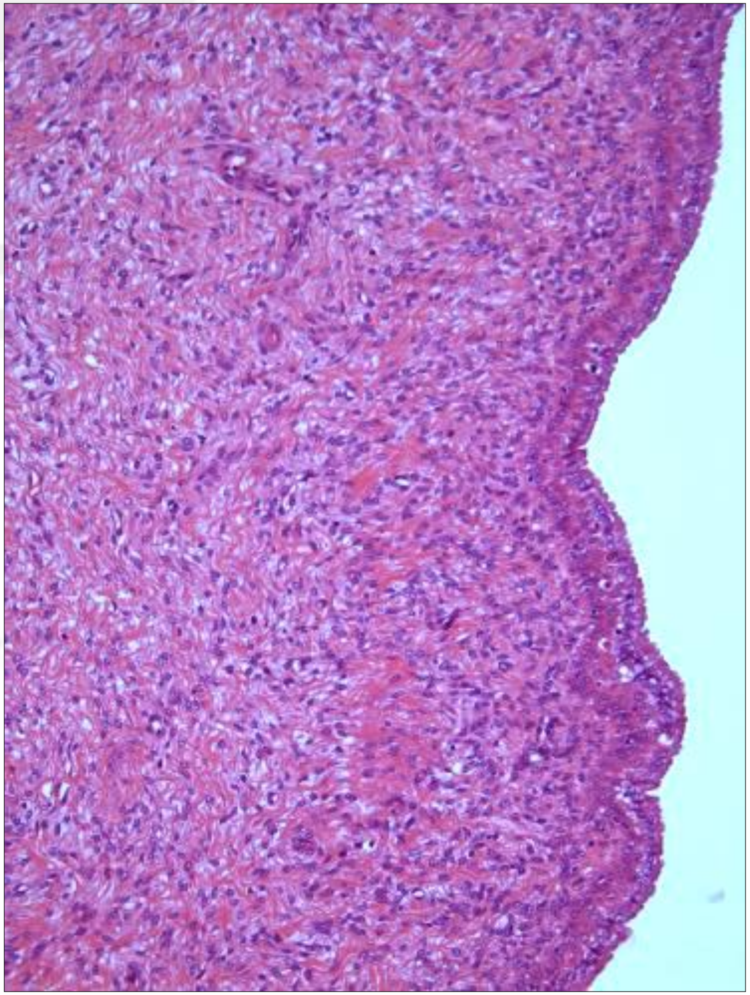


Anestrus: Ovary, Vagina

Ovary: small follicles,
but larger, occasionally corpora lutea

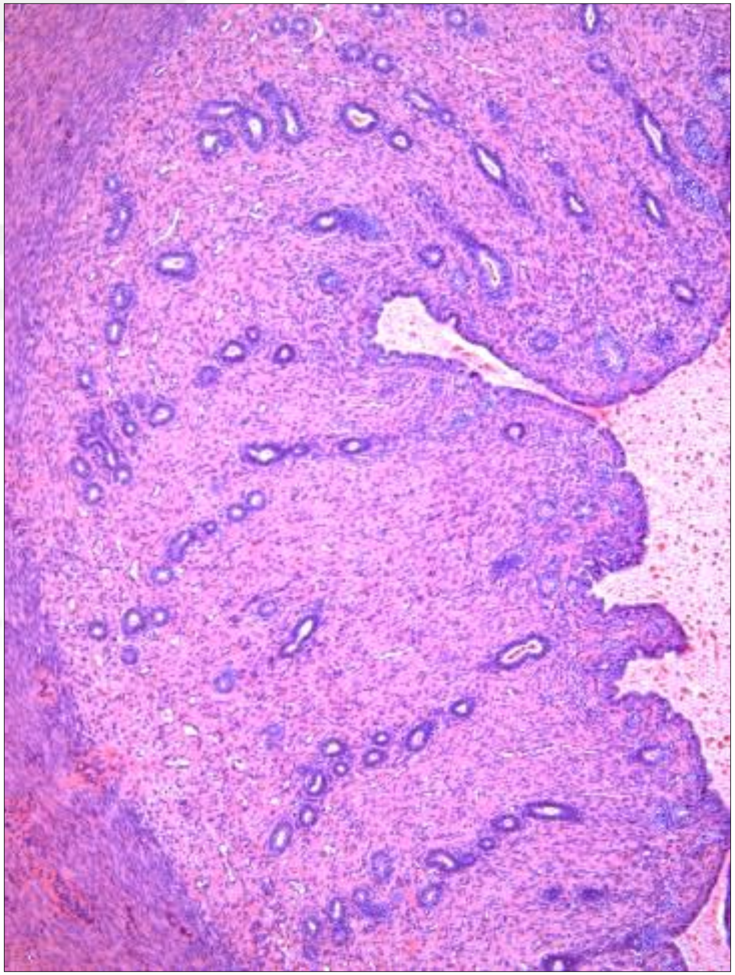


Vagina:
enlarged



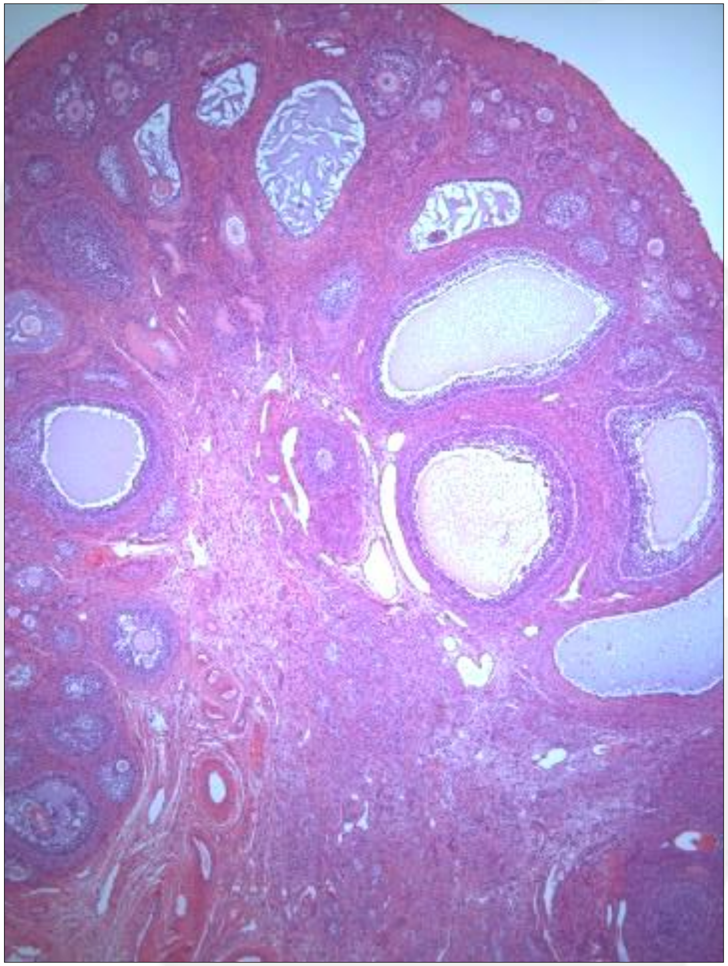
Anestrus: Uterus

Few atrophic glands present

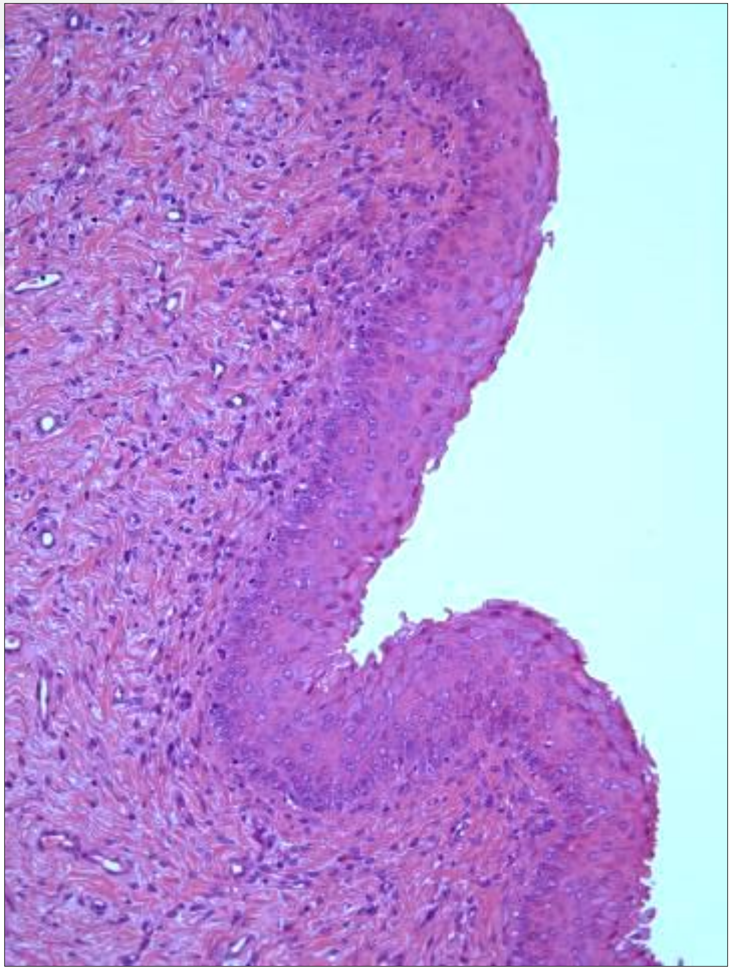


Proestrus: Ovary, Vagina

Ovary:
Large tertiary and Grafian follicles

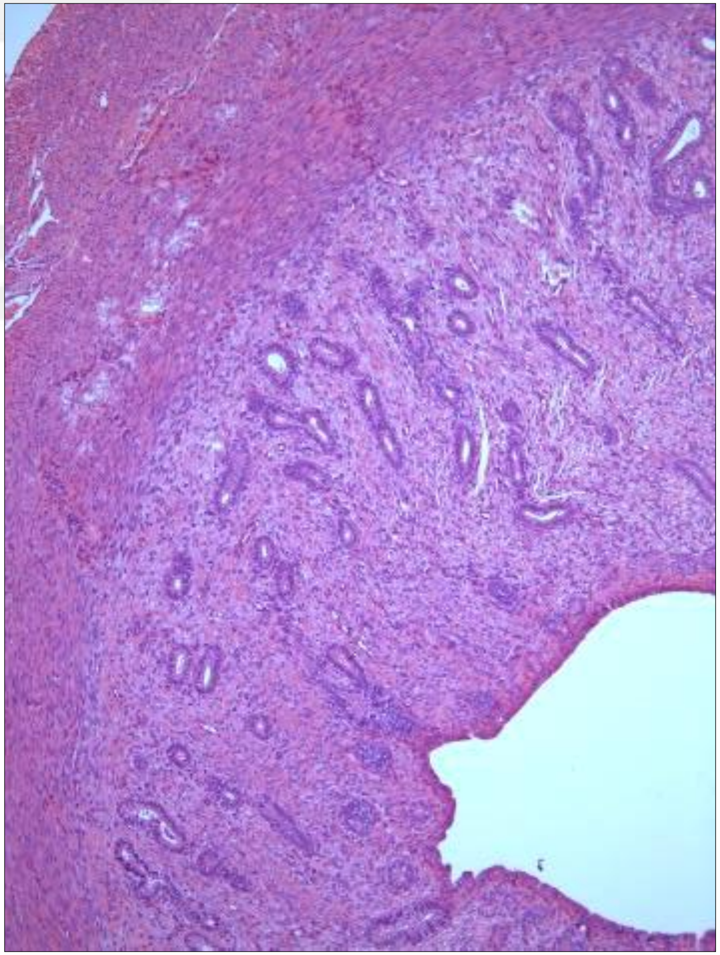


Vagina:
Thick epithelium



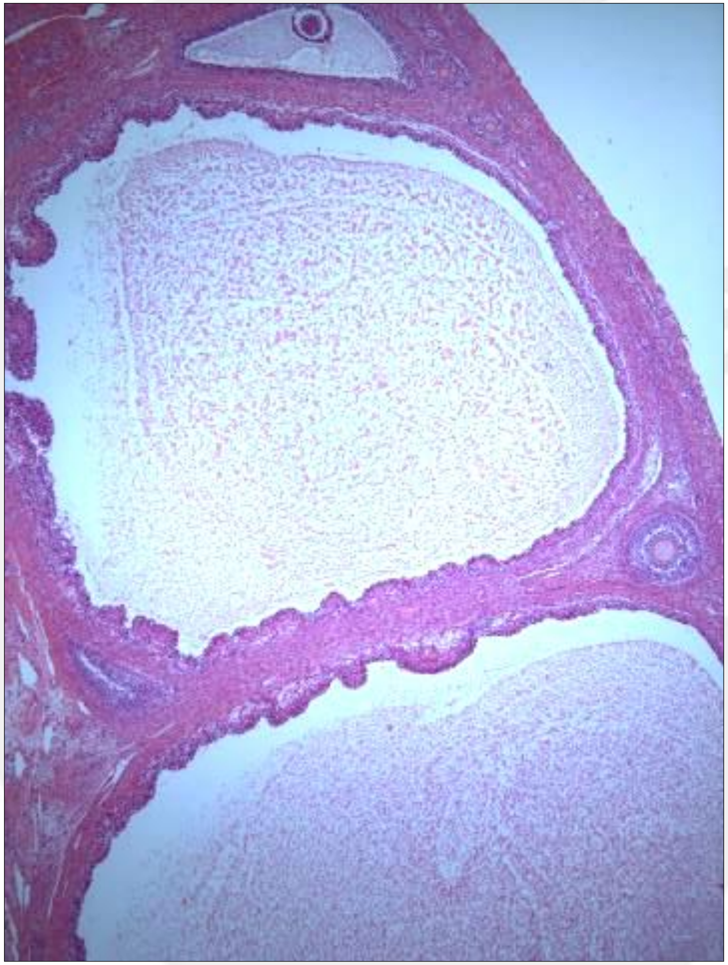
Proestrus: Uterus

Distended uterine horn and proliferating glands

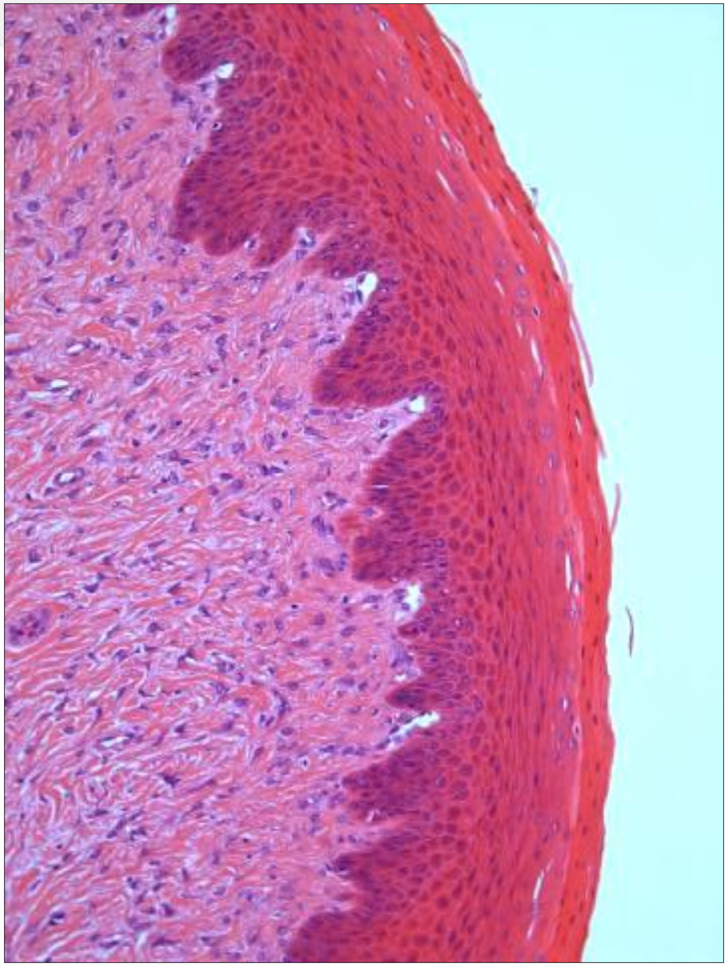


Estrus: Ovary, Vagina

Ovary:
Large Grafian follicles

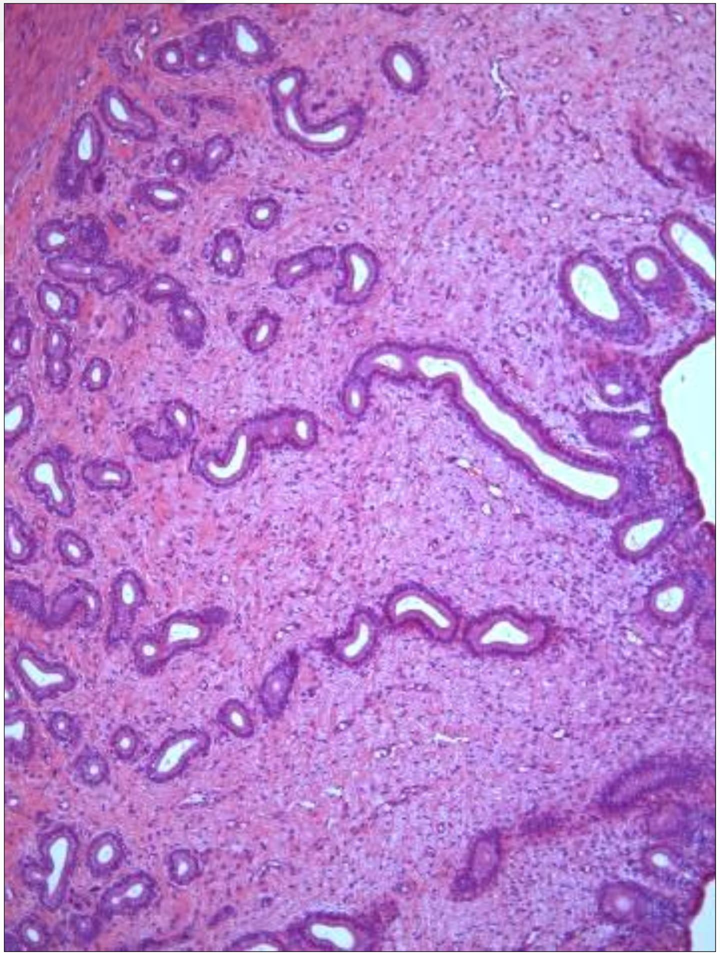


Vagina:
Thick epithelium with superfical cornification



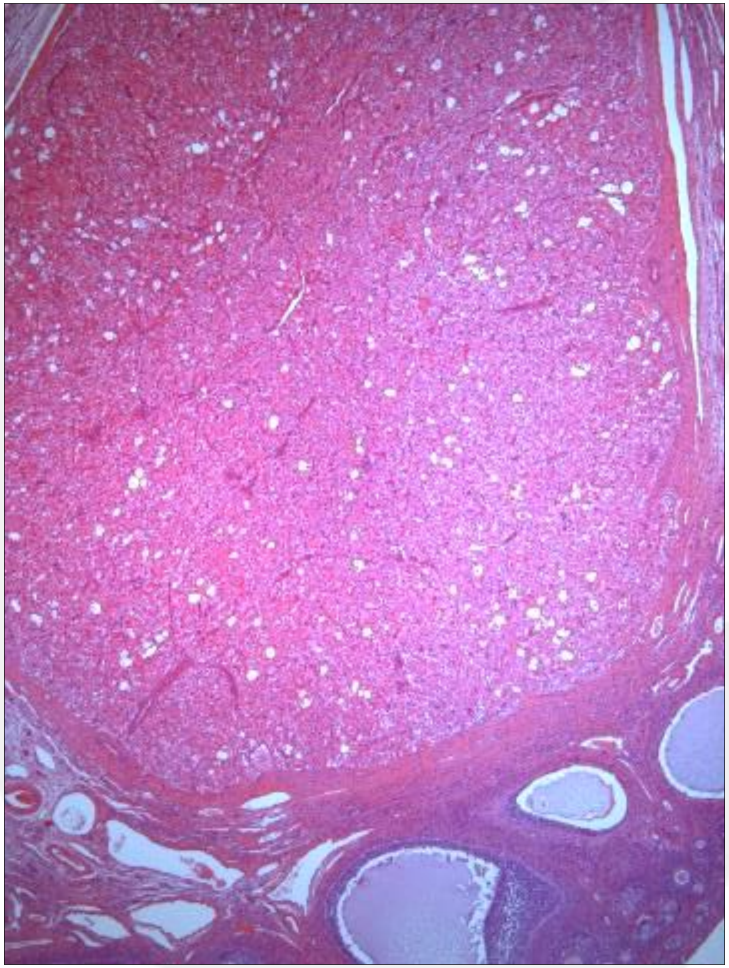
Estrus: Uterus

Distended uterine lumen and proliferating glands

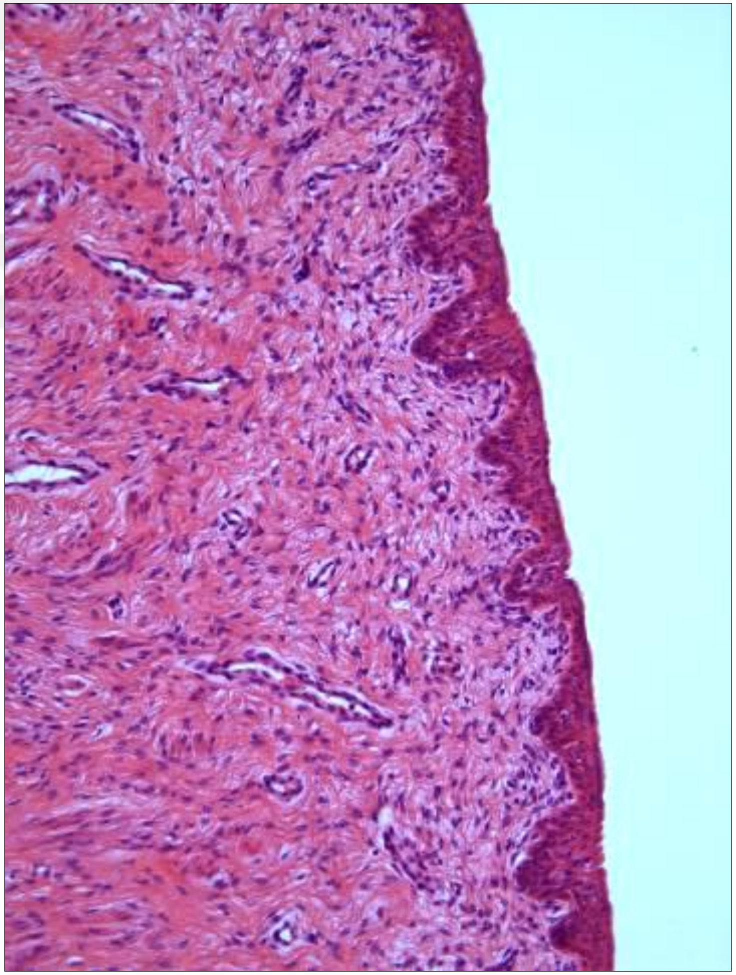


Metestrus: Ovary, Vagina

Ovary:
Large copora lutea

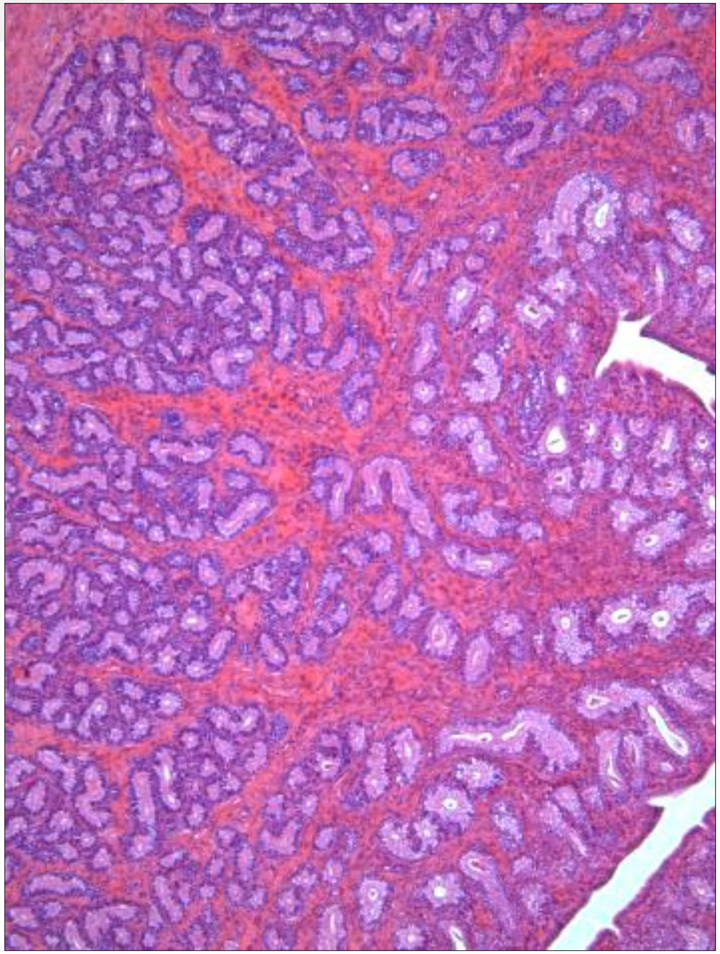


Vagina:
Thin epithelium



Metestrus: Uterus

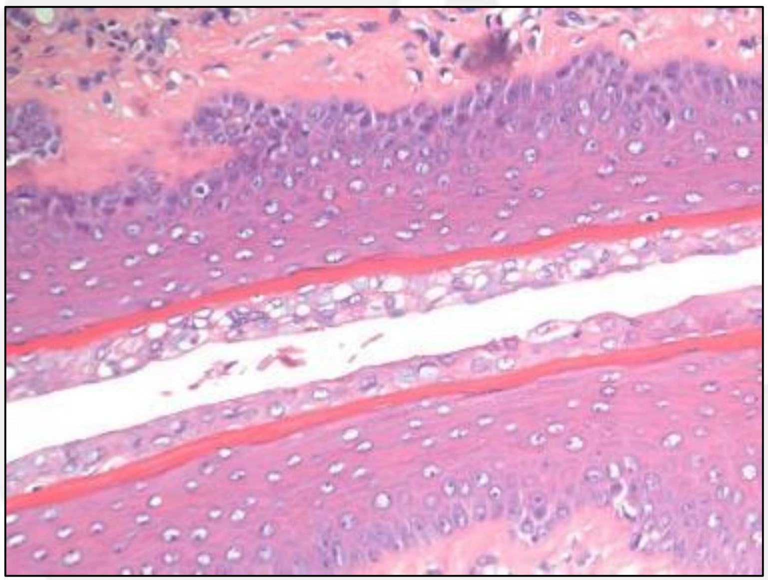
Glandular secretion



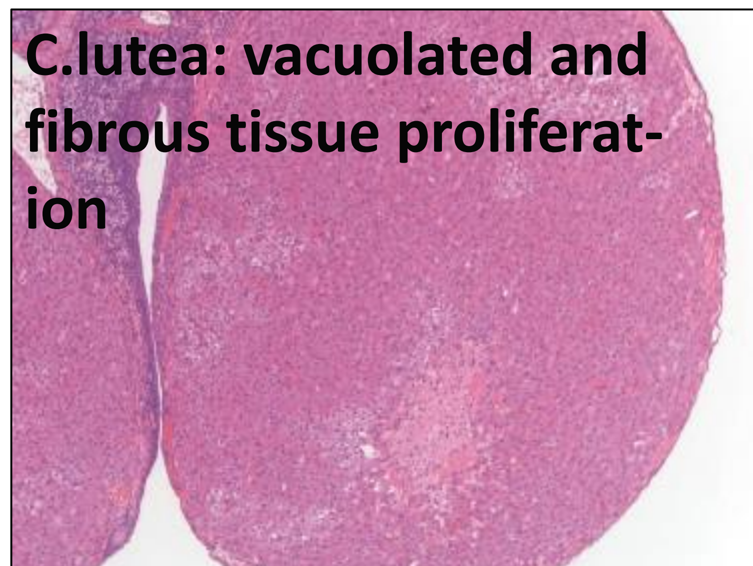
Female Rats

Normal Cycle: Rat – Proestrus

Vagina:
Mucification overlaying cornified layer, thick germinative epithelial layer



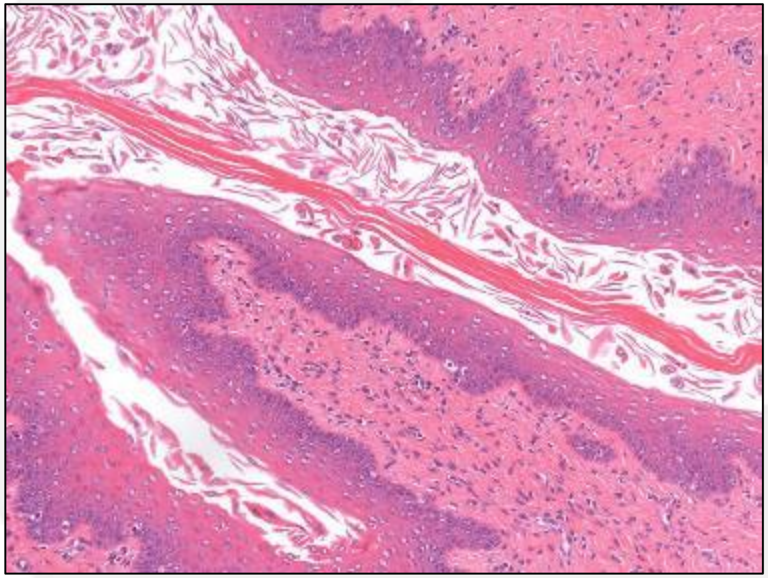
Horn: dilated

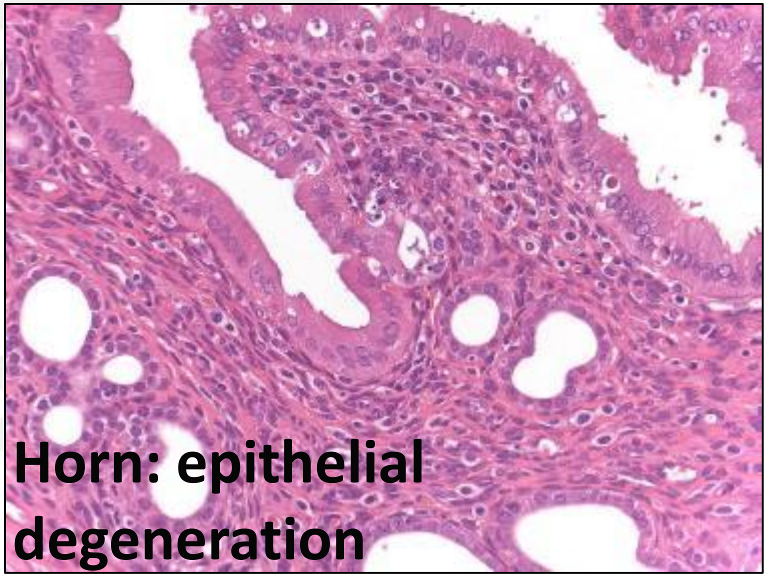


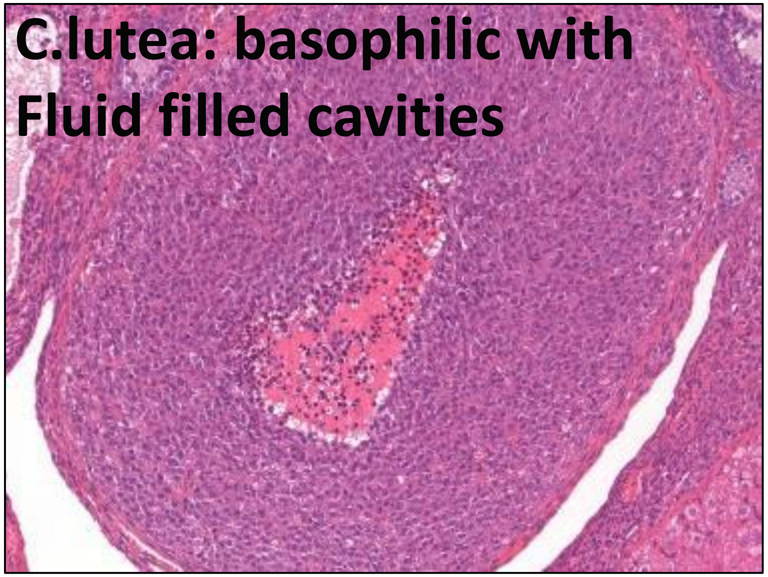
C.lutea: vacuolated and fibrous tissue proliferation

Normal Cycle: Rat – Estrus

Vagina:
Shedding of cornified layer

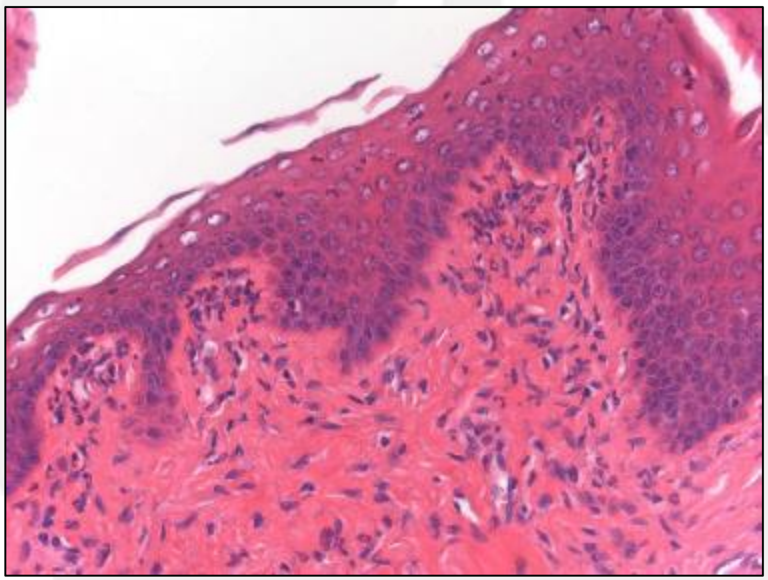


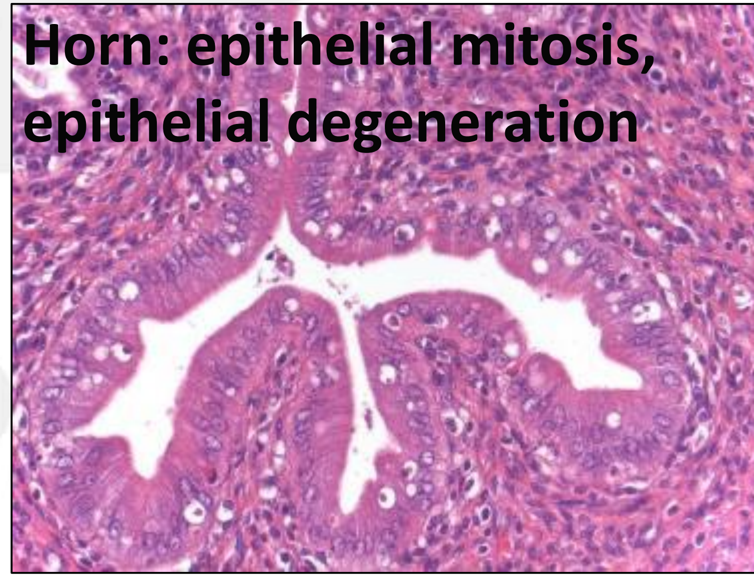


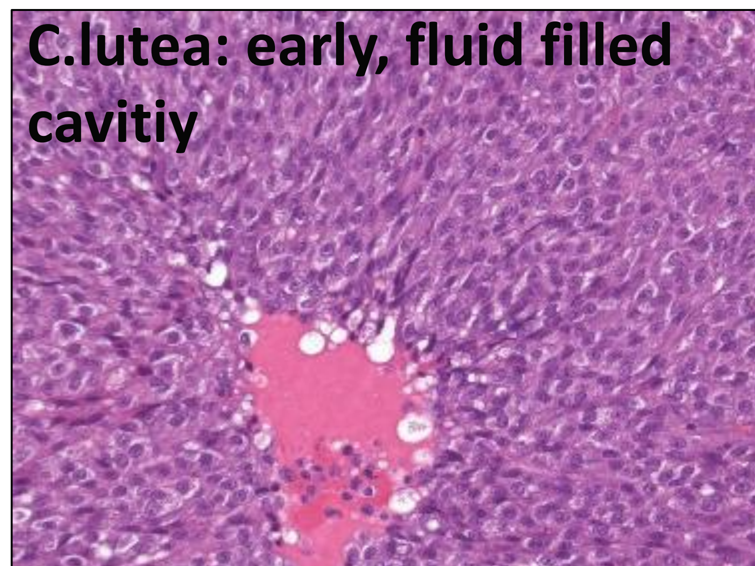


Normal Cycle: Rat – Metestrus

Vagina:
Shedding of cornified layer and infiltration with granulocytes

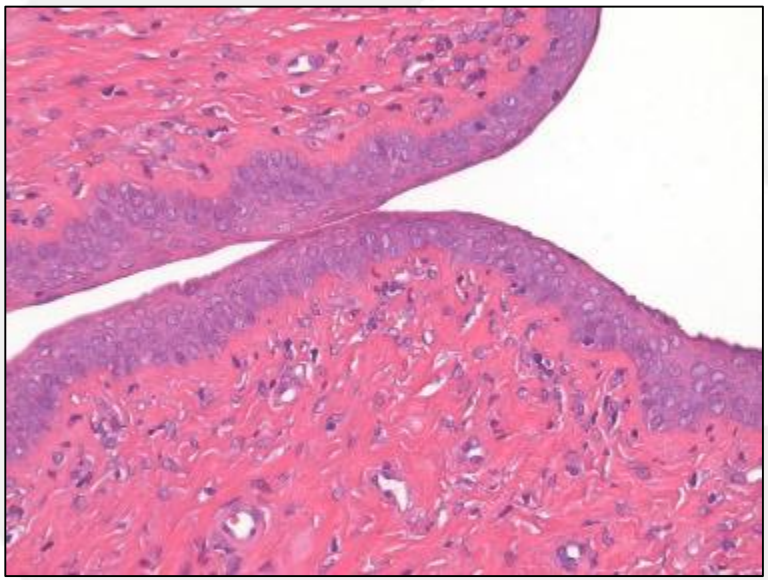


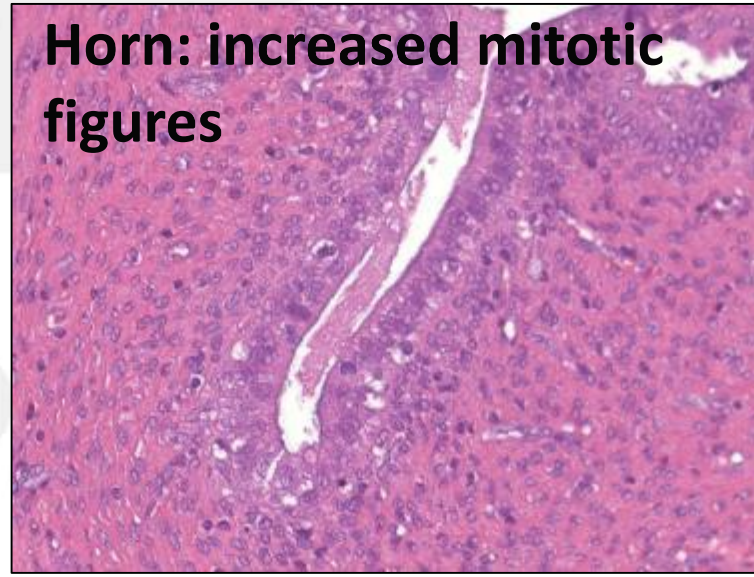


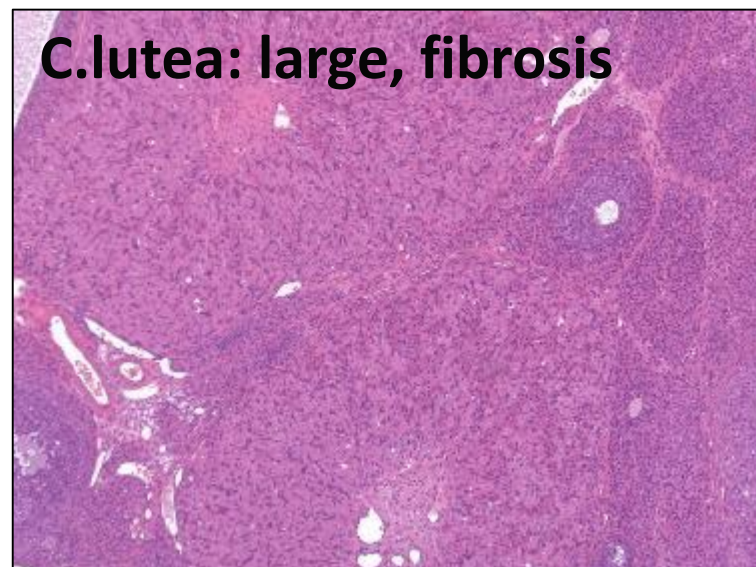


Normal Cycle: Rat – Diestrus

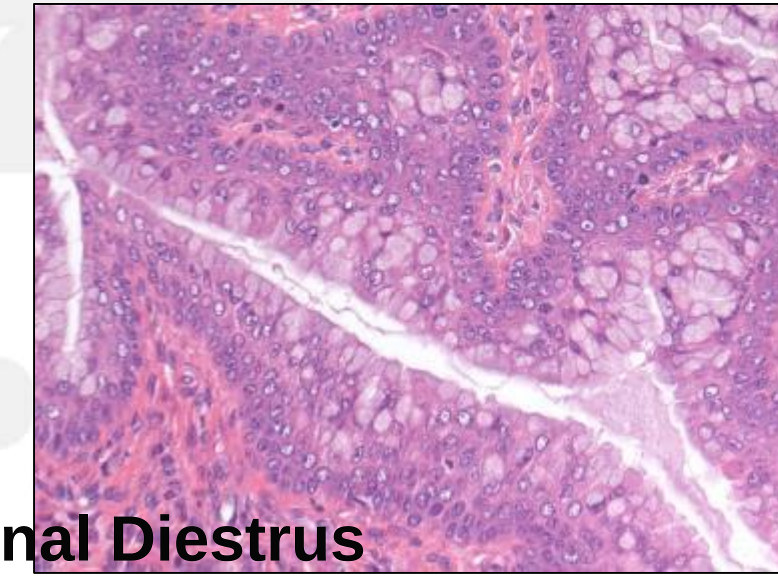
Vagina:
thick stratified epithelium,
No stratum granulosum



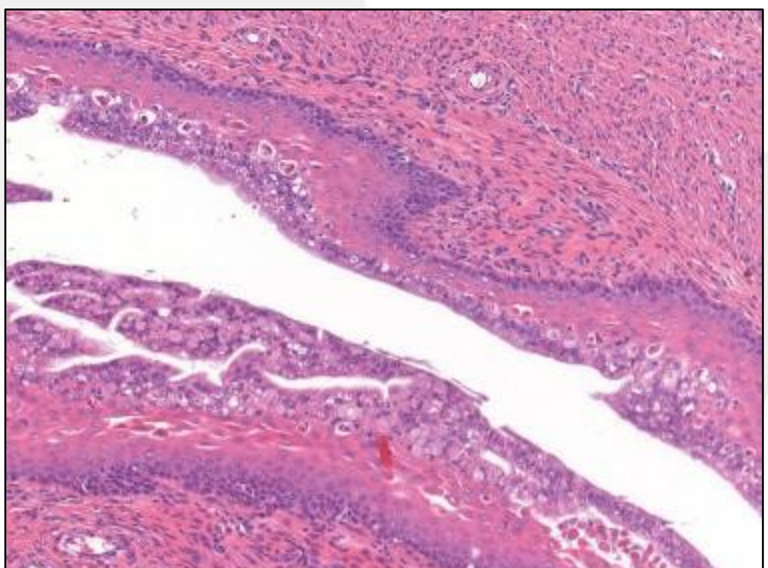
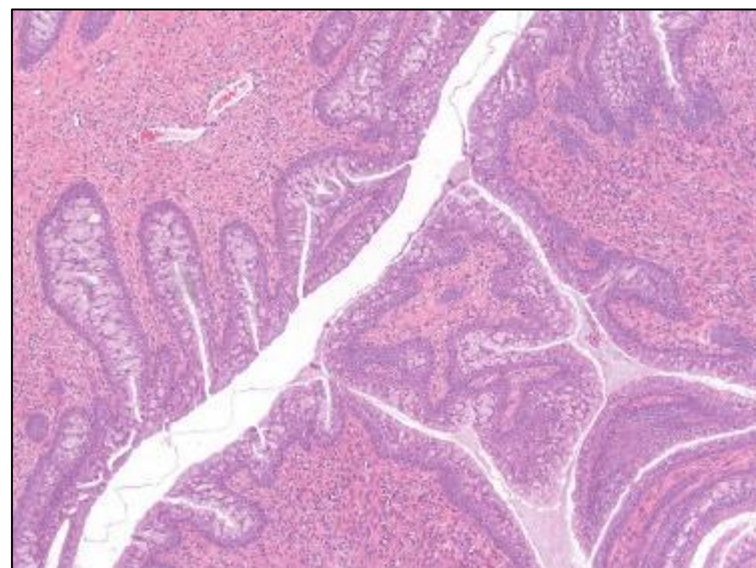




Normal Occuring Cyclic Changes: Rats

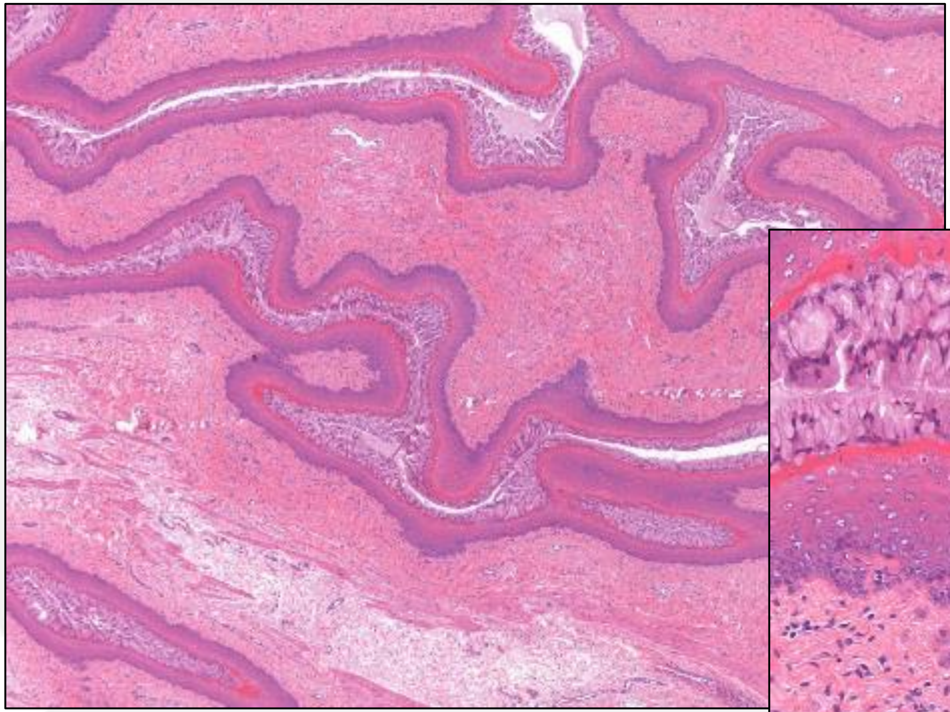



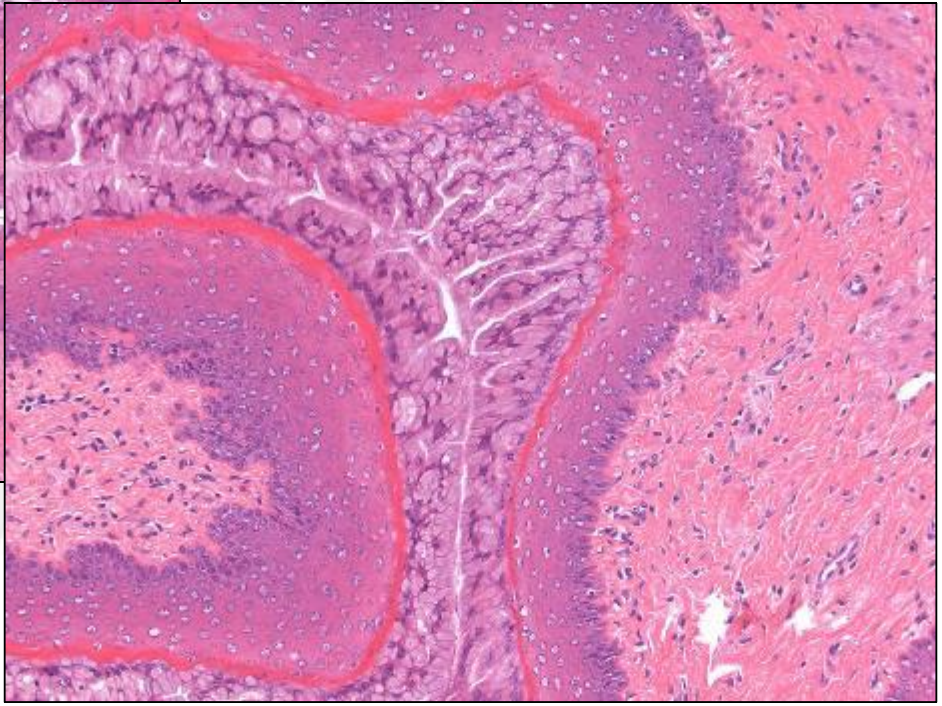
Vagina: Lactational Diestrus

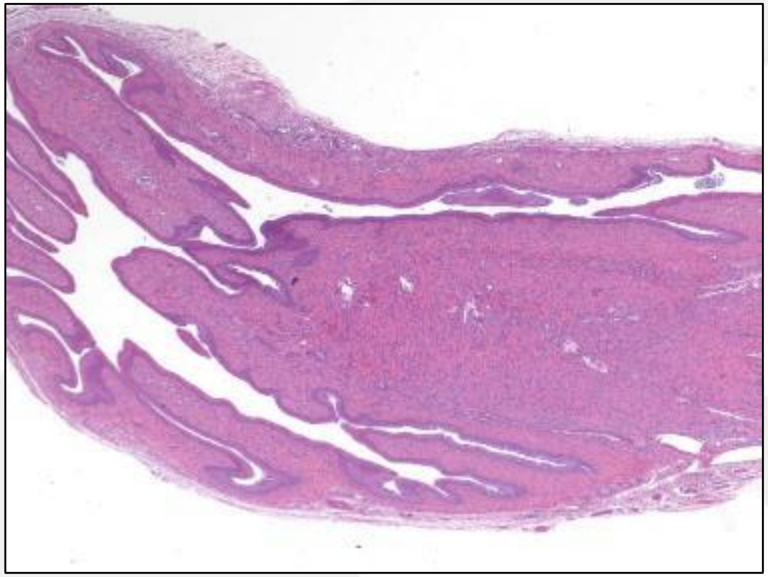
Natural Occuring Cyclic Changes: Rats

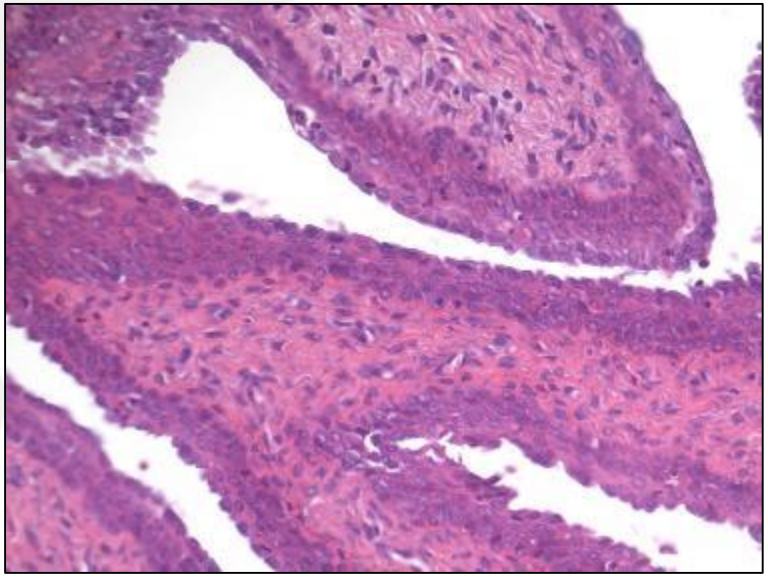
- **Vagina: Proestrus after Pregnancy with moderate mucification**

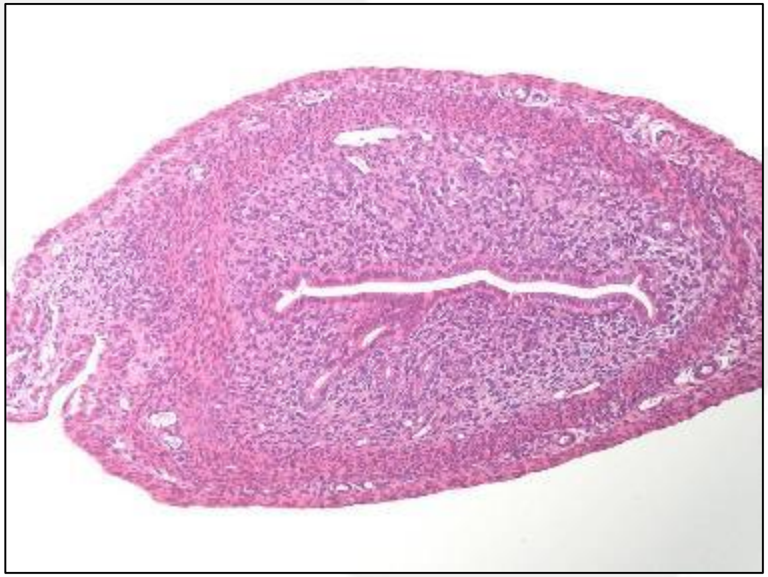


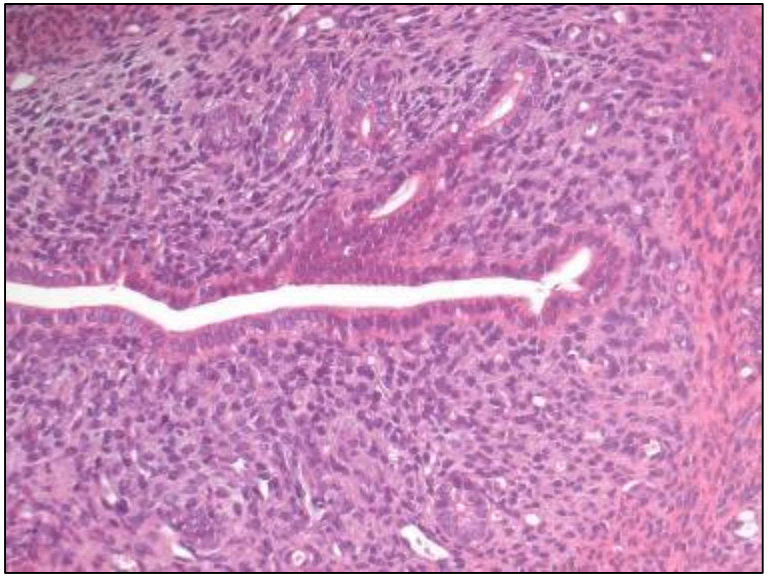


Immature Animal: Rat



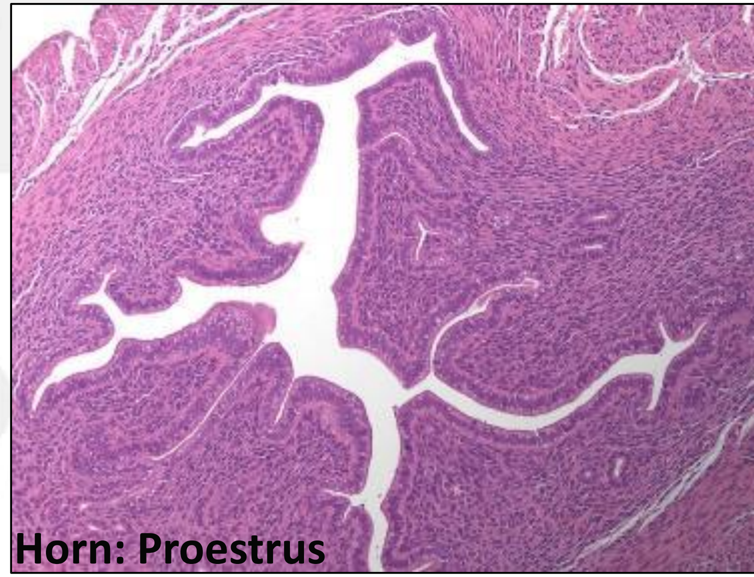


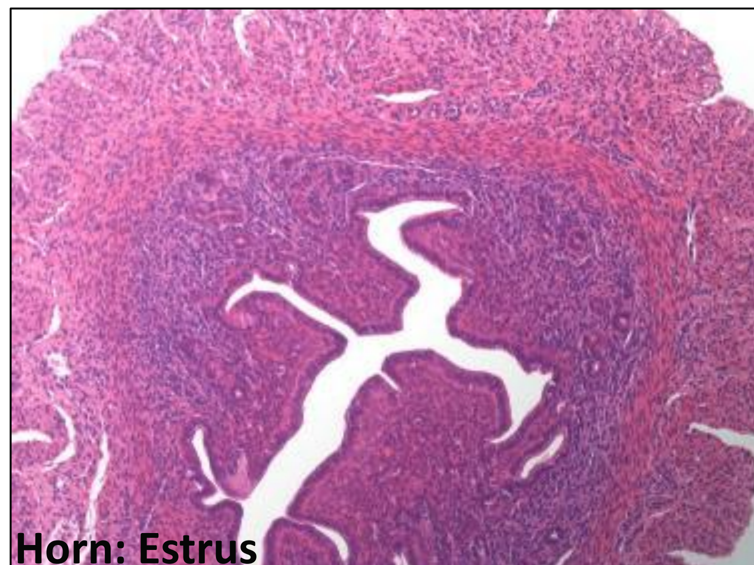




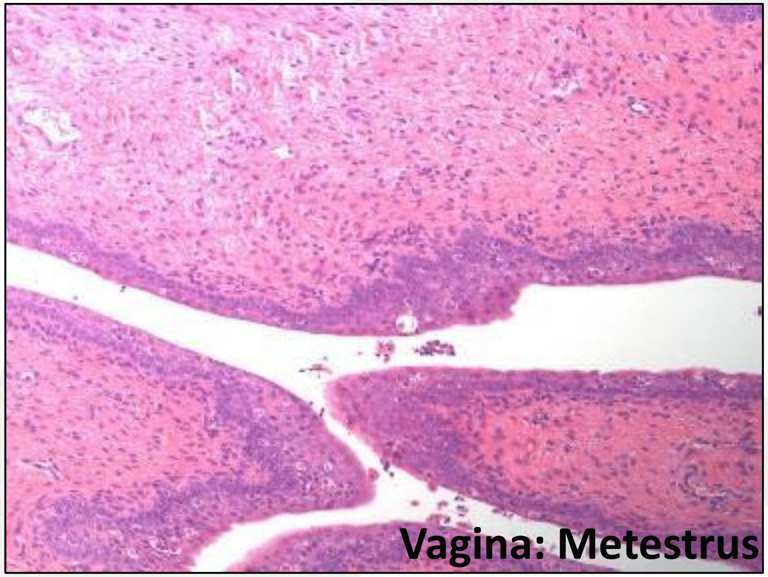
Female Mice

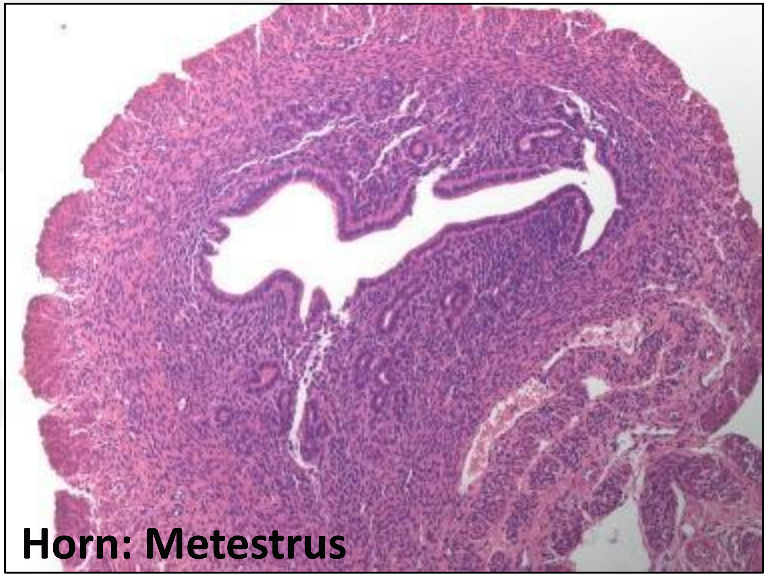
Normal Cycle: Mouse

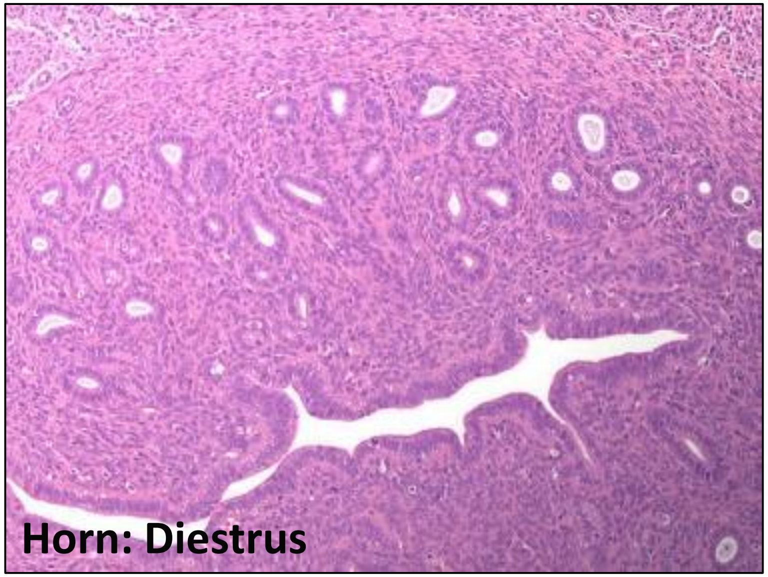




Normal Cycle: Mouse







Mammary Gland:
Female Monkeys

Mammary Gland

- **Macaques have 2 pectoral mammary glands**
- **5-7 lactiferous ducts exiting each nipple with varying degrees of communication between corresponding ductal and lobular units**
- **Occasional small clusters of glandular tissue in nipple**
- **Growth/differentiation depend on ovarian and local production of steroid hormones, GH or IGF**
- **Secretory stimuli include prolactin and placental lactogen**

Mammary Gland – Endocrine Regulation: Estrogen, Progesteron, Androgens

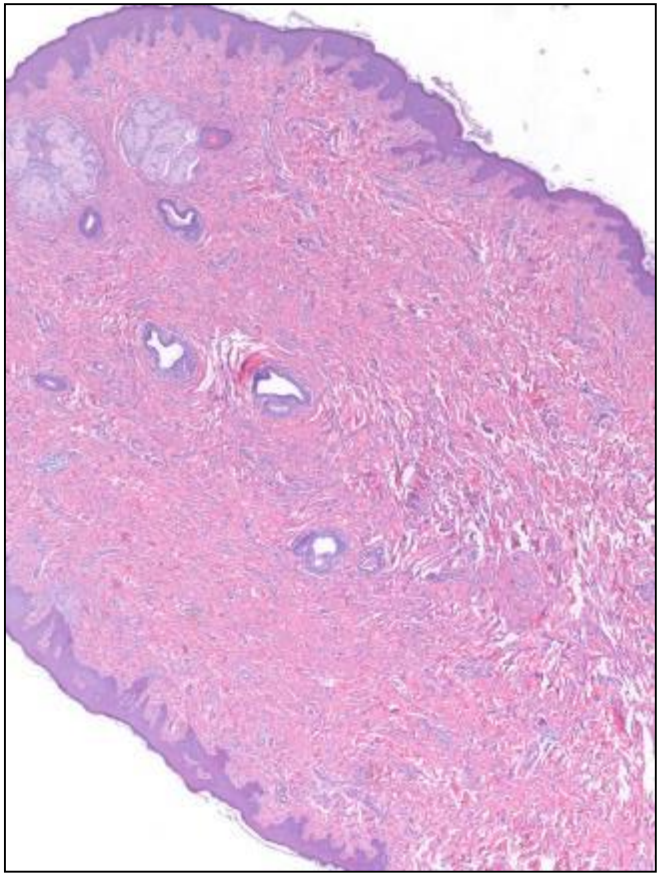
- Development depend on ovarian steroid production GH/IGF Axis
- Growth hormone (systemically and locally)
- GH and IGF are critical in proliferation and differentiation

Mammary Gland – Endocrine Regulation: Prolactin, Placental Lactogen, Tissue Hormones

- **Prolactin not obligate for mammary growth and development but required for lactation**
- **Exogenous administration causes insignificant increase**
- **Galactorrhea reported in conjunction with prolactin-producing neoplasms of the pituitary gland in macaques**
- **Placenta Lactogen (Somatomammotropin)**
- **GH-related placental lactogen derived (*placental lactogen* does not impair lactation)**
- **Intra-tissue production of sex steroids and growth factors is important (enzymatic systems for conversion of precursors to more bioactive estradiol (aromatase and steroid sulfatases)**

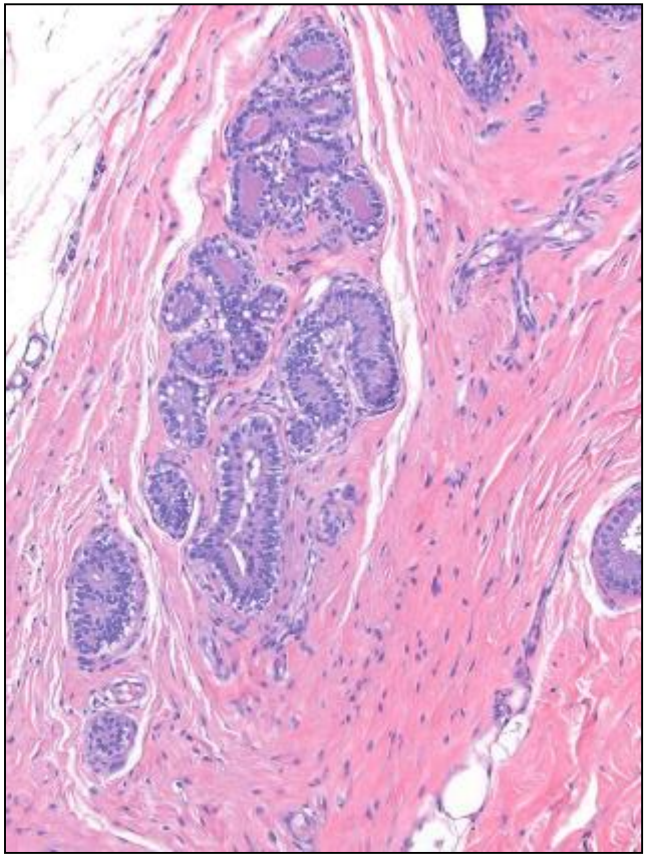
Mammary Gland – Puberty

- Nipple development in macaques is distinctive and precedes regular menstruation by several months
- Pubertal development of the breast starts with rudimentary ductal tree early in life, followed by elongation branching of major ducts
- Lobular development during puberty
- Pubertal development of mammary tissues in male macaques is not well described; but transient glandular development (gynecomastia) in more than 50% of normal adolescent males



Mammary Gland – Adult Non-Lactating

- Homogeneous pattern of mature type 2 lobules
- Estrogen receptors α and β with the latter being more abundant
- Effect of menstrual cycle on proliferation in breast is controversial: cycle-related changes are small
- Ductal tissues proliferate more during luteal phase
- Lobuloalveolar tissues epithelium with higher proliferation during late follicular phase



Mammary Gland – Lactation

- Gestation in macaques approx. 150 days
- Extensive growth and differentiation under estrogens, progestogens, chorionic gonadotropin, placental lactogen, prolactin
- Change in volume of glandular tissue is 10-20x, as a result of both epithelial proliferation and secretory distention of ductal and alveolar system
- Lobuloalveolar units markedly increase in number/size
- Macaques lactate for approx. 12 months and during this time, ovulation is suppressed
- Single offspring (neonate typically with strong preference for one nipple or the other

Mammary Gland – Senescence

- **Regression into ductal network with marked lobular atrophy and little proliferative activity**
- **Substantial variation in the amount of tissue remaining**
- **Estrogen and progesterone receptors expression persists (surgically postmenopause: at least 6-7 y)**
- **Breast is responsive to exogenous hormonal stimulation by estrogens and progestogens beyond 25 y**

Mammary Gland:
Female Dogs

Mammary Glands - General

- Like hair, uniquely mammalian
- Glands develop from distinctive mammary ridges running along both sides of the trunk of a mammalian embryo
- found in both sexes, but cease development in males well before puberty
- Glandular structure associated with a papilla (teat)
- May contain one or more duct systems
- Lactiferous sinus (milk sinus): cavity within the teat and glandular body
- Gland sinus: part of the milk sinus within the glandular body (teat sinus is part of the milk sinus within the teat)

Mammary Glands – Species Differences

Species	Number of mammae/teats	Localization	Teat Ducts
Rat/Mice/Hamster	10 and more	Thoracal, Abdominal	1
Guinea Pig	2	Inguinal	1
Rabbit	8-10	Thoracal, Abdominal	➢ 1
Dog	10 and more	Thoracal, Abdominal	➢ 20
Pig	Up to 18	Thoracal, Abdominal	2
Primates	2	Pectoral	up to 20

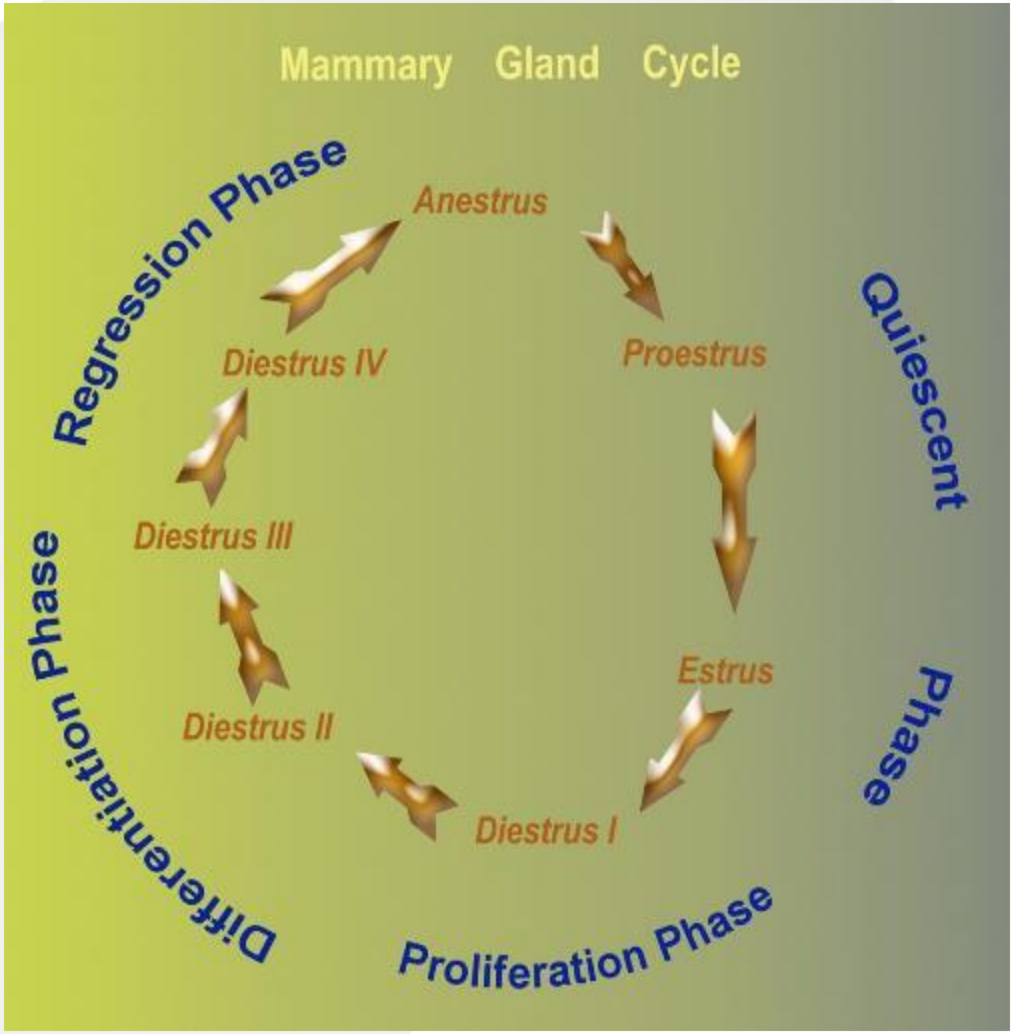
Mammary Gland – Cycling

Toxicologic Pathology
http://tpx.sagepub.com/content/38/6/969

Cyclic Morphological Changes in the Beagle Mammary Gland

Sundeep A. Chandra, J. Mark Cline and Rick R. Adler
Toxicol Pathol
2010 38: 969 originally published online 22 July 2010

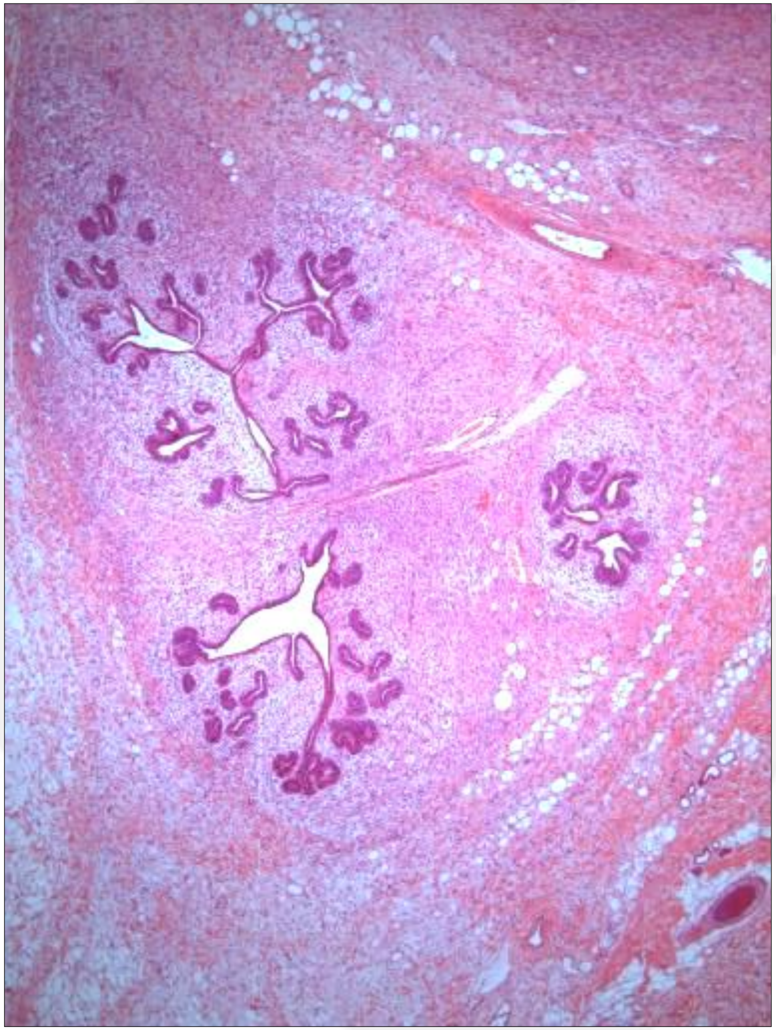
Figure 1. Schematic representation of the histologic changes in the mammary glands of sexually mature laboratory Beagles.



Chandra S A et al. Toxicol Pathol 2010;38:969-983

Mammary Gland – Cycling

Estrus:
Ductal and glandular proliferation



Mestrus:
More proliferation and dilatation of the ducts, buds appear to be basophilic