CONTINUING EDUCATION IN TOXICOLOGIC PATHOLOGY
REPRODUCTIVE SYSTEM

Third Conference

Society for Toxicologic Pathology in India
STPI

ORGANIZED BY SOCIETY FOR TOXICOLOGIC PATHOLOGY IN INDIA (STPI)

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The Atria Hotel, # 1, Palace Road, Bangalore - 560 001

Toxicologic Pathology of The Beagle Dog

Evaluation of the female reproductive system in toxicity Studies

Sundeep Chandra, BVSc, PhD, Dip. ACVP

Outline

- Estrous cycle – Histology
- Sexual maturity
- Hormone data
- Mammary gland (dog and rat)
- Test article induced changes
- Drug-induced estrogenic and antiestrogenic effects

The stages of the cycle

- Proestrus
  - bloody discharge/vulva edema/'in heat'/vaginal rugae
  - follicular growth
  - 1-2 weeks
- Estrus
  - receptive to male
  - follicular phase
  - Ovulation
  - metestrus phase –luteinization of postovulatory follicles
  - 1-2 weeks
- Diestrus
  - unreceptive to male
  - functional corpora lutea
  - mammary glands enlarge/pseudopregnancy
  - 2-3 months
- Anestrus
  - no specific clinical signs/genitalia & mammary minimum size
  - ovarian quiescence
  - 3-5 months

The Cycle in the bitch

- First estrus 8-14 months of age - 1.5 estruses/year
- Stages determined by - clinical signs; vaginal smears; hormone analysis, histology
- Macroscopic/microscopic observations - Changes occur gradually -End of one stage may closely resemble beginning of next stage
- In studies of ≤ 3 months duration, the animals may not have gone through estrous during the entire study
- With group sizes of 3-5/group, the chances of detecting an effect on the estrous cycle is very slim
- In general, more than 80% of the dogs are in the anestrus-diestrus stage, and a small percentage of dogs are immature (Chandra and Adler, 2008)

Toxicologic Pathology, 36: 944-949, 2008

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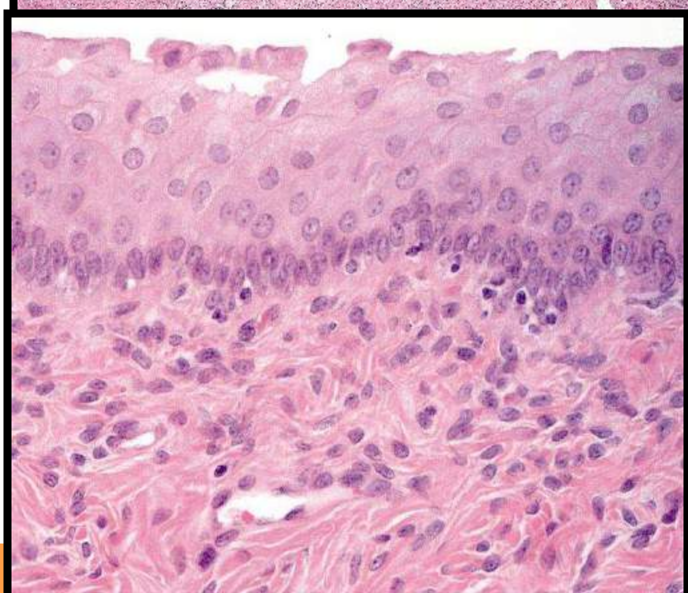
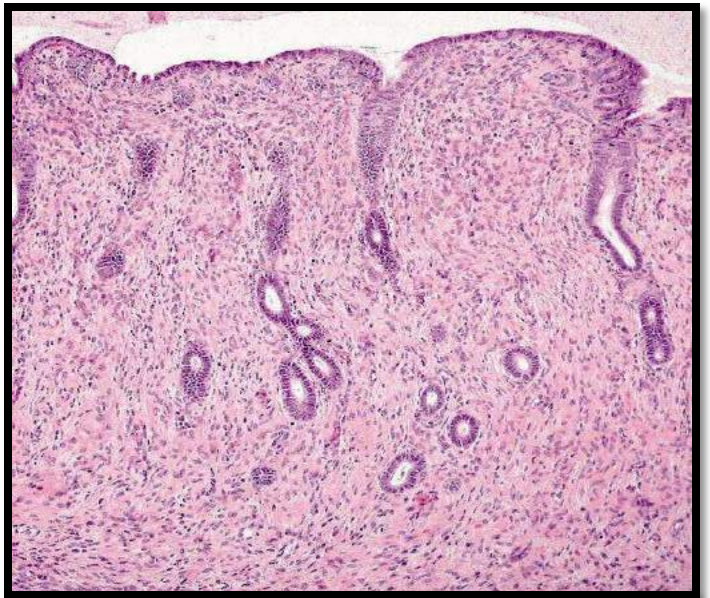
DOI: 10.1177/0192623308326150

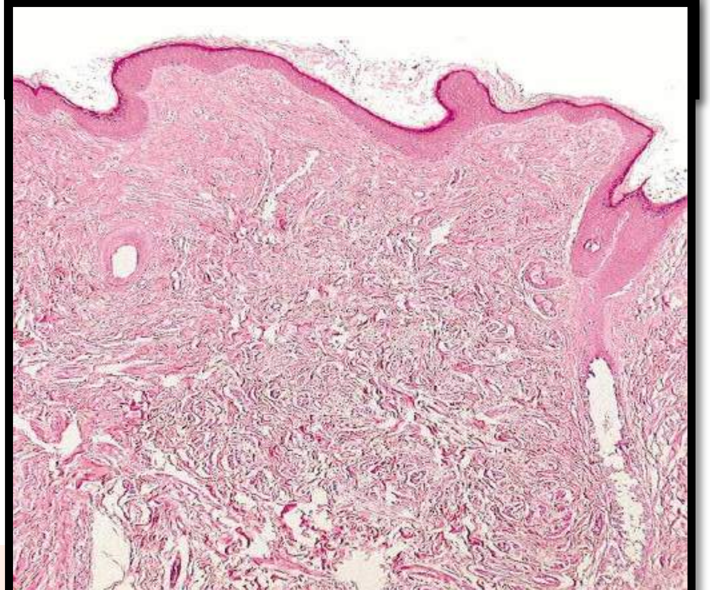
Frequency of Different Estrous Stages in Purpose-bred Beagles: A Retrospective Study

Sundeep A. Chandra and Rick R. Adler

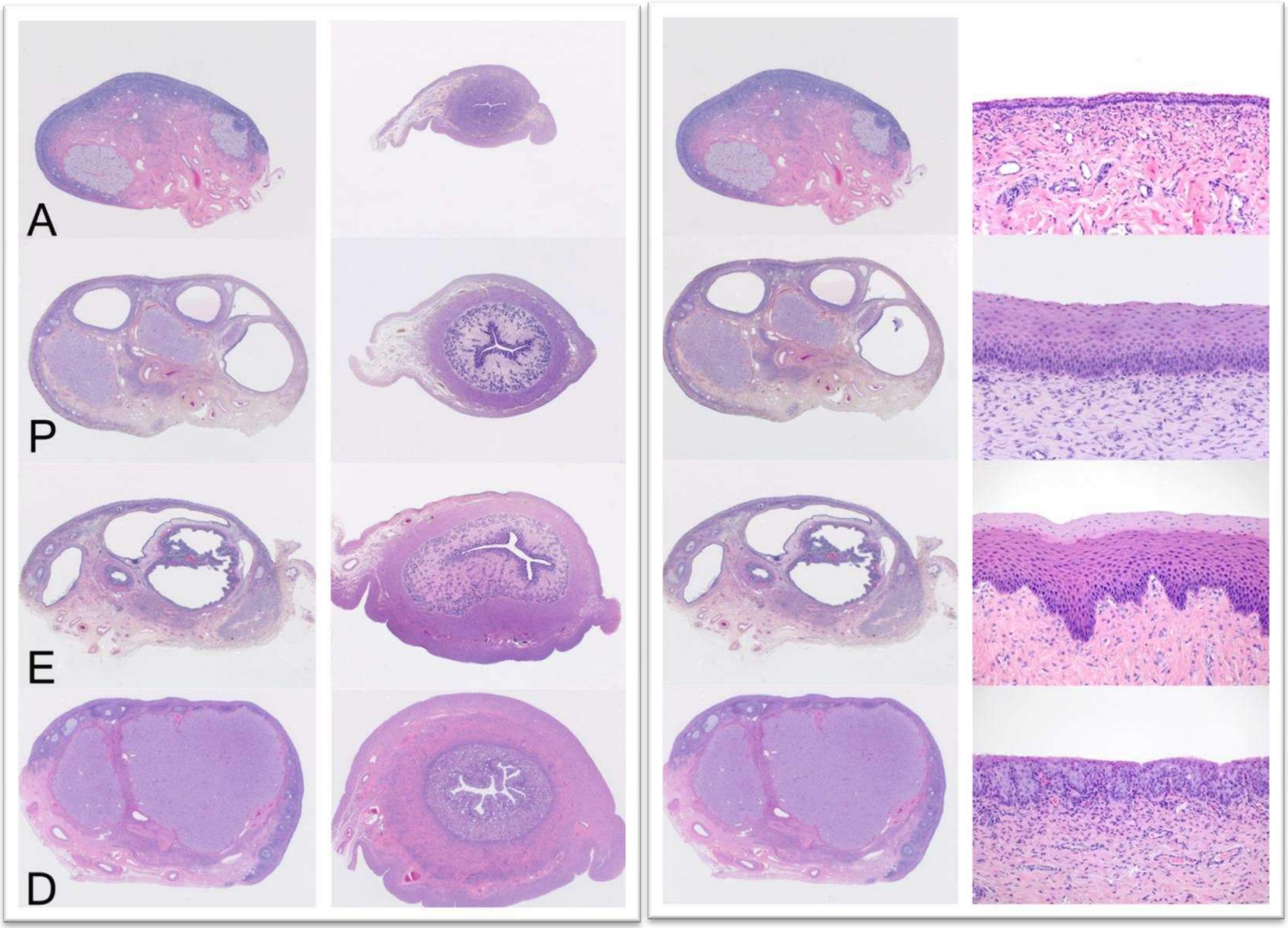
Immature dog





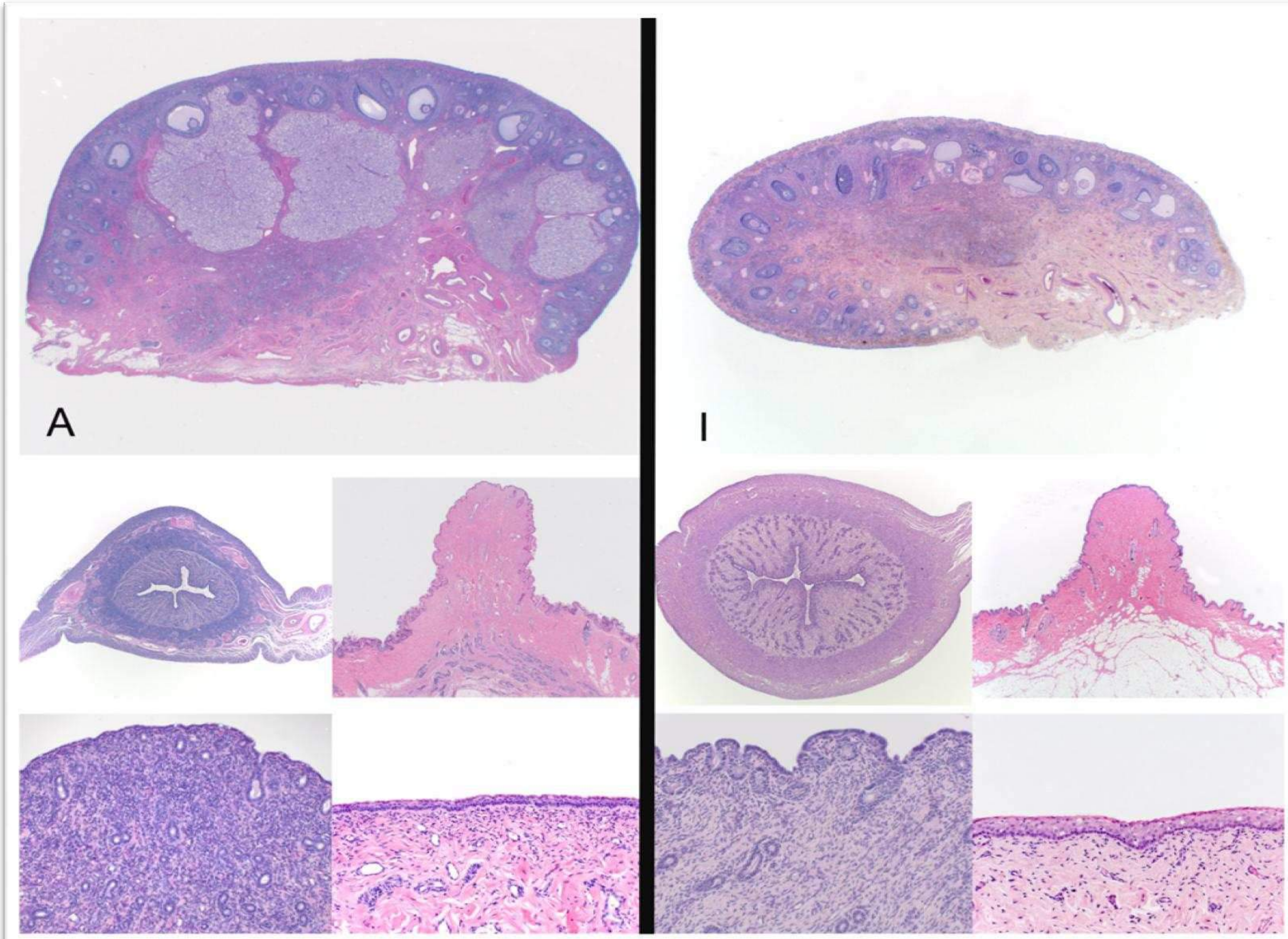


Staging the estrous cycle in dogs



GSK GlaxoSmithKline

Anestrus vs. immature



Conundrum of Immaturity

- Often a problem with dogs and primates
- In dogs, females undergo 1st estrous between 10 and 14 months of age.
- Not unusual to observe one immature dog in a study (even when 10-12 month old dogs are used)
- Small group size makes interpretation a challenge
- Morphologic differences between immaturity and atrophic changes?

Organ Weights

- STP – Position

•Most toxicities of the female reproductive tract can be adequately identified by light microscopy.

•Variability in age, sexual maturity, and stage of cycle in non-rodents may complicate or limit interpretation of reproductive organ weights.

•Weighing of reproductive organs is most valuable in sexually mature animals.

•*Weighing of other organs including female reproductive organs should be considered on a case-by-case basis*.

•Organ weight changes without macroscopic or microscopic correlation should be interpreted with caution.

Toxicologic Pathology, 35:751–755, 2007

ISSN: 0192-6233 print / 1533-1601 online
DOI: 10.1080/01926230701595300

Society of Toxicologic Pathology Position Paper: Organ Weight Recommendations for Toxicology Studies

Rani S. Sellers,[1] Daniel Morton,[2] Bindhu Michael,[3] Nigel Roome,[4] Julie K. Johnson,[5] Barry L. Yano,[6] Rick Perry,[7] and Ken Schafer[8]

Conundrum of Organ Weights

	Dose (mg/kg)	Ovary (g)
Vehicle	0	0.7030
GSK123456	15	0.8257
GSK123456	150	0.9257
GSK123456	1000	1.0913

Conundrum of Organ Weights

	Dose (mg/kg)	Stage of Estrous Cycle (Dog #)	Mean Ovary Wt (g)
Vehicle	0	Anestrus Anestrus Anestrus	0.7030
GSK123456	15	Diestrus Anestrus Anestrus	0.8257
GSK123456	150	Estrus Anestrus Anestrus	0.9257
GSK123456	1000	Estrus Estrus Proestrus	1.0913

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Recording – Reporting

- **Reporting in Toxicity Studies**
- Examine all reproductive tissues from individual dogs as a matching set
  - Understand inter-related morphology
- Is there a need to record normal cycle stages?
  - No need to record when all is normal
- Abnormal cycling often associated with lesions (recording cycle stages may aid provide clues to mechanism)
- Dogs/Monkeys (n=3/5) could skew the distribution
  - Rats ($n \geq 10$) deviation from control would be apparent
- Pre-treatment data is useful if reproductive issues are suspected

Hormone Analysis

- Know what you are measuring
- Single vs. multiple time points
- Is it real?
- Correlate with histology!

FSH

Data from female Beagles from a 1-month toxicity study

Single time point

	Control	30 mg/kg/day	100 mg/kg/day	600 mg/kg/day
Mean FSH (ng/mL)	7.00	⇩ 4.23	⇩ 6.63	⇩ 2.84
S.D.	4.64	4.27	3.35	1.75
N	5	3	3	5

FSH

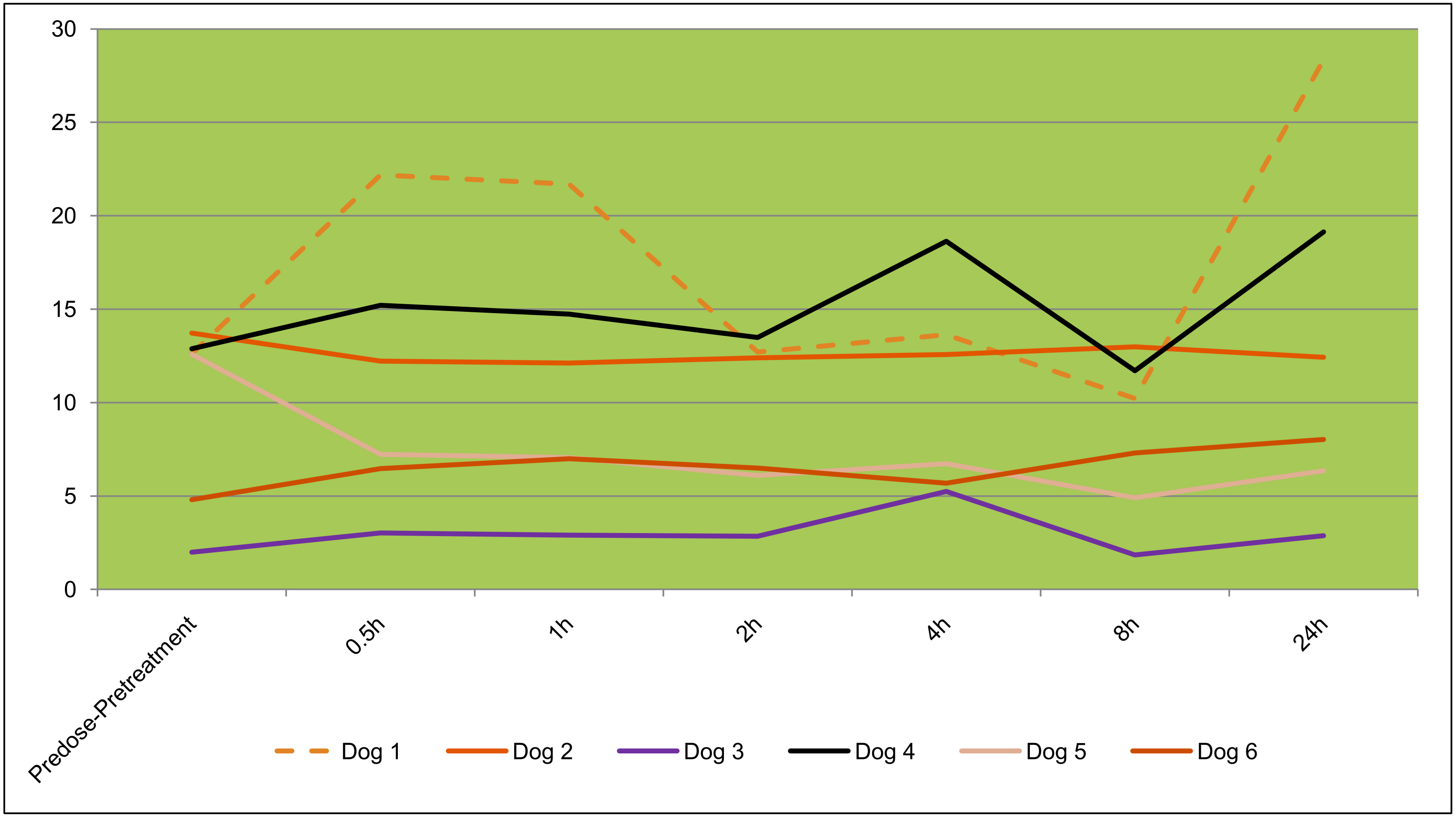
Data from female Beagles from a 1-month toxicity study

- This is ***pretreatment data*** from Day -15 prior to start of study!
- Limitations of using single time point

	Control	30 mg/kg/day	100 mg/kg/day	600 mg/kg/day
Mean FSH (ng/mL)	7.00	4.23	6.63	2.84
S.D.	4.64	4.27	3.35	1.75
N	5	3	3	5

Serum FSH levels in female “control” dogs – 24h profile

Range 1.84 – 28.34 (ng/mL) – Significant inter-animal variability



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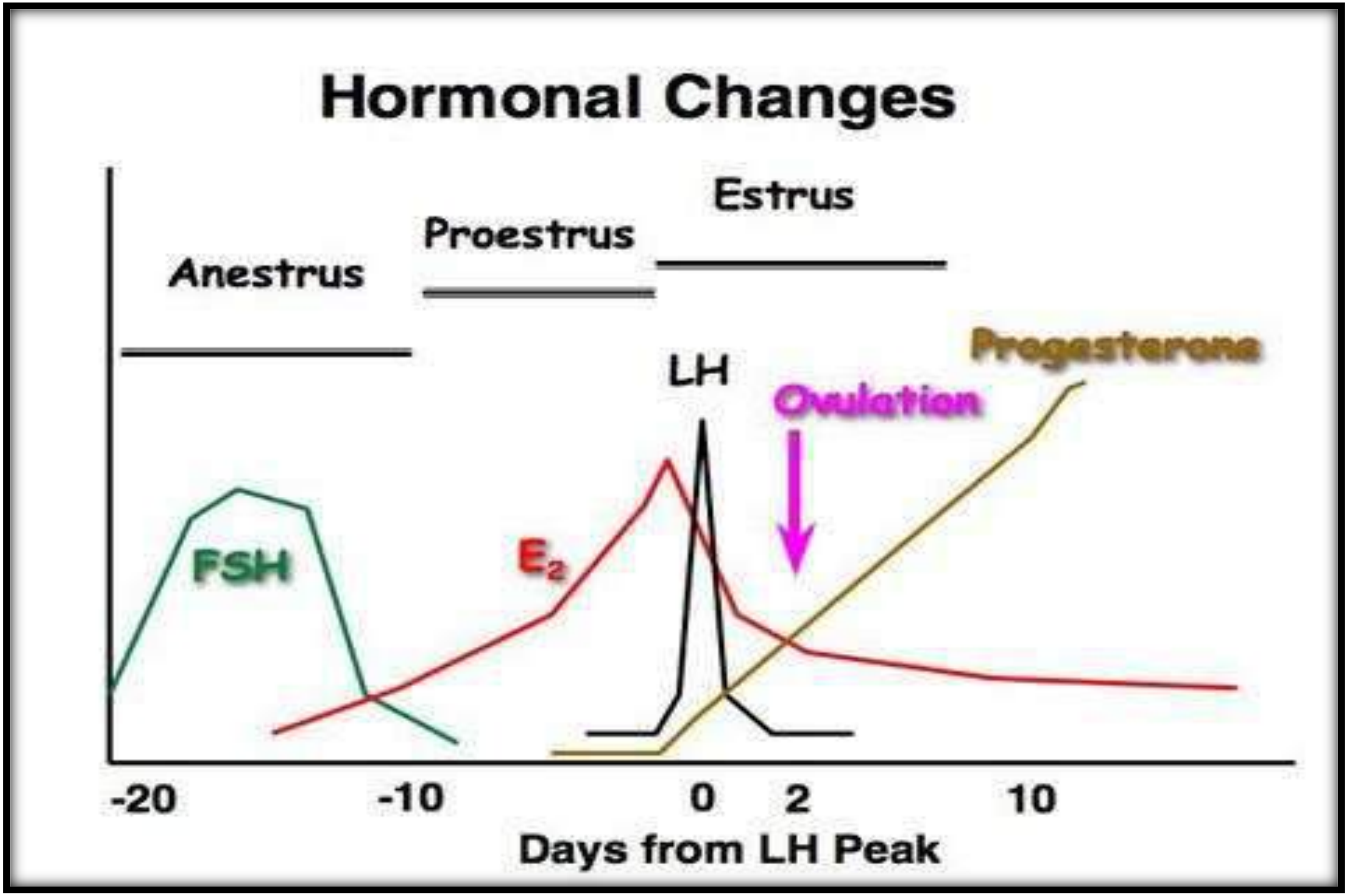
Canine Cycle



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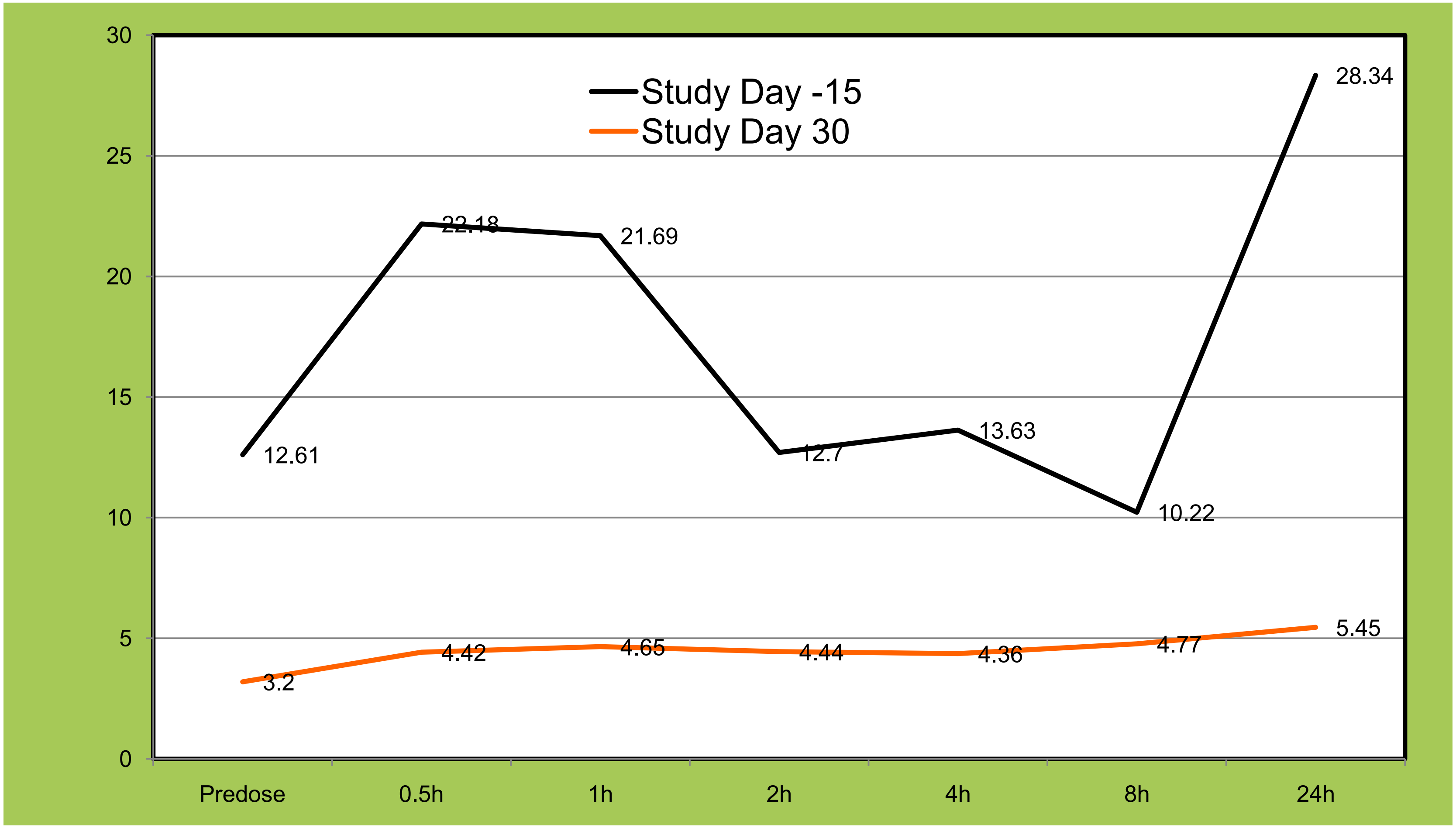
Serum FSH levels in a control Beagle dog (Dog No. 1)

Study Day -15 (Range 10.22-28.34 ng/mL)

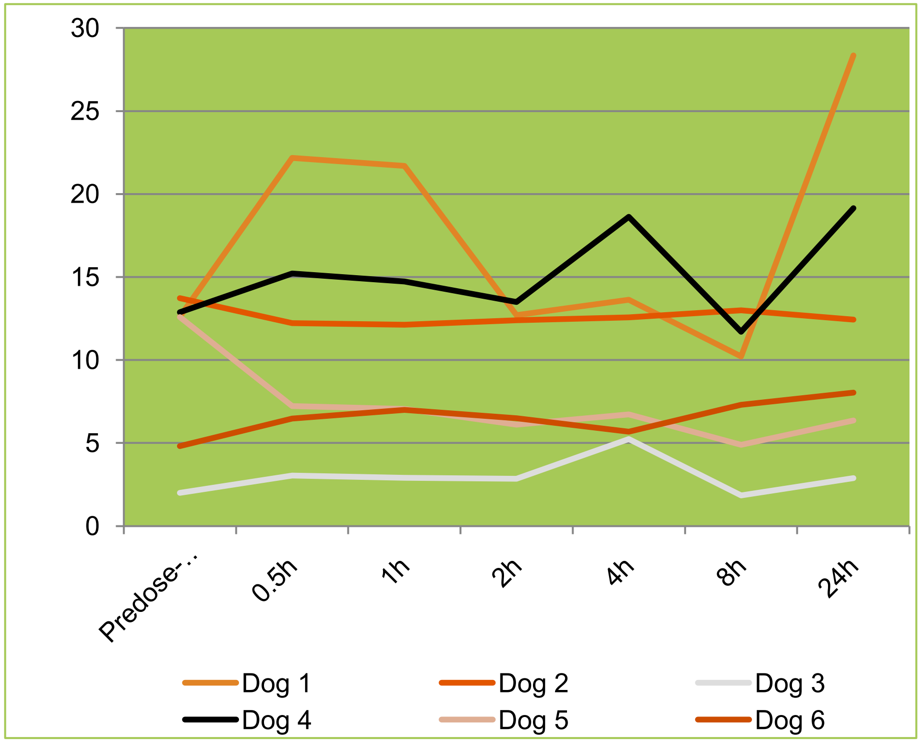
Study Day 30 (Range 3.20 – 5.45 ng/mL)

<u>Important to correlate hormone levels with stage of estrous</u>

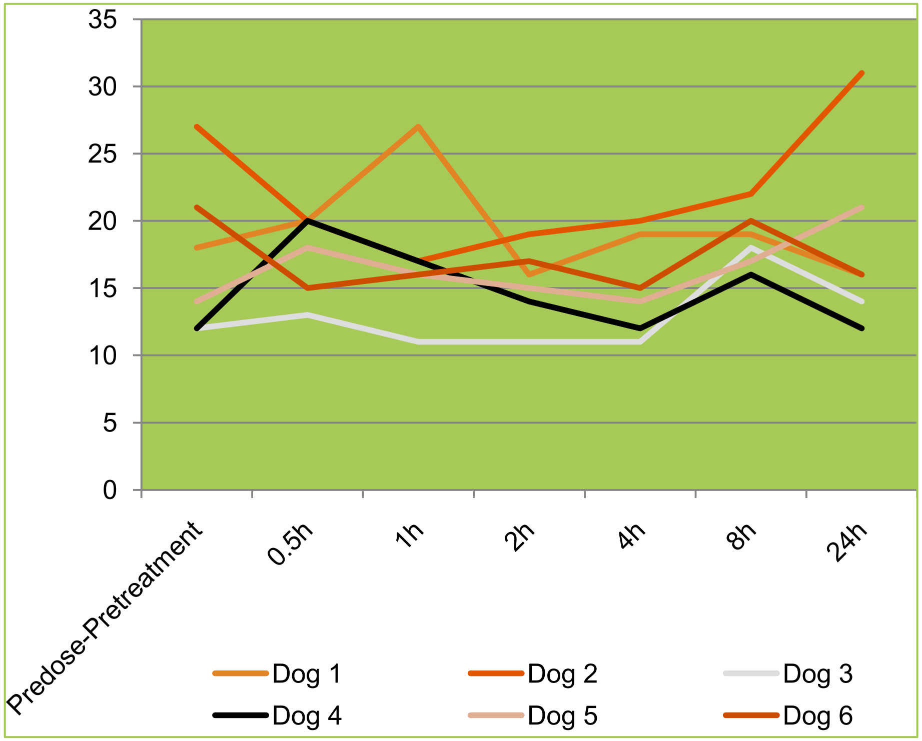
Pretreatment values are only useful if the stage of estrous is known



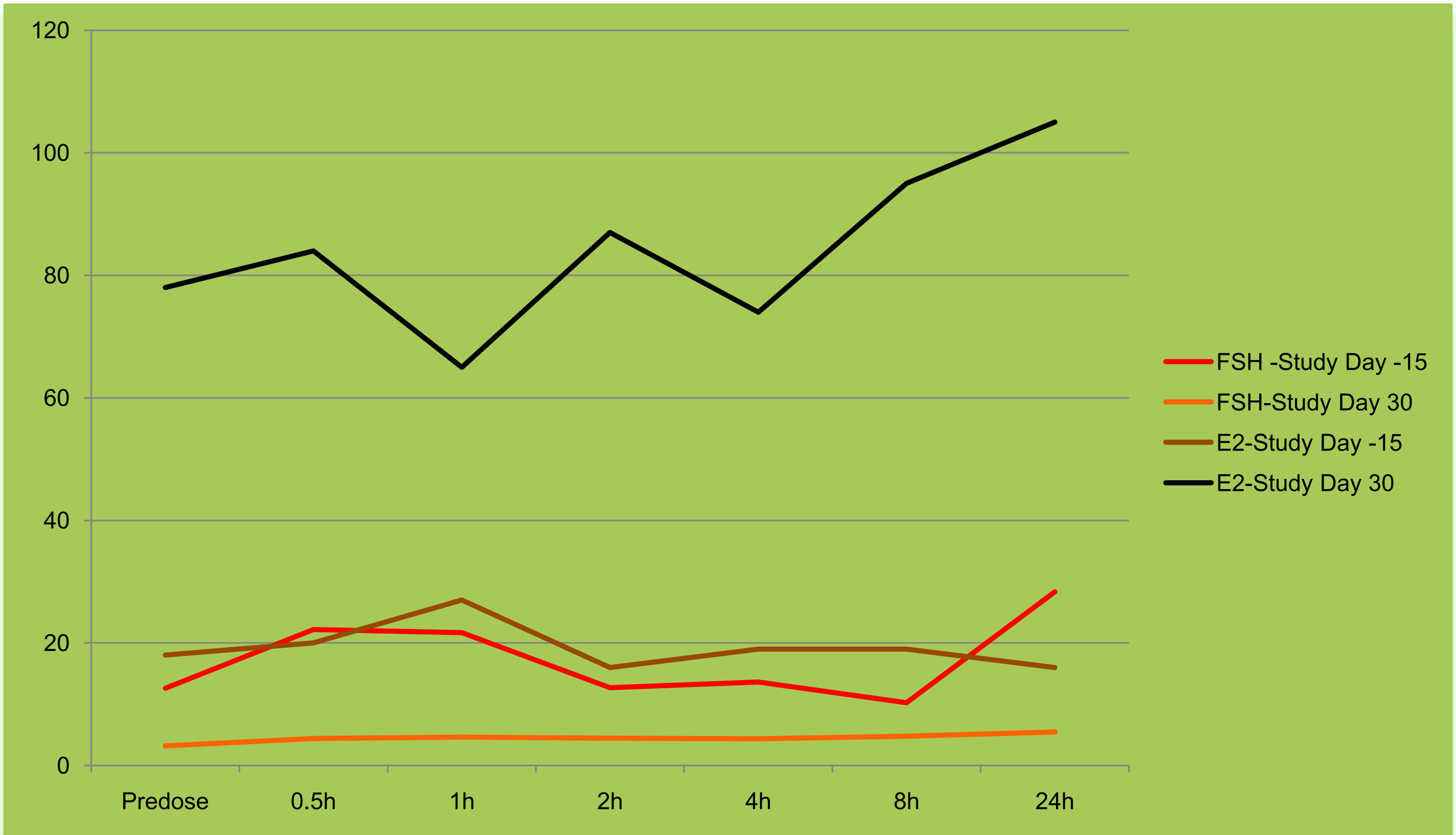
Serum FSH levels in female control dogs
Range 1.84 – 28.34 (ng/mL)



Serum 17-beta estradiol levels in female control dogs
Range 11-31 (pg/mL)



Serum FSH and estradiol levels in a control Beagle dog (Dog No. 1)
Important to correlate hormone levels with stage of estrous!



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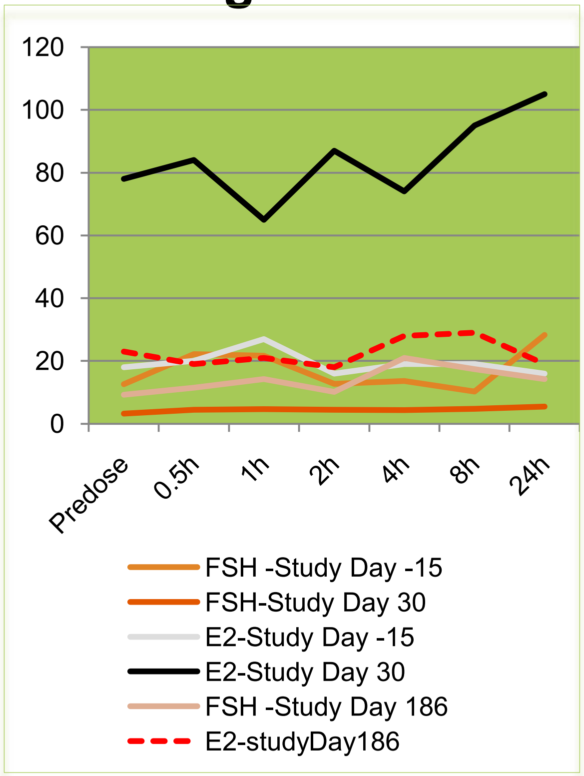
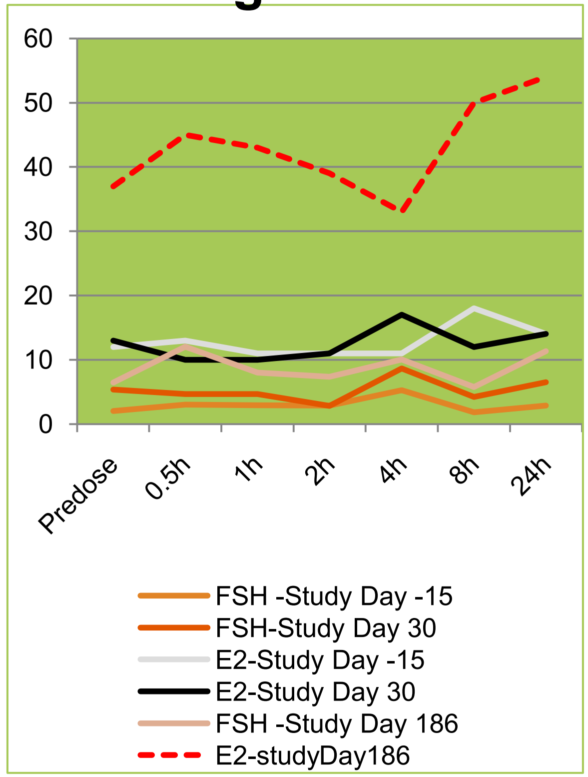
Relationship between organ weights and stage of estrous cycle.

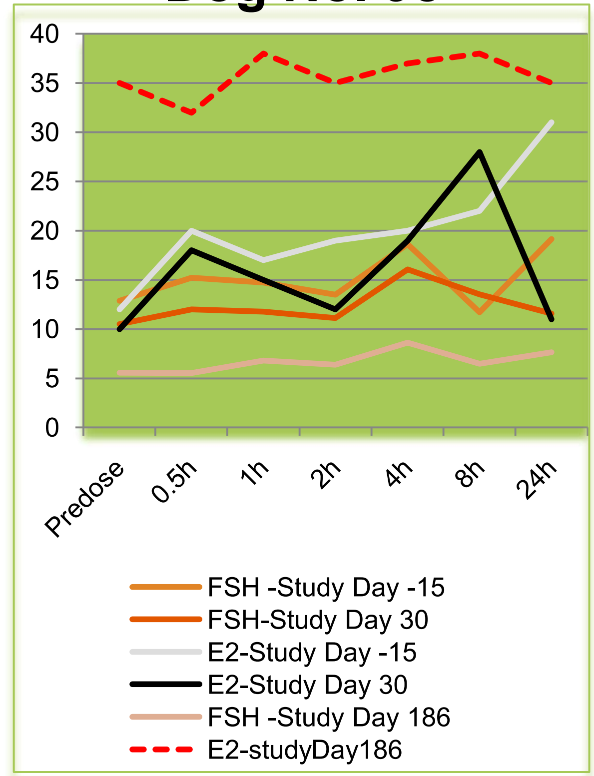
Dog No. 95



Dog No. 97



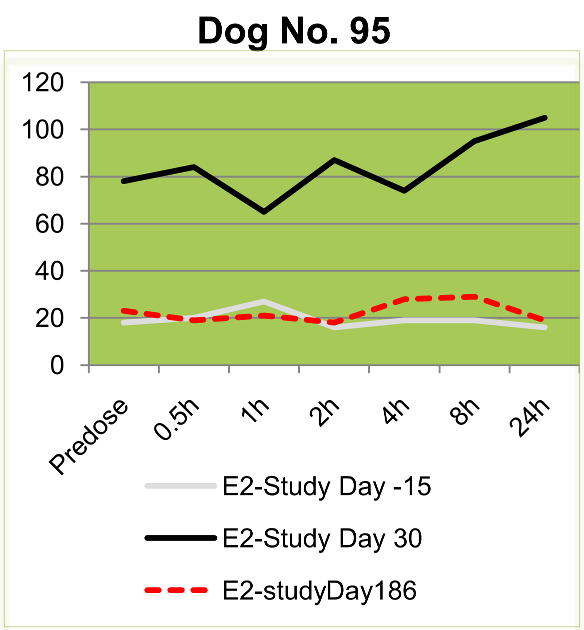
Dog No. 98

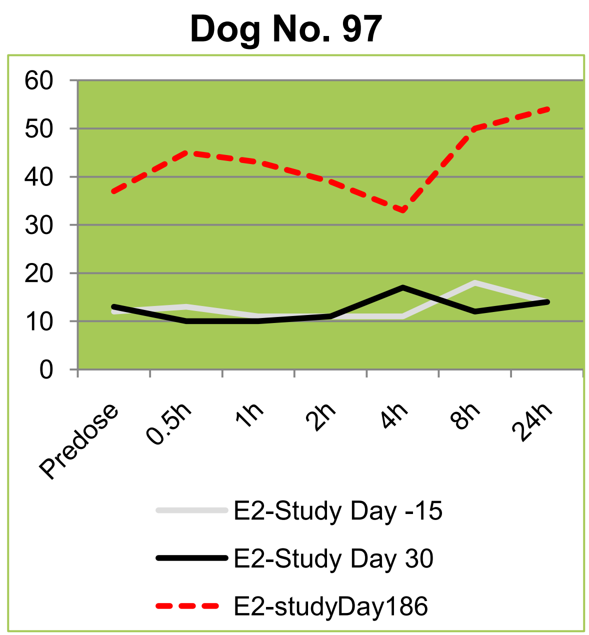


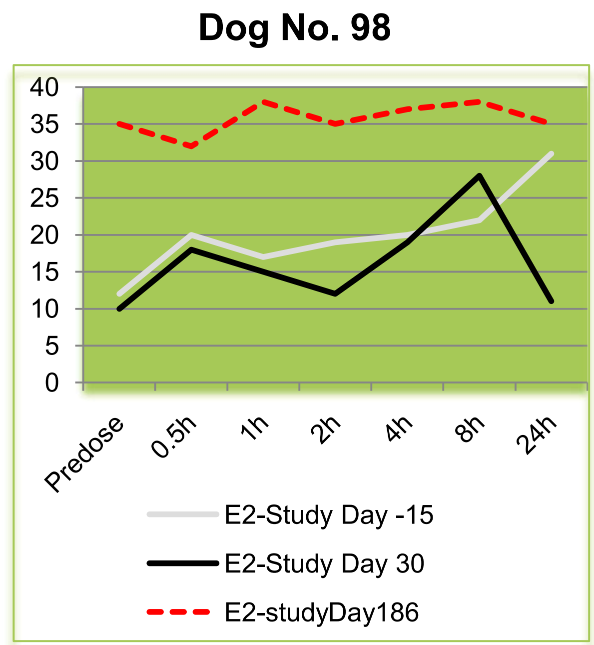
Organ weights - Study Day 186	Dog No. 95	Dog No. 97	Dog No. 98
Ovary (g)	0.88	1.58	1.818
Uterus (g)	3.06	12.97	21.35

GSK GlaxoSmithKline

Relationship between organ weights and stage of estrous cycle.



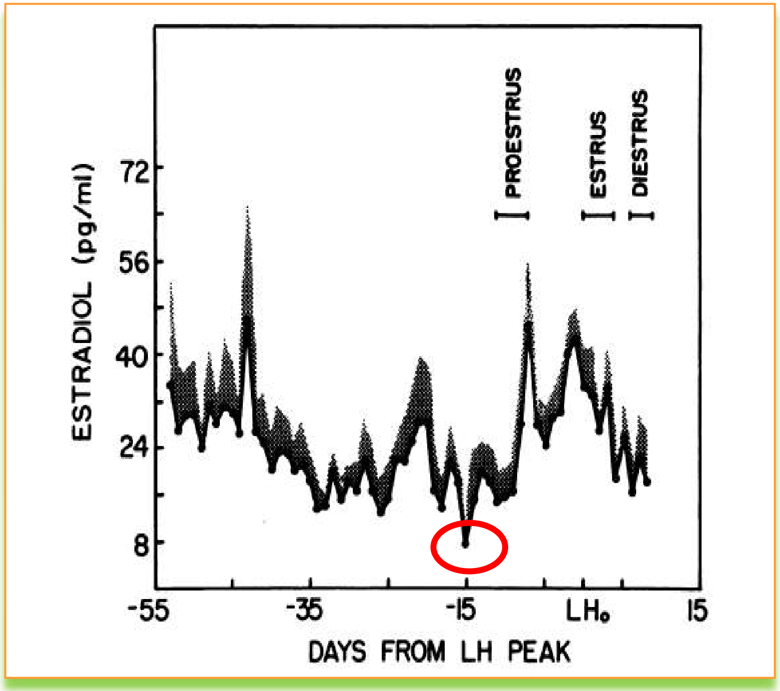


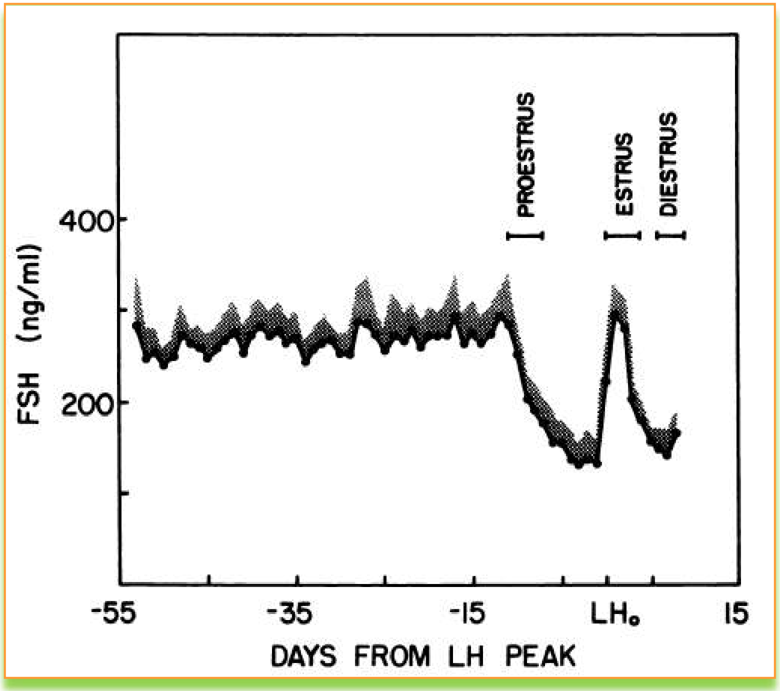


Organ weights - Study Day 186	Dog No. 95	Dog No. 97	Dog No. 98
Ovary (g)	0.88	1.58	1.818
Uterus (g)	3.06	12.97	21.35

Estradiol (AUC)	Dog No. 95	Dog No. 97	Dog No. 98
Day -15	434	359	339
Day 30	2253	315	466
Day 186	584	1154	877

Estradiol and FSH





Lowest (7.9 pg.) E2 concentration is 15 days prior to LH peak reaching 44.8 pg. in proestrus.

BIOLOGY OF REPRODUCTION 27, 1196–1206 (1982)

Concentrations of Reproductive Hormones in Canine Serum Throughout Late Anestrus, Proestrus and Estrus[1]

P. N. OLSON, R. A. BOWEN, M. D. BEHRENDT, J. D. OLSON and T. M. NETT[2]

Histopathology vs. hormone data

- Histology is more likely to detect a change in reproductive function than hormone measurement (subject to diurnal, cyclical and stress induced changes).
- Always correlate stage of cycle for each animal.
  - Do the vagina, cervix, uterus, ovary and mammary gland correspond? Consider them as a unit.
- Understand and recognize normal histology.
- Morphological changes can be easier to identify.

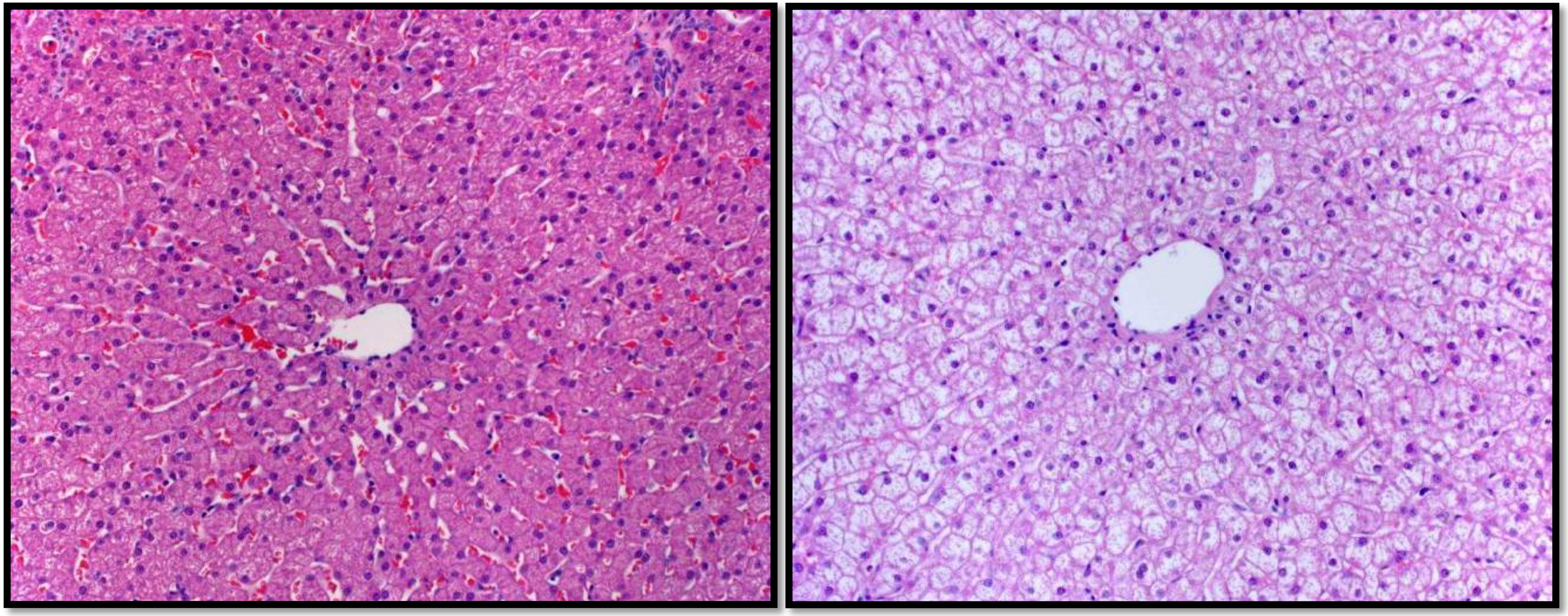
Points to consider

- Regardless of mechanism, chemically induced toxicity in the female reproductive tract generally causes hormonal imbalance
- The pattern of histopathological change in the ovaries, uterus and vagina reflects the disturbed hormonal profile and the tract needs to be examined <u>as a unit</u>
- Cyclicity, sampling consistency, immaturity are confounding factors for identifying changes.

The female Beagle in diestrus

Control female dog livers from the same study

(euthanized, processed, sectioned, & stained identically!)



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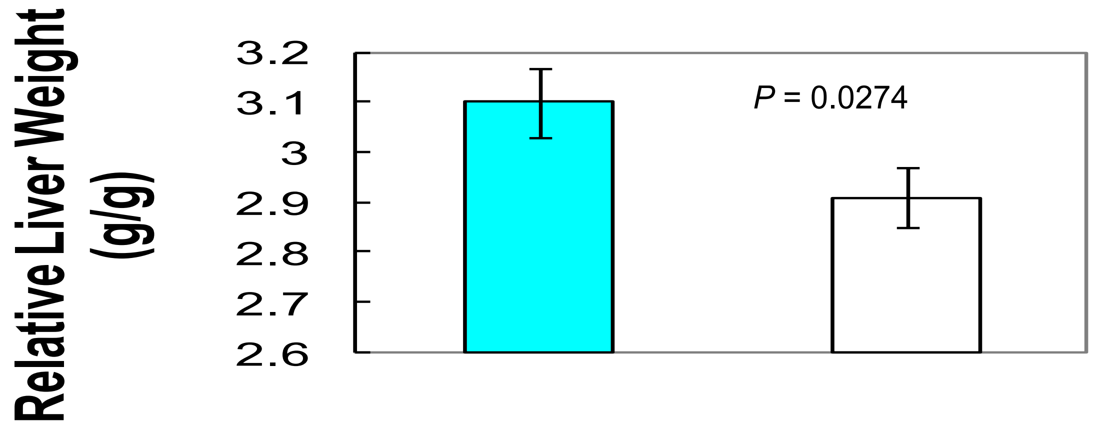
Liver weights



Relative liver weight (%) from control dogs in diestrus and in all other stages of the estrous cycle.

Dogs in diestrus vs. all other stages

Parameter and reference range	***Diestrus***		***All other stages***		***P-values for t-tests***	***% change in diestrus***
Cholesterol (mmol/L; 2.89-4.98)	**5.71**	**0.23***	4.23	0.11	*<0.0001 (<0.0001)*	***+ 35.0%***
Eosinophils (x10^9/L; 0.09-0.57)	**0.35**	**0.03**	0.24	0.02	*0.0035 (0.0532)*	***+ 45.8%***

Chemistry and hematology parameters that are elevated in beagle dogs during diestrus. Means ± SE's, and *P*-values for the significant differences of the means are shown. *P*-values after Hochberg multiplicity adjustment shown in parentheses. *Cholesterol was the only parameter outside the reference range for Beagle bitches.

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Dogs in diestrus vs. all other stages

Parameter and reference range	***Diestrus***	***All other stages***	***P-values for t-tests***	***% change in diestrus***
AST (U/L; 19-50)	28.6 1.0	33.3 0.9	*0.0011 (0.0190)*	***- 14.0%***
Chloride (mmol/L; 109-116)	111.2 0.3	112.2 0.3	*0.0224 (0.3800)*	***- 0.9 %***
Hemoglobin (g/L; 148-191)	158.4 1.9	171.7 1.9	*<0.0001 (<0.0001)*	***- 7.8%***
Hematocrit (%; 42-57)	46.1 0.5	49.9 0.6	*<0.0001 (<0.0001)*	***- 7.6%***
RBC (x10^12/L; 6.17-8.28)	6.78 0.07	7.35 0.08	*<0.0001 (<0.0001)*	***- 7.8%***

Chemistry and hematology parameters that are decreased in beagle dogs during diestrus. Means ± SE's, and *P*-values for the significant differences of the means are shown. *P*-values after Hochberg multiplicity adjustment shown in parentheses.

Effect of Estrous Cycle Phase on Clinical Pathology Parameters in Beagle Dogs

Cynthia J. Willson[1,2], Sundeep A. Chandra[2], Carie L. Kimbrough[2] and Holly L. Jordan[2]
[1]North Carolina State University College of Veterinary Medicine, Raleigh, NC, USA; [2]Safety Assessment, GlaxoSmithKline, RTP, NC, USA

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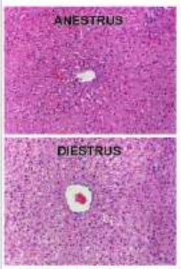
Abstract

The duration of diestrus in dogs is considerably longer (about 75 days) than in most species and is characterized by high circulating progesterone and growth hormone levels that may influence clinical pathology parameters. The influence of the phase of the reproductive cycle on clinical pathology parameters has been reported in many species, but there is a relative paucity of information in the dog. Such differences may potentially confound interpretation of data in toxicity studies, which often have small group sizes. The objective of this retrospective study was to investigate differences in clinical pathology data in dogs in diestrus relative to dogs in other phases of the estrous cycle. For 86 healthy control females from 23 toxicity studies (age range: 11-22.5 months), estrous cycle stage was determined by histological examination of reproductive tissues. Serum chemistry, hematology, and urinalysis parameters were compared using two-tailed t-tests. For Beagles in diestrus (n = 38), serum cholesterol was 35.0% higher ($P < 0.0001$), AST was 14.0% lower ($P = 0.0011$), chloride was 0.9% lower ($P = 0.0224$), eosinophils were 45.8% higher ($P = 0.0035$), hemoglobin and red blood cell count were 7.8% lower ($P < 0.0001$), and hematocrit was 7.6% lower ($P < 0.0001$) than dogs in all other stages combined (anestrus, proestrus, estrus, and immature; n = 48). Urine parameters did not differ significantly between the two groups. Interpretation of clinical pathology data in female dogs should be performed with due consideration given to the stage of the estrous cycle.

Introduction



- Toxicity studies typically contain small numbers of dogs (3-5 per group), each of which may differ in the stage of the estrous cycle.
- We have observed that livers from control (non-drug-treated) Beagle dogs during diestrus (luteal, or post-ovulatory phase) have a rarefied appearance (*left, lower*) compared to livers from control dogs in anestrus (*left, top*). The changes are similar to the vacuolar change and hepatocyte swelling seen with glycogen accumulation from steroid hepatopathy in dogs.
- Diestrus is unique in the dog because serum progesterone (P4) levels peak and remain elevated, whether the bitch is pregnant or not, for ~75 days.
- During diestrus, mammary glands produce growth hormone and pseudopregnancy occurs ± mammary gland secretory activity and ± nesting behavior.
- OBJECTIVE: **The objective of this retrospective study was to investigate differences in clinical pathology data (chemistry, hematology, and urinalysis) and liver weight in purpose-bred Beagle dogs in diestrus relative to dogs in other phases of the estrous cycle.**
- SIGNIFICANCE: Differences in clinical pathology parameters, in addition to liver changes, in dogs in different stages of the estrous cycle may be important for interpretation of drug or toxicity studies.

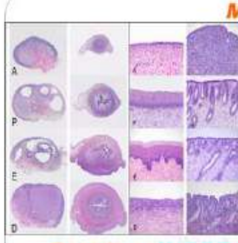
Methods

Study animals: Retrospective study using non-drug-treated control dogs (3-5 per study) from 23 toxicity studies (2002 – 2008). Studies were conducted in accordance with current guidelines for animal welfare and internal IACUC guidelines.

Samples: At the end of the study (2 or 4 weeks duration), urine and blood were collected for measurement of routine clinical chemistry (Olympus AU640e, Melville, NY, USA) profile and hematology (Advia 2120, Siemens, Norwood, MA, USA). Age range: 11 - 22.5 months (average 14.38 months). Weight range: 5.05 – 10.13 kg (average 6.87 kg).

Statistical analyses: There were no significant differences in parameters among anestrus, immature, proestrus, and estrus, so these were combined. Means for dogs in diestrus vs. all other stages were compared using two-tailed t-tests (Proc T-TEST) corrected for multiple comparisons using SAS v. 9.1 (Cary, NC, USA). P-values both with and without Hochberg multiplicity adjustment are shown.

Methods



Stage of estrous cycle was determined by histological examination of H&E-stained sections of ovaries, uterine horns, vagina, cervix, and mammary tissue (*Chandra & Adler 2008*) for 86 control dogs from 23 studies. Each study included ≥ 1 female in the control group in diestrus at necropsy. 38 dogs were classified as being in diestrus. 48 dogs were classified as all other stages other than diestrus (34 in anestrus, 7 were immature, 4 in estrus, and 3 in proestrus).

Results: INCREASED during DIESTRUS

Parameter and reference range	Diestrus	All other stages	P-values for t-tests	% change in diestrus
Cholesterol (mmol/L; 2.89-4.98)	**5.71** ± 0.23*	4.23 ± 0.11	<0.0001 (<0.0001)	+ 35.0%
Eosinophils (x10^9/L; 0.09-0.57)	0.35 ± 0.03	0.24 ± 0.02	0.0035 (0.0532)	+ 45.8%

Chemistry and hematology parameters that are elevated in beagle dogs during diestrus. Means ± SE's, and P-values for the significant differences of the means are shown. P-values after Hochberg multiplicity adjustment shown in parentheses. *Cholesterol was the only parameter outside the reference range for Beagle bitches.

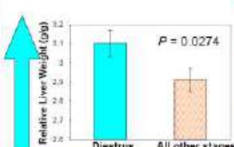
Cholesterol

- Cholesterol peaks at 5-7 weeks after estrus (therefore during diestrus) in Beagle bitches (*Tarumi et al. 1988*).
- Increased cholesterol may be directly related to P4, as both peak during diestrus.

Eosinophils

- Eosinophils migrate into the submucosa of the GI tract, mammary gland, thymus, and uterus (*Rothenberg and Hogan 2006*).
- In tissues, eosinophils express growth factors (TGFα, TGFβ, and EGF).
- During estrus, eosinophils infiltrate uterine tissue and reach peak numbers in uterus (humans, rats, mice). During diestrus, eosinophils degranulate & ↓ in numbers in the uterus.
- Eosinophilia is a feature of hypoadrenocorticism/ hypoaldosteronism.
- Perhaps the increased peripheral blood eosinophils during diestrus is related to continued production and/or prolonged survival in blood but lack of migration into tissues, or due to P4 interference with aldosterone.

Results: INCREASED during DIESTRUS



Relative liver weight (liver weight: body weight) from dogs in diestrus and in all other stages of the estrous cycle.

Relative liver weight increased during diestrus in beagle dogs.

- OVX'd Beagles given the progestin medroxyprogesterone acetate develop steroid-induced hepatopathy with large, glycogen-rich hepatocytes (*Selman et al. 1995*).
- Estrogen &/or P4 may be binding to glucocorticoid receptors in the liver or interacting with glucocorticoids to ↑ liver glycogen deposition and therefore ↑ liver mass.

Results: DECREASED during DIESTRUS

Parameter and reference range	Diestrus	All other stages	P-values for t-tests	% change in diestrus
AST (U/L; 19-50)	28.6 ± 1.0	33.3 ± 0.9	0.0011 (0.0190)	- 14.0%
Chloride (mmol/L; 109-116)	111.2 ± 0.3	112.2 ± 0.3	0.0224 (0.3800)	- 0.9 %
Hemoglobin (g/L; 148-191)	158.4 ± 1.9	171.7 ± 1.9	<0.0001 (<0.0001)	- 7.8%
Hematocrit (%; 42-57)	46.1 ± 0.5	49.9 ± 0.6	<0.0001 (<0.0001)	- 7.6%
RBC (x10^12/L; 6.17-8.28)	6.78 ± 0.07	7.35 ± 0.08	<0.0001 (<0.0001)	- 7.8%

Chemistry and hematology parameters that are decreased in beagle dogs during diestrus. Means ± SE's, and P-values for the significant differences of the means are shown. P-values after Hochberg multiplicity adjustment shown in parentheses.

Chloride

- Progesterone (P4) is known to have anti-aldosterone effects in humans and rats
- P4 is a competitive inhibitor of aldosterone receptor → Less K^+ secretion → Less reabsorption of Na^+ & Cl^-.
- Women also have been shown to have ↓ serum Na^+ & Cl^- mid-luteal phase compared to the mid-follicular phase (*Chapman et al. 1997*).

Red blood cell parameters

- In a study of several breeds of dogs, hematocrit ↓ from **46%** at pro-estrus to:
  - **40%** at day 60 of diestrus in non-pregnant dogs, or to
  - **35%** at day 60 in pregnant dogs (*Günzel-Apel et al. 1997*).
  - Perhaps this is due to increased plasma volume, as dogs are pseudopregnant during diestrus.

Summary and Conclusion

Compared to dogs in all other estrous cycle stages, dogs in diestrus had:

- No difference in urinary clinical pathology parameters (data not shown)
- *INCREASED*:
  - 35% ↑ serum cholesterol
  - 46% ↑ blood eosinophils
  - 7% ↑ relative liver weight
- *DECREASED*:
  - 14% ↓ AST
  - 1% ↓ chloride
  - 8% ↓ red blood cell parameters

Stage of estrous cycle should be considered during toxicity studies using dogs, especially for hormonally or metabolically active compounds or compounds affecting glycogen modulation, cholesterol, eosinophils, or red blood cells.

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Mammary Gland

Toxicologic Pathology, 000: 1-15, 2010

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Cyclic Morphological Changes in the Beagle Mammary Gland

Sundeep A. Chandra[1], J. Mark Cline[2], and Rick R. Adler[1]

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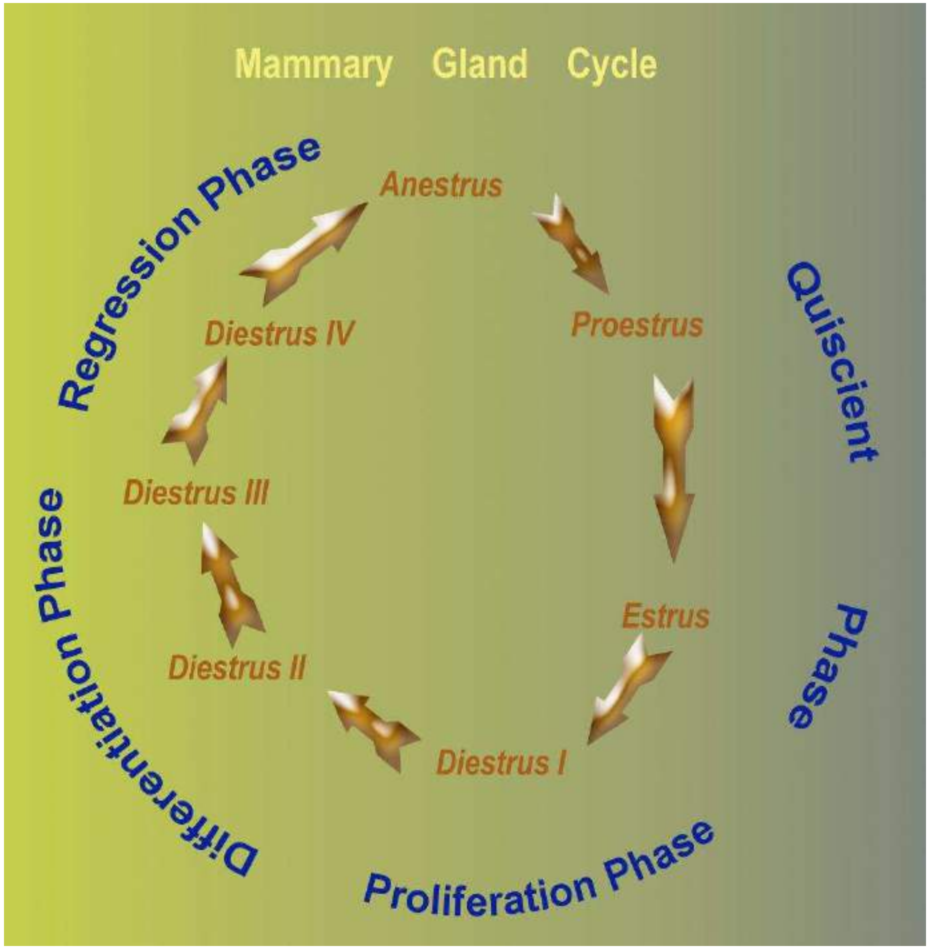
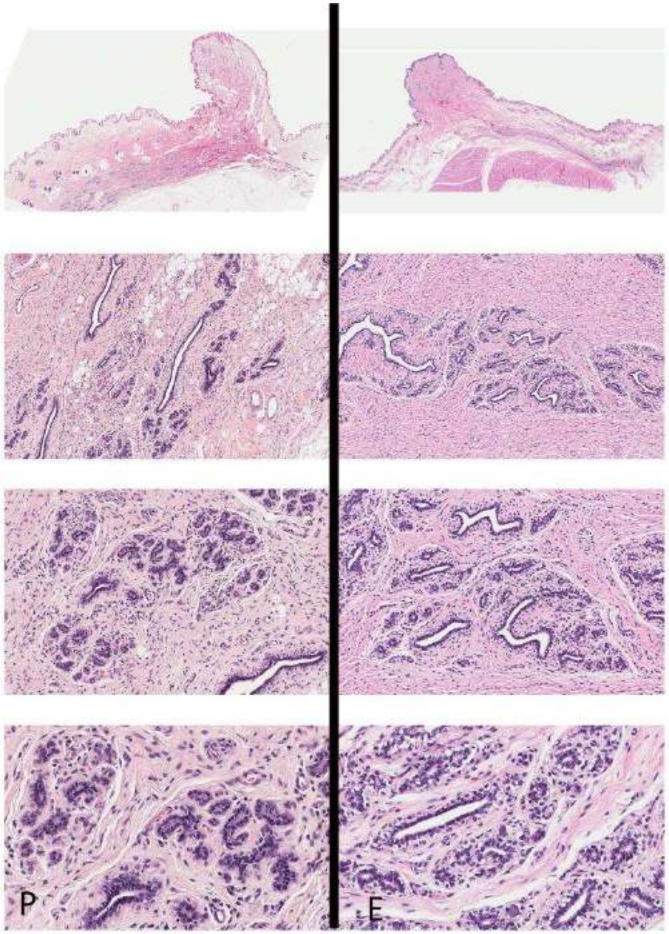
[2] *Wake Forest University School of Medicine, Winston-Salem, NC 27157-1040*

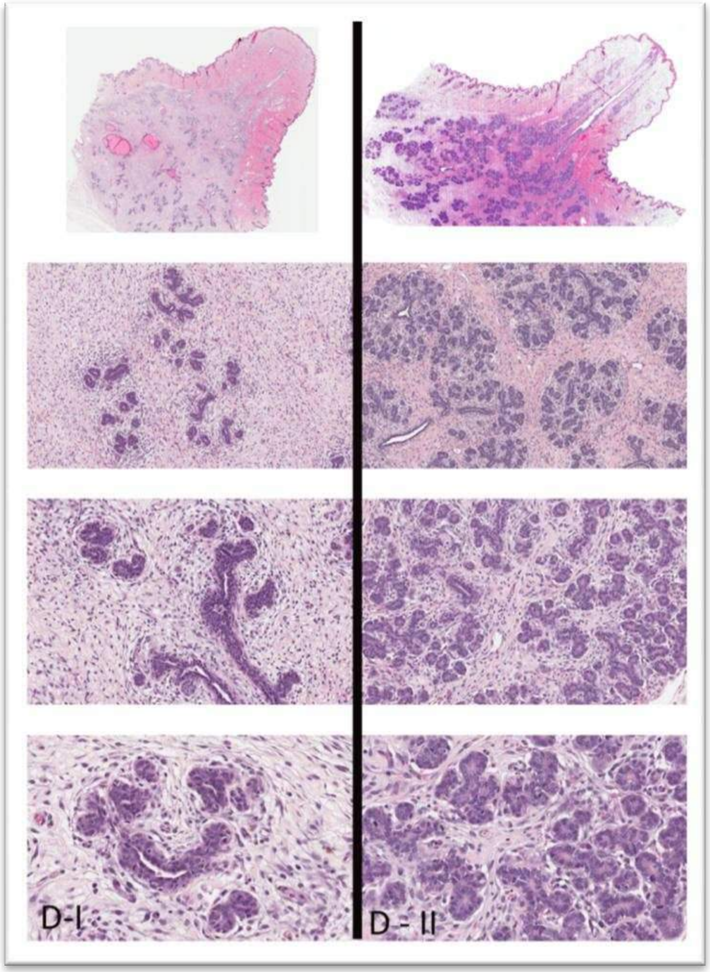
Mammary gland

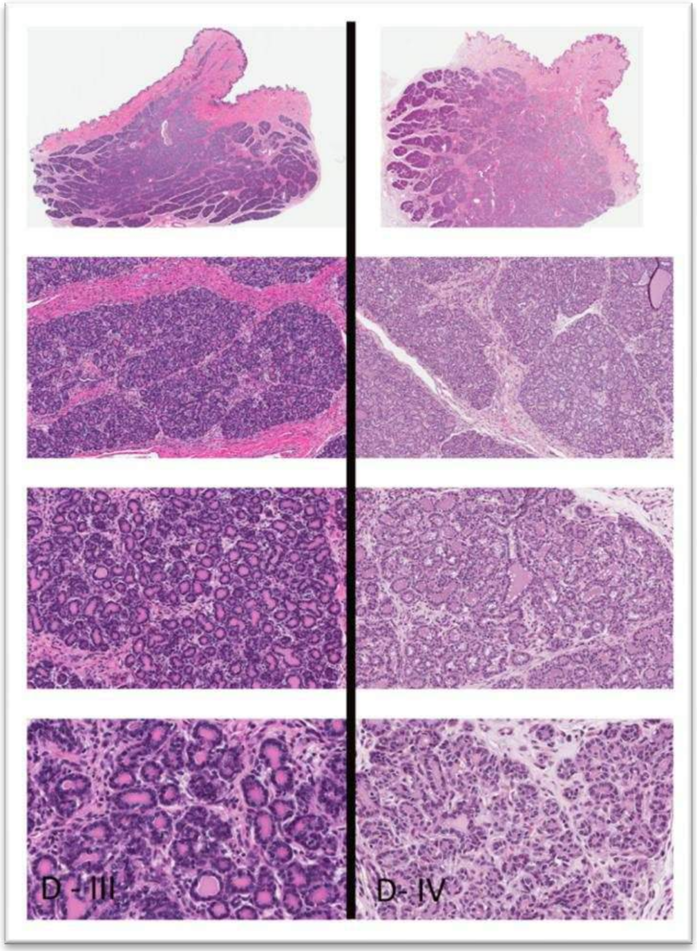


Mammary gland in Proestrus and Estrus

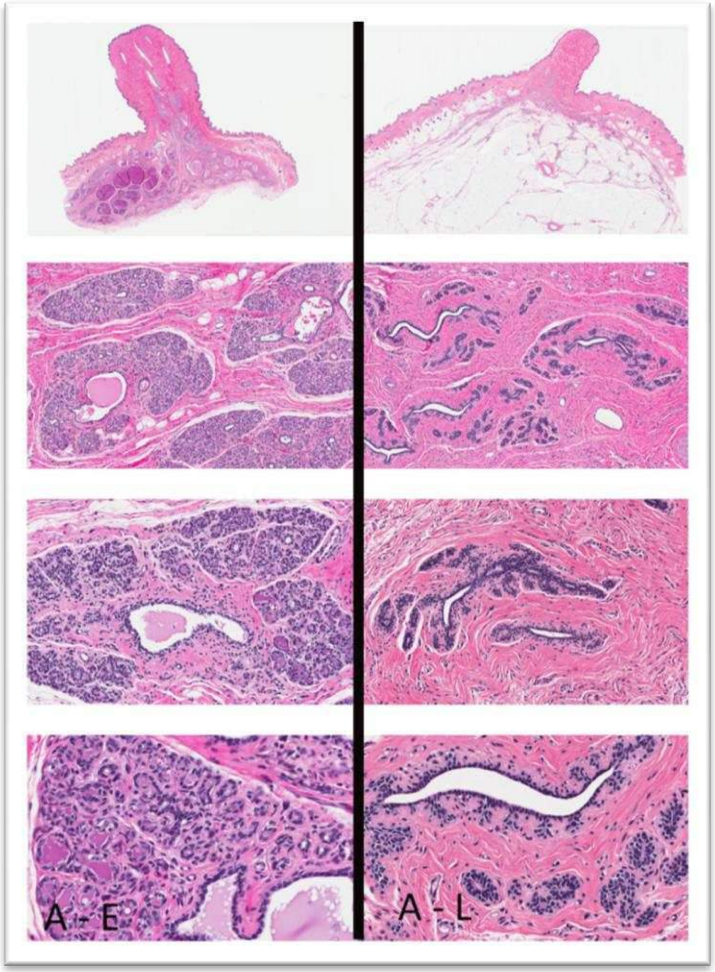


Mammary gland in Diestrus (I-IV)





Mammary gland in Anestrus (early and late)

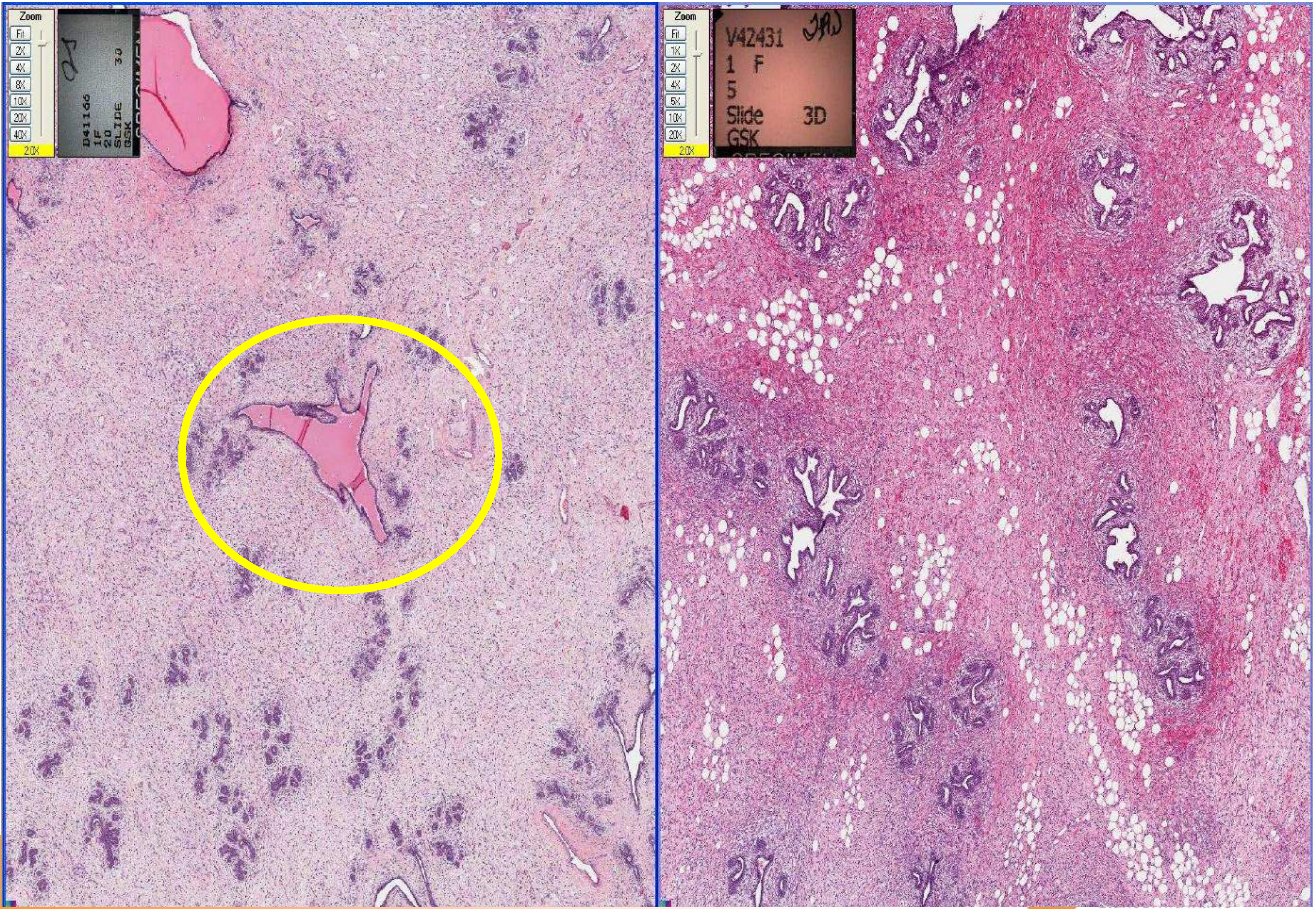


GSK GlaxoSmithKline

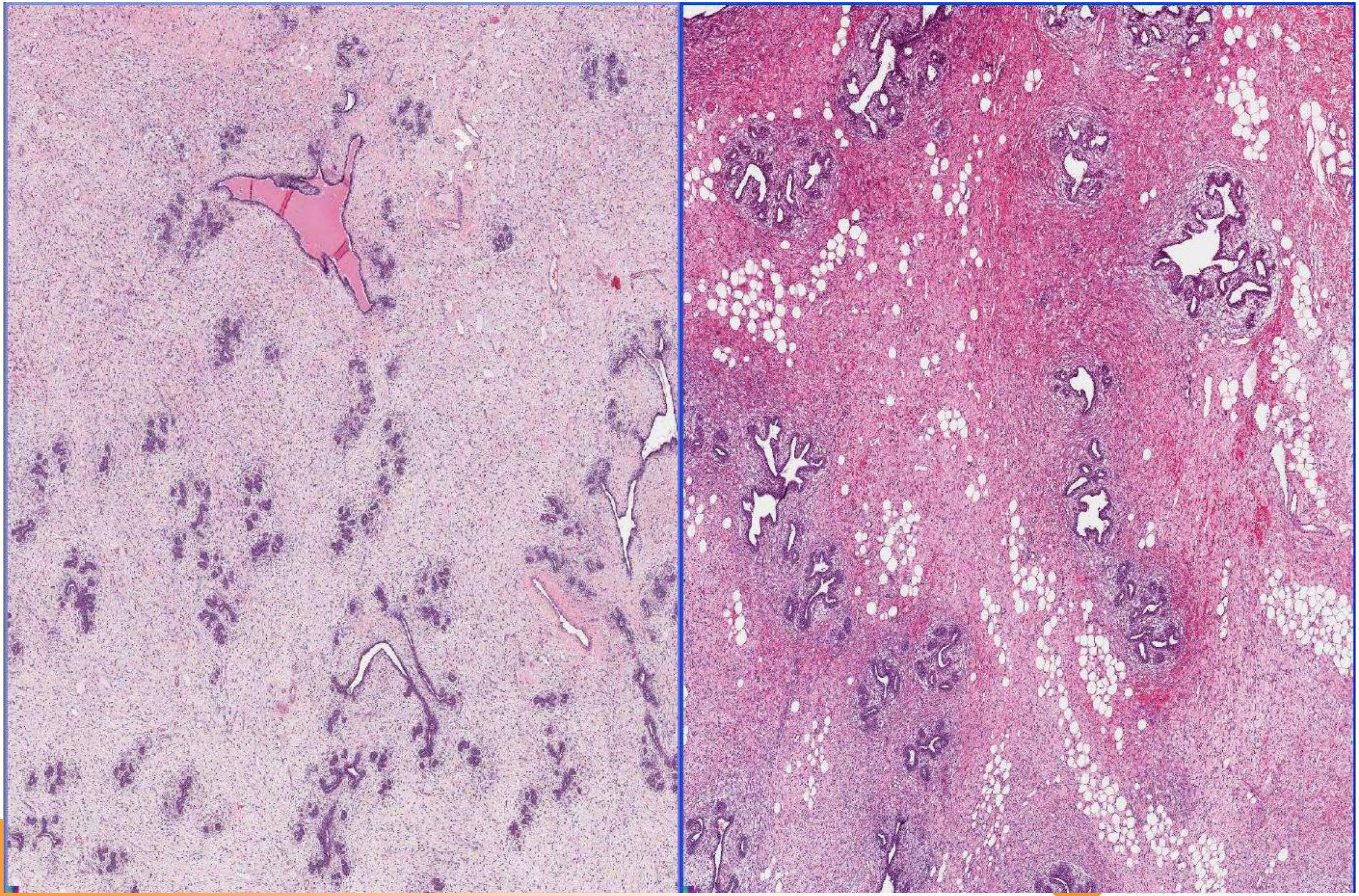
Mammary Gland

- Comparison of glandular changes noted in Diestrus I with that noted in the glands of dogs entering the first estrus cycle
- Morphologically very similar

Diestrus I vs. First Estrus



Diestrus I vs. First Estrus



Sexual dimorphism of the mammary gland in rats

Toxicologic Pathology, 35:199–207, 2007

ISSN: 0192-6233 print / 1533-1601 online
DOI: 10.1080/01926230601156260

The Rat Mammary Gland: Morphologic Changes as an Indicator of Systemic Hormonal Perturbations Induced by Xenobiotics

JULIA N. LUCAS,[1] DANIEL G. RUDMANN,[2] KELLY M. CREDILLE,[2] ARMANDO R. IRIZARRY,[2] AUGUSTINE PETER,[1] AND PAUL W. SNYDER[1]

Toxicologic Pathology, 33:711–719, 2005

ISSN: 0192-6233 print / 1533-1601 online
DOI: 10.1080/01926230500343902

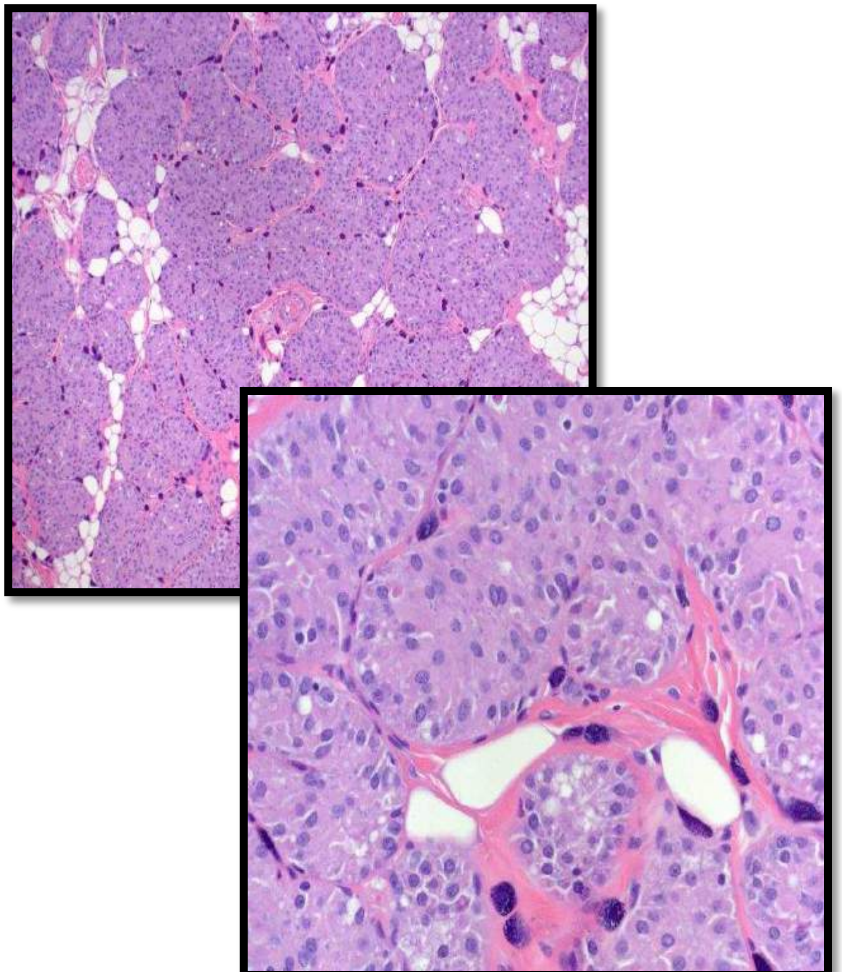
Androgen Dependent Mammary Gland Virilism in Rats Given the Selective Estrogen Receptor Modulator LY2066948 Hydrochloride

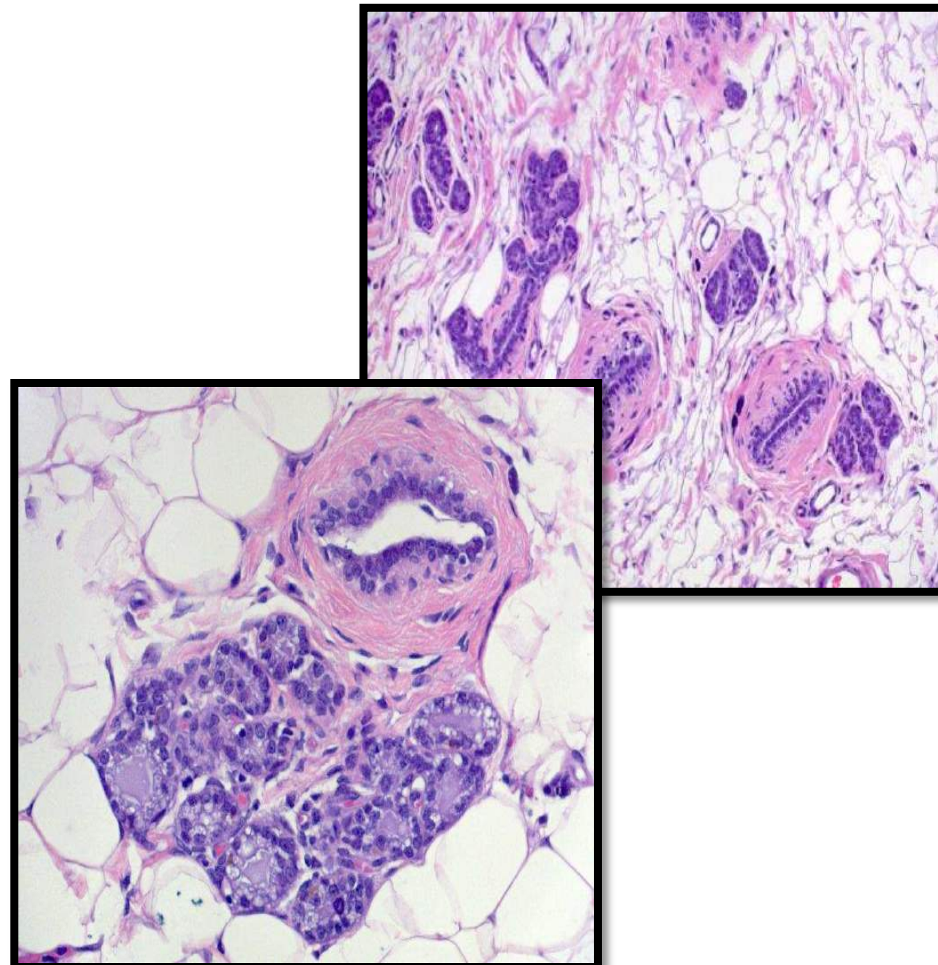
DANIEL G. RUDMANN,[1] ILENE R. COHEN,[2] MICHELLE R. ROBBINS,[2] DAVID E. COUTANT,[3] AND JUDITH W. HENCK[4]

TABLE 1.—Morphologic differences in mammary glands from sexually mature rats.

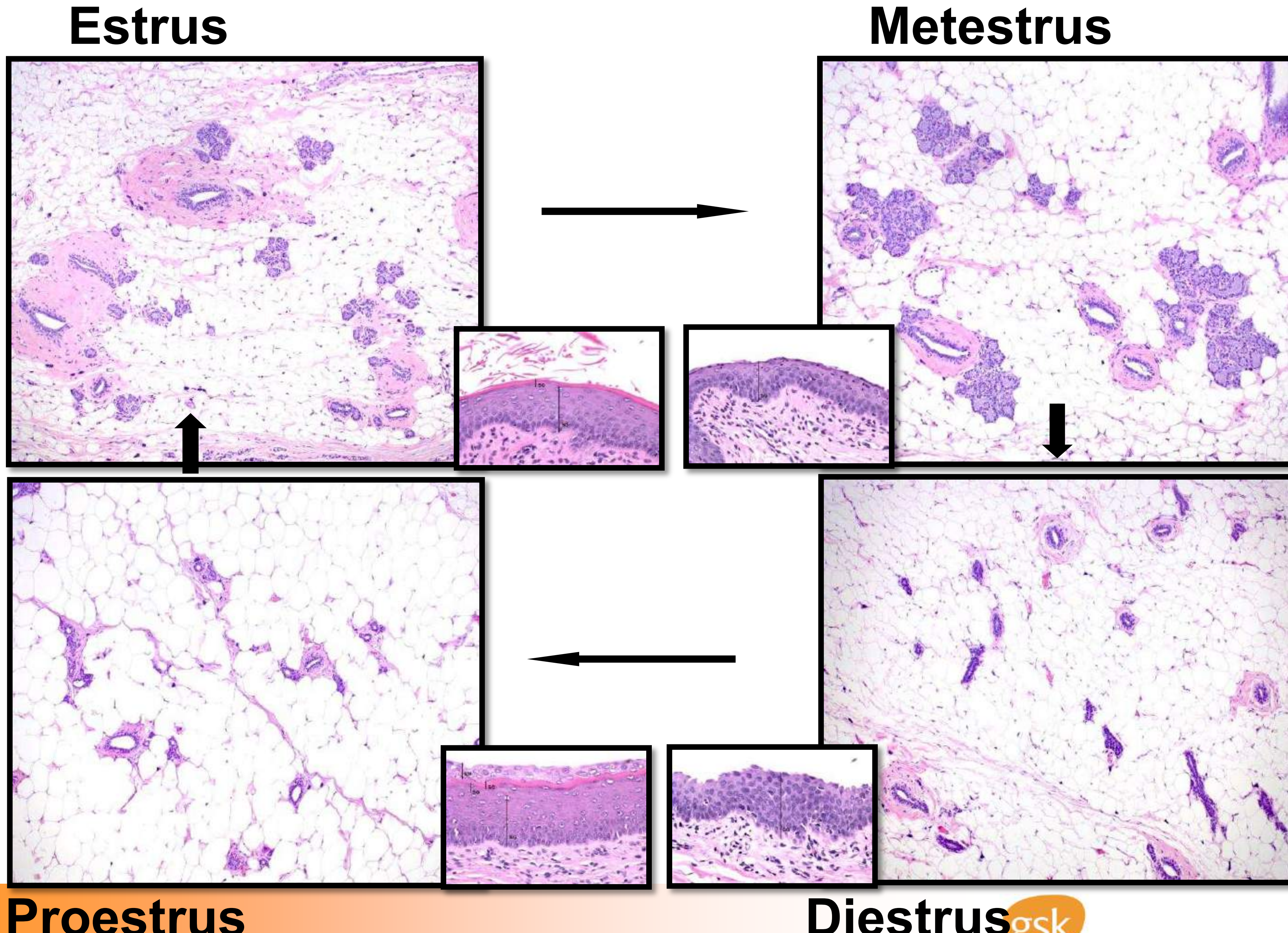
Morphology	Male rat	Female rat
Overall structure	Lobuloalveolar	Tubuloalveolar
Duct		
Number	Low	High
Epithelium	Pseudostratified or stratified cuboidal or short columnar	Simple cuboidal
Epithelial cytoplasm	Abundant, eosinophilic, vacuolated	Scant, basophilic, nonvacuolated
Epithelial apoptosis	Frequent	Rare
Luminal space	Not usually evident	Prominent
Alveolus		
Number	High, contiguous lobules	Low, centered on ductules
Epithelium	Pseudostratified or stratified cuboidal or short columnar	Simple cuboidal
Epithelial cytoplasm	Abundant, eosinophilic, vacuolated	Scant, basophilic, nonvacuolated
Epithelial apoptosis	Frequent	Rare
Luminal space	Not usually evident	Prominent

Normal male vs. Normal female





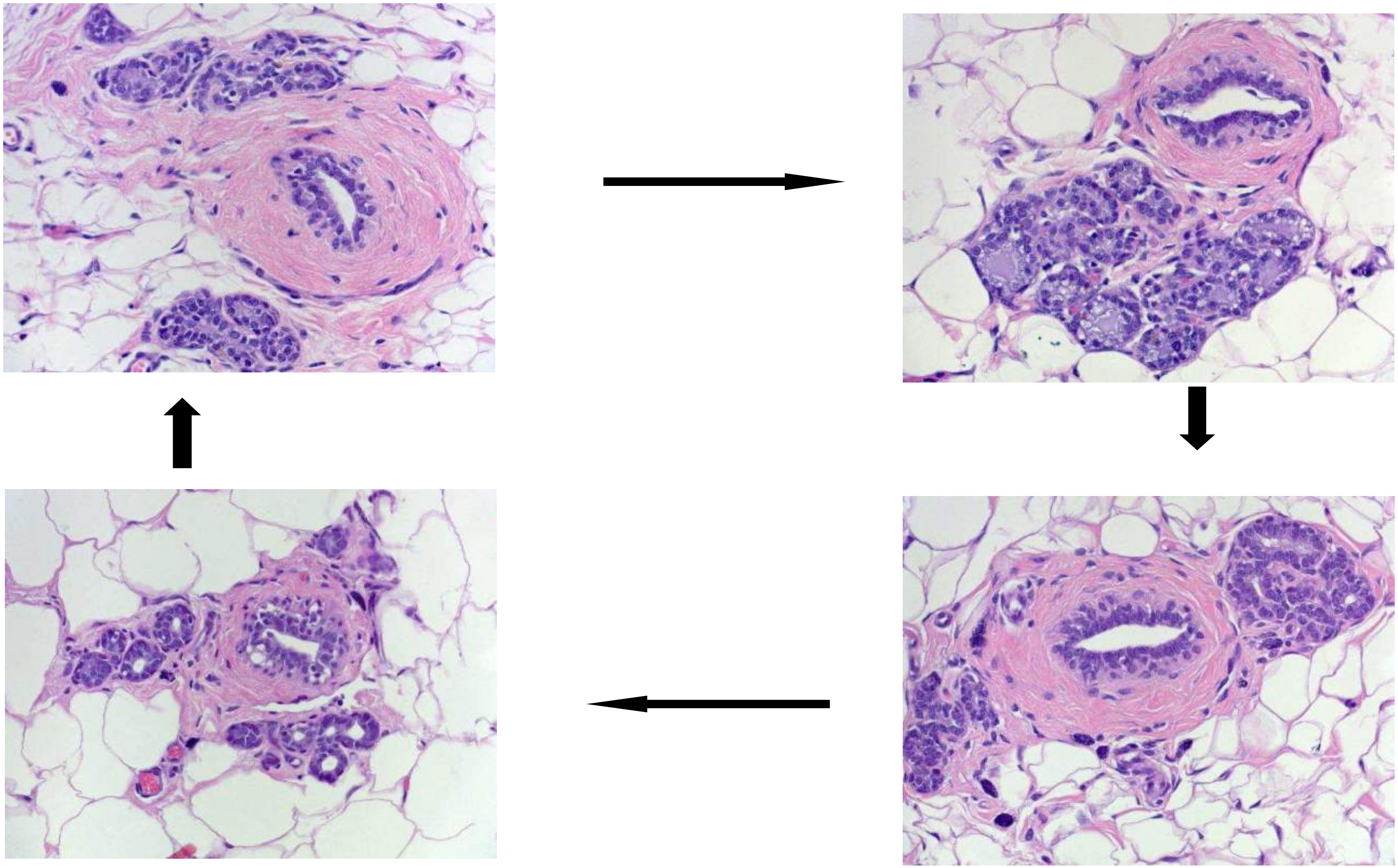
- Male - mostly lobules of alveoli.
- Female - predominance of ducts and few alveoli.



GlaxoSmithKline

Estrus

Metestrus

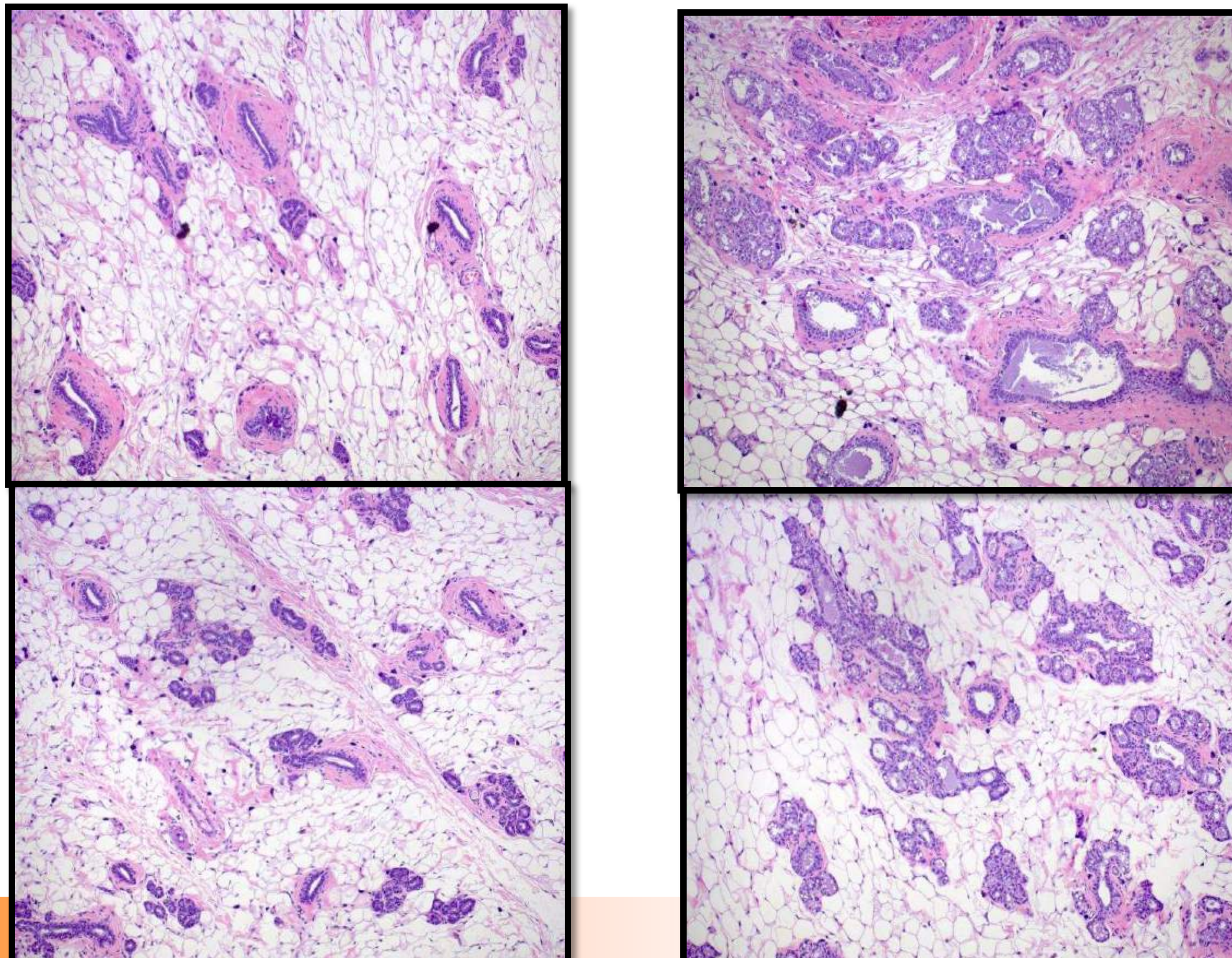


Proestrus

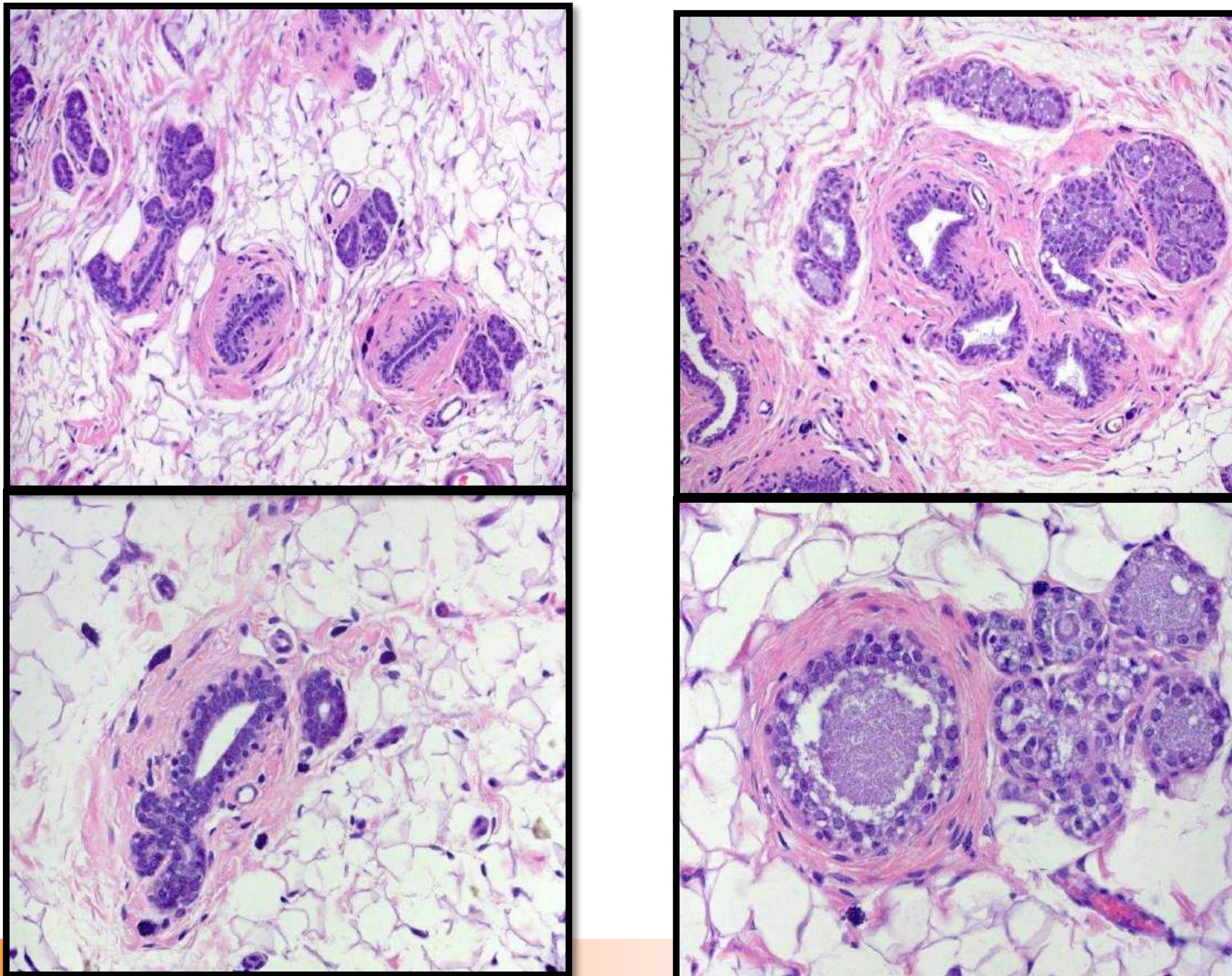
Diestrus

Normal and Virilization

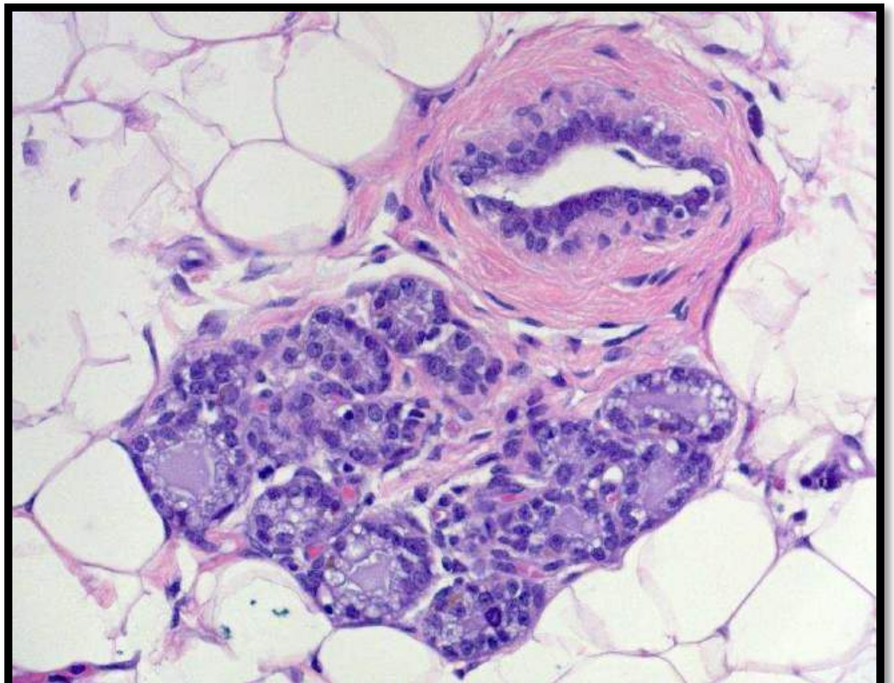
Virilization refers to the development of male sex characteristics in a female.

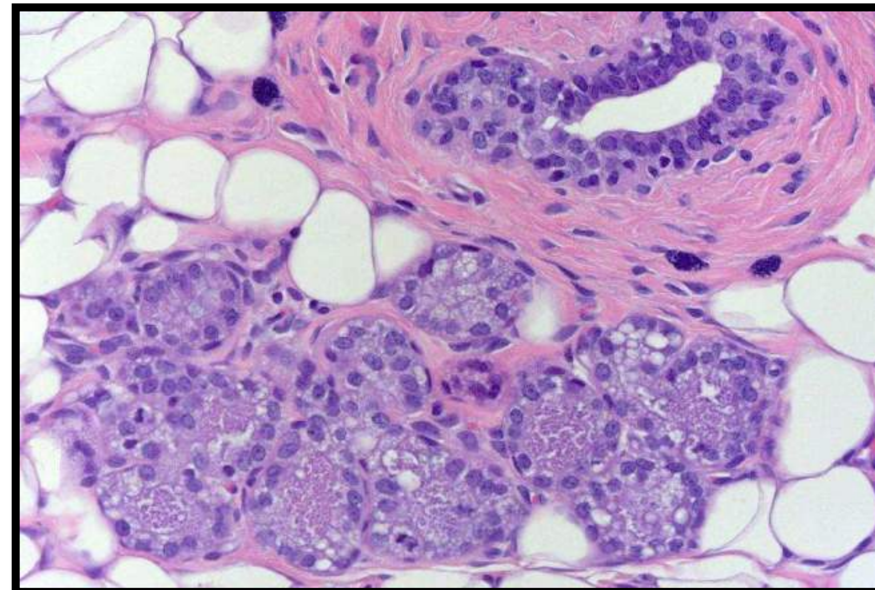


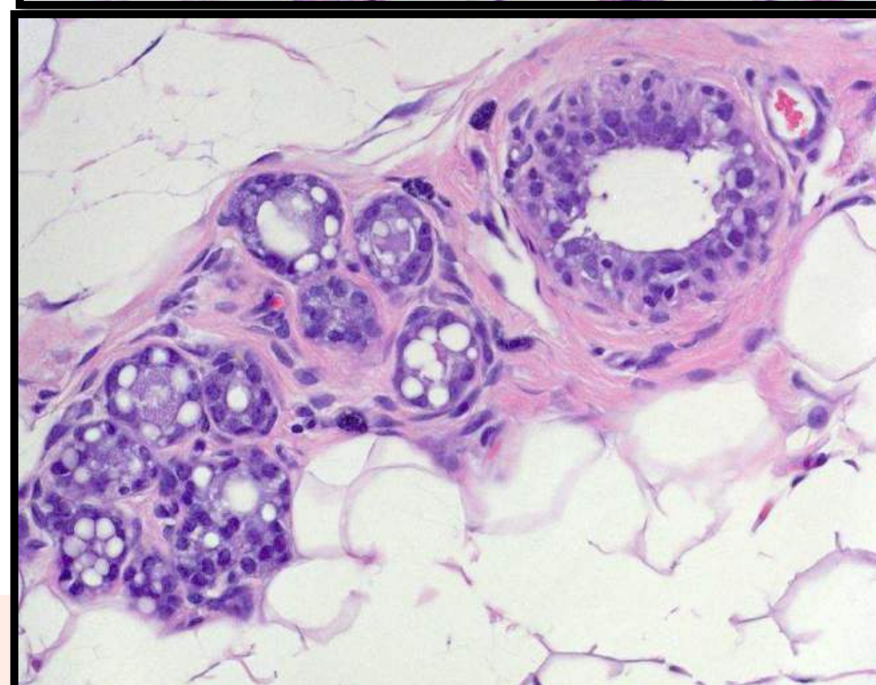
Normal and Virilization



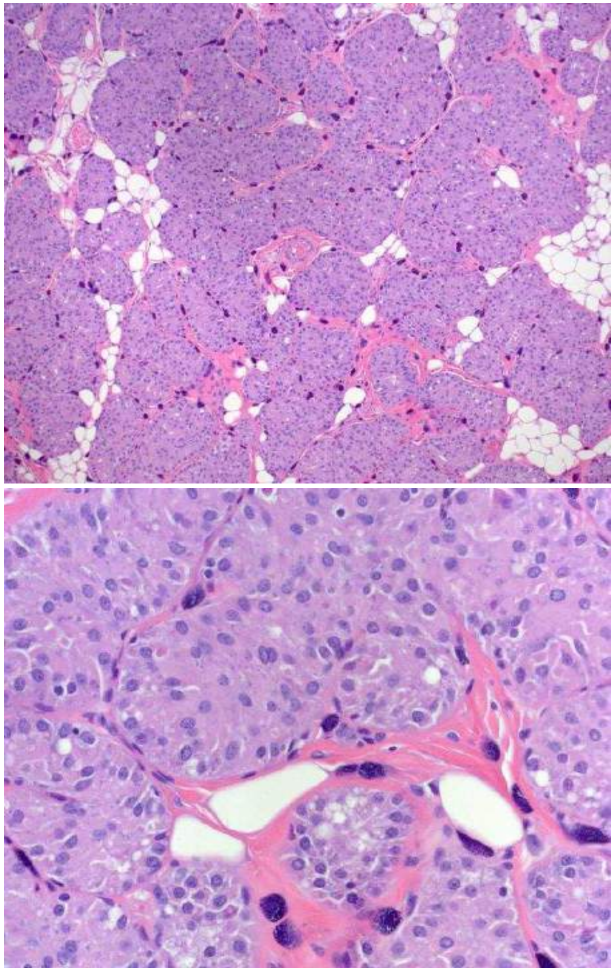
Metestrus vs Virilization

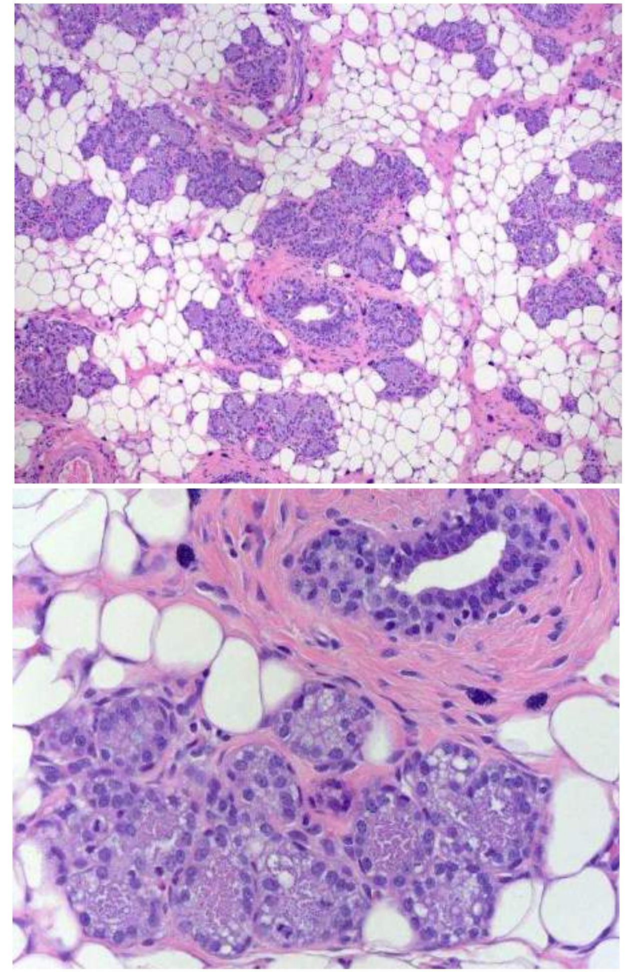




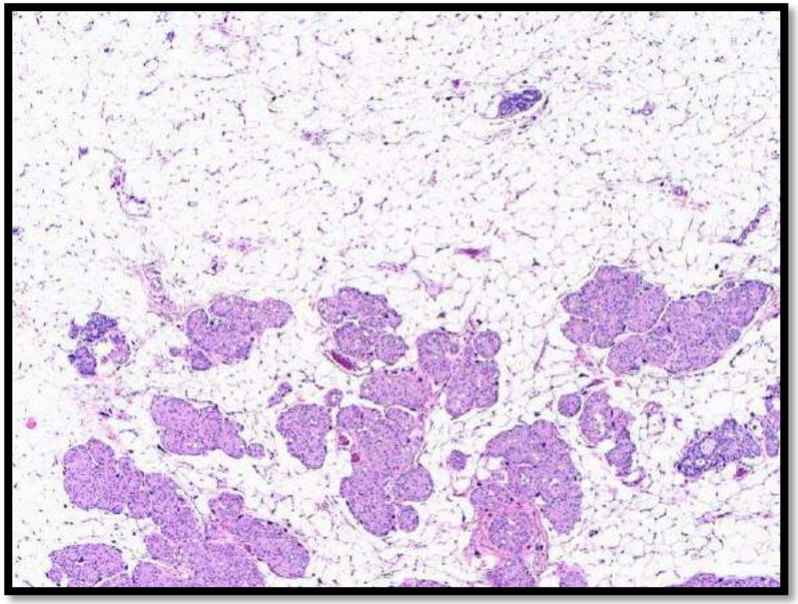


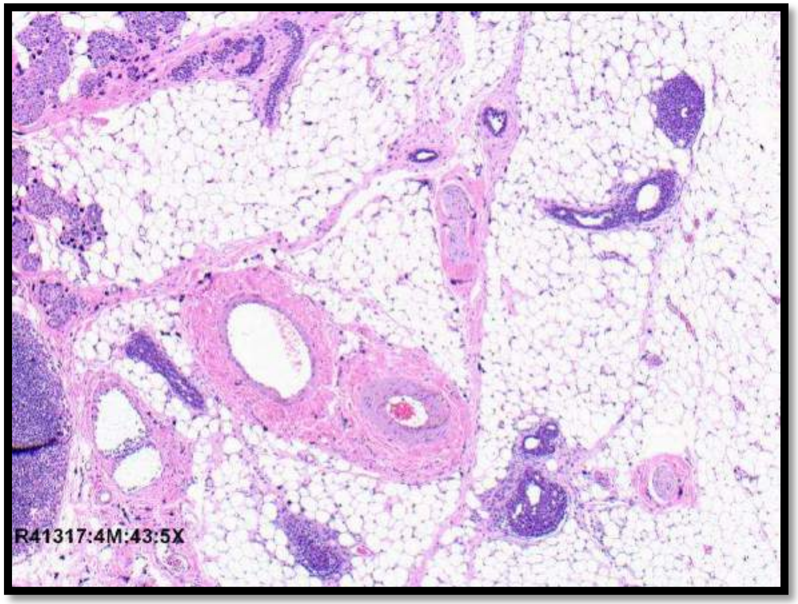
Normal Male vs. Virilization (females)

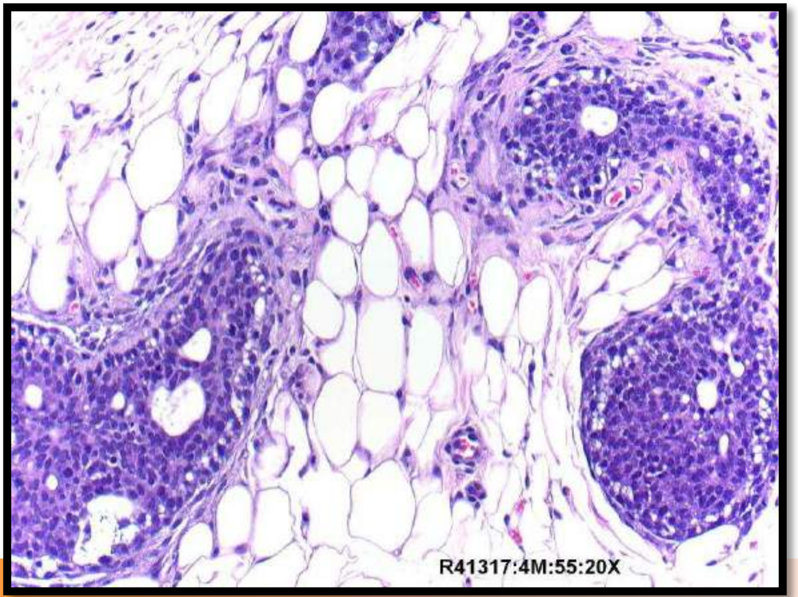


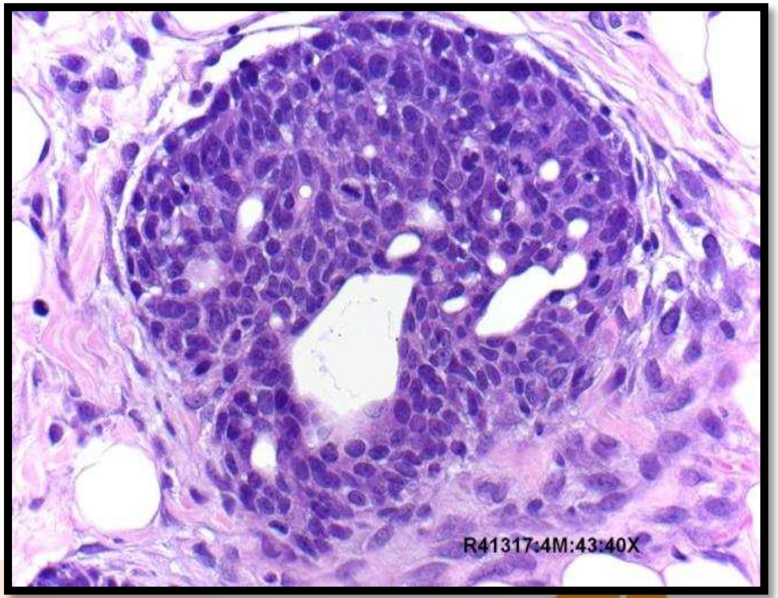


Male mammary gland - Feminization

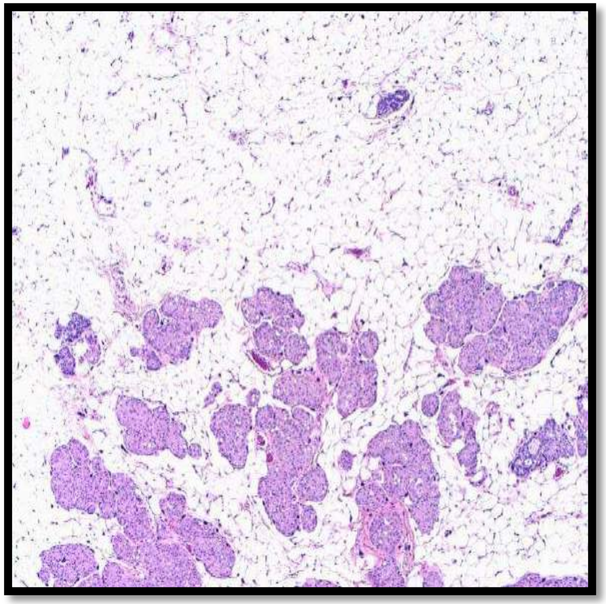


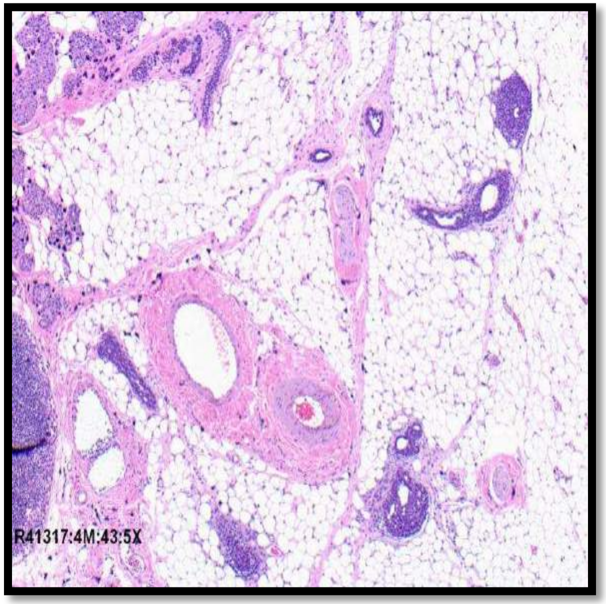


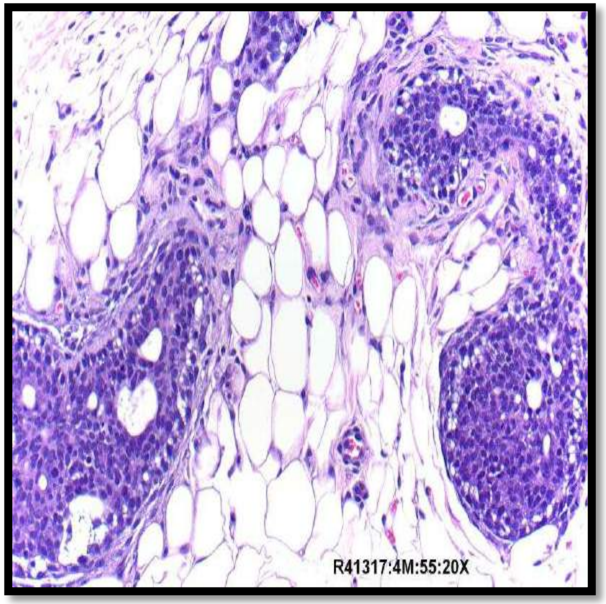




Male rat mammary gland changes given SERM







Toxicologic Pathology, 33:711–719, 2005

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Androgen Dependent Mammary Gland Virilism in Rats Given the Selective Estrogen Receptor Modulator LY2066948 Hydrochloride

Daniel G. Rudmann,[1] Ilene R. Cohen,[2] Michelle R. Robbins,[2] David E. Coutant,[3] and Judith W. Henck[4]

GlaxoSmithKline

Test article-related findings

SERM

Birth Defects Research (Part B) 80:225–232 (2007)

Review Article

Histologic Changes in Ovary, Uterus, Vagina, and Mammary Gland of Mature Beagle Dogs Treated With the SERM Idoxifene

Sabine Rehm,* Henk A. Solleveld, Samm T. Portelli, and Patrick J. Wier

GlaxoSmithKline

SERM
Selective Estrogen Receptor Modulators

- Treatment/prevention of breast cancer and osteoporosis
- Effects of SERMs may vary greatly, dependent on species and cell type
- Idoxifene – (similar to tamoxifen) –
  - Toxicity testing in rats, mice, dogs and monkeys
  - Rodents: 2 years; Dogs/Monkeys: 1 year

	ER Antagonist	ER Agonist
Tamoxifen	Mammary Gland	Endometrium/Vagina
		Bone
Raloxifene (Evista)	Mammary Gland	Bone
	Endometrium/Vagina	

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SERM - Control vs. Treated

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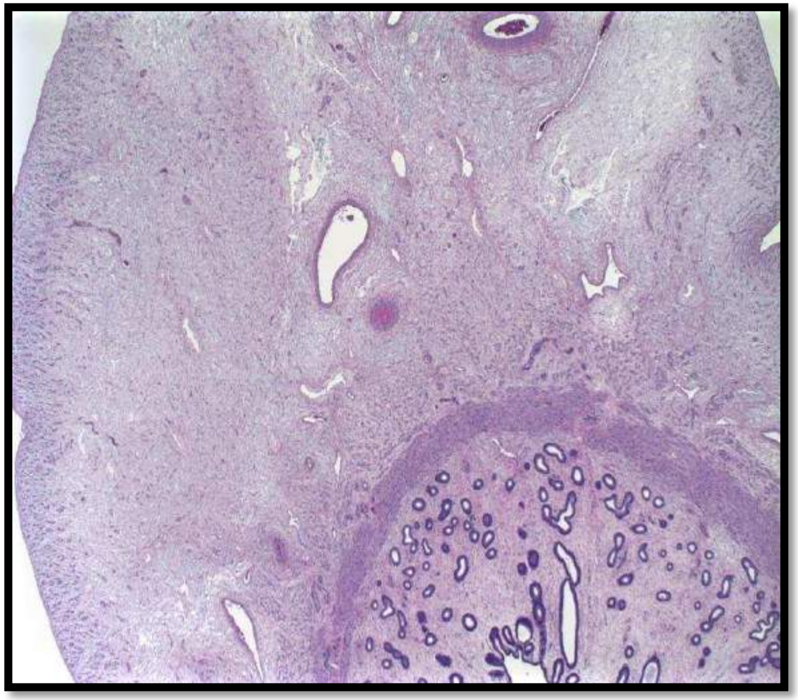
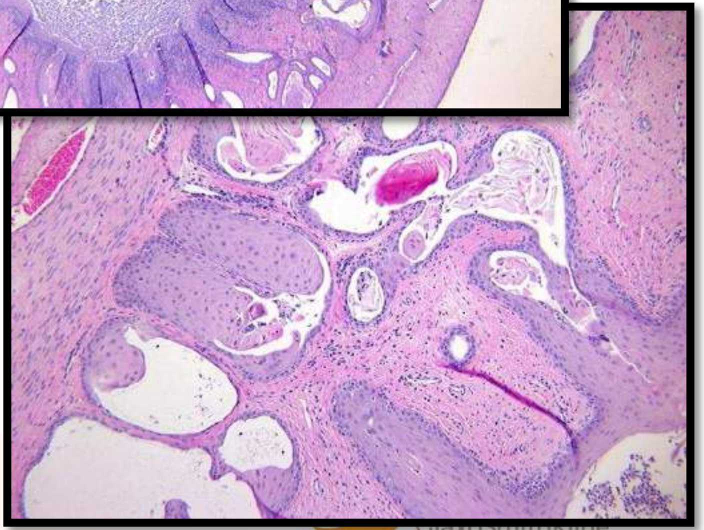
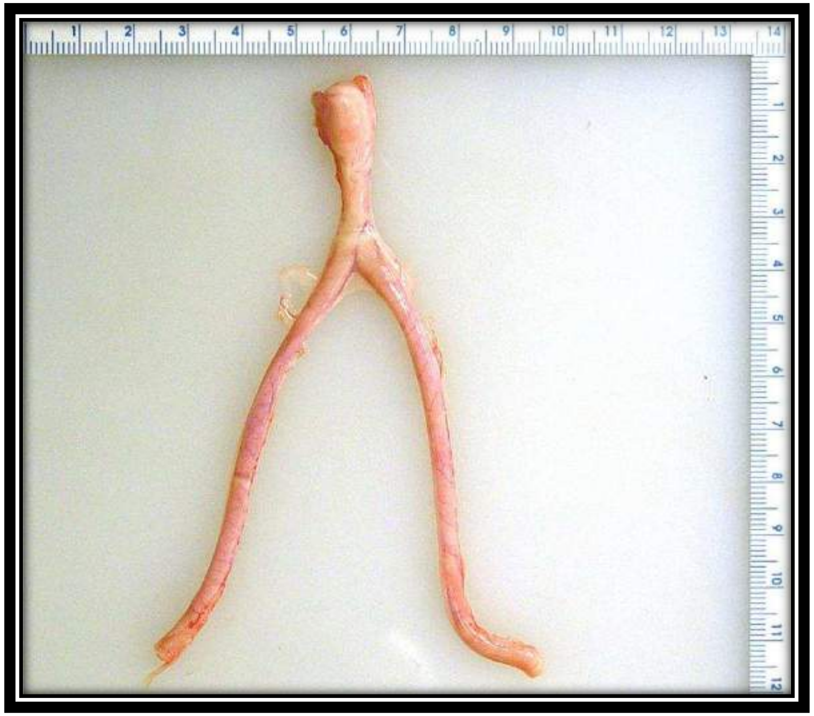
GlaxoSmithKline

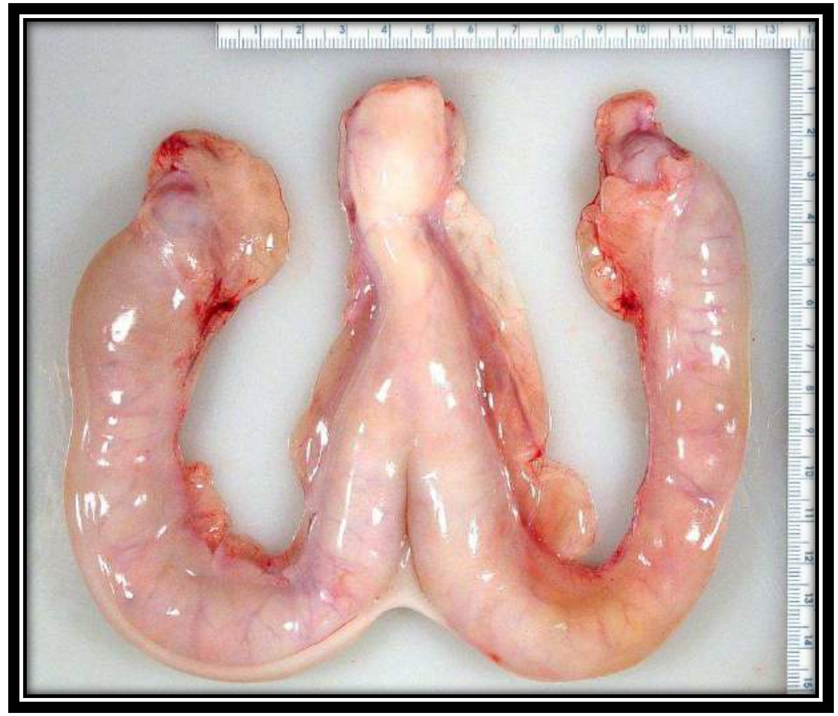
SERM – Uterine Effects
(1month vs. 3 month toxicity study)





SERM – Uterus – 13 week study





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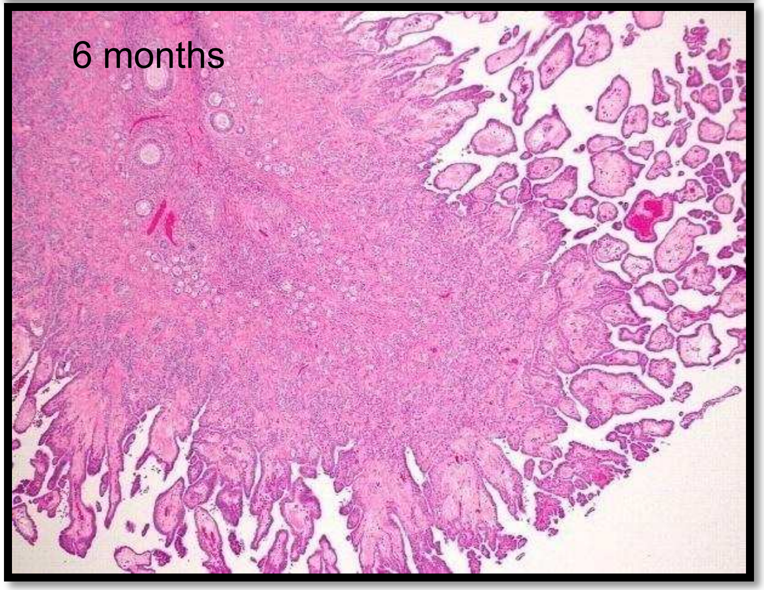
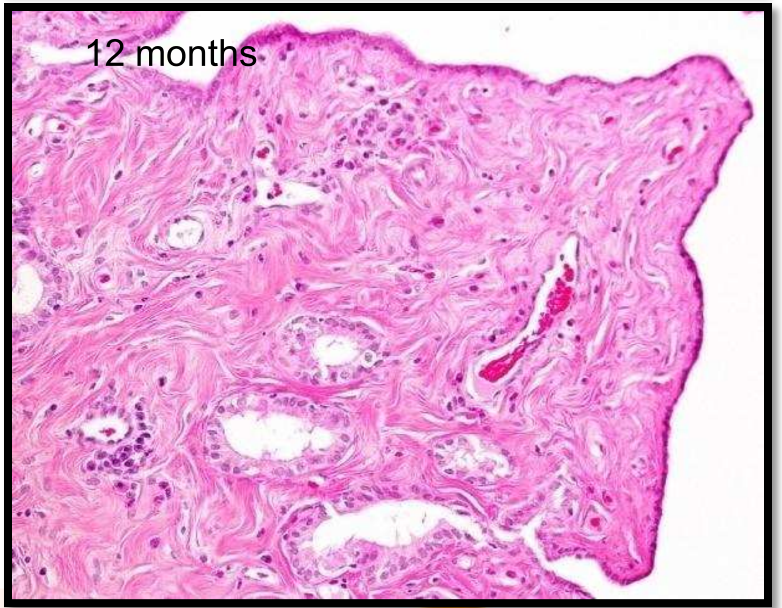
gsk GlaxoSmithKline

Ovary

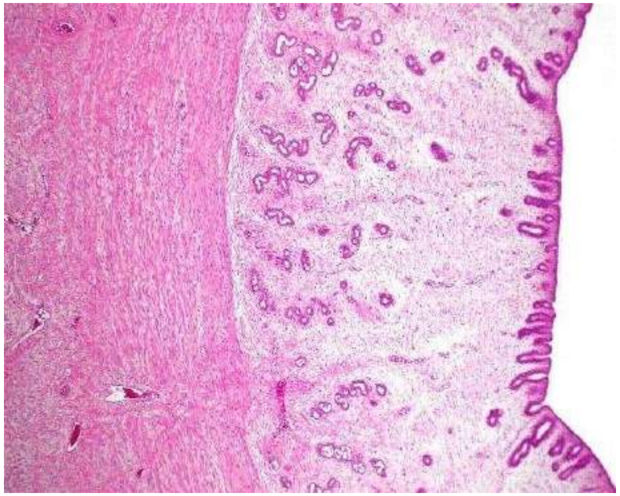




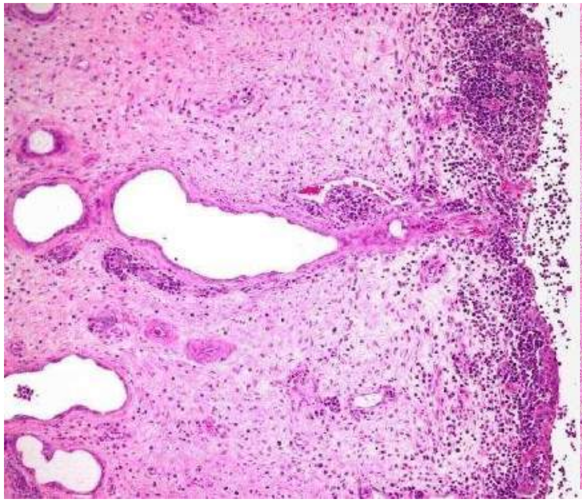
Images: Dr. S. Rehm

GSK GlaxoSmithKline

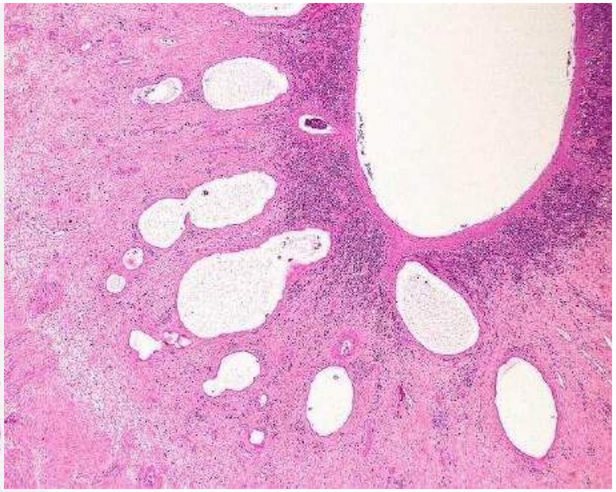
Uterus



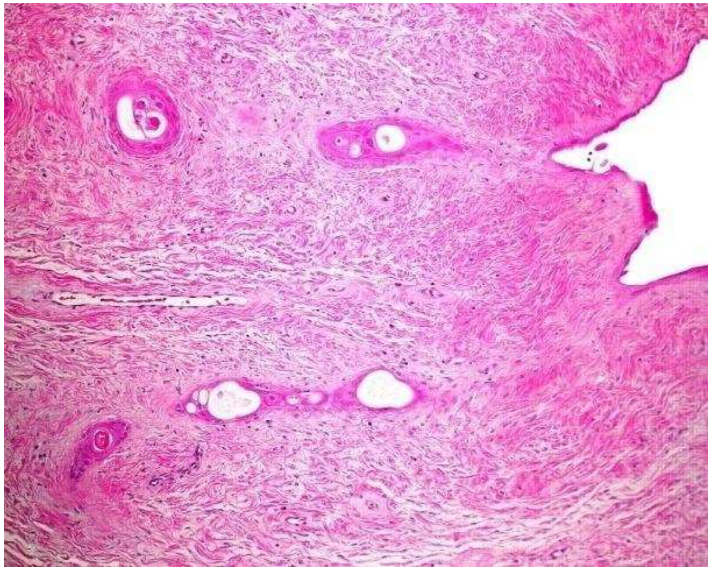
1 month



1 month



6 months

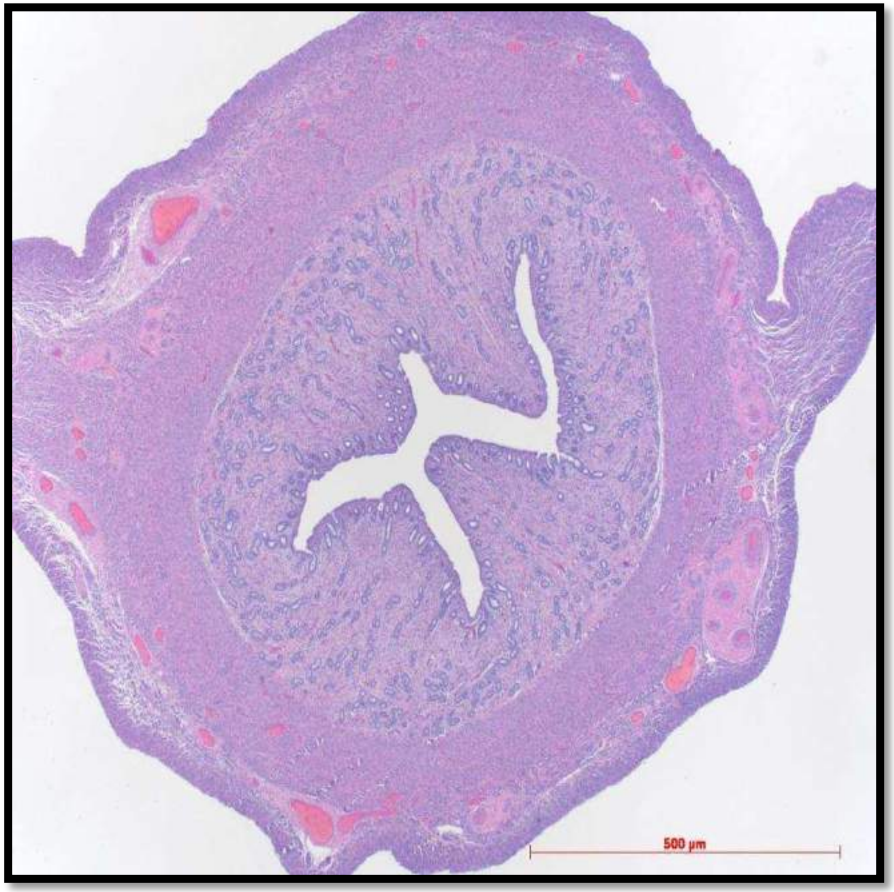


12 months

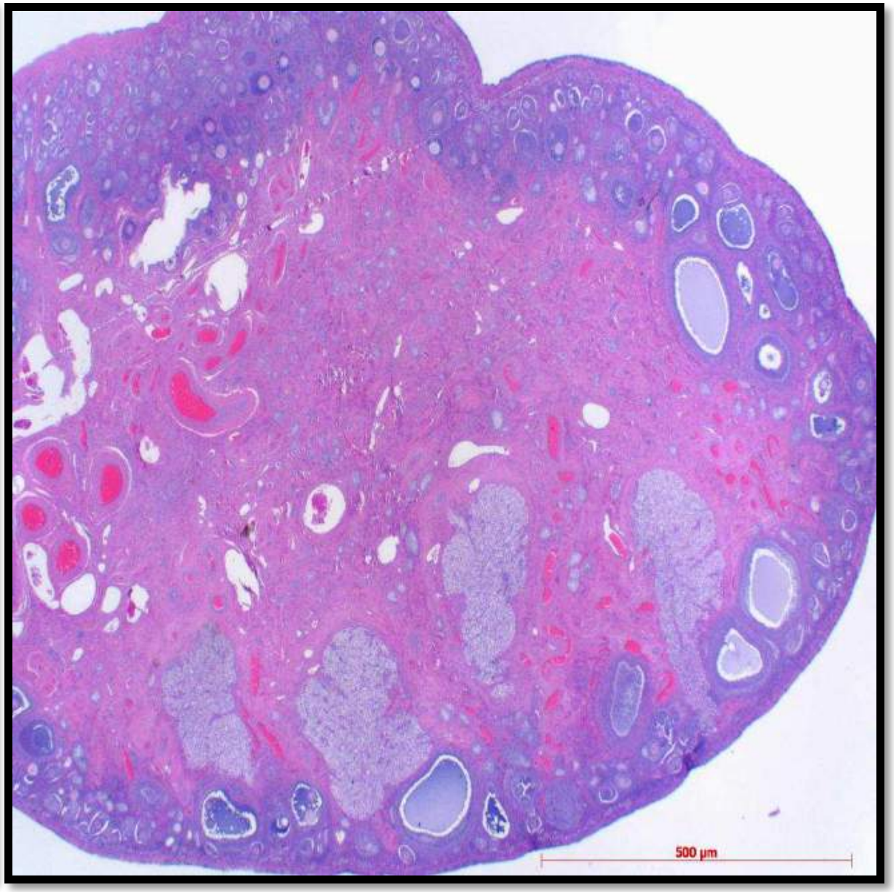
12 months

GlaxoSmithKline

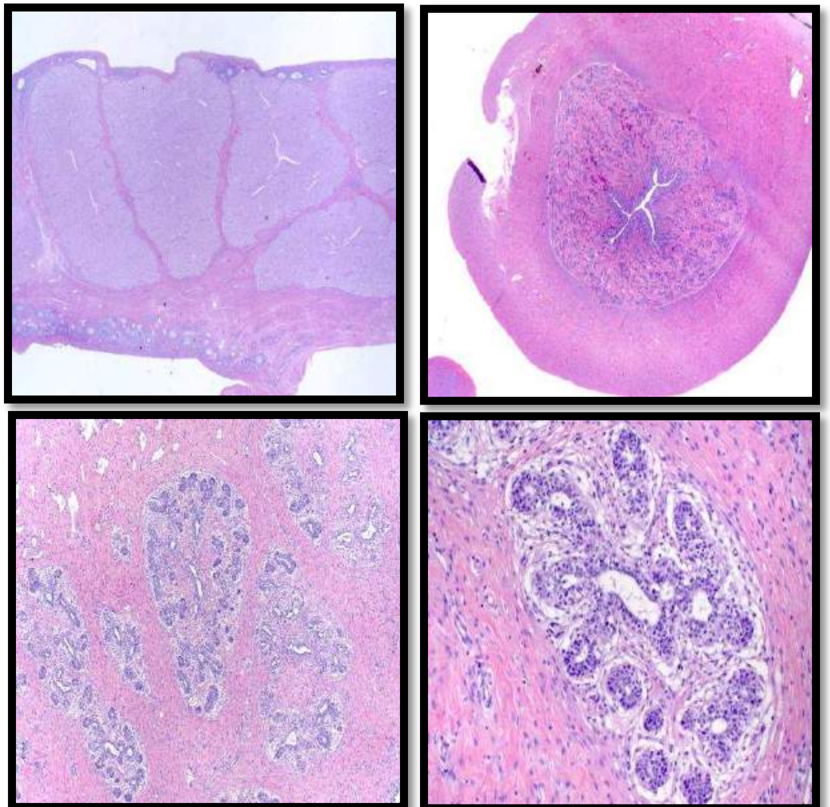
Uterus – Androgenic effects

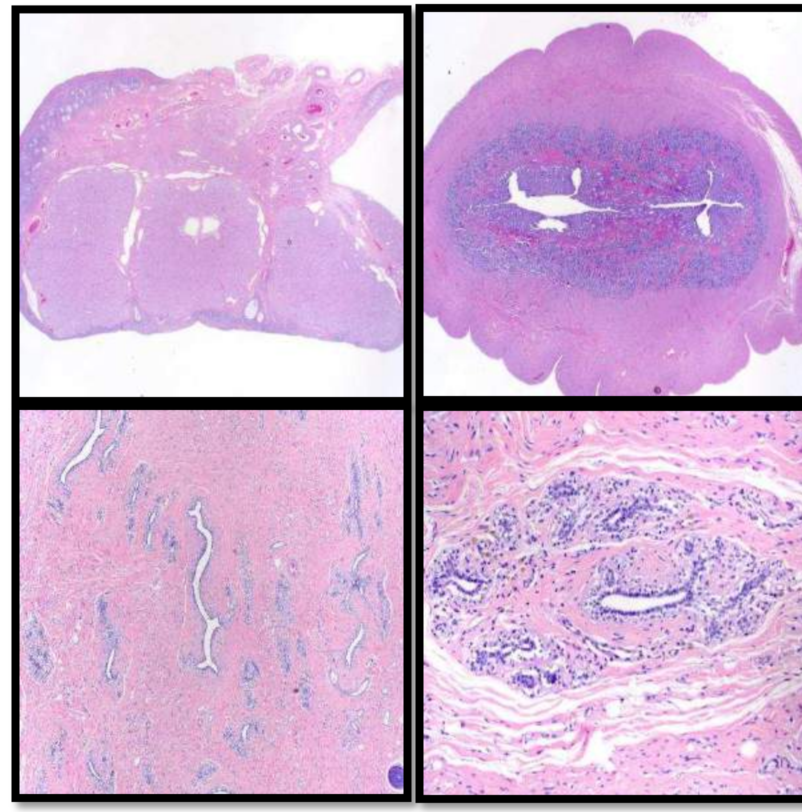


Ovary – Androgenic effects

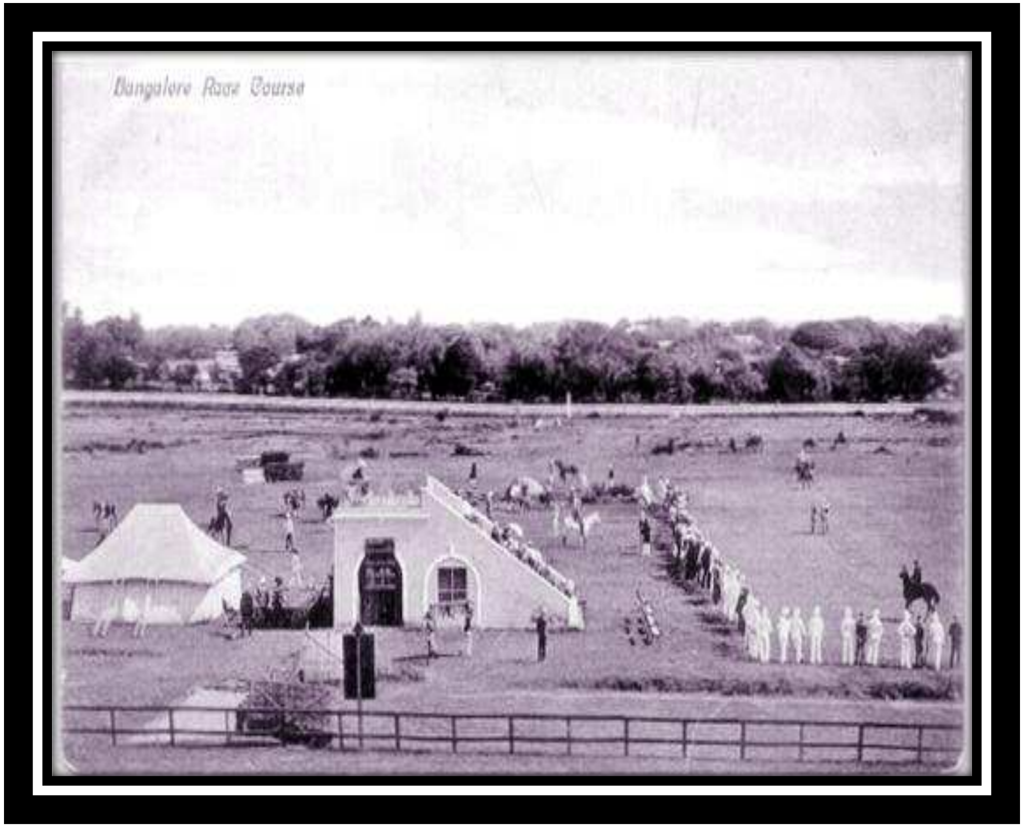


Control vs. Treated





Bangalore - Circa 1946



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Questions/comments/suggestions

Sundeep.a.chandra@gsk.com