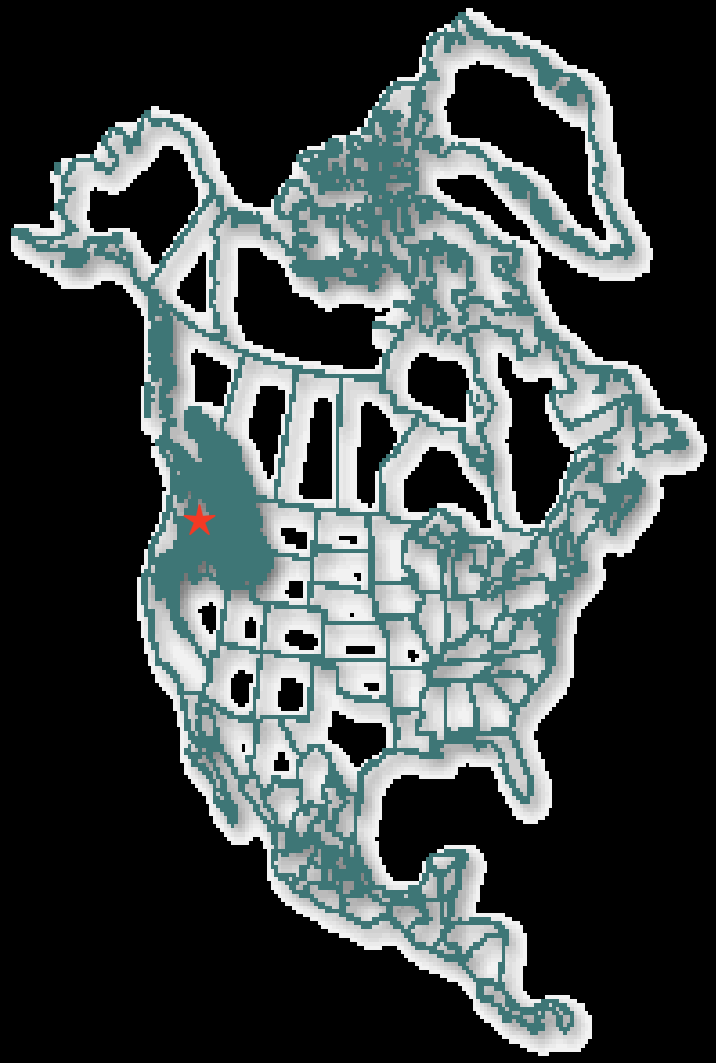
MAMMALIAN REPRODUCTIVE PHYSIOLOGY

Ned J. Place, Ph.D., M.D.

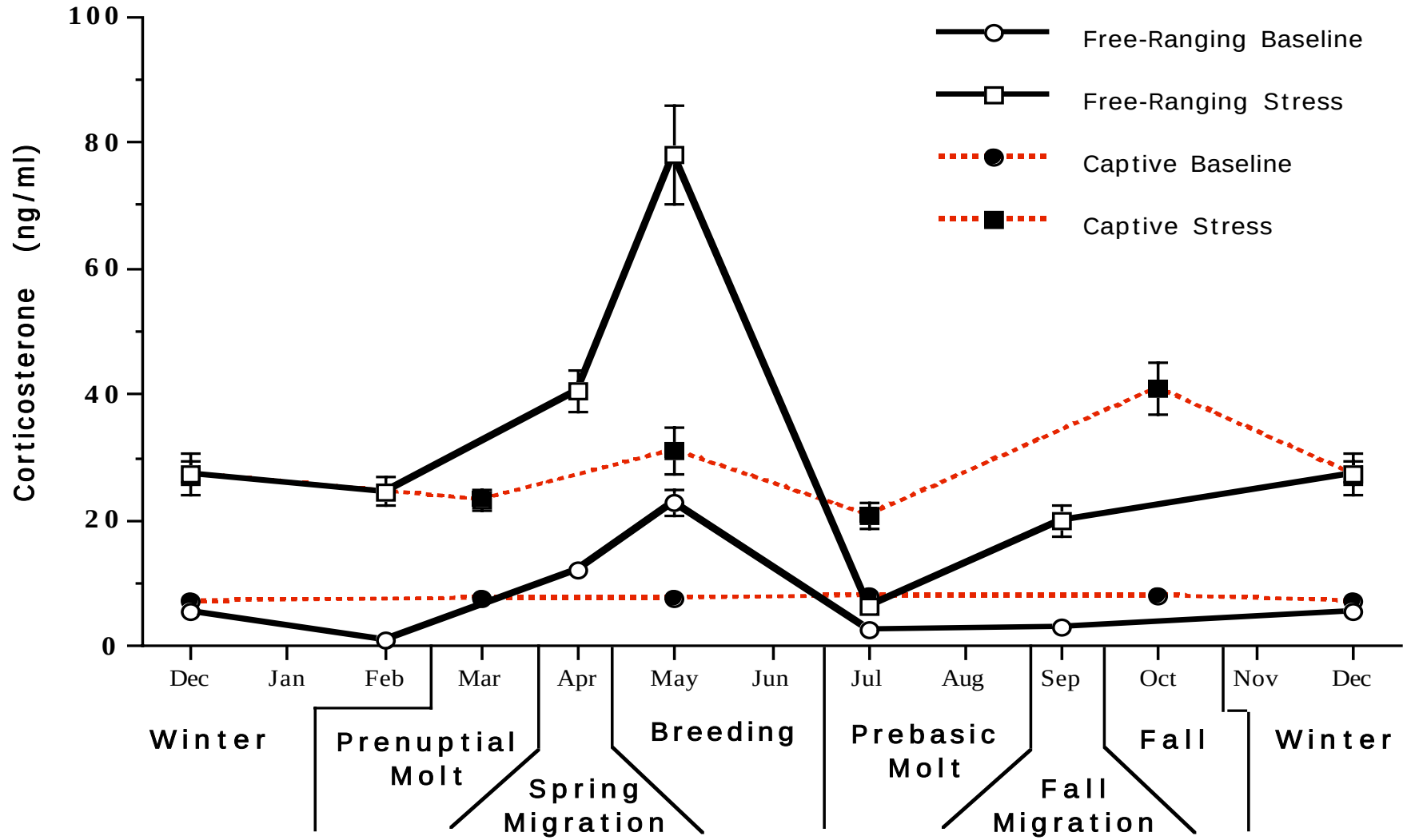
Cornell University
College of Veterinary Medicine
Population Medicine & Diagnostic Sciences

Environmental endocrinology of the yellow-pine chipmunk, *Tamias amoenus*



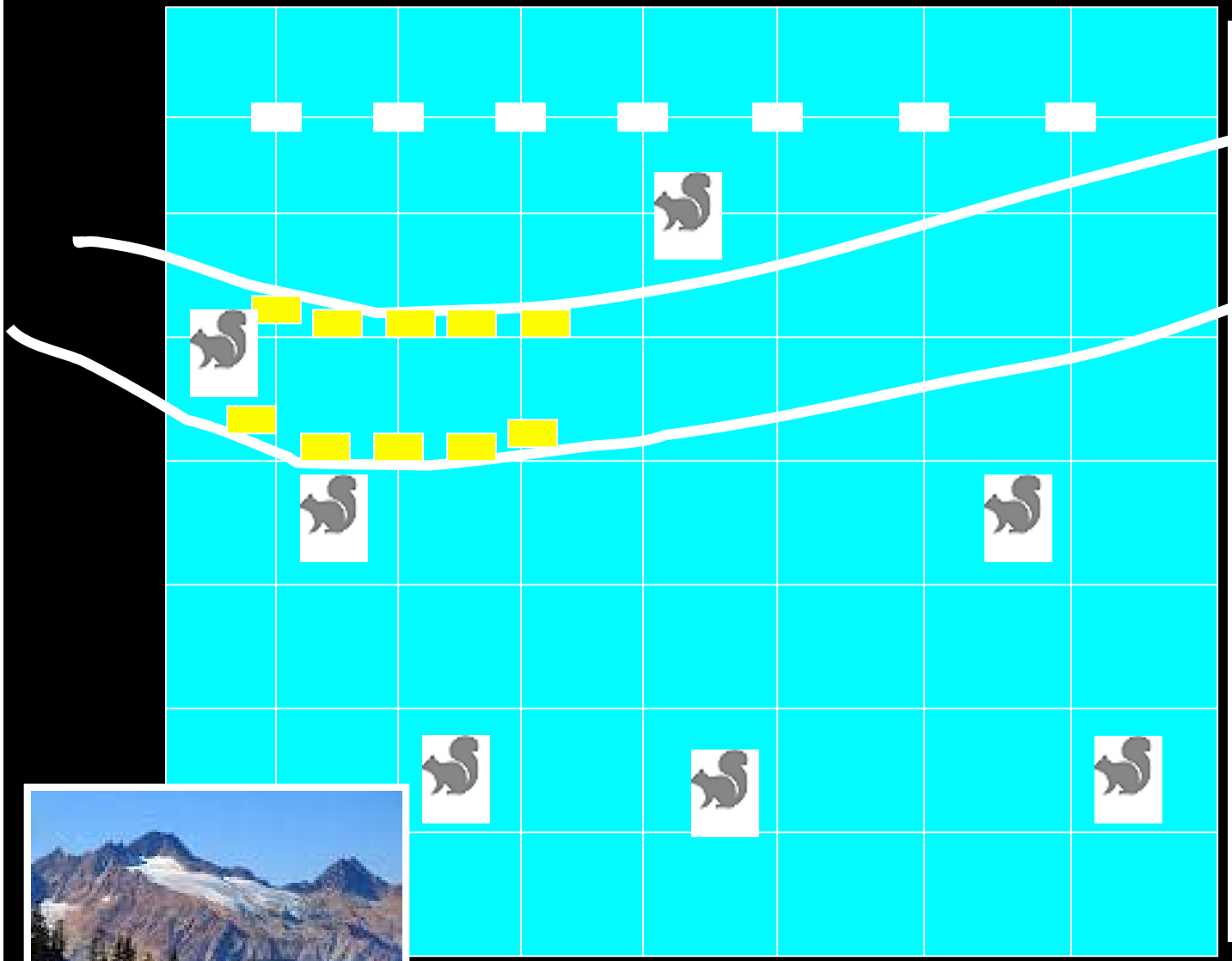
PhD research, University of Washington

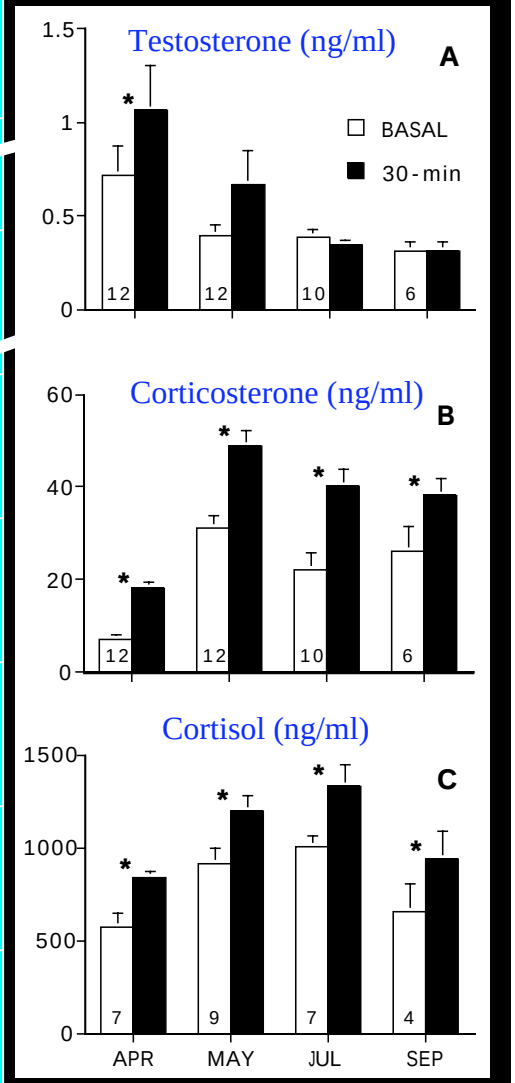
Yearly Corticosterone Cycle in White-Crowned Sparrows



Romero & Wingfield, 1999

Standard Grid Trapping & Stress Series





Stress series

Standard trapping

Reproductive and somatic aging in female Siberian hamsters

The Three Ages of Woman
Gustav Klimt, 1905

Postdoc research with Irving Zucker, University of California, Berkeley → now at Cornell as PI

How does seasonal suppression of reproductive physiology affect reproductive aging?

Spring

Summer

Rapid maturation
1st breed at 2-3 months
Low over-winter survival

Delayed maturation
1st breed at 7-9 months
Hi over-winter survival

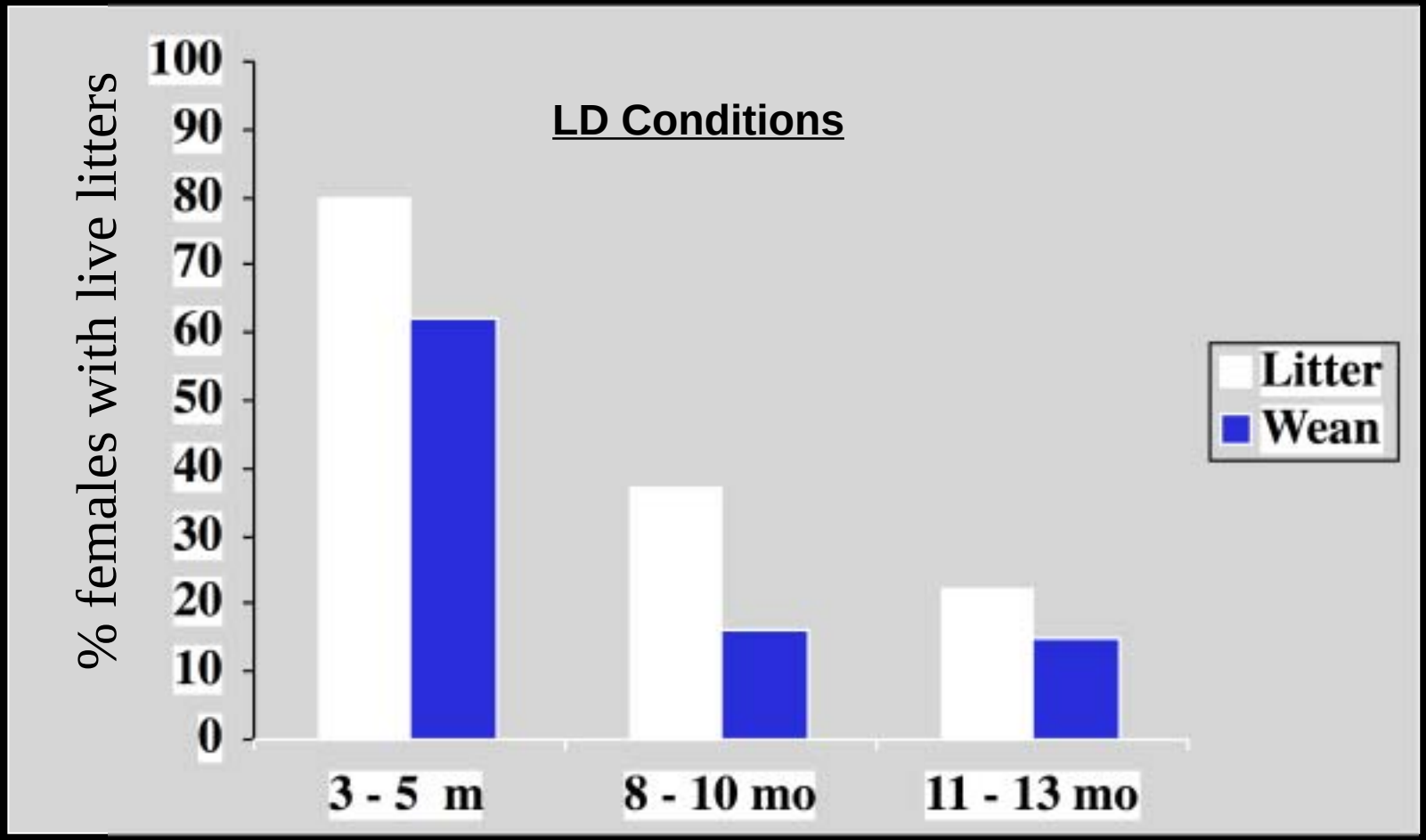
Siberian Hamsters

Phodopus sungorus

Responses to Short or Decreasing Day Lengths:

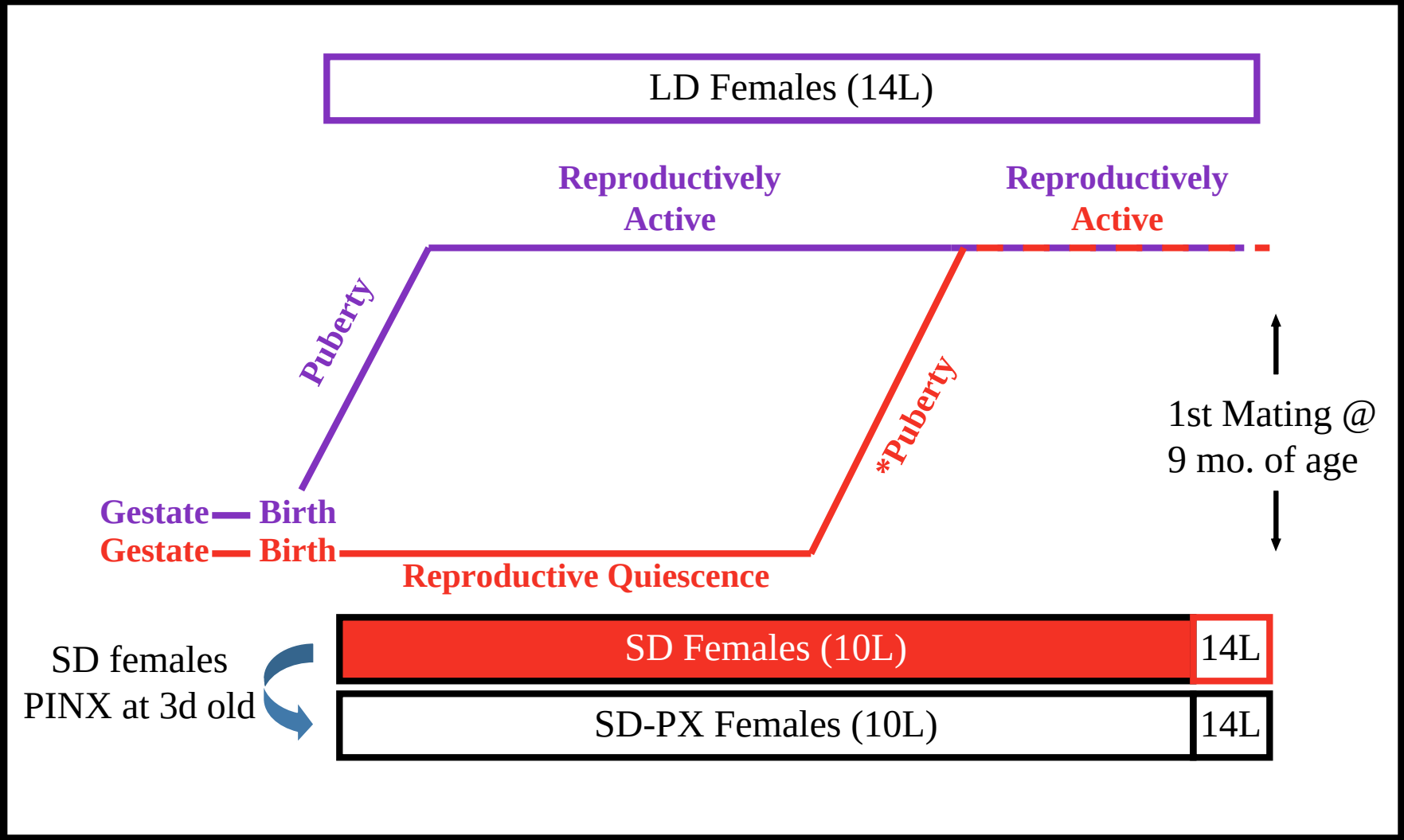
- Longer duration of nighttime melatonin
- Pre-puberty:
  - Delayed onset of sexual maturity
  - Primary winter pelage
  - Increase body mass slowly
- Post-puberty (following long days):
  - Regression of reproductive organs
  - Molt into winter pelage (d/t decreasing prolactin)
  - Decrease food intake and lose body mass

Significant signs of reproductive aging are evident in hamsters at an age when summer-born females first breed

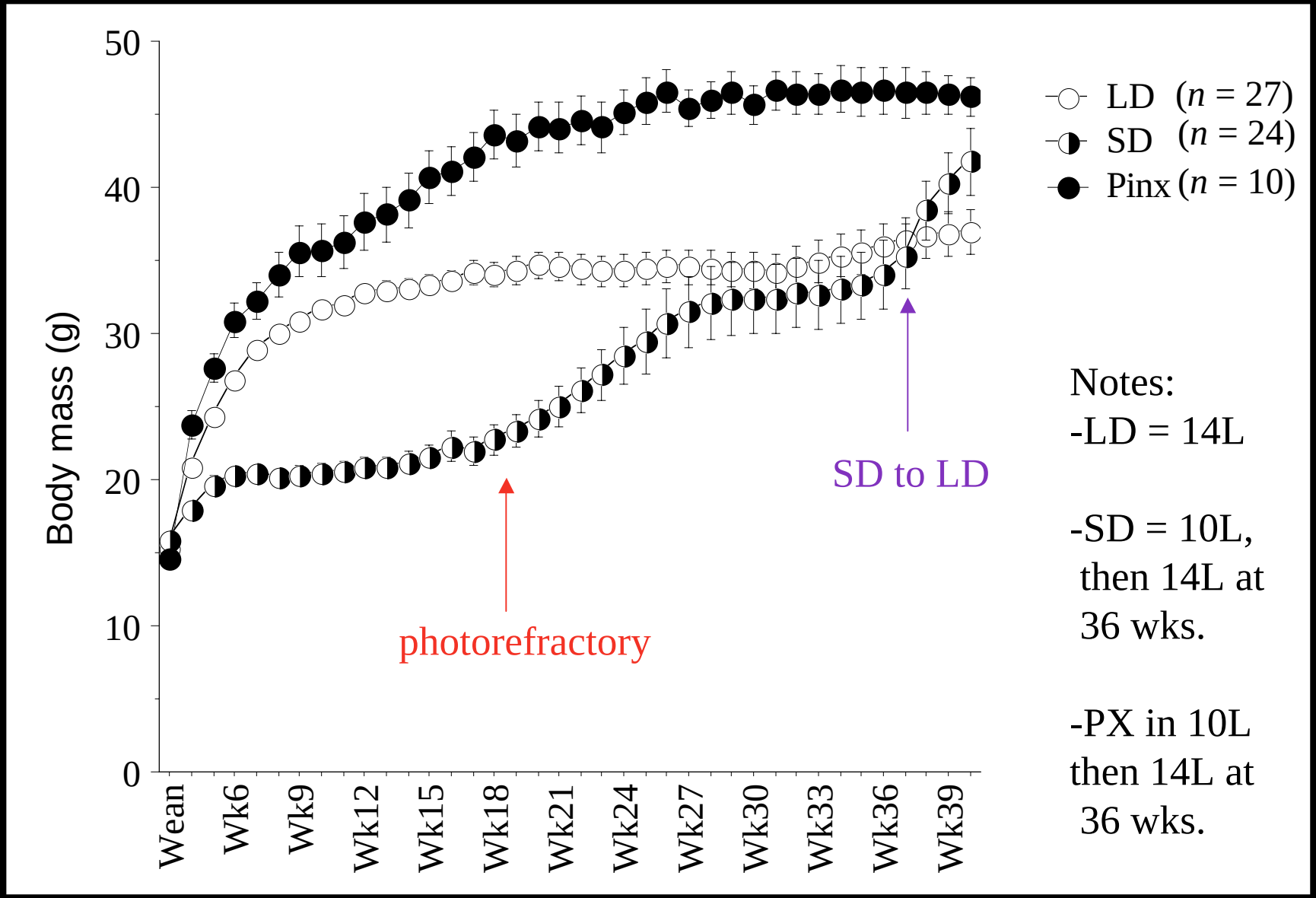


Reduced litter & weaning success by 8 to 10 months of age in dwarf hamsters (*Phodopus*) in continuous LD (Edwards *et al*. 1998)

Study Design: Middle-Aged Female Hamsters With Marked Differences in Photoperiodic & Reproductive Histories



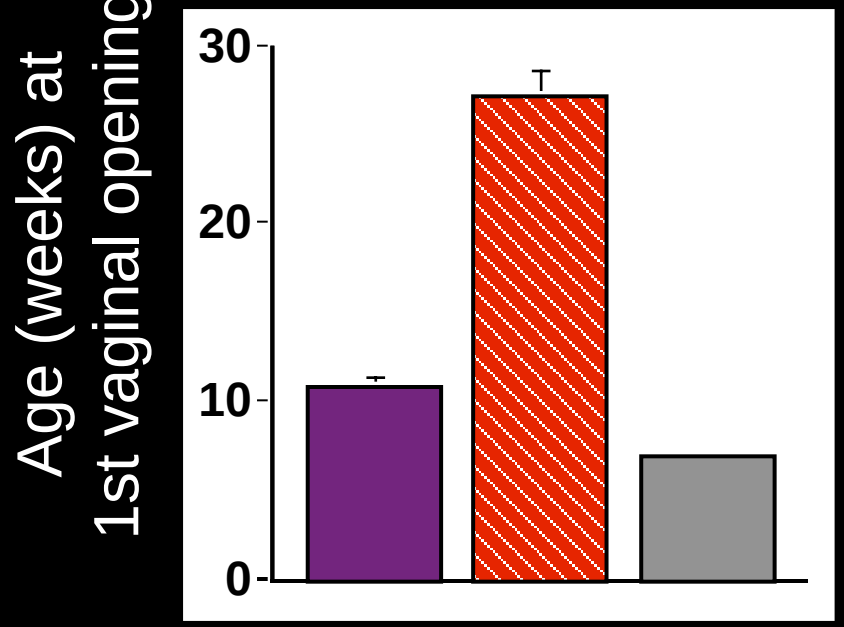
Effect of Photoperiod and Neonatal Pinealectomy on Body Mass
- slower somatic growth in SD due to decreased food intake

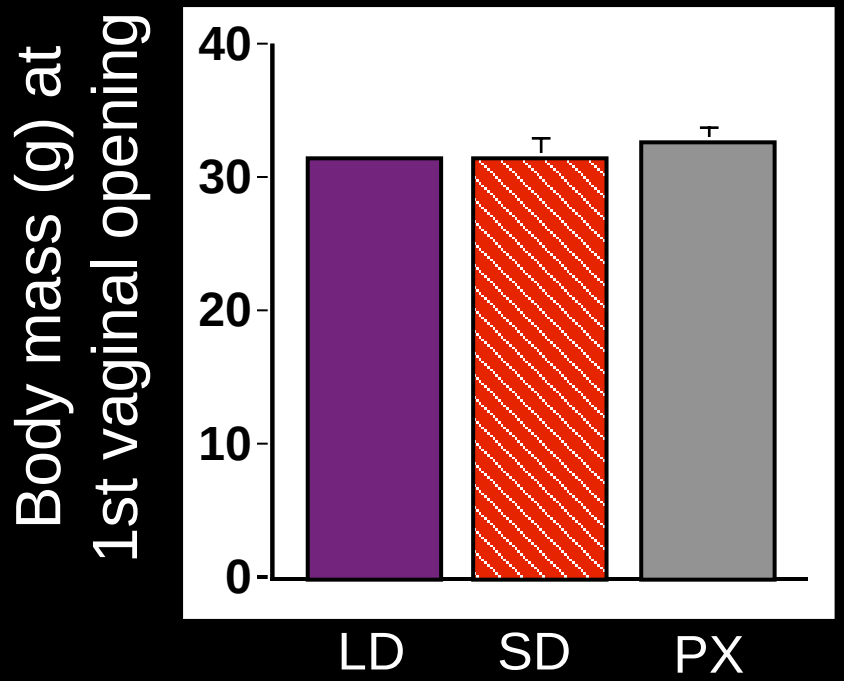


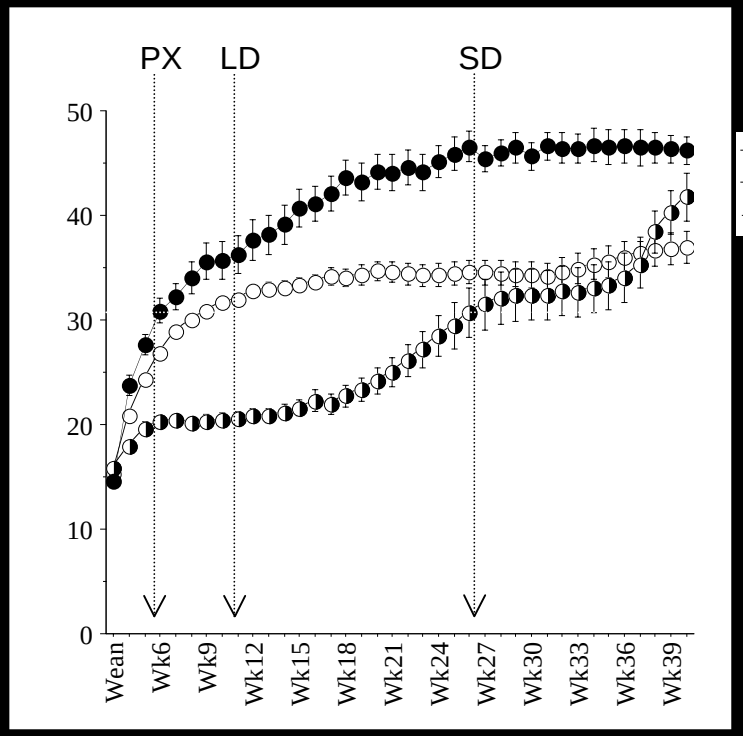
Notes:
-LD = 14L

-SD = 10L, then 14L at 36 wks.

-PX in 10L then 14L at 36 wks.

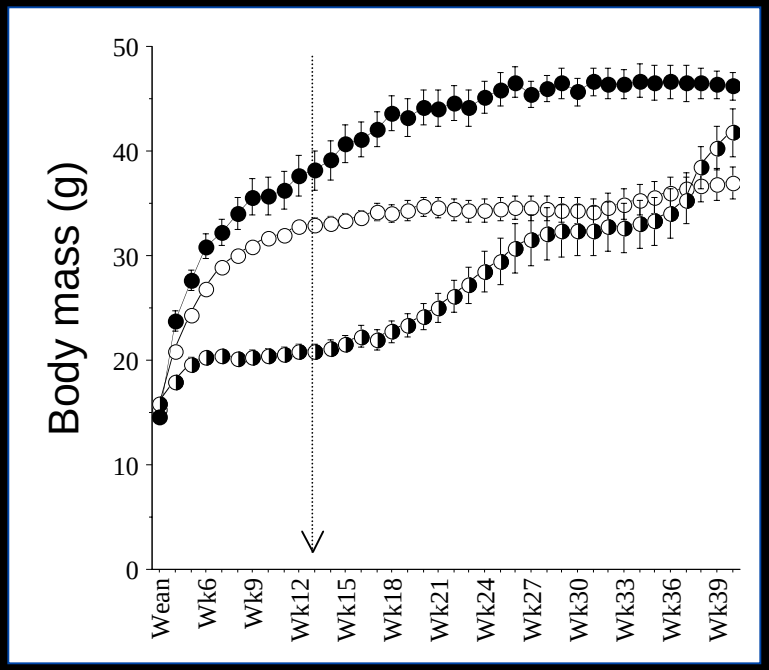


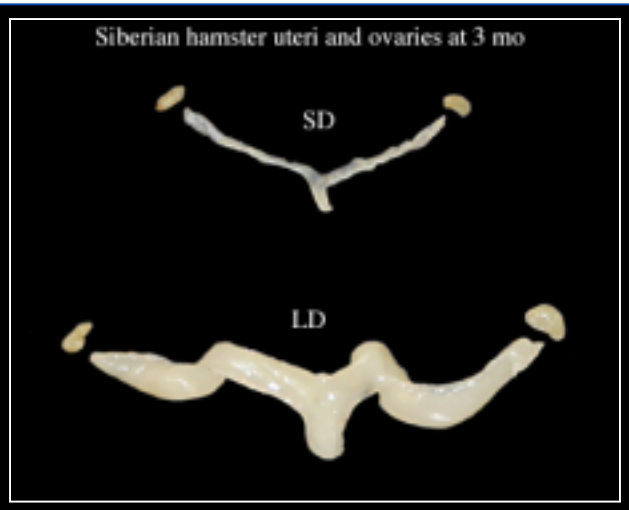


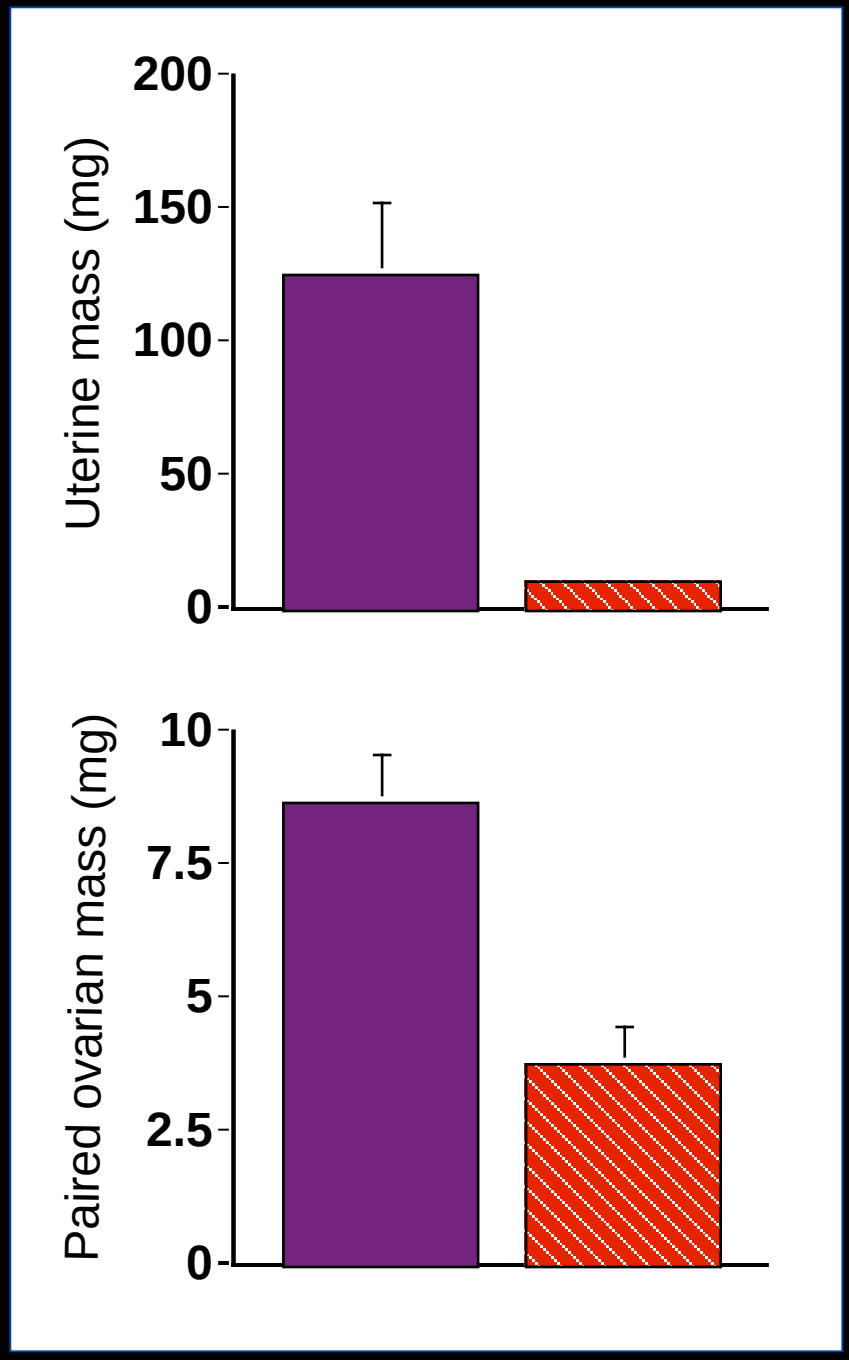


NO Body Mass Difference
At First Vaginal Opening

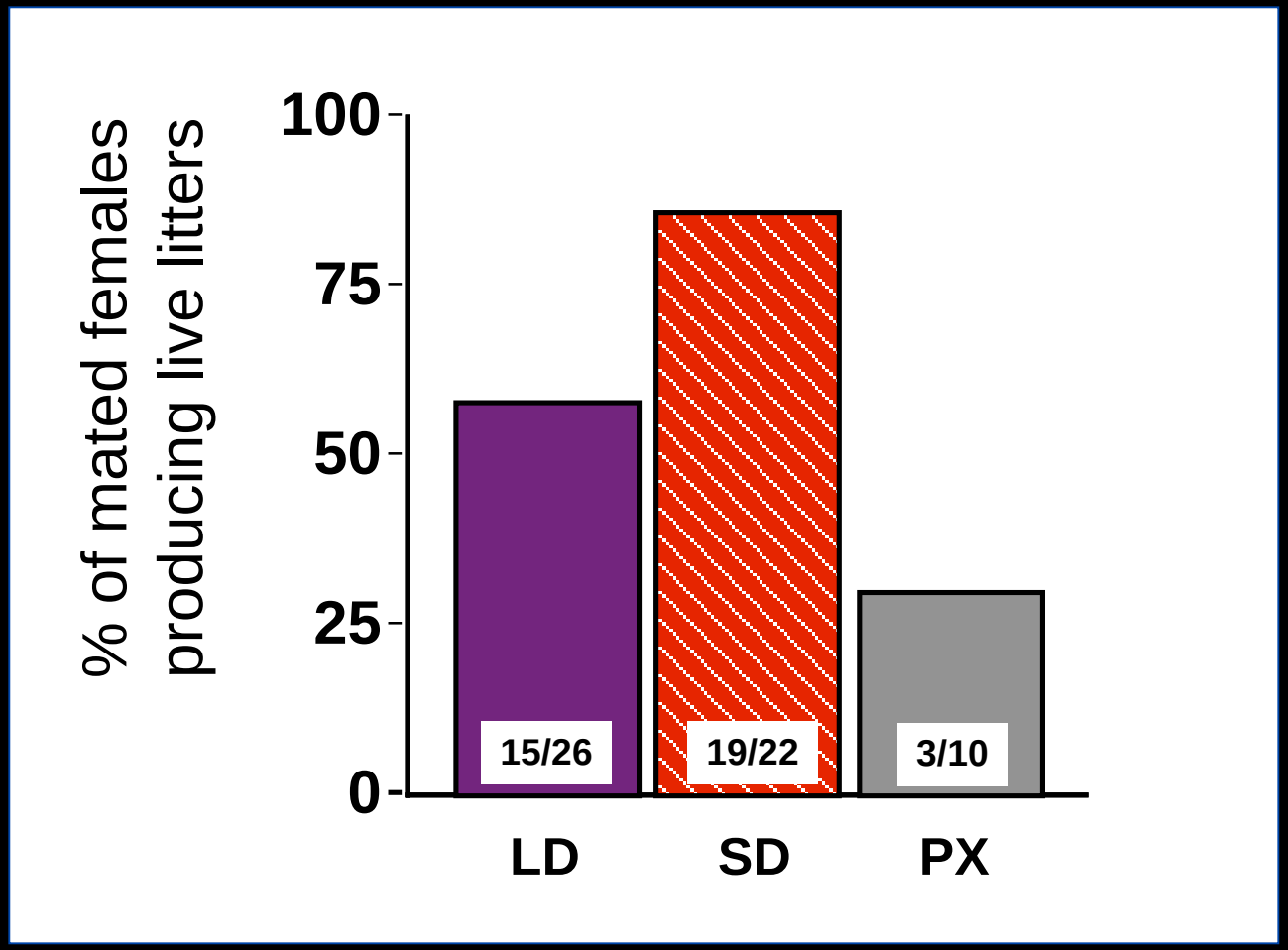
Body, uterine, and paired ovarian mass in LD and SD Siberian hamsters at 13 weeks of age







Effect of Photoperiod and Pinealectomy on Litter (and weaning) Success in 9 Month-old Female Siberian Hamsters



Equal Weaning Success (%), but …

SD females wean larger pups and their litters are male biased as compared to LD female pups (controlled for litter size)

Table 1. Mean pup body mass (g) ± STD at weaning from LD and SD females, limited to litters of equal sizes (six or seven pups). Representation of these litter sizes was identical in the LD and SD groups. Sample sizes are given in parentheses.

	LD	SD	*P* value
Male	15.3 g ± 1.0 (22)	16.3 g ± 1.2 (41)	*0.002**
Female	14.2 g ± 1.0 (29)	15.3 g ± 1.0 (24)	*0.005**
M:F Ratio	0.76:1	1.71:1	*0.040*[†]

* *t*-test; log-transformed data

[†] Fisher's Exact Test

1. Two-way ANOVA for pup mass v. photoperiod and sex
a. significant effect of photoperiod: SD > LD; P < 0.0001
b. significant effect of sex: M > F; P < 0.0001
c. interestingly, NO interaction effect: P = 0.85

Photoperiod and Reproductive Failures

	LD	SD	SD-PX
# Paired	27	23	10
# Mating	26 (96%)	22 (96%)	10 (100%)
# Pregnant (% of mated)	22 (85%)	19 (86%)	7 (70%)
# Failed Pregnancies	7	0 *	4
Maternal Deaths	2	0	1
Complete Stillborn Litters	3	0	2
Complete losses *in utero*	2	0	1

Are post-conception failures d/t age-associated uterine dysfunction?

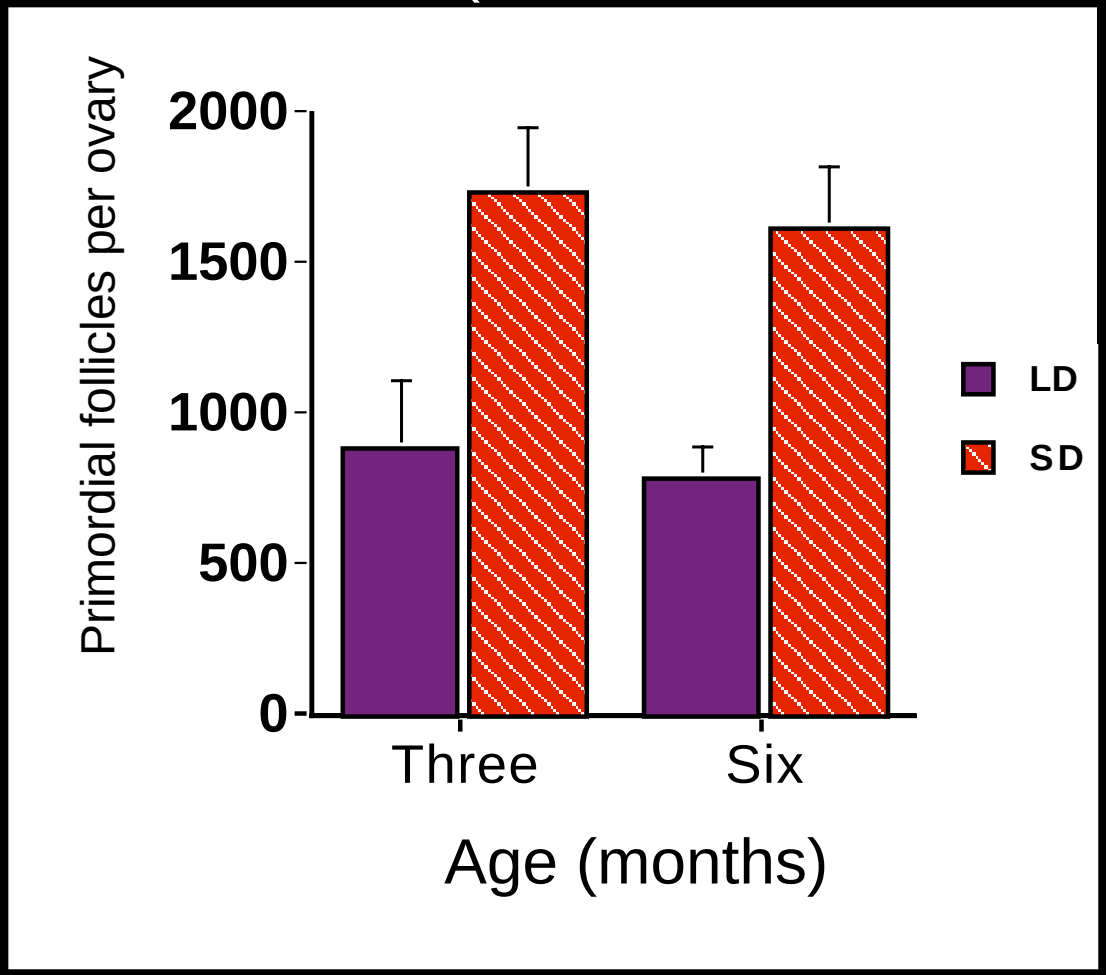
1. Effects of photoperiod on uterine ER expression.
2. Effects of photoperiod on uterine contractile-associated proteins (e.g., connexin43).

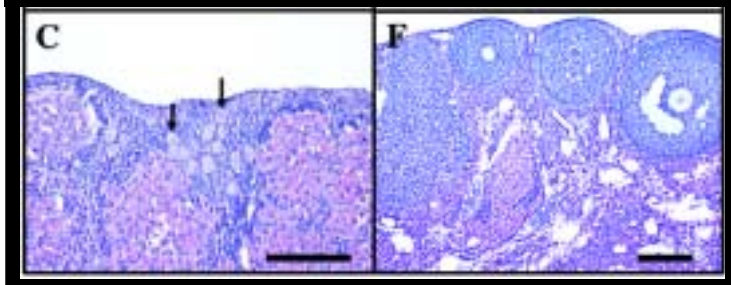
Summary: Effects of SD on Reproductive Aging

- Pregnancy rates: SD = LD ≥ PX
- # Implant sites: SD ≈ LD ≈ PX
- Litter success: SD > LD > PX
- Weaning Success: SD = LD = PX
- Pup Size at weaning: SD > LD
- Pup Sex Ratio: SD - male biased;
  LD - female biased

SD Deceleration of Ovarian Aging

Primordial Follicles: SD >> LD-females
(at 3 and 6 months of age)



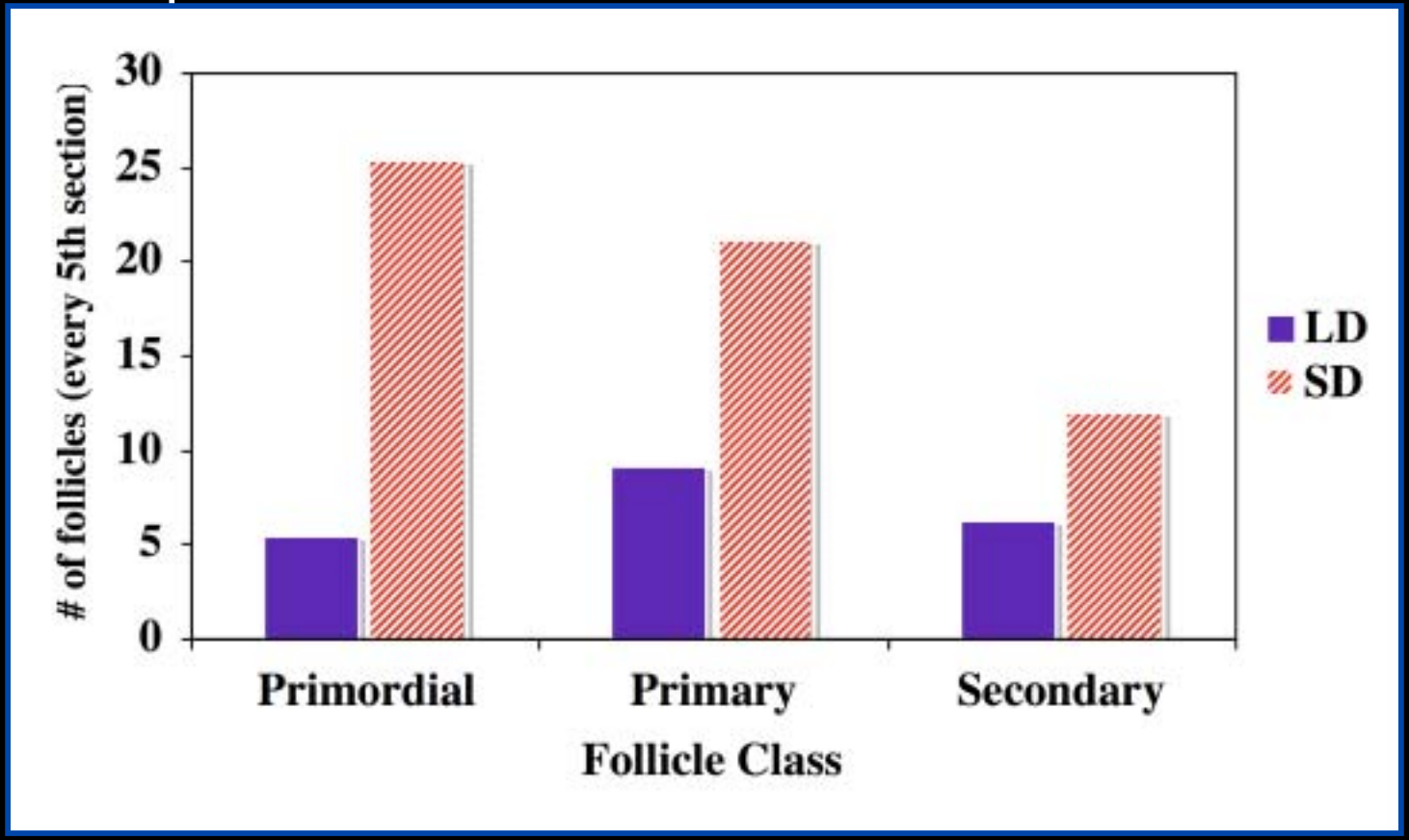


SD LD

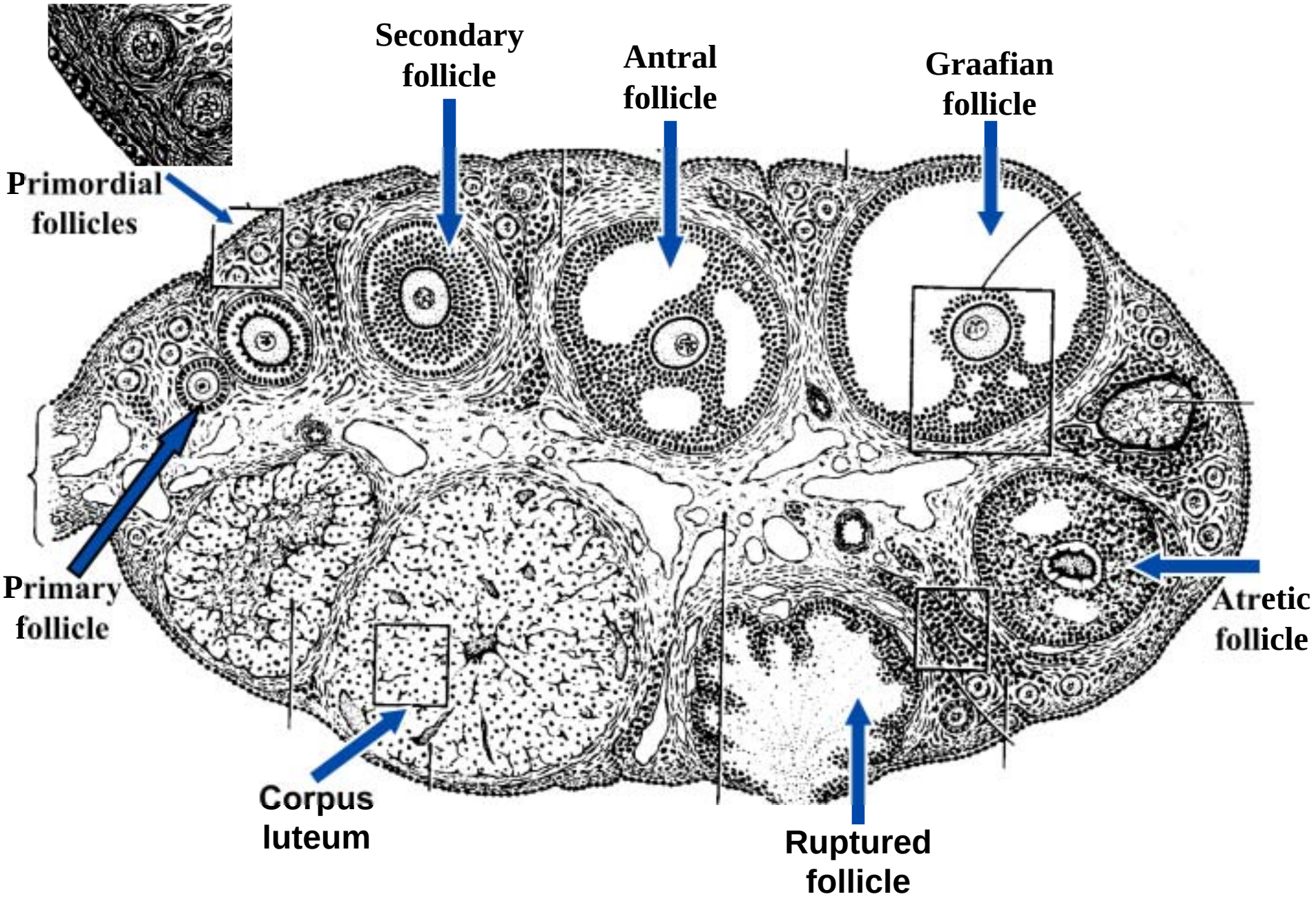
3 months-old

Preservation of primordial follicles persists in SD females at 1 year of age

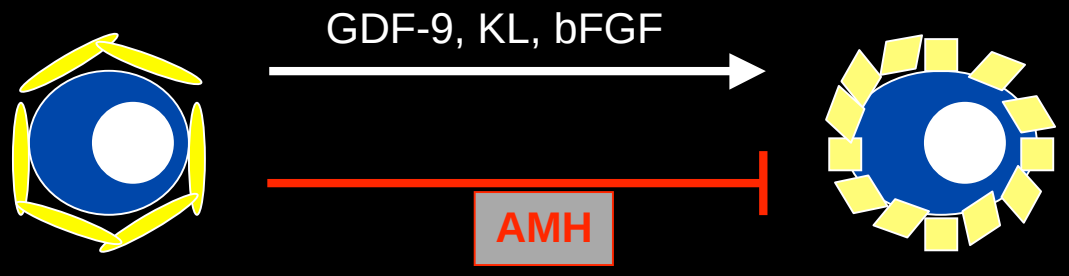
despite their transfer to LD at 8 months old



Follicle classes in folliculogenesis



Signals / factors that stimulate or inhibit primordial follicle activation

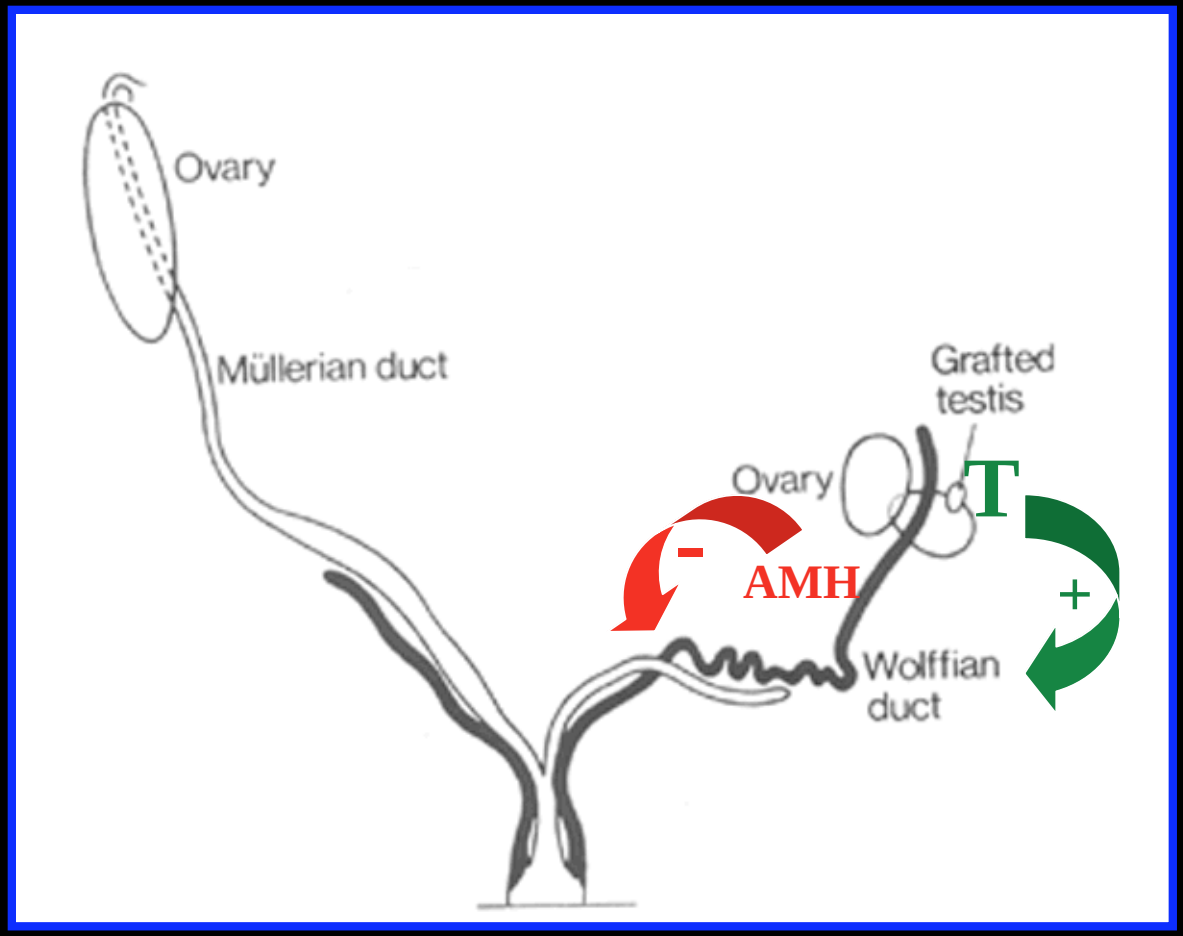


primordial follicle

primary follicle

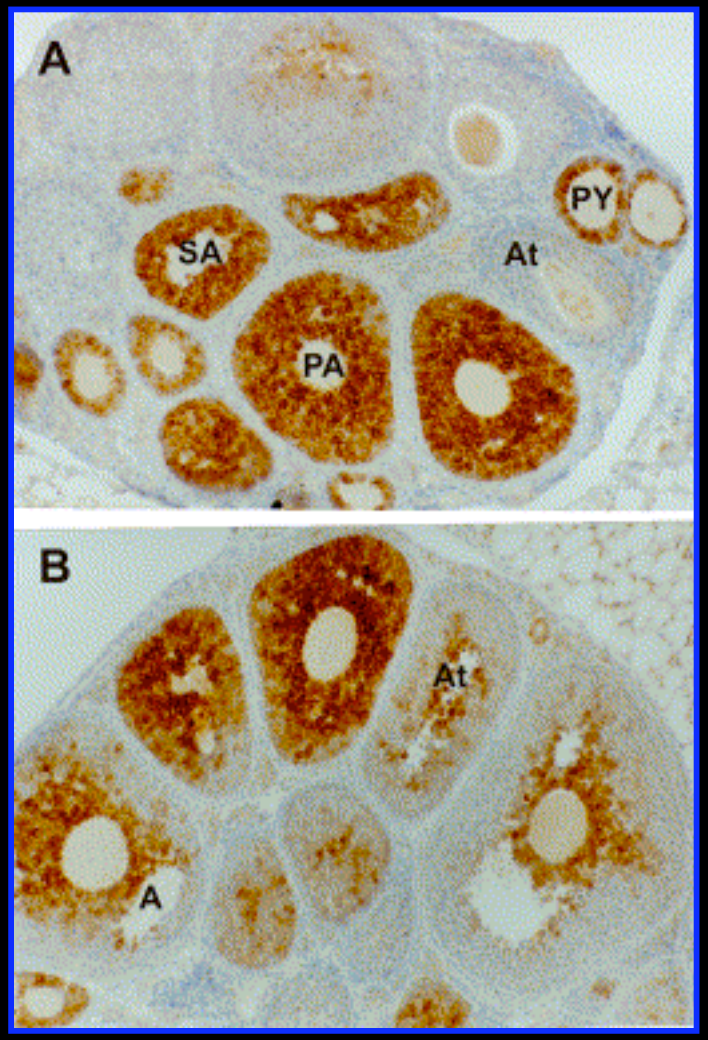
GDF-9: growth determination factor-9
KL: kit ligand
bFGF: basic fibroblast growth
AMH: anti-Müllerian hormone

Jost Model
Sexual Differentiation of the Reproductive
Fetal Testes Produce AMH

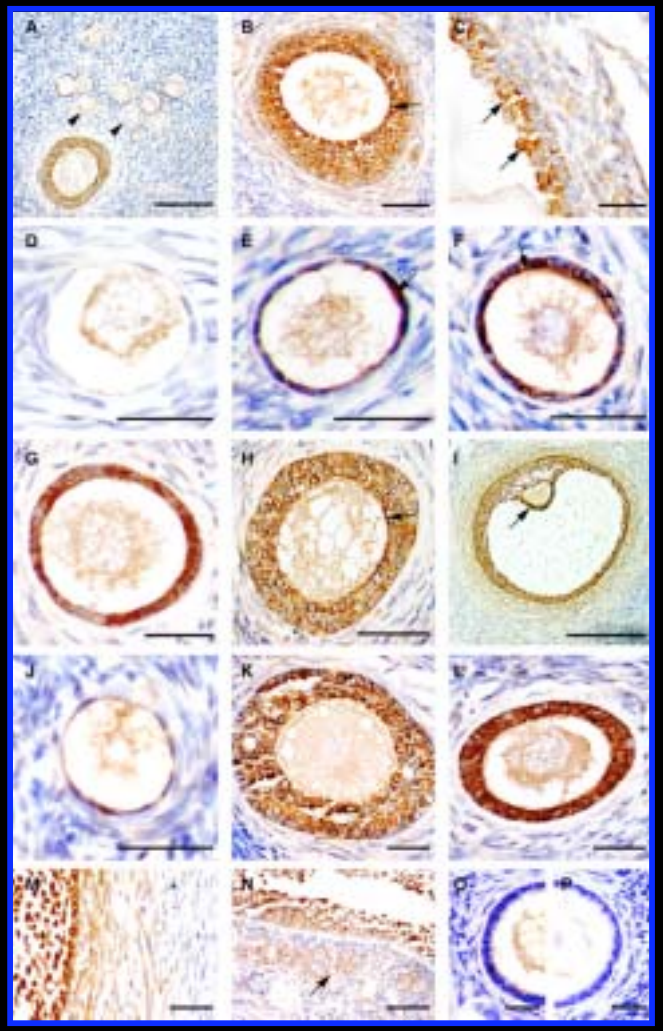


J.D. Wilson

Granulosa cells of growing ovarian follicles also produce AMH

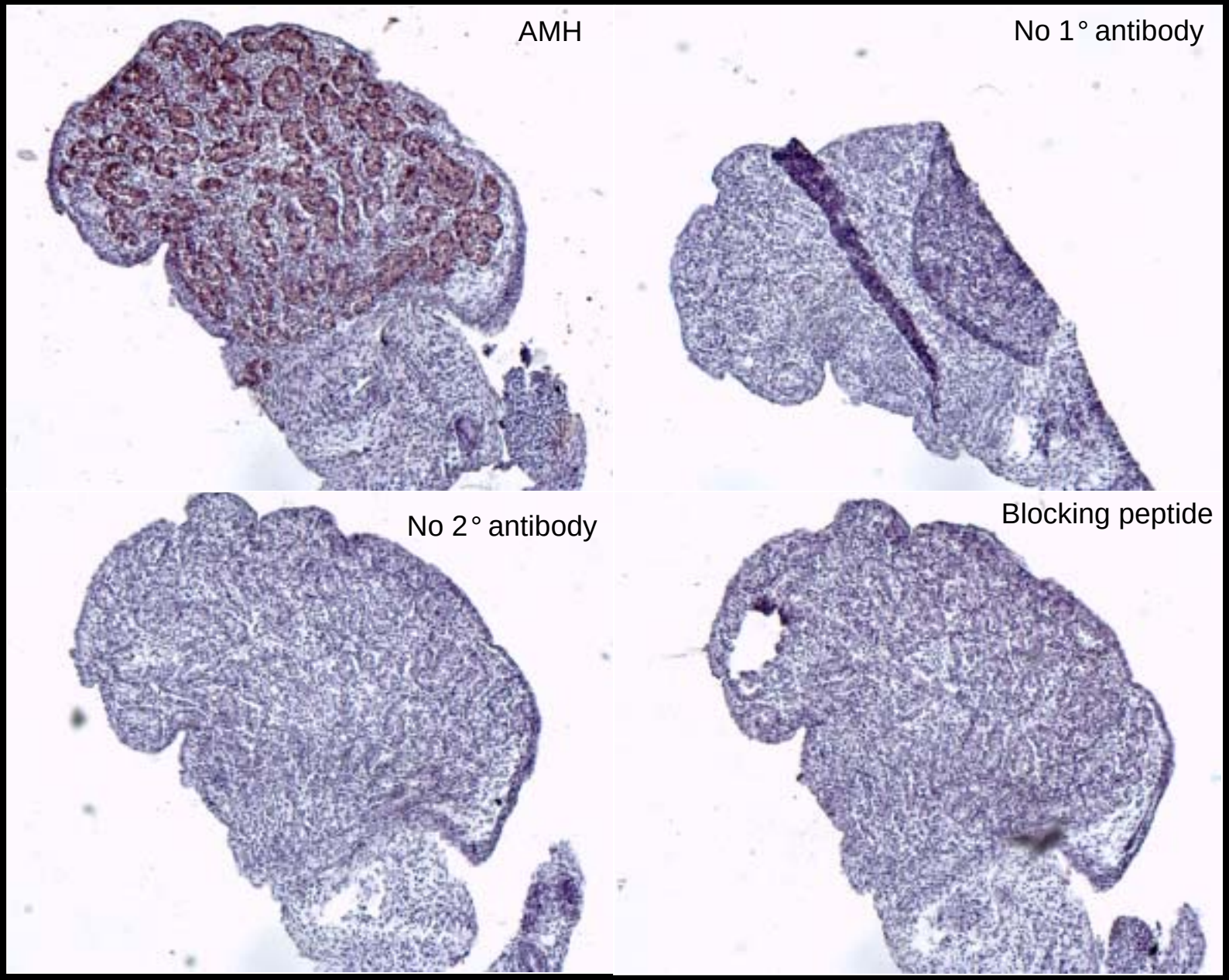


mouse ovary

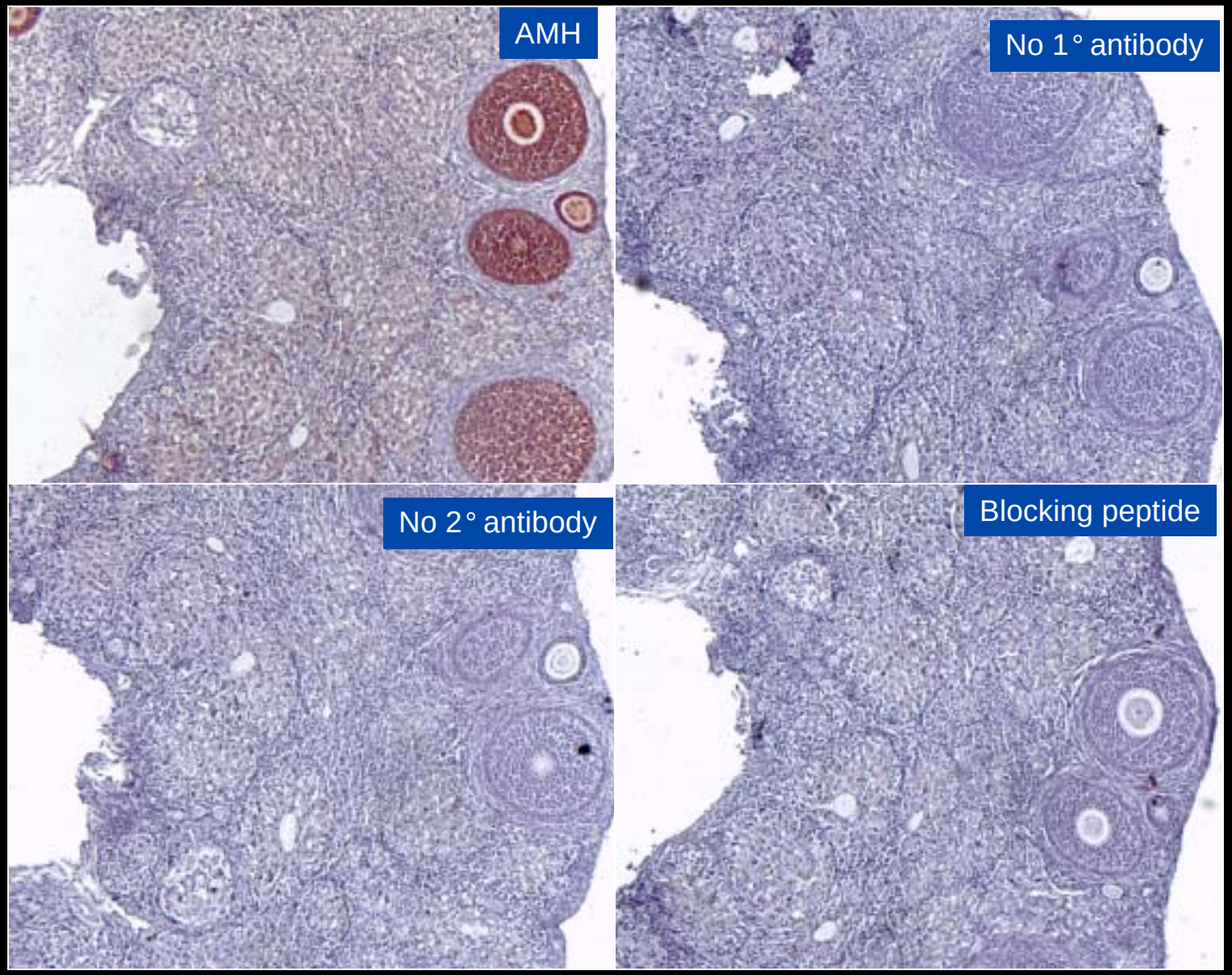


human ovary

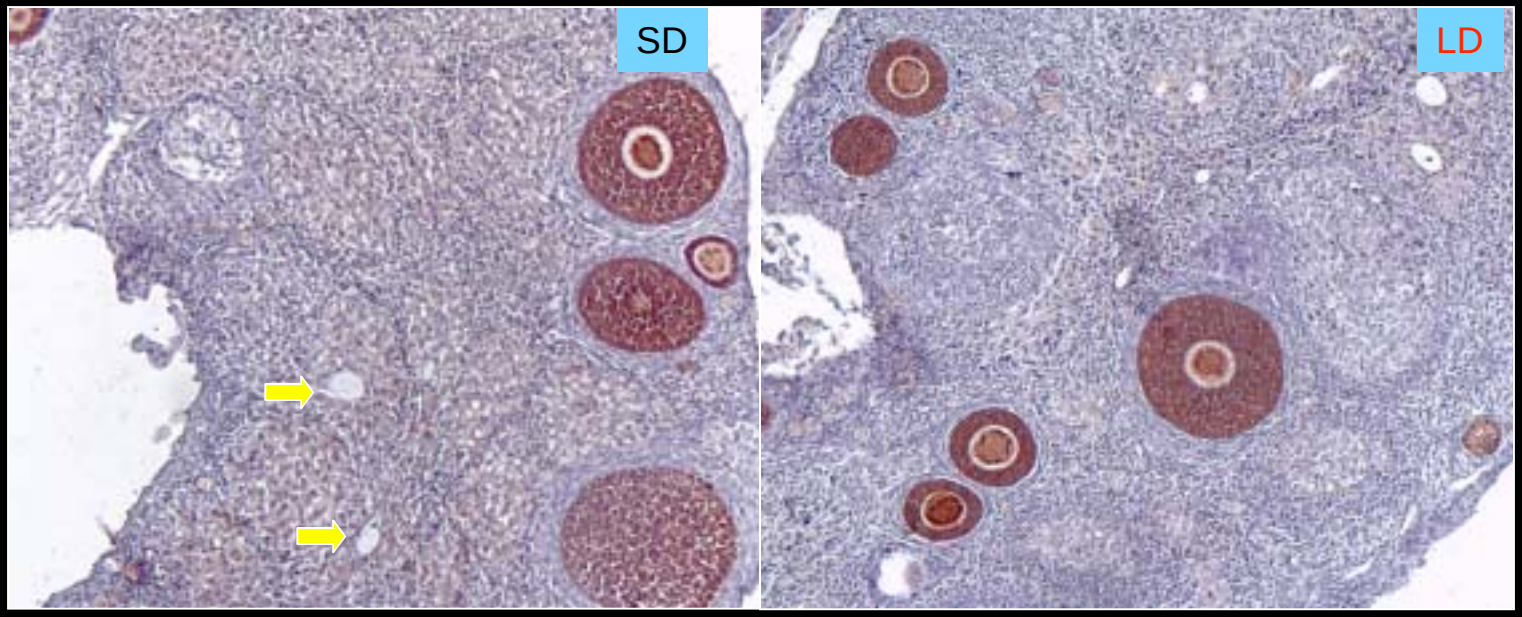
Neonatal Siberian hamster testis - positive control for AMH



Short Day Siberian hamster ovary - AMH antibody specificity



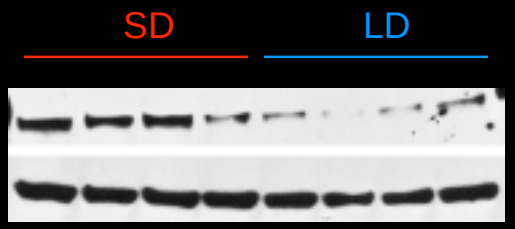
AMH staining in **Short Day** v. **Long Day** Siberian hamster ovaries
-SD and LD: staining in granulosa cells (GCs) of growing follicles.
-SD only, staining of hypertrophied GCs of atretic follicles.

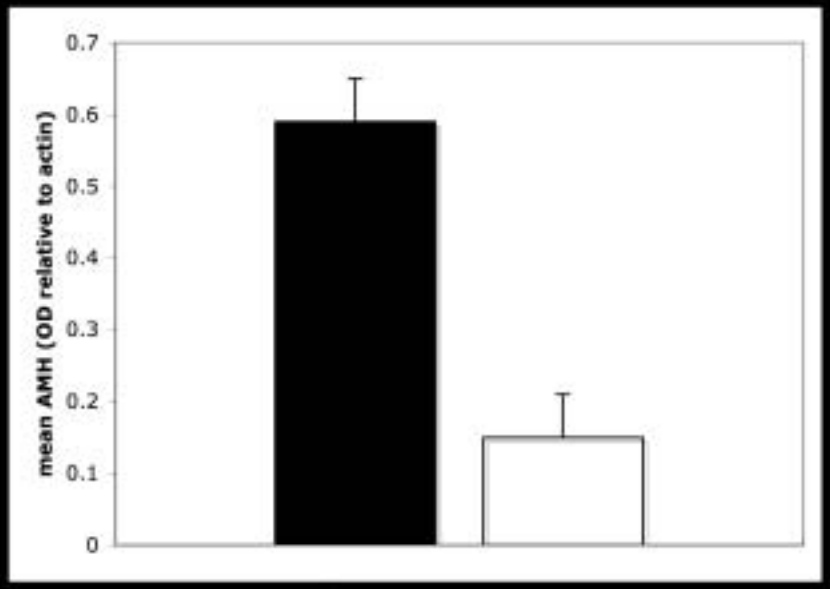


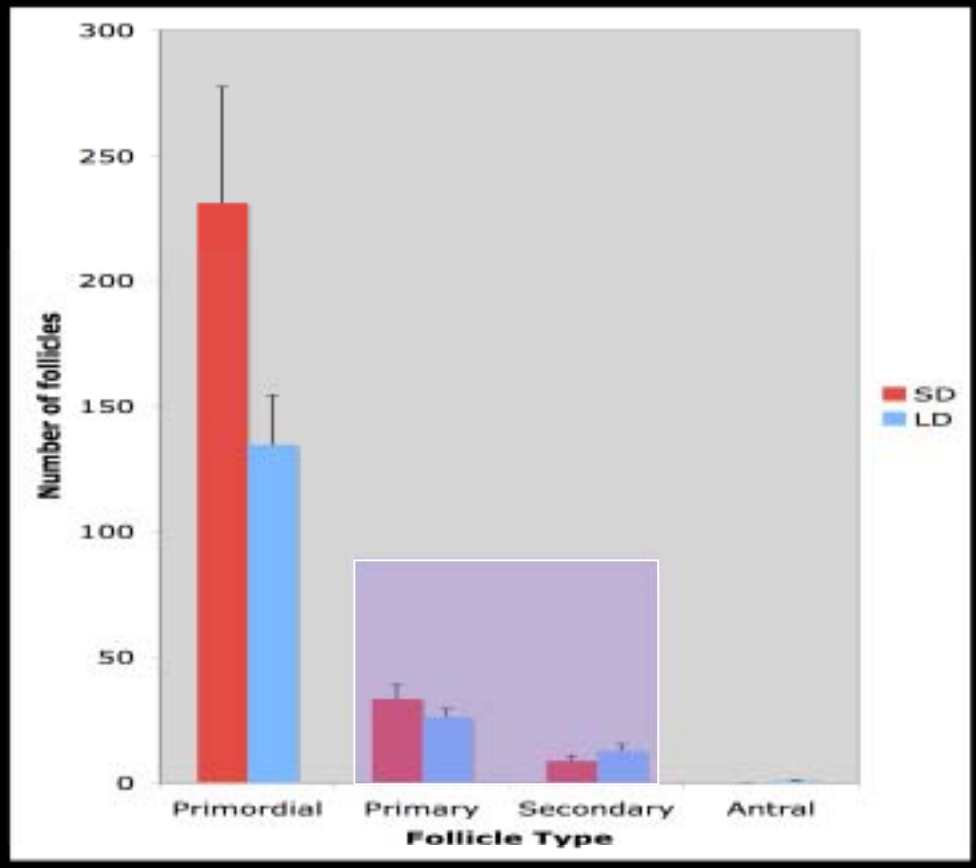
so called “hypertrophied granulosa cells of atretic follicles ” consistently show modest staining
➡ oocytes have degenerated

Ovarian AMH Summary

1. SD ovaries have 2X's primordial follicles as LD.
2. AMH (*inhibits follicular activation*) uniformly high in SD ovaries.
3. Variable AMH protein content in LD ovaries.
4. Similar numbers of 1º and 2º follicles in SD and LD
   - probably not the source of differences in ovarian AMH.

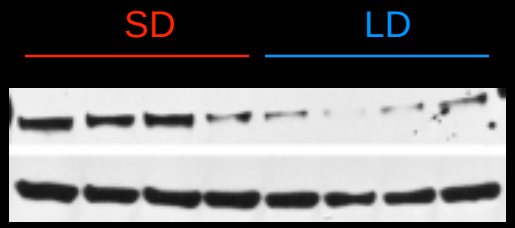


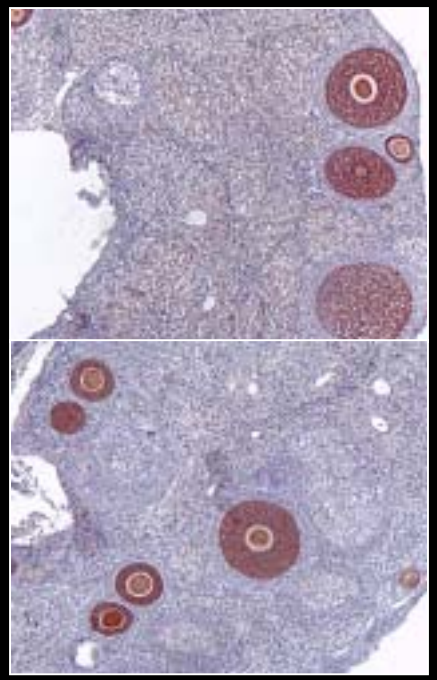




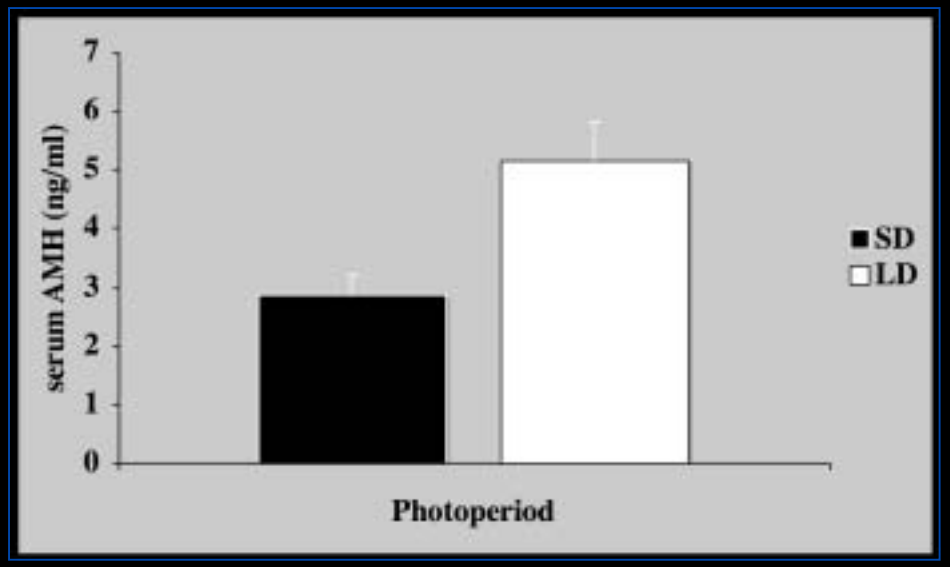
Timonin et al. Reproduction 2006

Whereas AMH is higher in SD than LD ovaries,





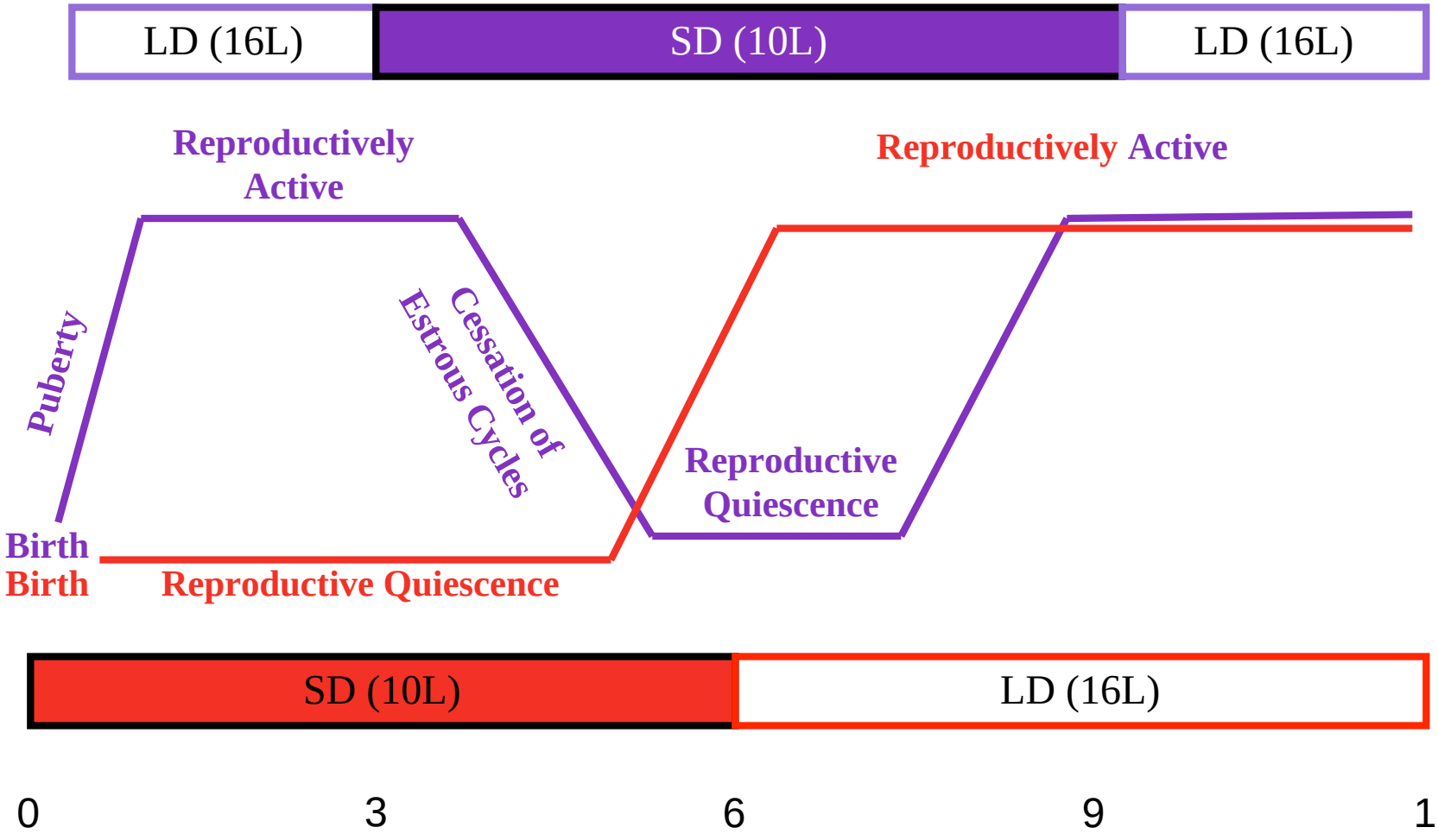
serum AMH is lower in SD than LD females.



- ***SD ovaries are smaller and structures that produce AMH might be poorly vascularized.***
- ***AMH may have paracrine/autocrine effects on follicular activation.***

Ongoing Research

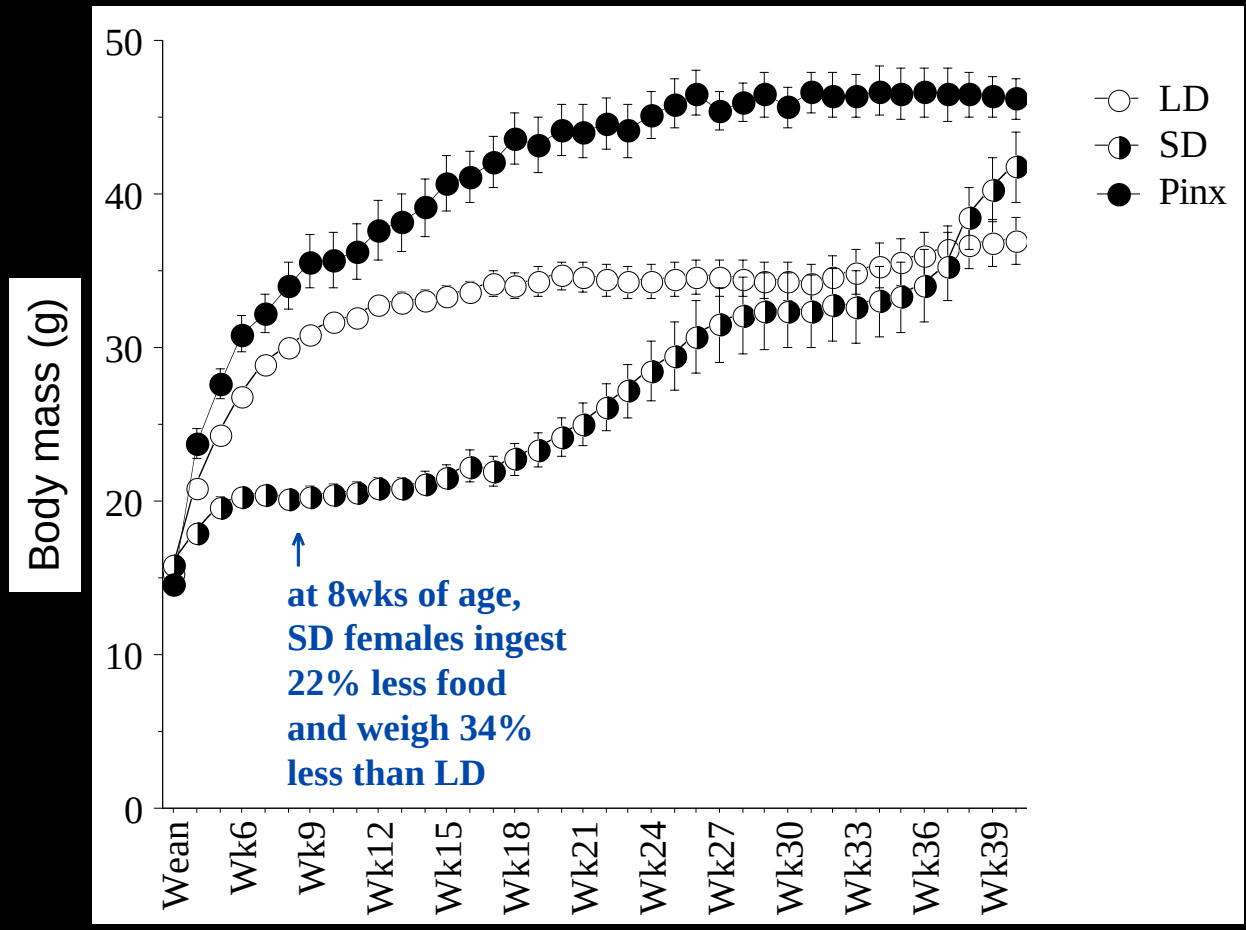
Does Reproductive Quiescence **After** Puberty Slow Aging?
-Transfer LD females to SD after puberty; mate at 1 y.o.
-Is decelerated aging d/t delayed puberty or repro quiescence?



Future research

-Food restrict LD females to match intake of SD females

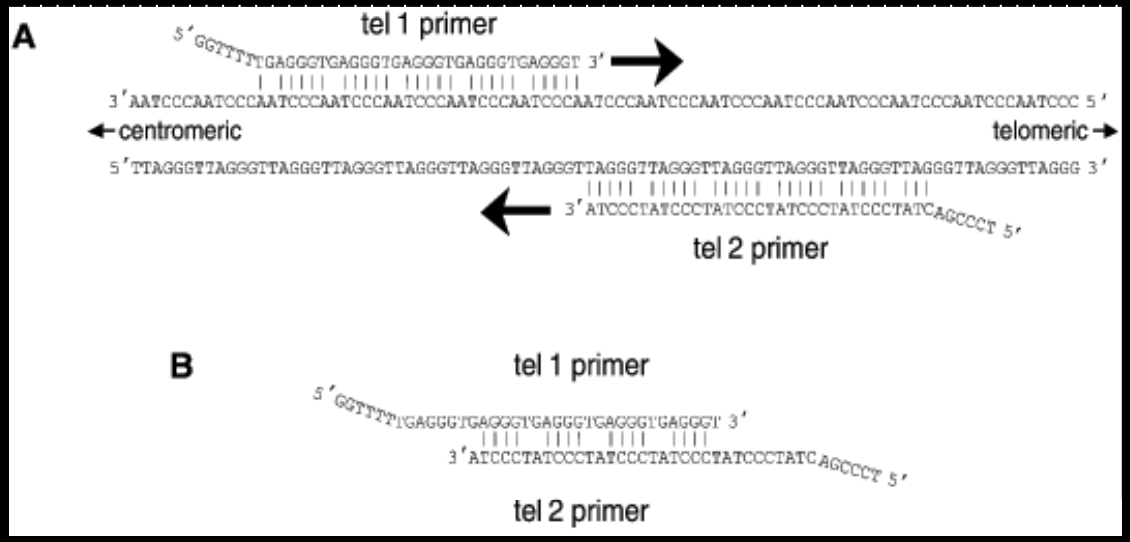
-Is the intensity and duration of CR adequate to delay aging?

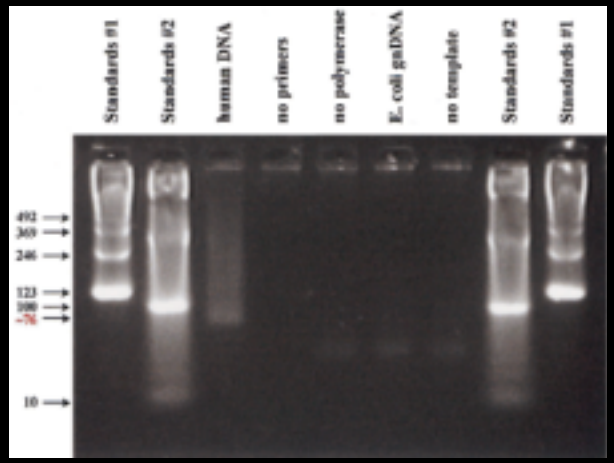


An integrative approach:
Somatic Aging and Reproductive Aging

- Biomarkers of Aging for Future studies:
  - Uterine aging: Cellular Senescence (SA-βgal), Telomere Lengths, Estrogen Receptors, and Connexin43.
- Immune function:
  - T-cell mediated antibody response.
  - delayed-type hypersensitivity response.
- Wound healing.

Telomere Length $(TTAGGG)_n$ as a Biomarker of Photoperiodic-induced Deceleration of Aging





Cawthon 2002

Is attrition of telomeres slower when hamsters are held in SD?
-unfortunately, telomeres do NOT appear to become shorter with age in Siberian hamsters.

Telomeres did NOT shorten with age in a predictable manner in Siberian hamsters?

- Validated with groups of widely disparate ages (1 v. 16 months). No age difference in spleen, uterus, WBC's.
- Alternatives:
  - Cellular senescence (SA-βgal).
  - Accumulation of DNA double-strand breaks (Sedelnikova et al., 2004)
  - Longevity studies - very costly and other logistical hurdles.

Postdoc research with Steve Glickman, University of California, Berkeley
→ now a collaborator and occasional visitor

Field Station for the Study of Behavior, Ecology, and Reproduction

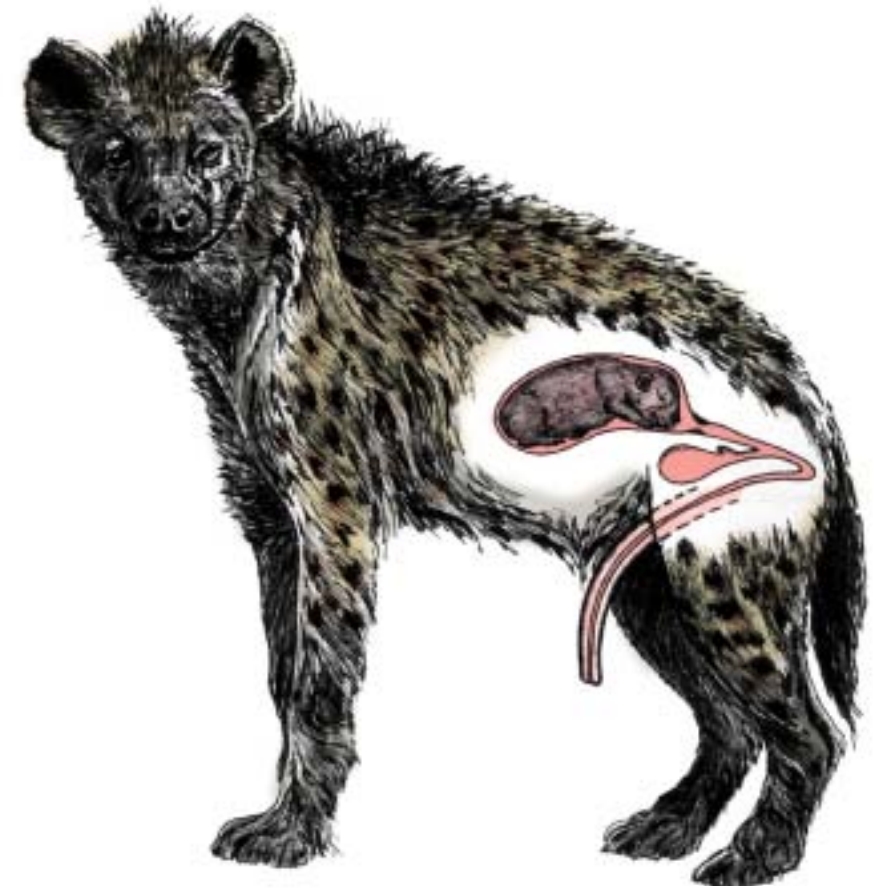
The Berkeley Hyenas

Collecting Cubs in Kenya

"The female spotted hyena is unique among mammals in displaying an extraordinary degree of masculinization of the external genitalia …"

"… [the labia majora are] fused to form a scrotum and the clitoris is hypertrophied to form a fully erectile pseudopenis, similar in size and anatomy to the male penis"

Frank and Glickman, 1994



Drawing by Christine Drea

Spotted Hyenas: Important Facts

1. Social organization:
   - Clan w/ matrilineal lines & immigrant males.
2. Dominance hierarchies exist between matrilineal lines (and within litters).
3. Adult females and their cubs are dominant to all adult males (females generally larger and more aggressive than males).
4. Social hunters and competitive feeders.

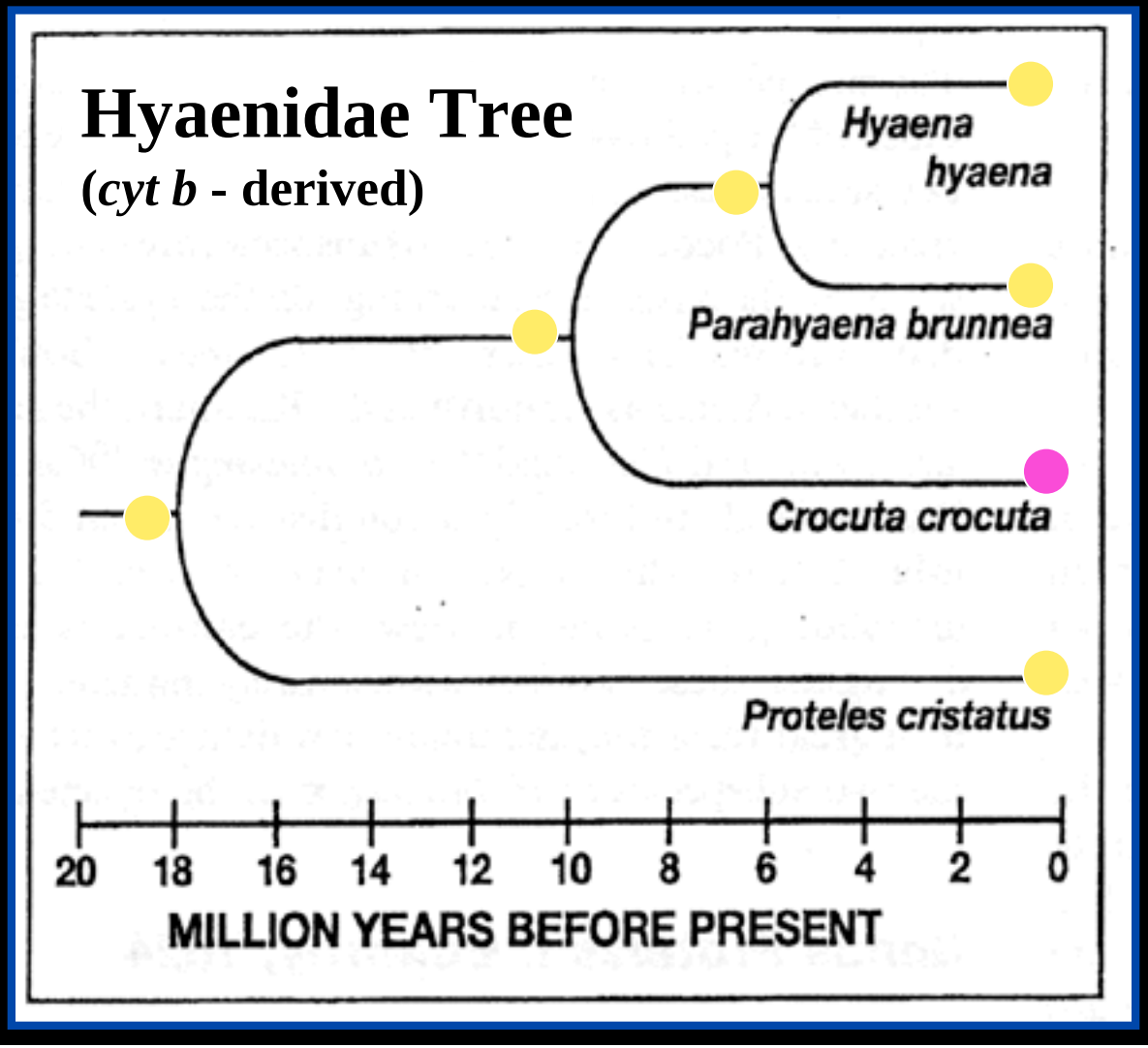
Aardwolf

Striped hyena

Spotted hyena

Brown hyena

Masculinization of female genitalia: When, How, and Why?

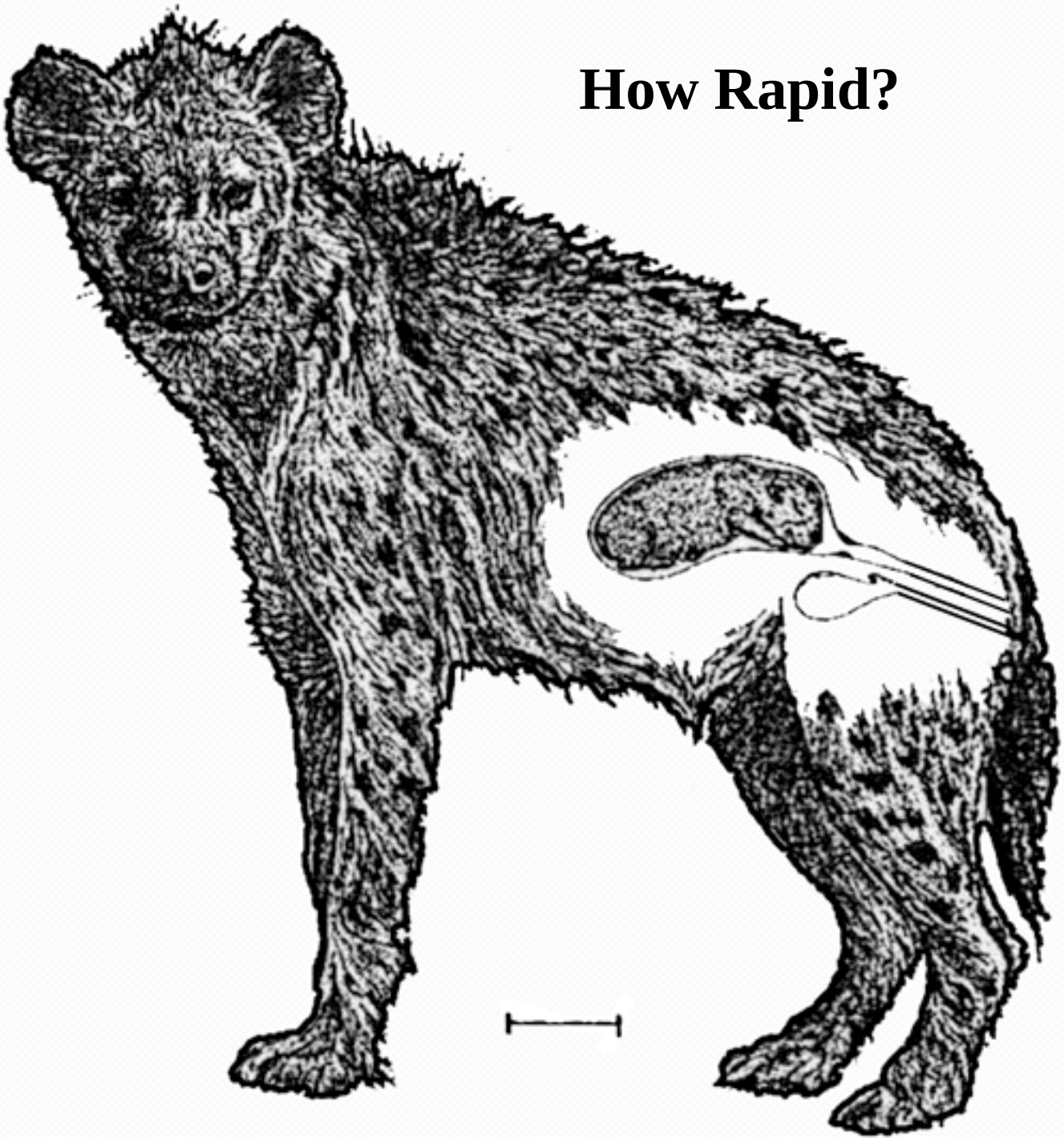


"typical" females (+ distal vagina)

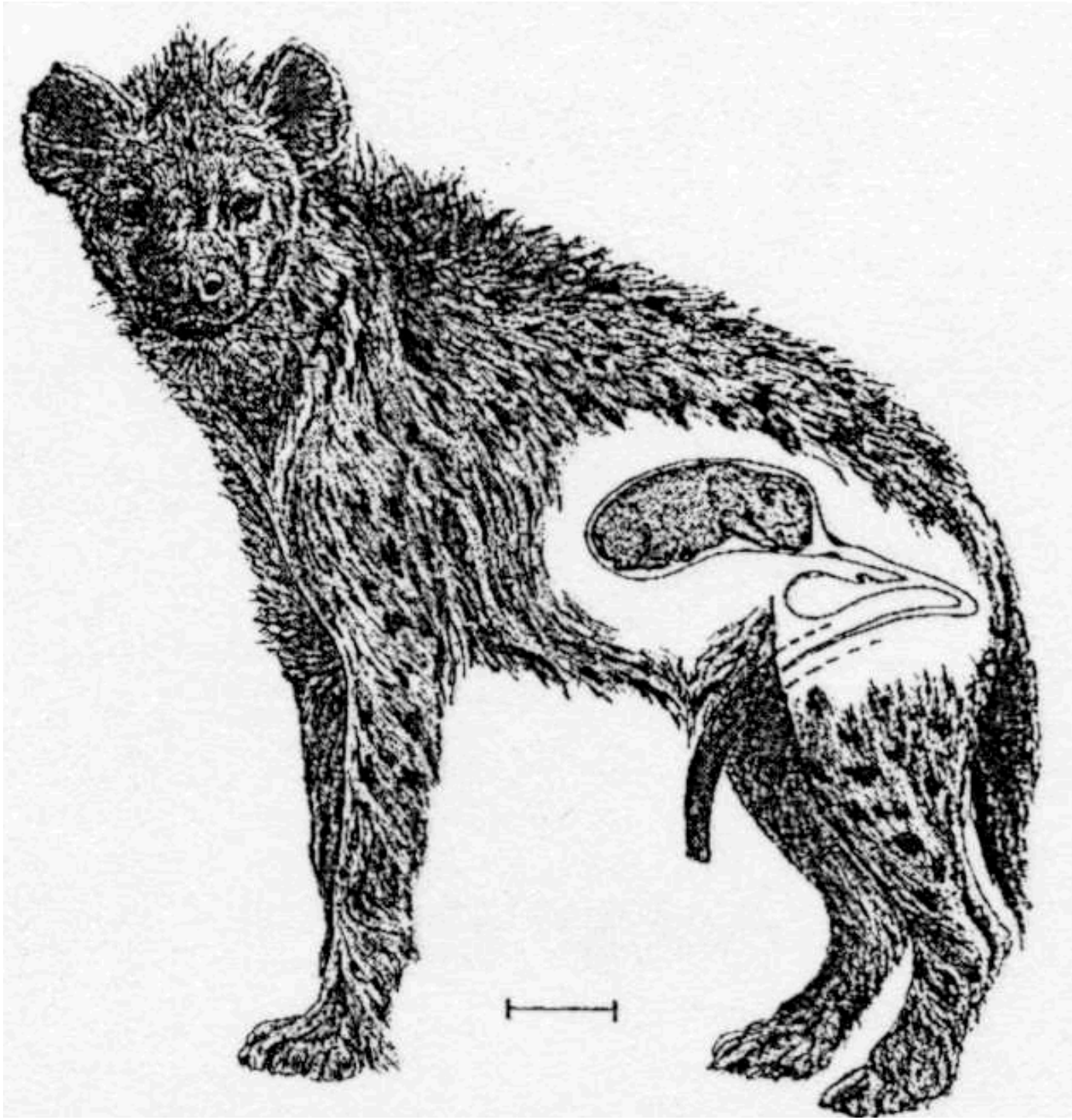
masculinized females (No distal vagina)

Jenks & Werdelin, 1998

How Rapid?



The Ancestral Hyena?



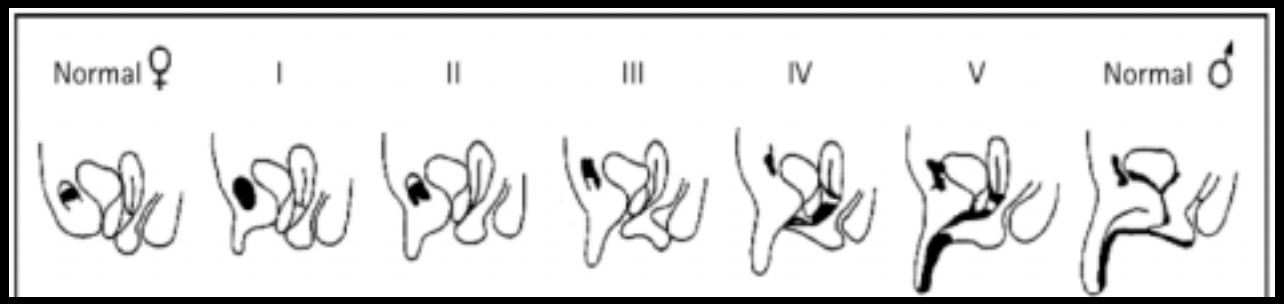
The Spotted Hyena (Today)

Spotted Hyena

Striped Hyena

Male

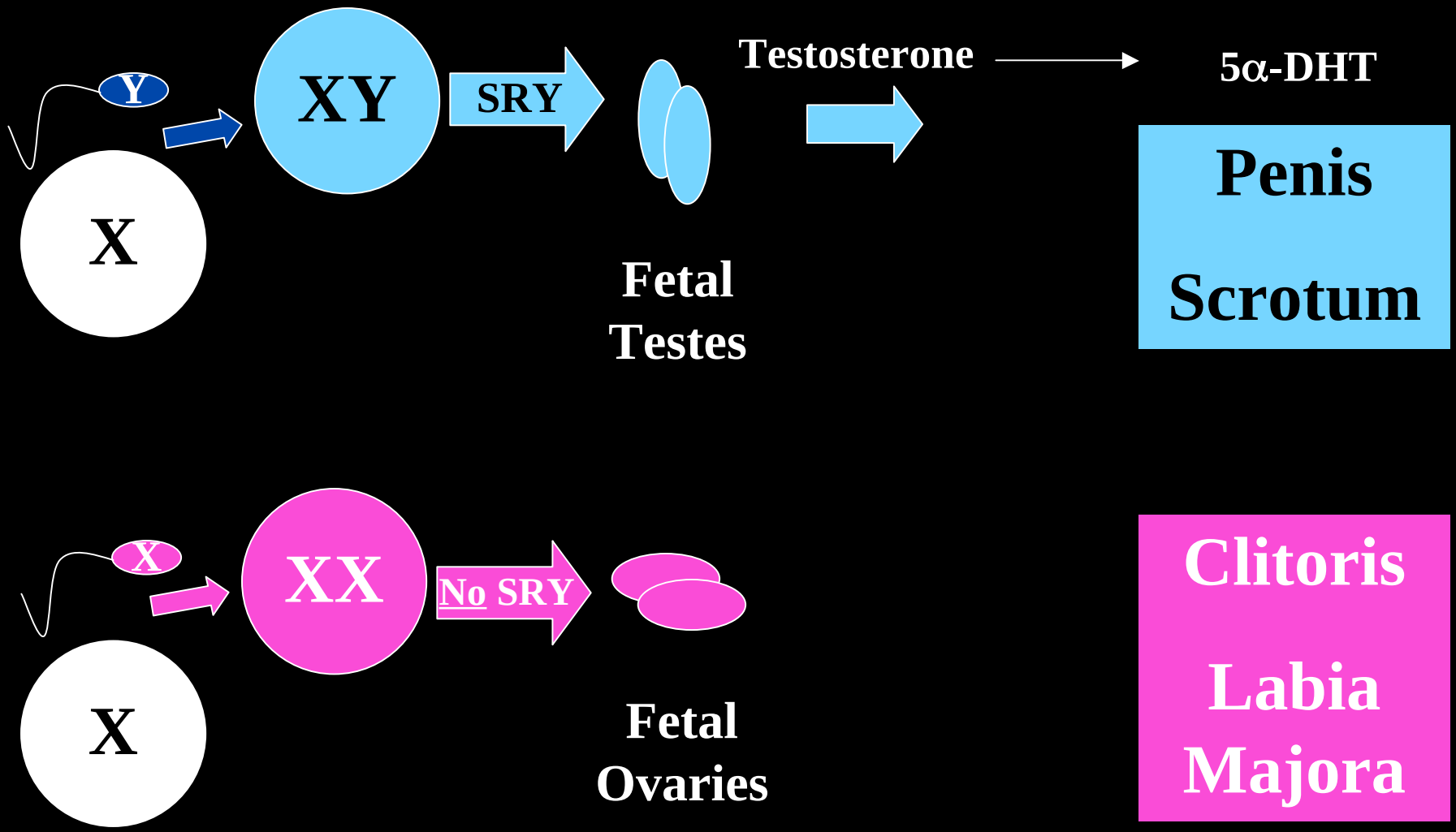
Female



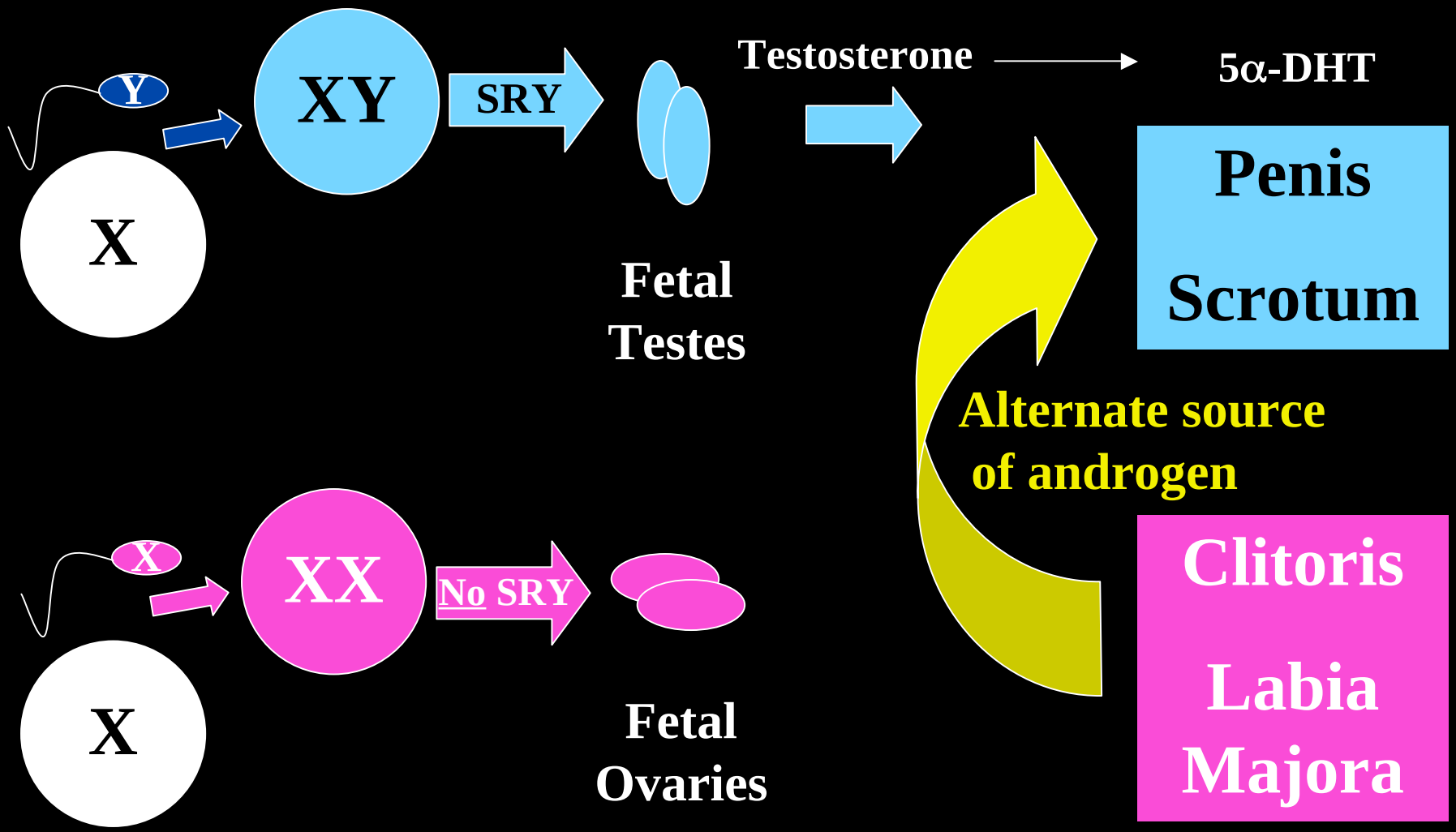
HOW?

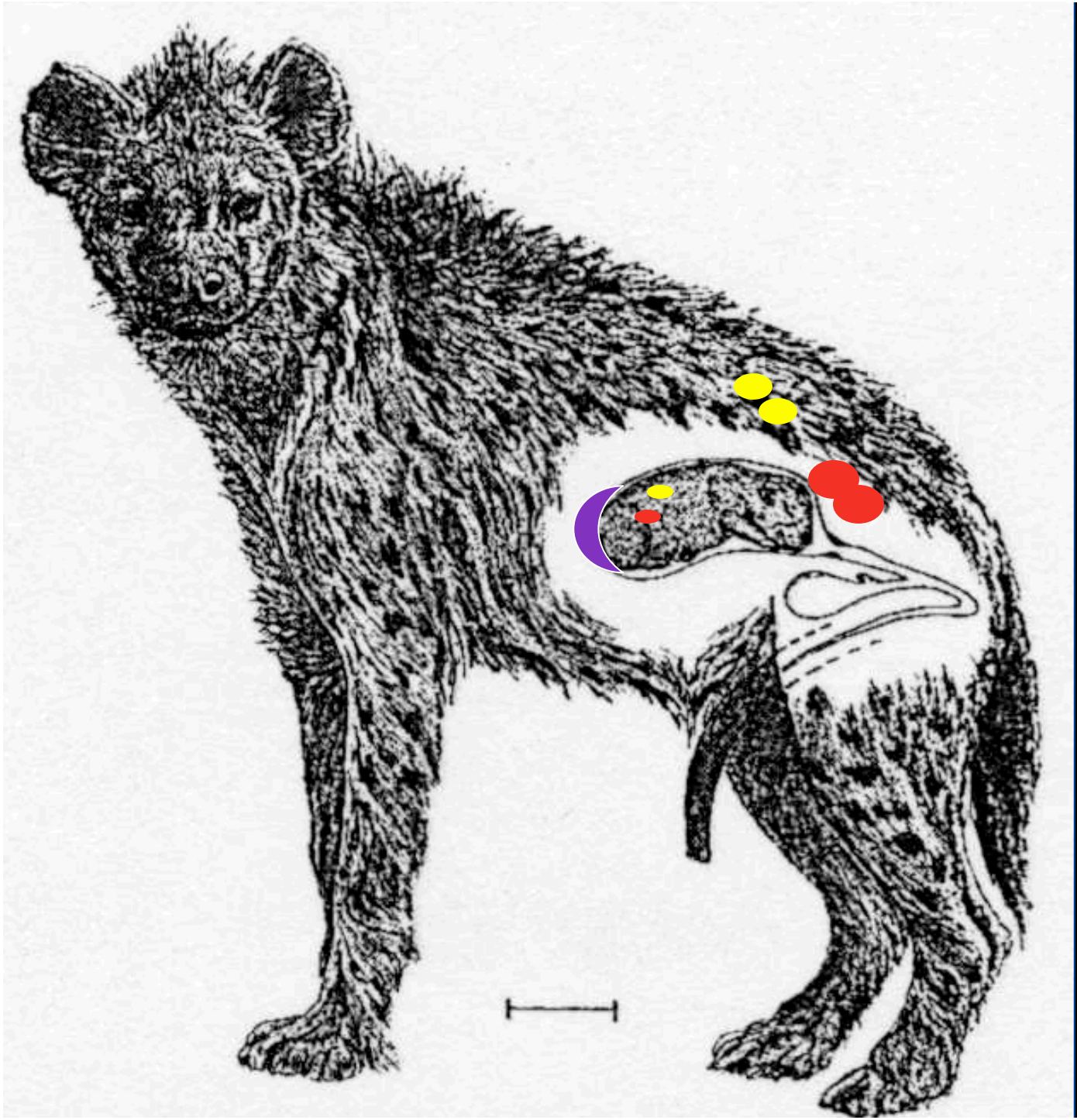
Putative mechanism for masculinization of female genitalia - spotted hyenas and other mammals.

Contemporary Model of Eutherian Sexual Differention (Jost; Wilson)



Model of Female Masculinization (Experimental, Pathological, Natural)





Potential Sources of Prenatal Androgens

Ovaries
Maternal/Fetal

Adrenals
Maternal/Fetal

Placenta

Over production of androgens by the fetal adrenals

INFANT FEMALE

AMBIGUOUS GENITALIA

CONGENTIAL ADRENAL HYPERPLASIA

Or, failure of the placenta to convert androgens to estrogens

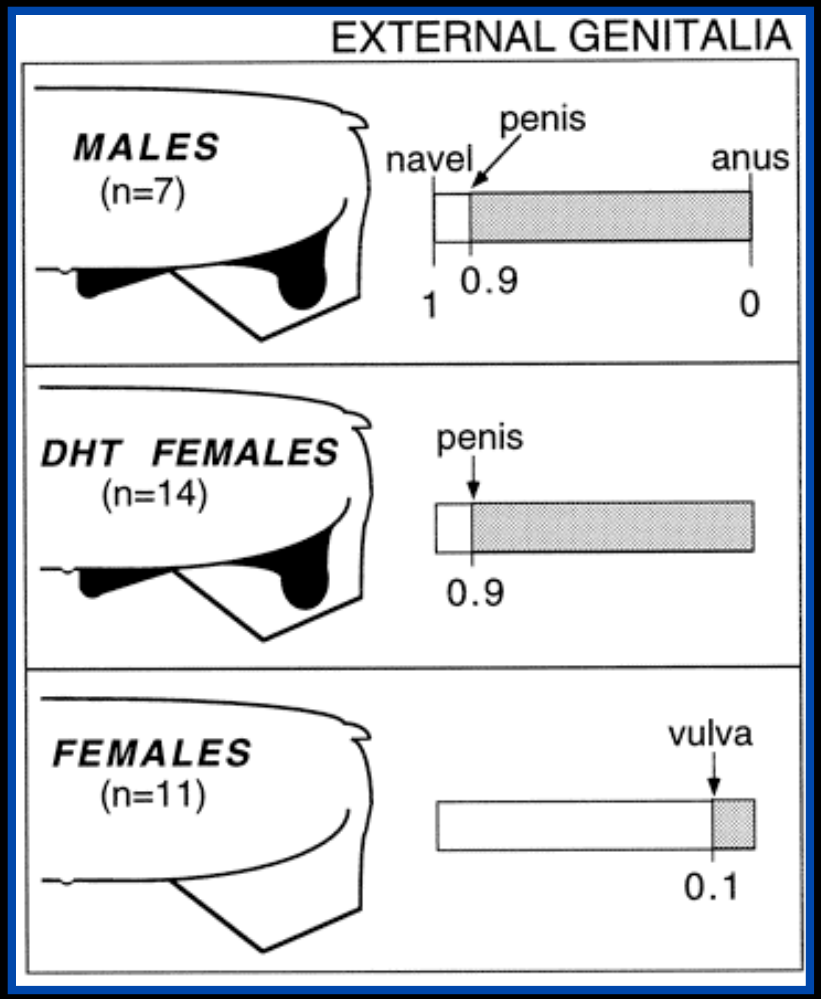
INFANT FEMALE

AMBIGUOUS GENITALIA

Placental Aromatase Deficiency

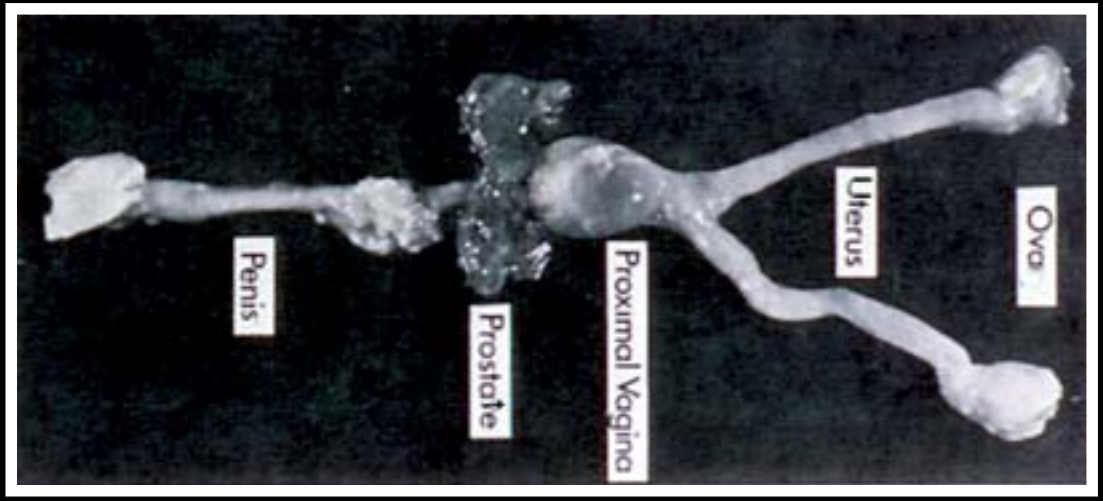
EXPERIMENTAL VIRILIZATION

-effects of exogenous androgens



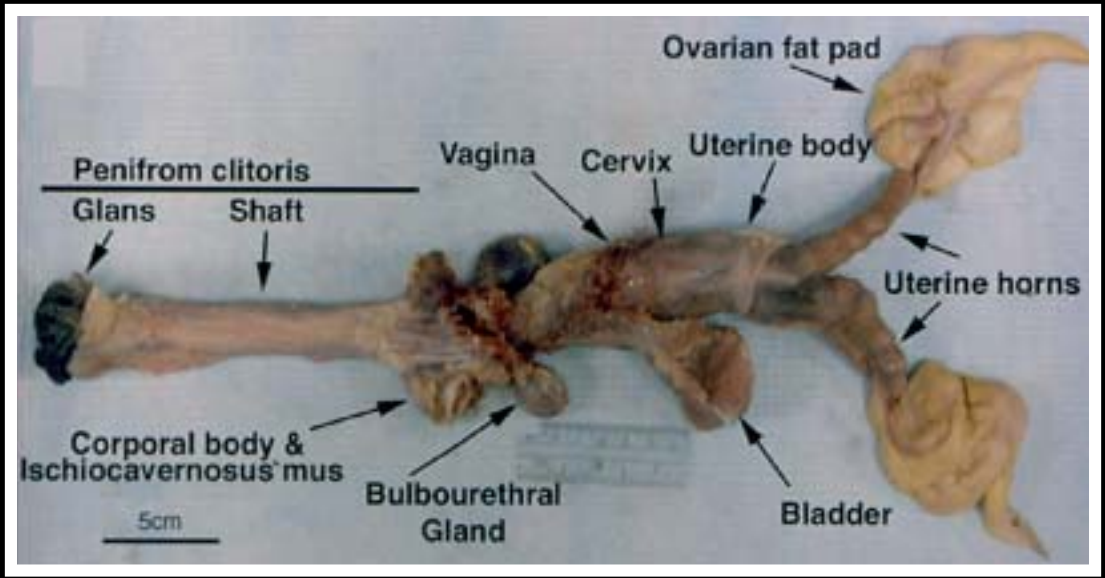
Masek *et al.*, (1999)

Experimental and natural masculinization in two carnivore species



Urogenital System of "Androgenized" Female Beagle

Beach, Buehler & Dunbar, Behav Neural Biol, 1983



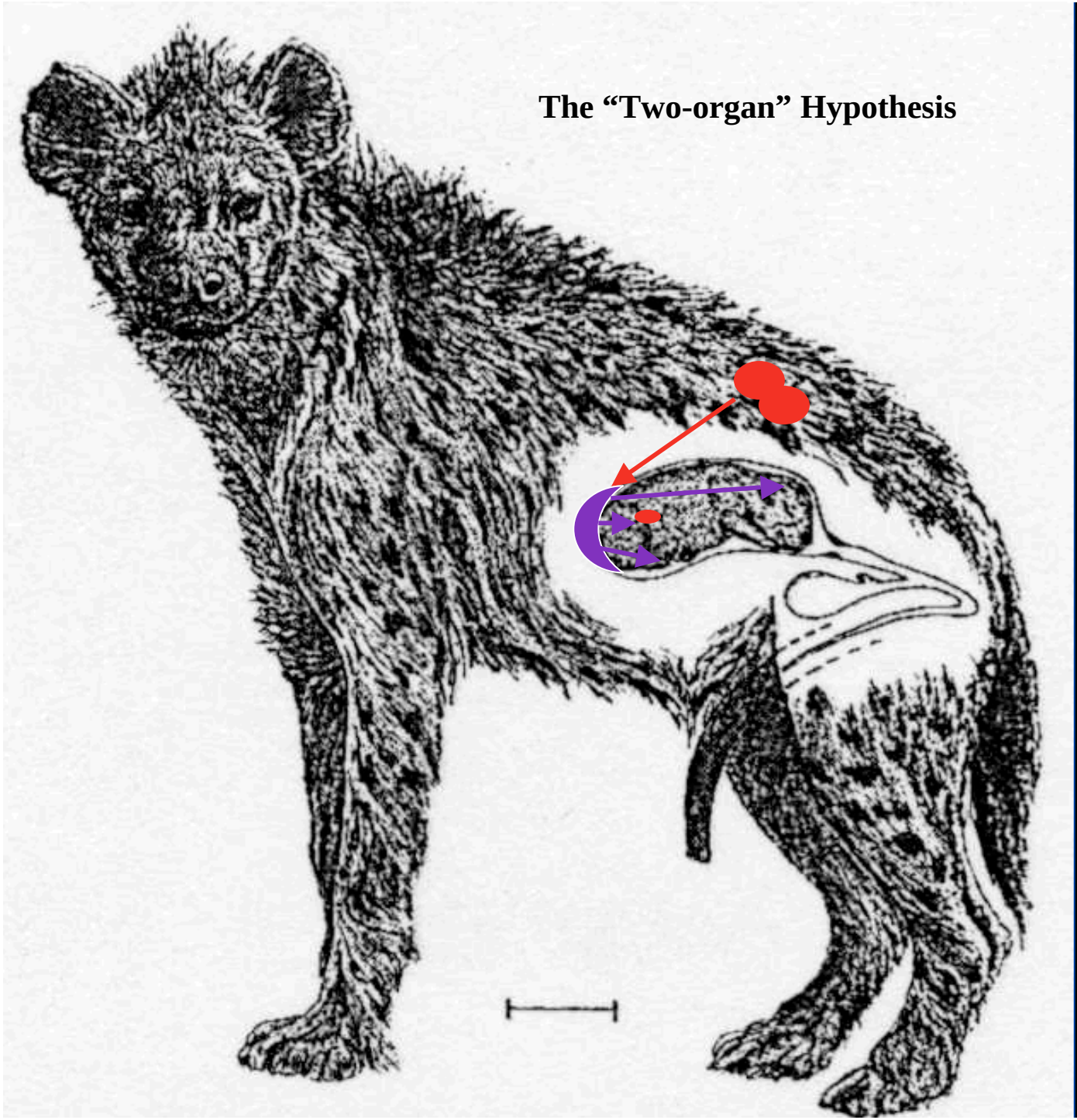
Urogenital System of "Normal" Female Spotted Hyena

Cunha, et al., *J Morph*, 2003

External Genitalia of the Spotted Hyena

Natural Masculinization to the Extreme

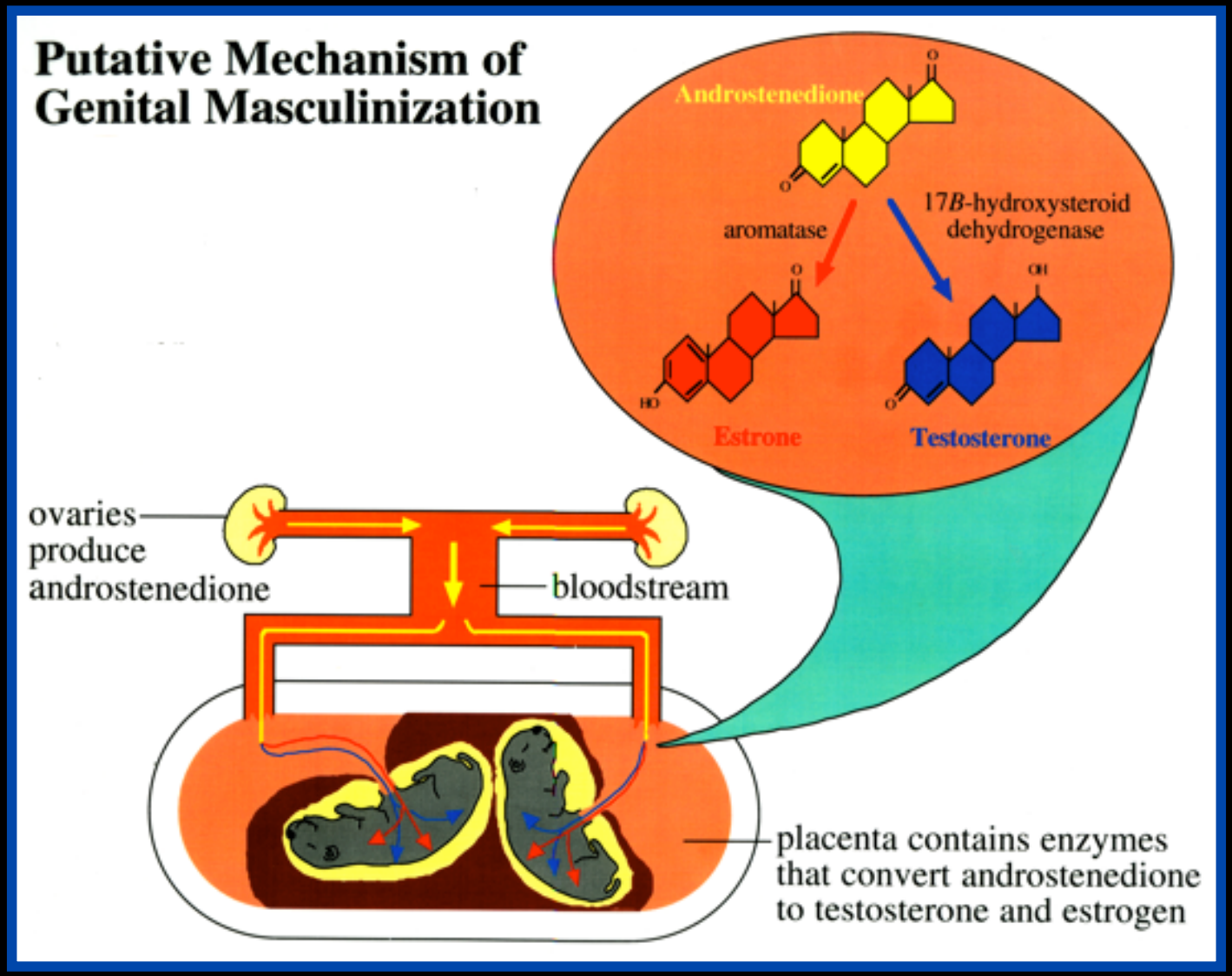
The "Two-organ" Hypothesis



Maternal
Ovaries
⇒A4
Androstenedione

Placenta
⇒**T**, E1
Testosterone
or
Estrone

A Theory of "Masculinization"



Licht et al, J Reprod Fertil, 1992; Yalcinkaya et al, Science, 1993

Two Clinical Reports: When a Human Placenta Acts Like a Hyena Placenta

Journal of Clinical Endocrinology and Metabolism

1991:

A New Cause of Female Pseudohermaphroditism: Placental Aromatase Deficiency

MAKIO SHOZU*, KAZUTOMO AKASOFU, TAKENORI HARADA, AND YOSHI KUBOTA

Department of Obstetrics and Gynecology, Kanazawa University School of Medicine, Takara-machi 13-1, Kanazawa 920, Japan

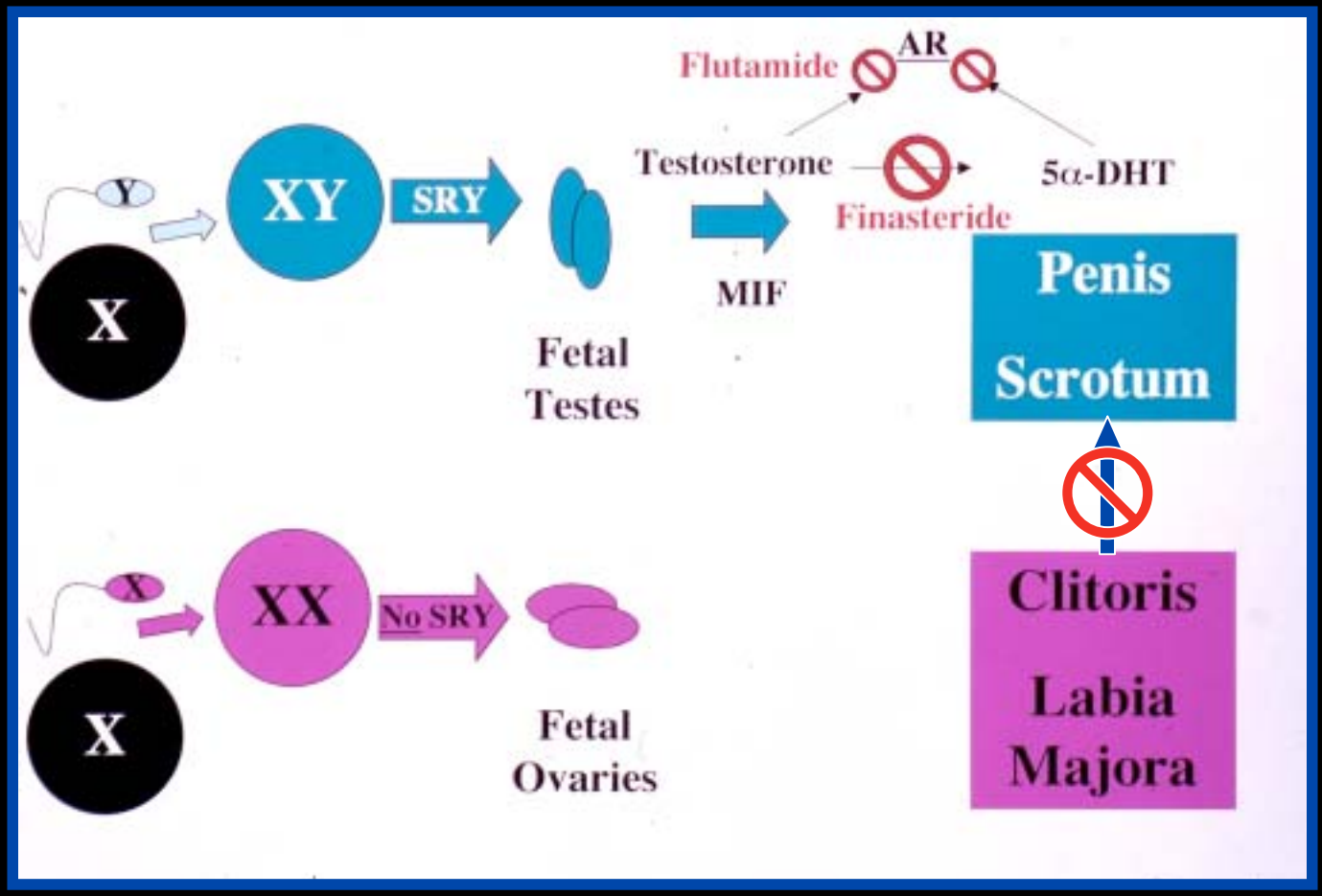
1994:

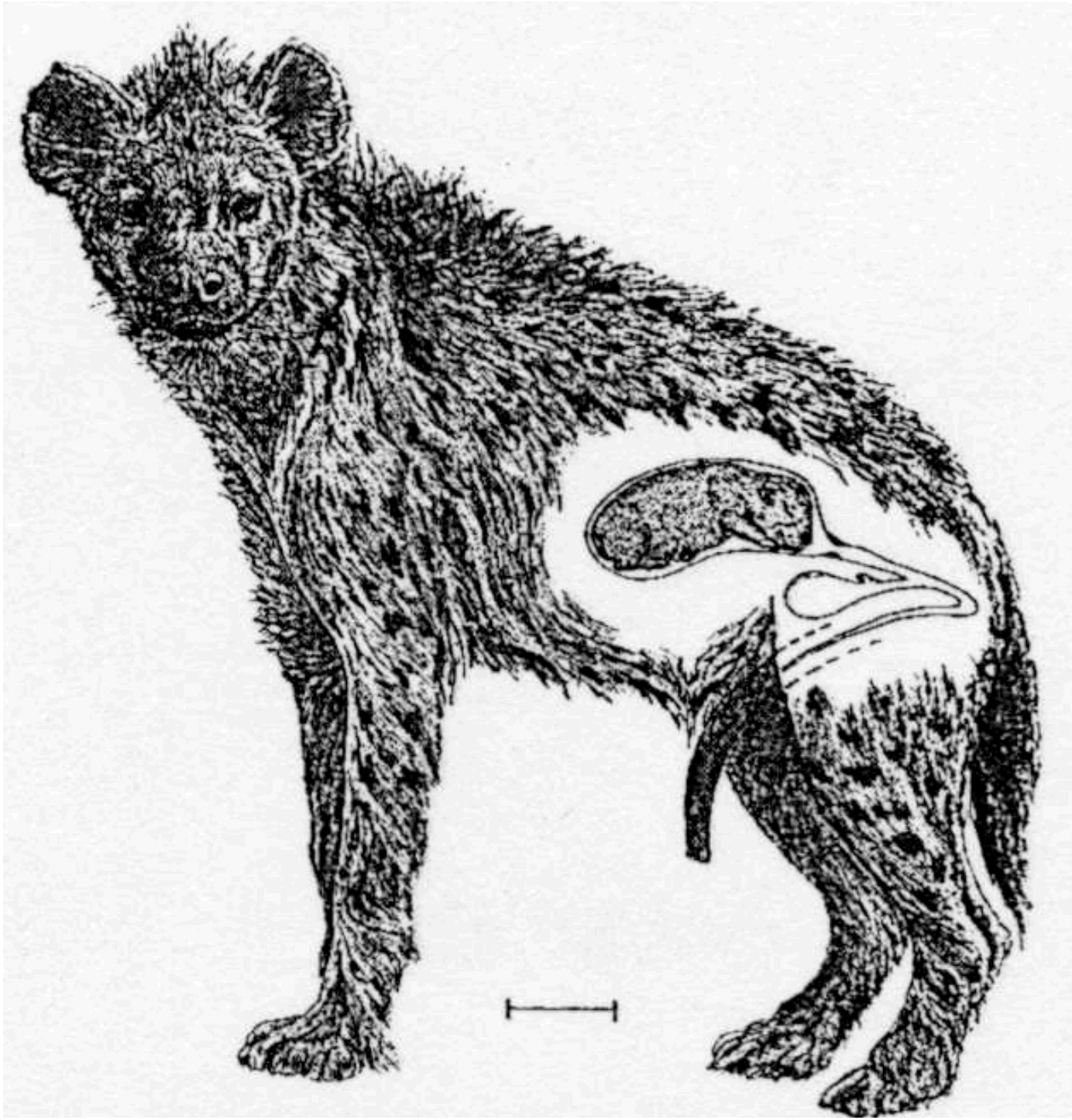
A Syndrome of Female Pseudohermaphrodism, Hypergonadotropic Hypogonadism, and Multicystic Ovaries Associated with Missense Mutations in the Gene Encoding Aromatase (P450arom)*

FELIX A. CONTE, MELVIN M. GRUMBACH, Y. ITO, CAROLYN R. FISHER, AND EVAN R. SIMPSON

Department of Pediatrics, University of California (F.A.C., M.M.G.), San Francisco, California 94143-0106; the Cecil H. and Ida Green Center for Reproductive Biology Sciences and the Departments of Obstetrics/Gynecology and Biochemistry, University of Texas Southwestern Medical Center (Y.I., C.R.F., E.R.S.), Dallas, Texas 75235

Testing the Maternal Ovary-Placenta Hypothesis →Treat with Anti-Androgens prenatally



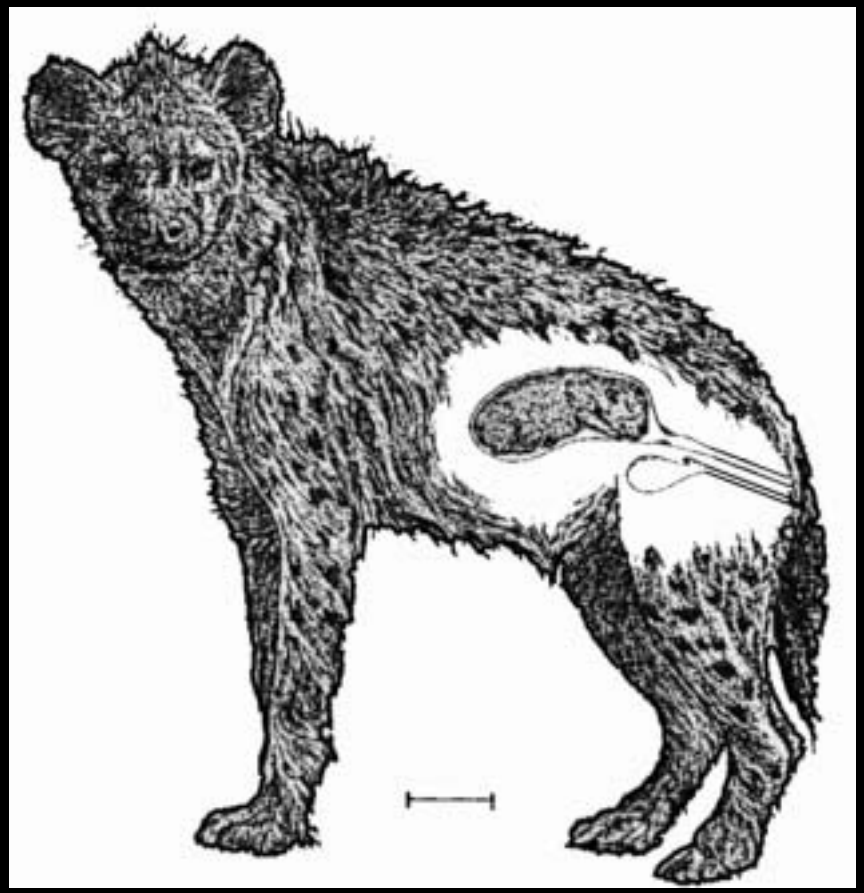


MOM

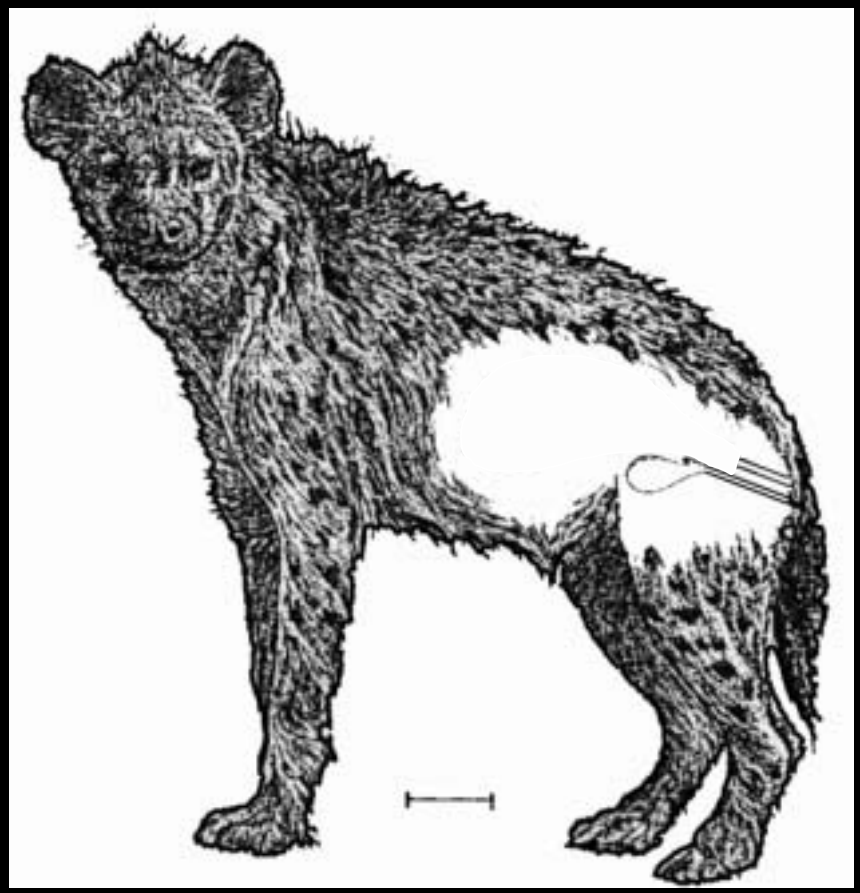
**Treated w/ Flutamide & Finasteride (*Anti-androgens)*

Expected outcomes based on similar expts and observations of other species (e.g., *AR*$^{-/-}$)

Daughters?



Sons?



Changes in spotted hyena genitalia associated with prenatal anti-androgens

Untreated Female

(AA-female similar gross appearance)

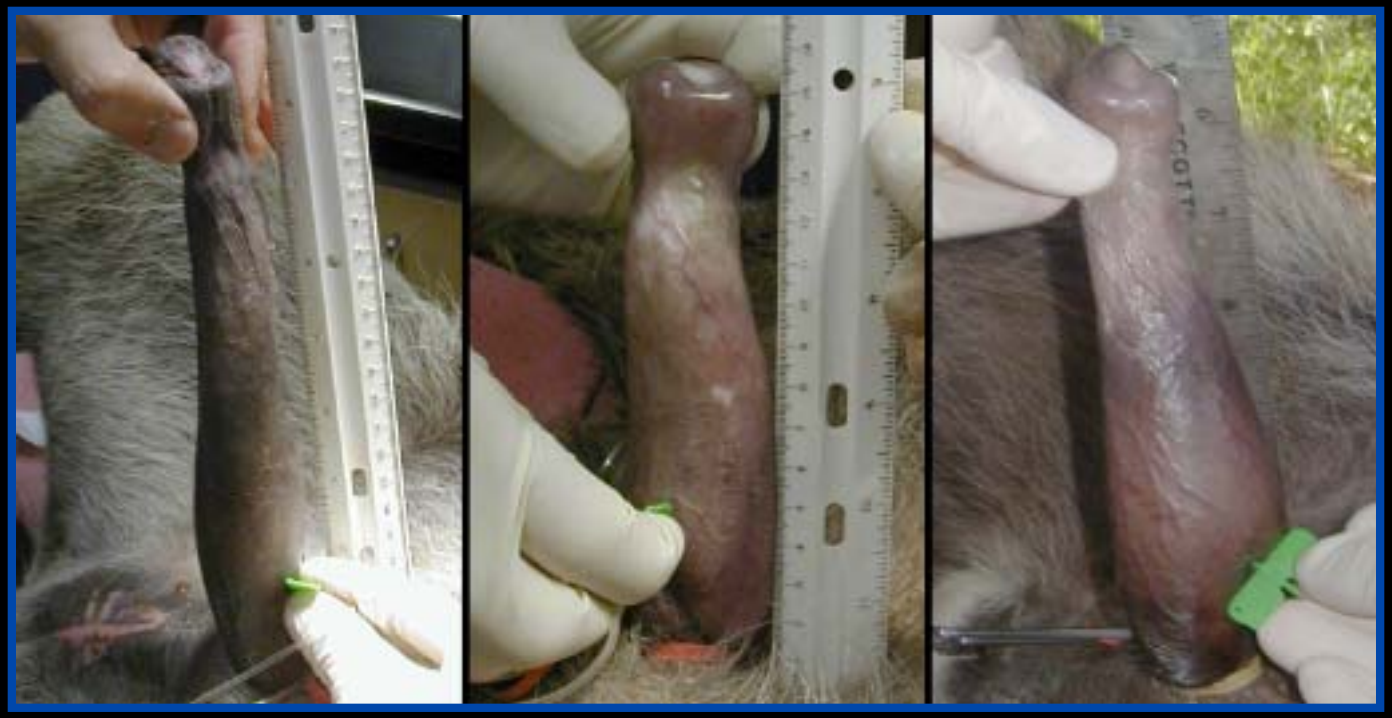
Anti-androgen Male

Untreated Male

Drea *et al.* (1998)

Anatomical Factors That May Impact Mating 1

Artificial erections further emphasize the effects of prenatal anti-androgens on male genitalia and explain why AA-males are functionally sterile.

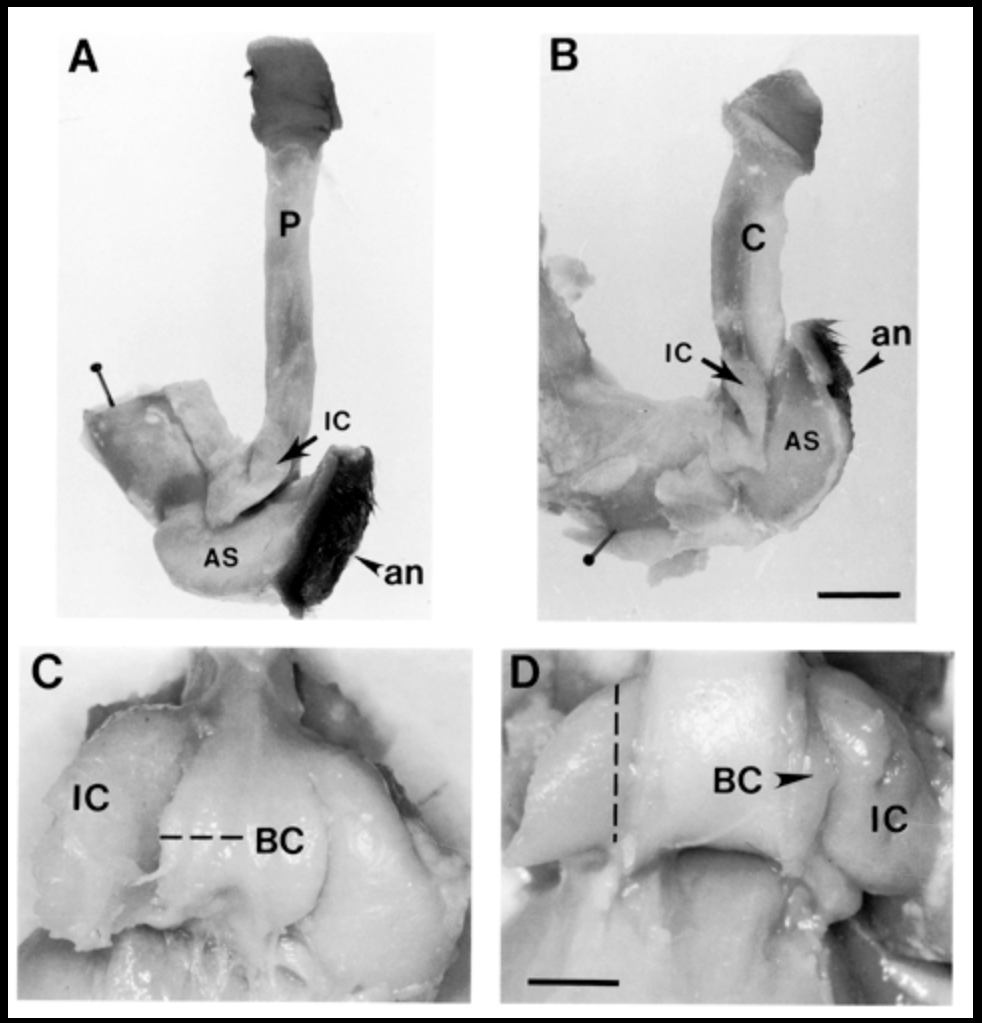


Anatomical Factors That May Impact Mating 2

Prenatal Anti-androgens 'Feminize' Bulbocavernosus Muscles

Max width of bulbocavernosus was 84% larger in male than female neonates

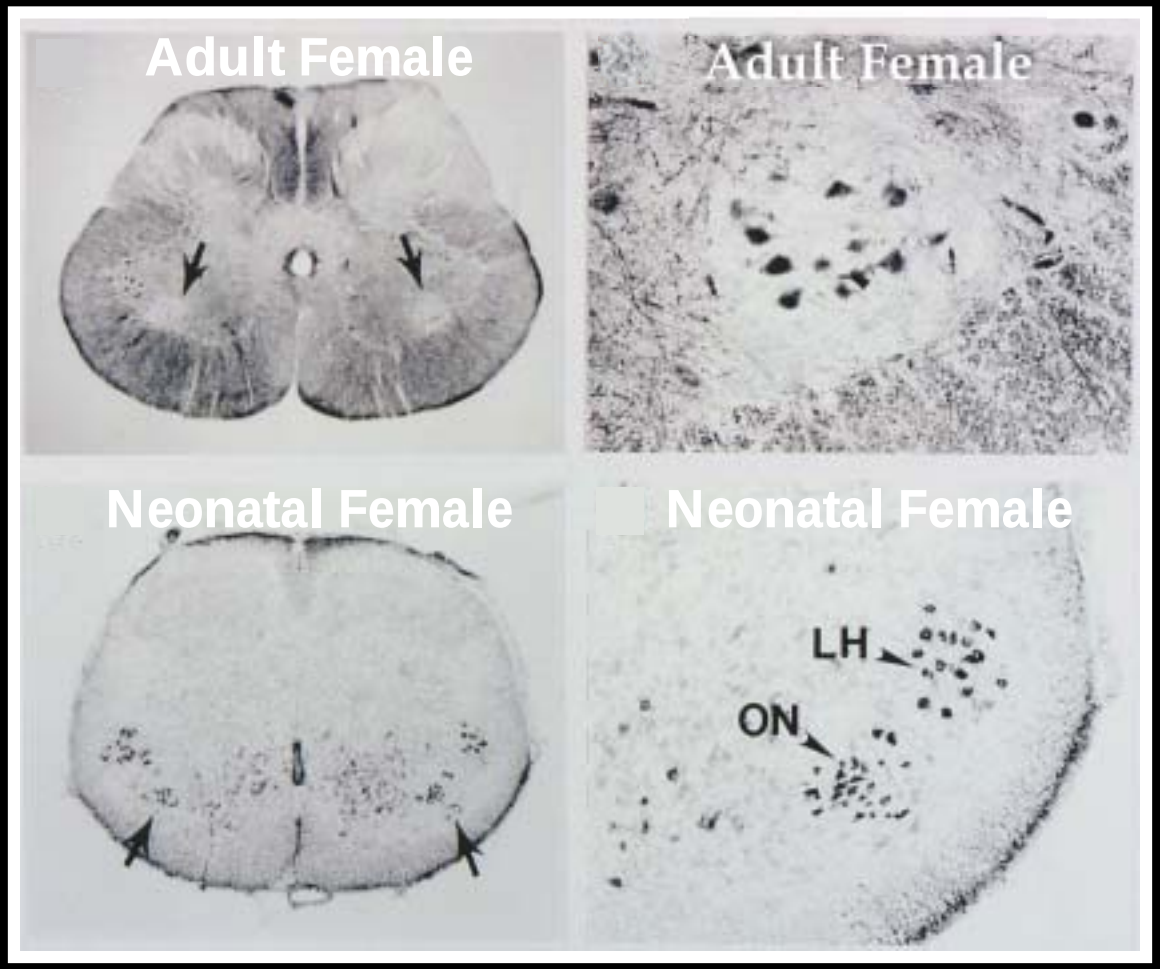
Max width of bulbocavernosus muscle of AA-treated male neonates was similar to that of female neonates

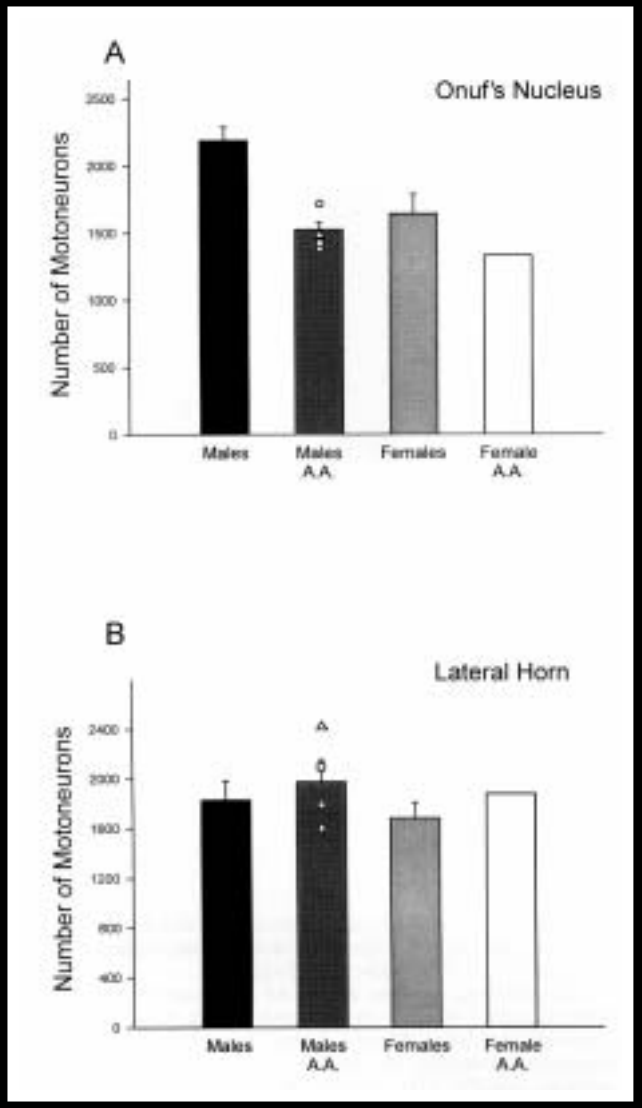


Forger et al., *J Comp Neurol*, 1996

Anatomical Factors That May Impact Mating 3

Prenatal Anti-androgens 'Feminize' Spinal Motoneurons



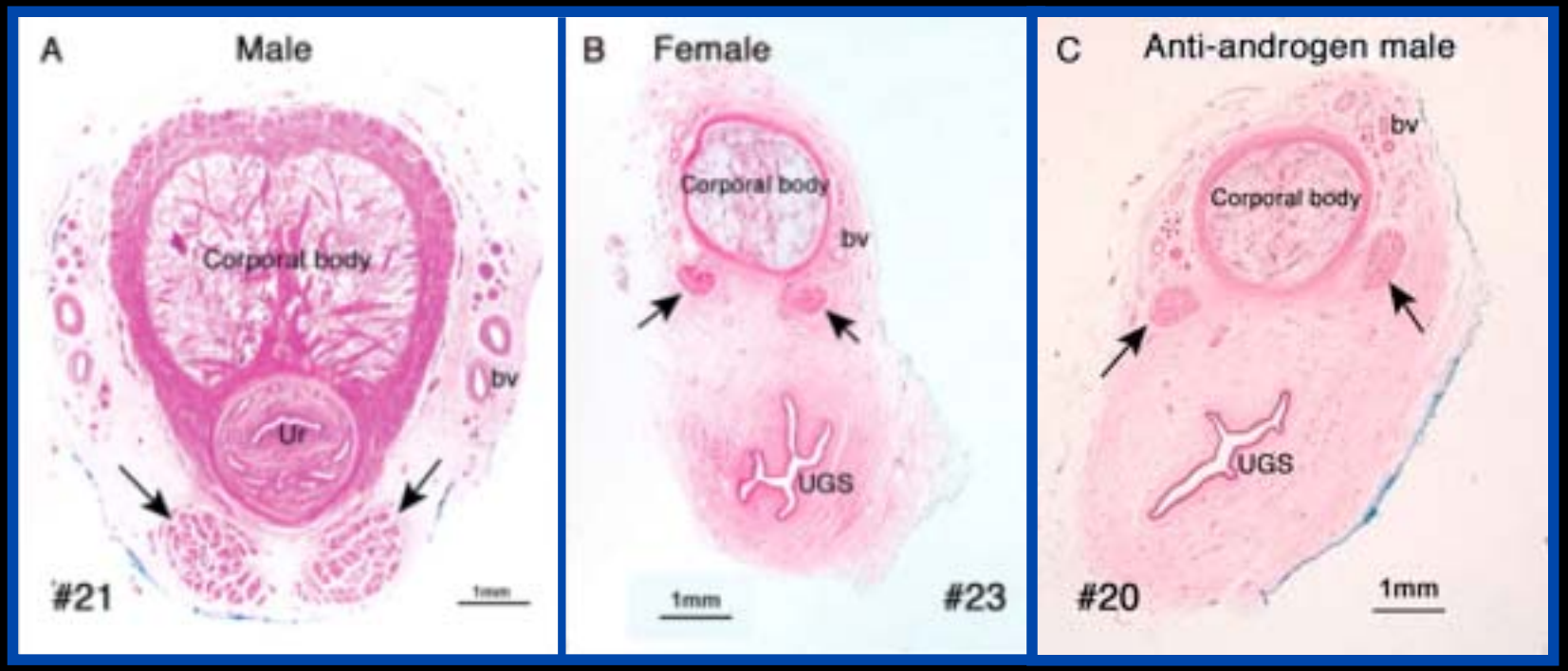


Forger, et al., *J Comp Neurol*, 1996

Anatomical Factors That May Impact Mating 4

Prenatal Anti-androgens 'Feminize' Penile Retractor Muscles

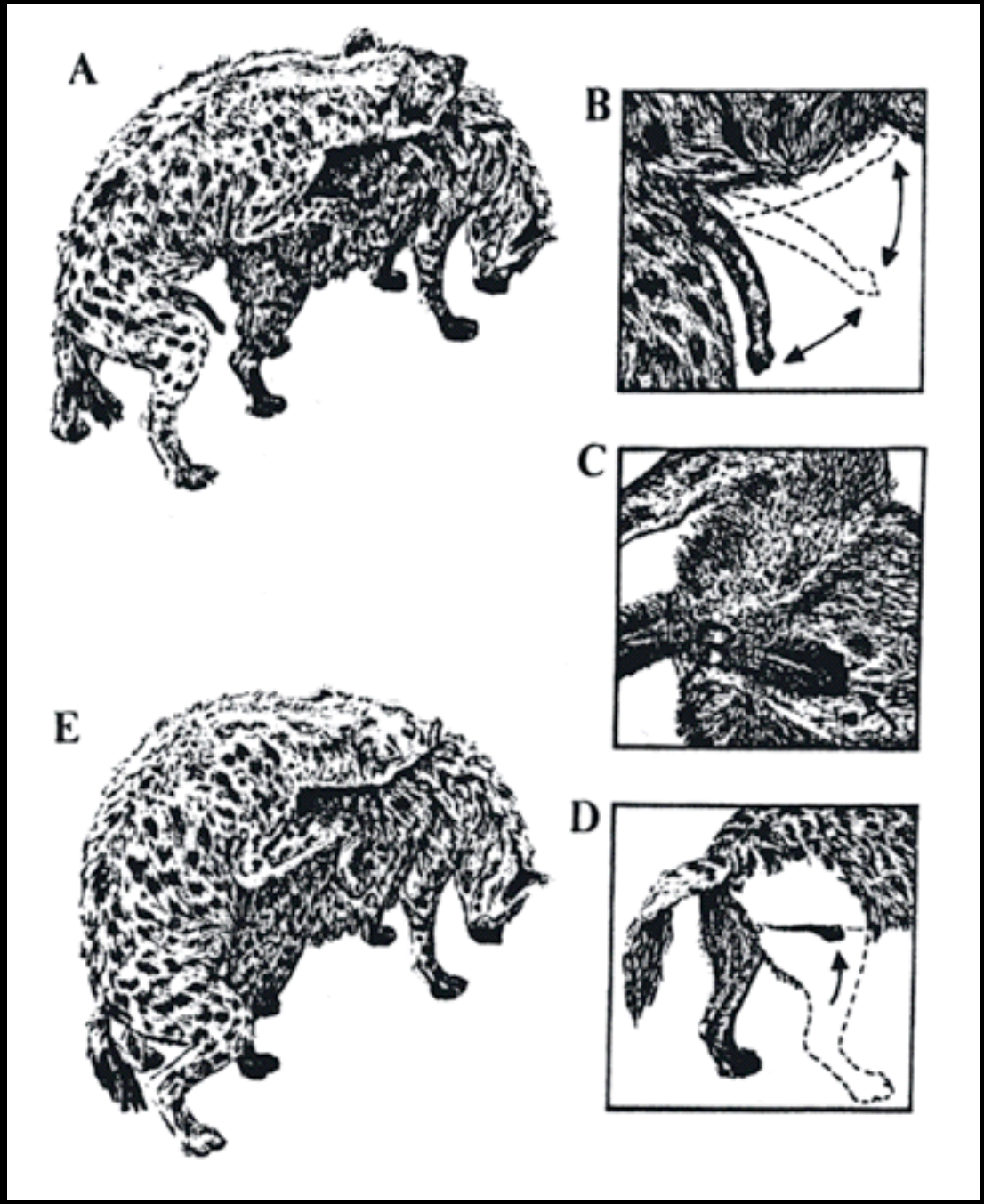
(as long as female spotted hyenas are the reference point)



1. **Retractor muscles dorsal to urethra**
2. **Tunica limited to corporal body**
3. **Voluminous UG sinus (urethra) surrounded by loose connective tissue**

Cunha *et al.* (2005)

A. Mounting



B. Penile "Flips"

C. Ventral View

E. Intromission

D. Retracted Clitoris

Drea *et al.* (1999)

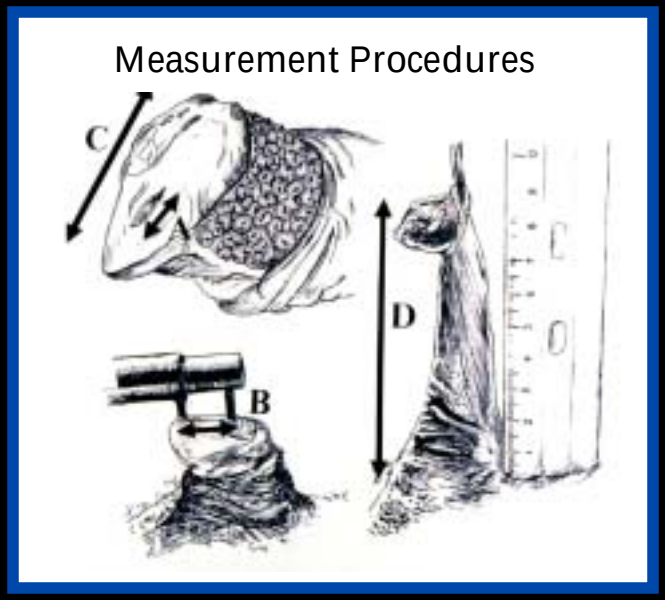
Why persistence by the male is important

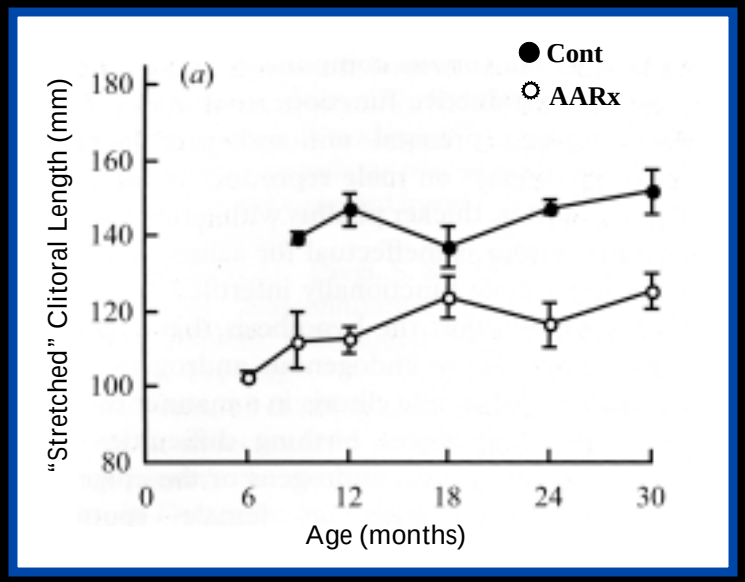
Spotted Hyena Mating

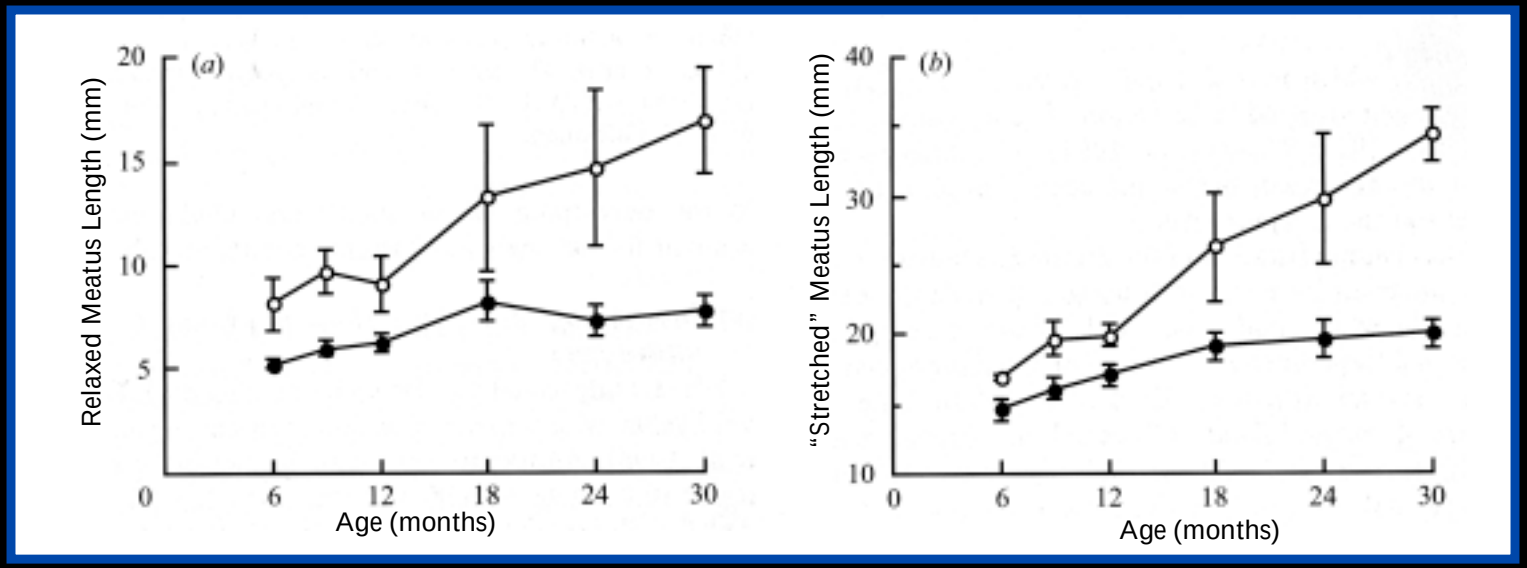
AARx-male (*Gremlin*)

Untreated-male (*Bramble*)

Effects of Anti-Androgen Treatment *in Utero* on Clitoral Morphology





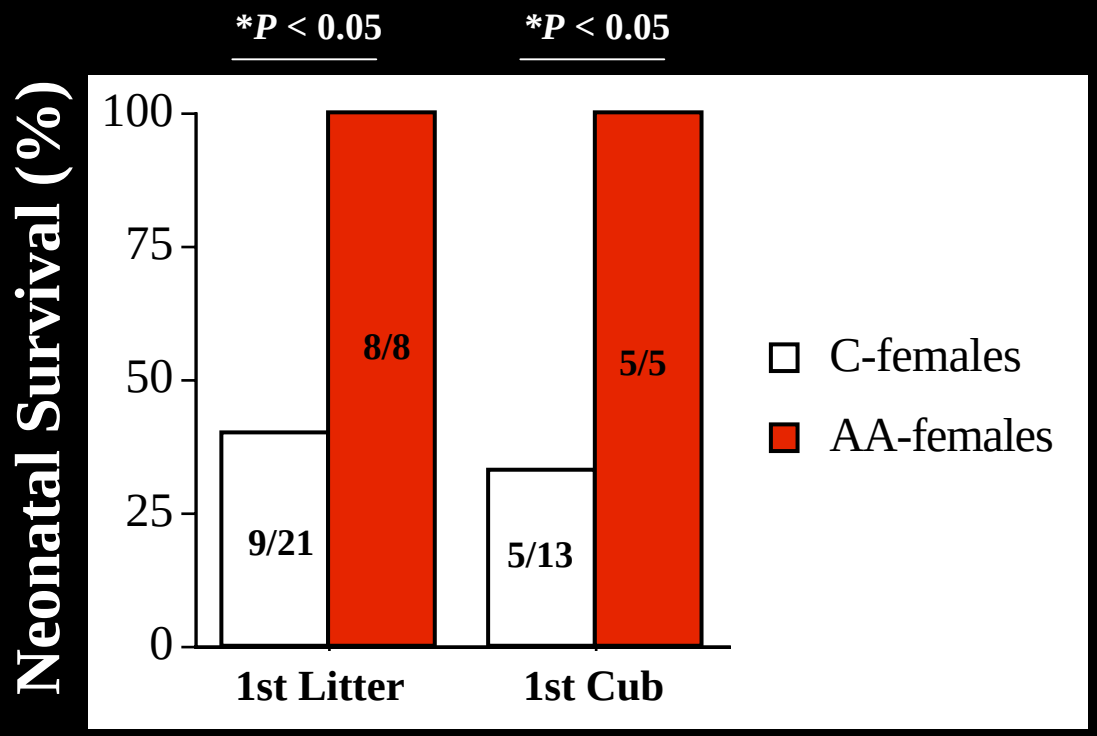


Drea *et al.* (2002)

Birthing via the clitoris

The 1.1-1.5kg fetal hyena traverses a tortuous course before emerging from the tip of the clitoris…

Improved Reproductive Success in Female Offspring Treated *in utero* with 'Anti-androgens'



Modest changes associated with big effects

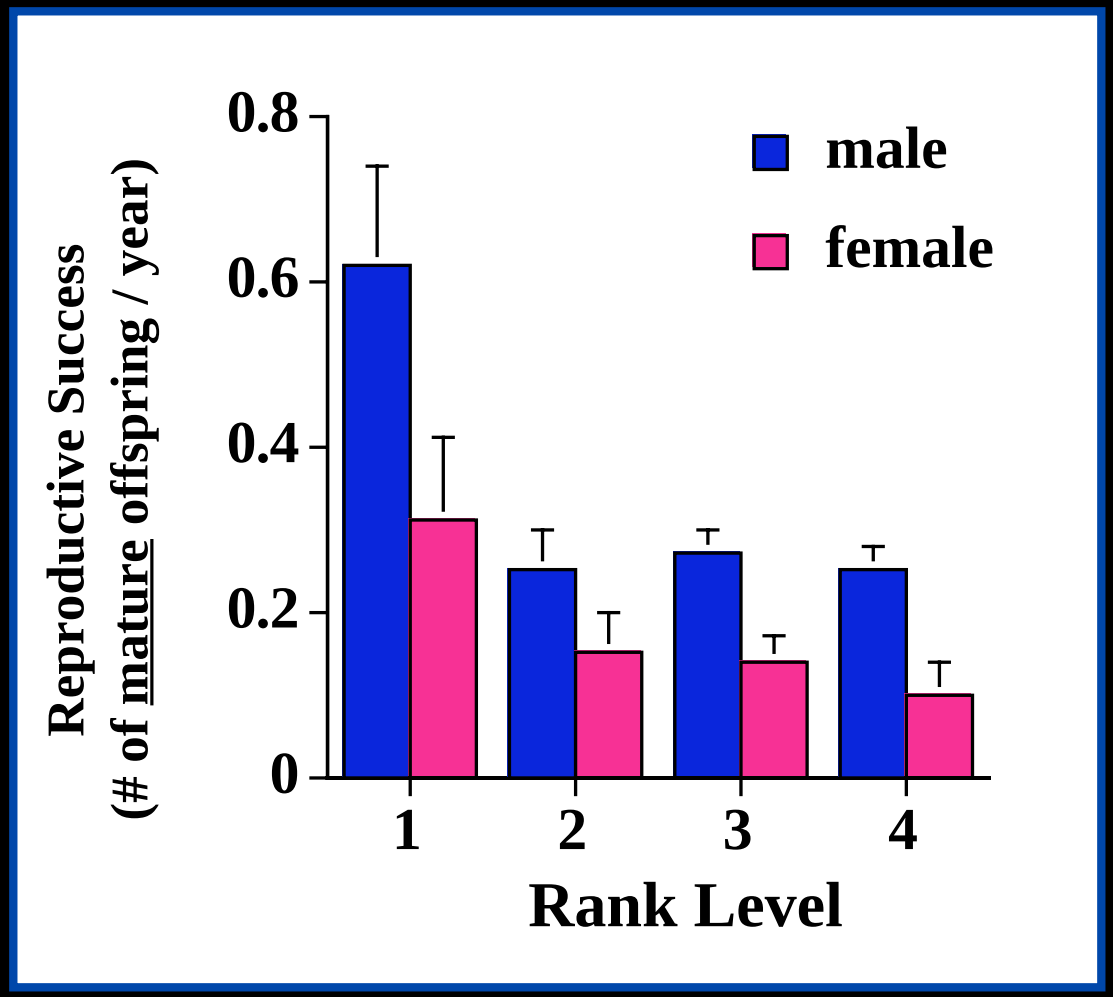
Drea *et al.* (2002)

WHY?

Adaptive or unselected by-product.

Regardless, fitness costs must be counter-balanced by gains.

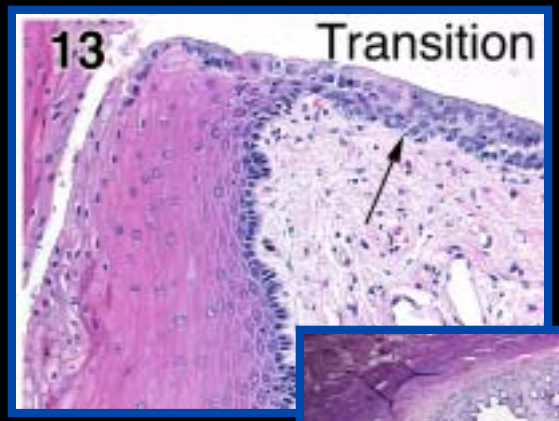
Rank and Reproductive Success in Female Spotted Hyenas

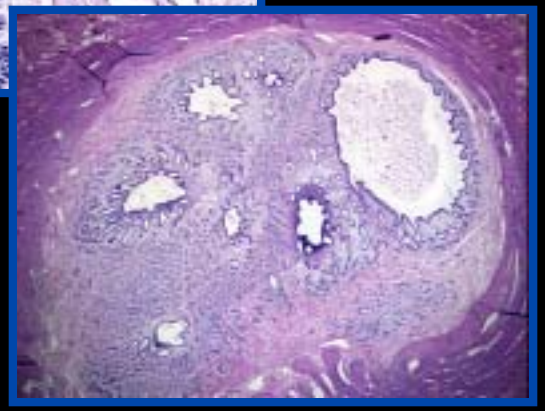


Frank *et al.*, (1995)

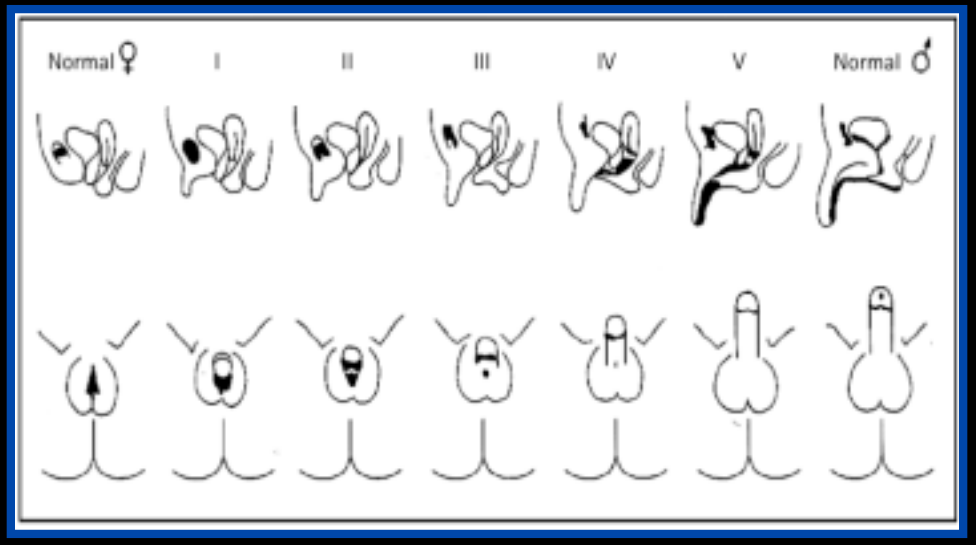
Impact of Anti-Androgen Studies on Ultimate Causation

- Competition-aggression hypothesis - Racey & Skinner; Gould & Vrba; Frank et al.
- Sexual mimicry hypothesis - Kruuk; Muller & Wrangham
- Chastity belt hypothesis - Hofer & East
- Siblicide hypothesis - Hofer & East
- Social inclusion hypothesis - Kruuk; Roughgarden
- Sperm competition - Holekamp





If modest changes to the female external genitalia improve fertility and fecundity, why isn't the spotted hyena's clitoris a little less masculinized?



Possible Answer: constraints on females

No mechanism may exist in hyenas by which the components of the masculinized phenotype can be decoupled, i.e., female aggression/dominance and genitalia

Your Thoughts and Questions