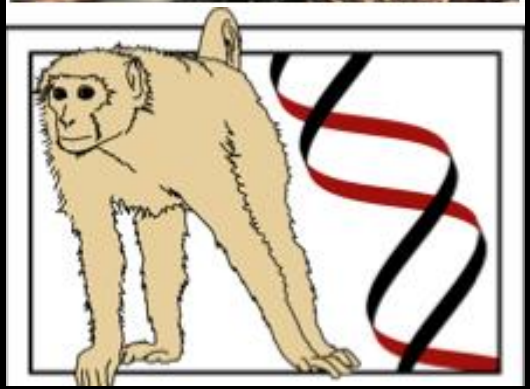

Female Marmoset Reproductive Cycles and How to Control Them

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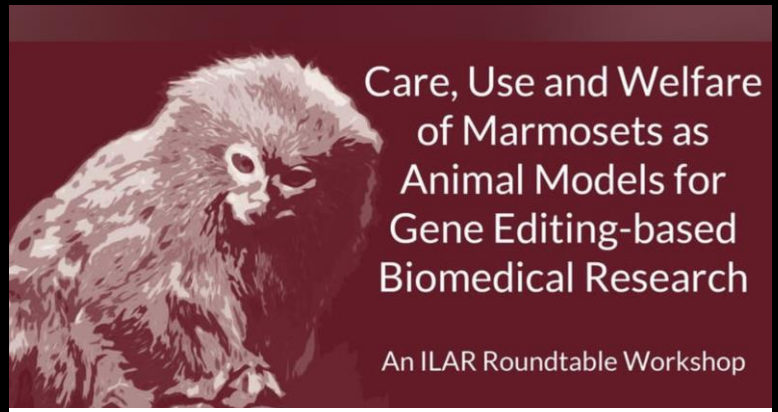

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Female Marmoset Reproductive Cycles and How to Control Them

1. Focus on laboratory housed female marmosets in their prime reproductive years

2. Female marmosets exhibit New World primate-typical reproductive endocrinology

3. Social and pharmacological control points in female marmoset reproduction

4. Endocrine profiles defining an ovarian cycle

5. Post-partum ovulation

6. Two methods of reliable cycle control contributing to timed ovarian stimulation protocols for oocyte retrieval and in vitro fertilization

Female Marmoset Reproductive Cycles

Female marmoset reproduction (laboratory housed)

- typical of anthropoid primates, have ovarian not estrous cycles
  - menses are absent, no menstrual cycles

- onset of puberty, 1st elevations in circulating estradiol (E_2)
  - about 8-10 months

- first ovulation, 1st >10 ng/ml elevations in circulating progesterone (P_4)
  - about 13-14 months in non-familial mixed sex groups/pairs
  - by 18-20 months for most daughters in families
  - commonly ovulate 2-3 oocytes per cycle

- first parturition
  - about 18-20 months in non-familial, mixed sex groups/pairs
  - prime reproductive years, 2-6 years of age

- reproductive senescence, no 'menopause'
  - close to end of lifespan: reduced litter size, intermittent/absent ovulatory cycles
  - no loss of E_2 or androgens; hormonally active ovaries even with intermittent or absent ovulatory cycles

Female Marmoset Reproductive Cycles: New World Primate Endocrinology Similar But Different to Old World Primates (OWP)

Female marmoset specialized endocrinology

1. Marmosets exhibit typical New World primate endocrine traits
2. Pituitary gonadotropin release and action

- target cells only express LHCG receptor type 2, binds only CG

- pituitary gonadotropes produce and release chorionic gonadotropin (CG) beta subunit, LH beta subunit is absent

- marmoset pituitary CG beta transcriptional regulation is similar to LH in OWP and differs from marmoset placental CG beta transcriptional regulation, effectively a two-promoter, one gene system in marmosets

- outcome: CG functions as the marmoset's LH
  - rapidly rising E_2 generates an ovulation-inducing CG surge
  - CG supports post-ovulatory corpus luteum (CL) function

- FSH beta subunit production and release maintained
  - an alternate distal gene promoter site to that in humans may result in different transcriptional regulation of FSH beta in marmosets

Adams et al. (2011) J Mol Endocrinol. 47:285-298.
Kutteyil et al. (2017) Gen Comp Endocrinol. 246:331-336.

Female Marmoset Reproductive Cycles: New World Primate Endocrinology Similar But Different to Old World Primates

Female marmoset specialized endocrinology (contd.)

Kraynak et al. (2018) In, "The Common Marmoset in Captivity and Biomedical Research", Eds. Marini RP, Wachtman LM, Tardif SD, Mansfield K, Fox JG. Elsevier Science

3. steroid binding hormones

- extra-cellular
  - minimal cortisol binding globulin (CBG), circulating progesterone (P_4) is mostly unbound or 'free'
  - typical levels of sex hormone binding globulin (SHBG), circulating estradiol (E_2) and testosterone (T) are mostly bound
- intra-cellular
  - increased expression of hsp27 competes with estrogen receptor alpha (ERa) to bind E_2, diminishing transcription complex formation
  - even when E_2 does form a transcription complex with either ERa or ERbeta (ERb), increased expression of estrogen response element-binding protein (ERE-BP or hnRNP C-like 1) competes with ERa or ERb assembled transcription complexes for binding to ERE-DNA binding sites around or within target genes, further diminishing E_2-driven gene expression
  - increased expression of FKBP51 diminishes androgen and progesterone receptors binding T and P_4, respectively, reducing transcription complex formation

Female Marmoset Reproductive Cycles: New World Primate Endocrinology Similar But Different to Old World Primates

Female marmoset specialized endocrinology (contd.)

3. steroid binding hormones

Barriers are overcome by increased circulating levels of E_2, T and P_4
(as well as other steroid hormones)

- extra-cellular
  - minimal cortisol binding globulin (CBG), circulating progesterone (P_4) is mostly unbound or 'free'
  - typical levels of sex hormone binding globulin (SHBG), circulating estradiol (E_2) and testosterone (T) are mostly bound
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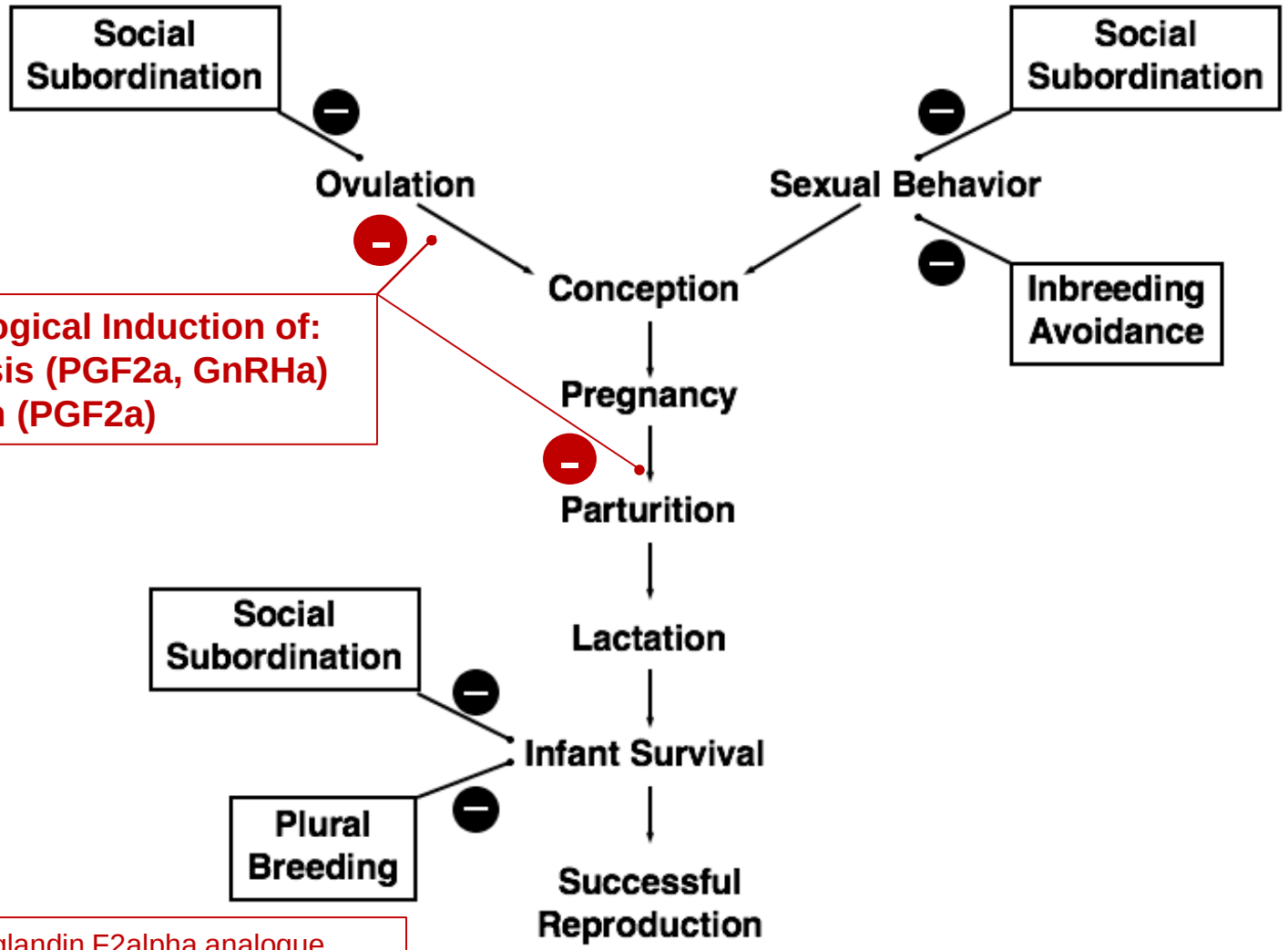
  - increased expression of FKBP51 diminishes androgen and progesterone receptors binding T and P_4, respectively, reducing transcription complex formation

Social and Pharmacological Control of Adult Female Marmoset Reproduction

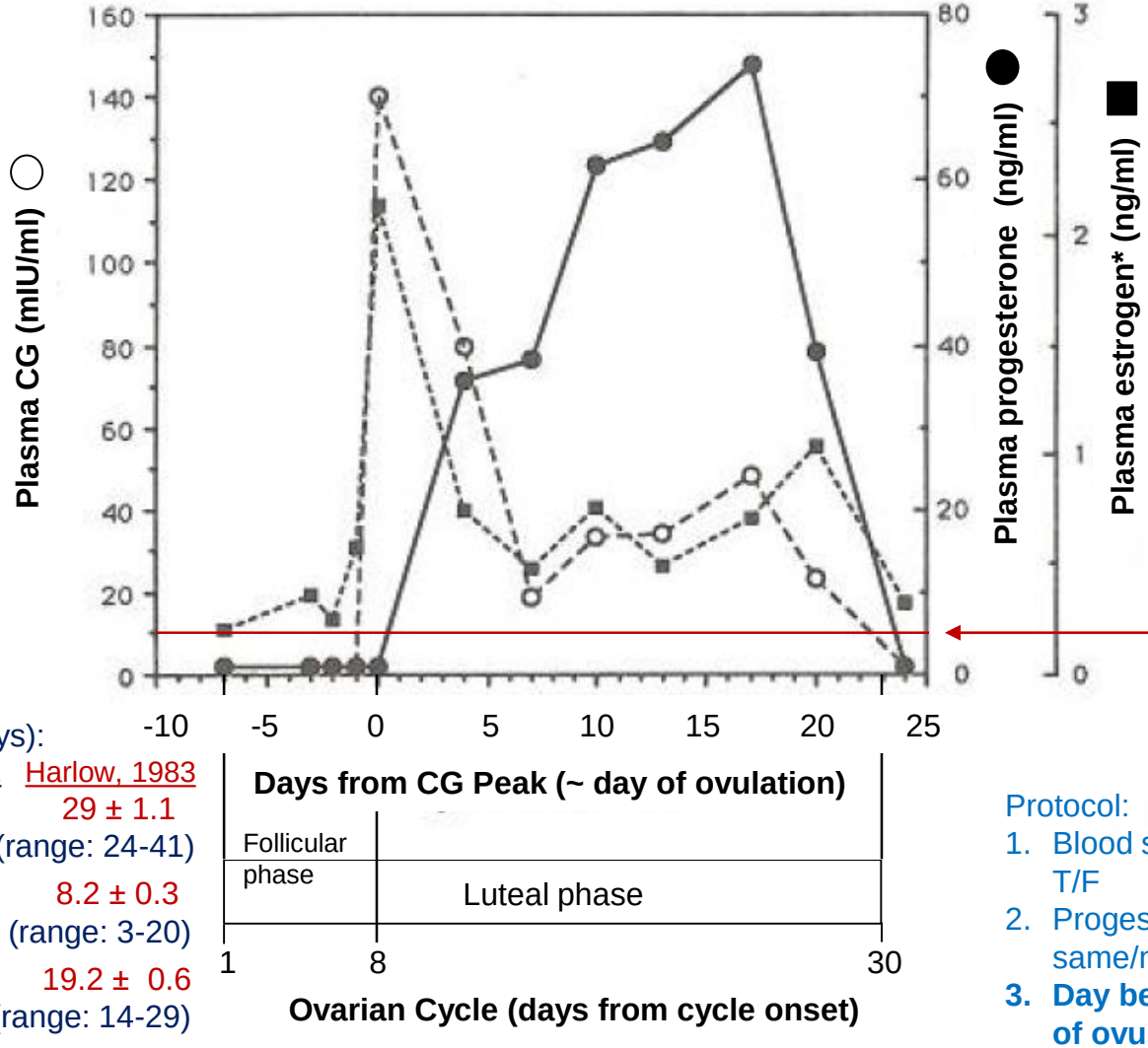


PGF2a = prostaglandin F2alpha analogue, cloprostenol
GnRHa = gonadotropin-releasing hormone antagonist, antide

Adapted from: Abbott, Digby, Saltzman (2009) in, "Reproductive Skew in Vertebrates", Ed. Hager R, Jones CB. Cambridge University Press, Cambridge., pp.337-368.

Example of Hormonal Profiles Across a 30-day Ovarian Cycle in an Adult Female Marmoset

*** Estrogen = estradiol (E_2) plus antibody partial cross-reactivity with estrone (E_1)**



Plasma progesterone: ●

> 10 ng/ml = luteal phase

≤ 10 ng/ml = follicular phase

Average duration (days):

	Harding, 1982	Harlow, 1983
- ovarian cycle	30 ± 3.8	29 ± 1.1 (range: 24-41)
- follicular phase	8.8 ± 3.7	8.2 ± 0.3 (range: 3-20)
- luteal phase	21.5 ± 2.2	19.2 ± 0.6 (range: 14-29)

Protocol:

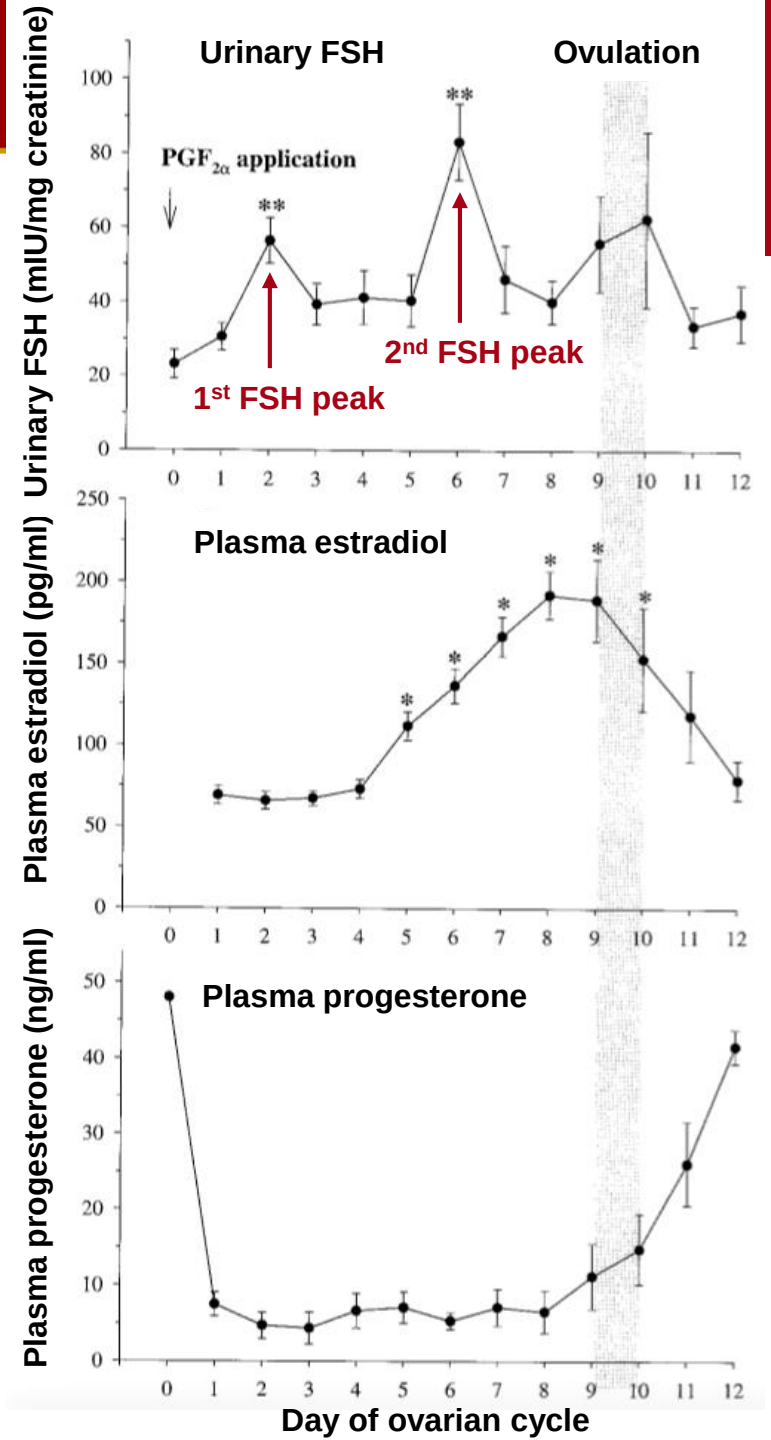
1. Blood sample twice weekly, M/Th, T/F
2. Progesterone (P_4) EIA results same/next day
3. **Day before P_4 > 10 ng/ml = day of ovulation**

Harding et al. (1982) J Med Primatol. 11:43-51

Harlow et al. (1983) J Zool Lond. 201:273-282

Abbott (1992), in "Reproductive Biology of South American Vertebrates" Ed. Hamlett, WC. Springer-Verlag, New York, pp.245-261.

Follicle Stimulating Hormone (FSH) Profile During the Follicular Phase of the Marmoset Ovarian Cycle

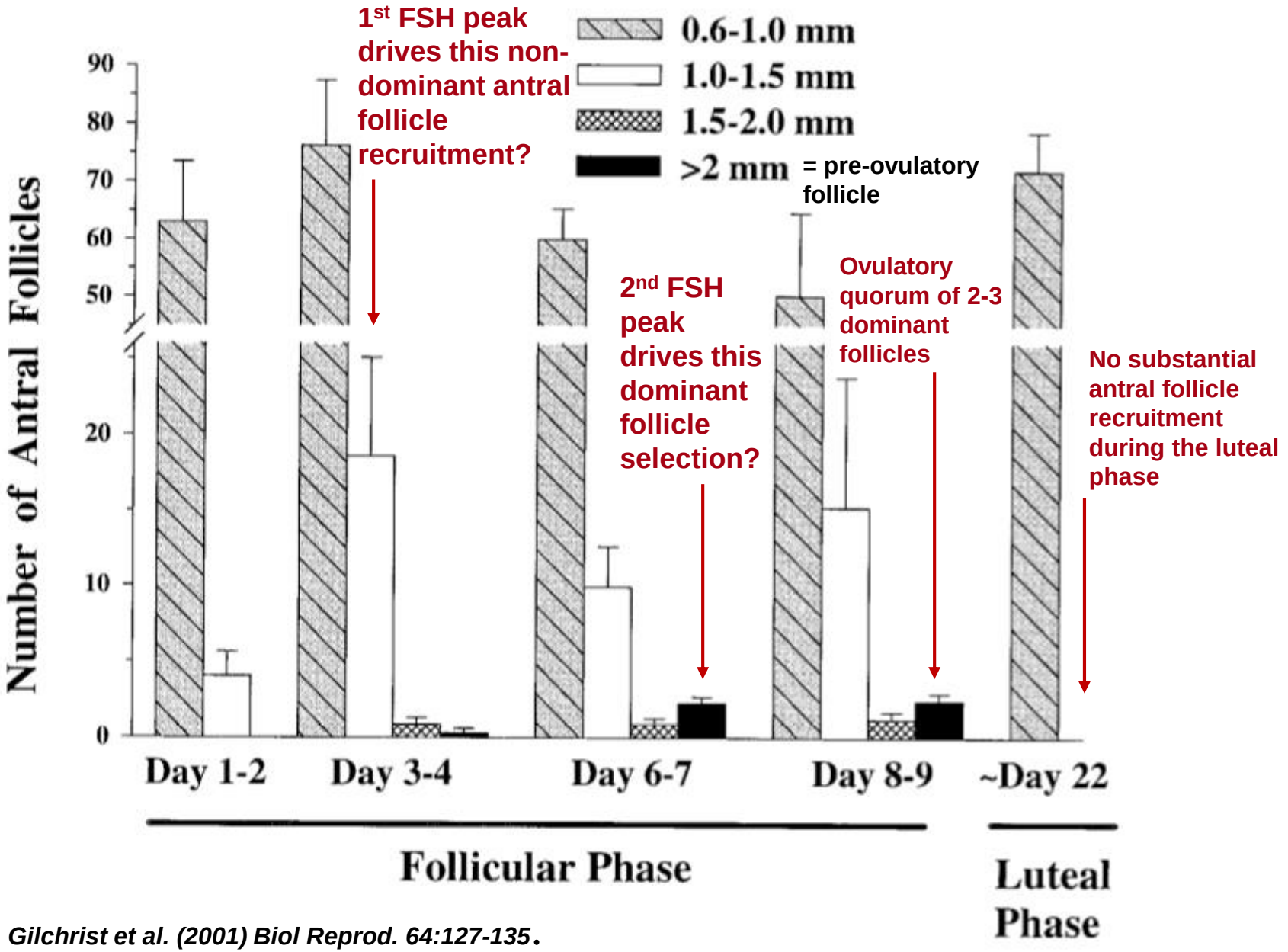


Marmosets exhibit two FSH peaks before the pre-ovulatory gonadotropin surge.

Women exhibit one FSH peak before the pre-ovulatory gonadotropin surge.

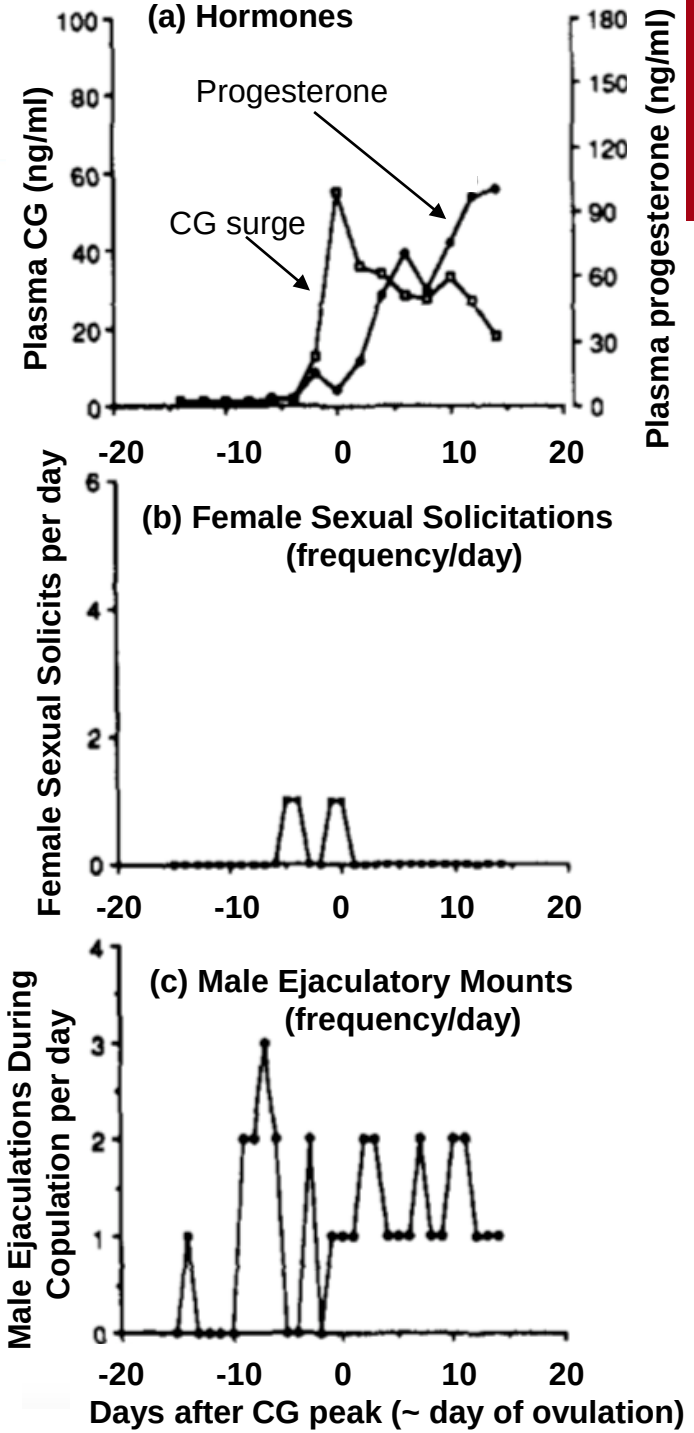
Gilchrist et al. (2001) Biol Reprod. 64:127-135.

Possible Relationship of FSH to Antral Follicle Development During the Marmoset Ovarian Cycle



Gilchrist et al. (2001) Biol Reprod. 64:127-135.

Example of Post-Partum Ovulation and Conception in a Marmoset: No Lactation Inhibition, No Estrus



Days to post-partum preovulatory CG surge (~ day of ovulation): 20.7 ± 4.3 (n=13)

% conceived at post-partum ovulation = 38%

McNeilly et al. (1981) J Reprod Fertil. 62:353-360.

Days to post-partum preovulatory CG surge (~ day of ovulation): 13.8 ± 1.3 (n=6)

% conceived at post-partum ovulation = 50%

Dixson and Lunn (1987) Physiol Behav. 41:577-583.

Control Timing of Onset of Marmoset Ovarian Cycles

Rationale:

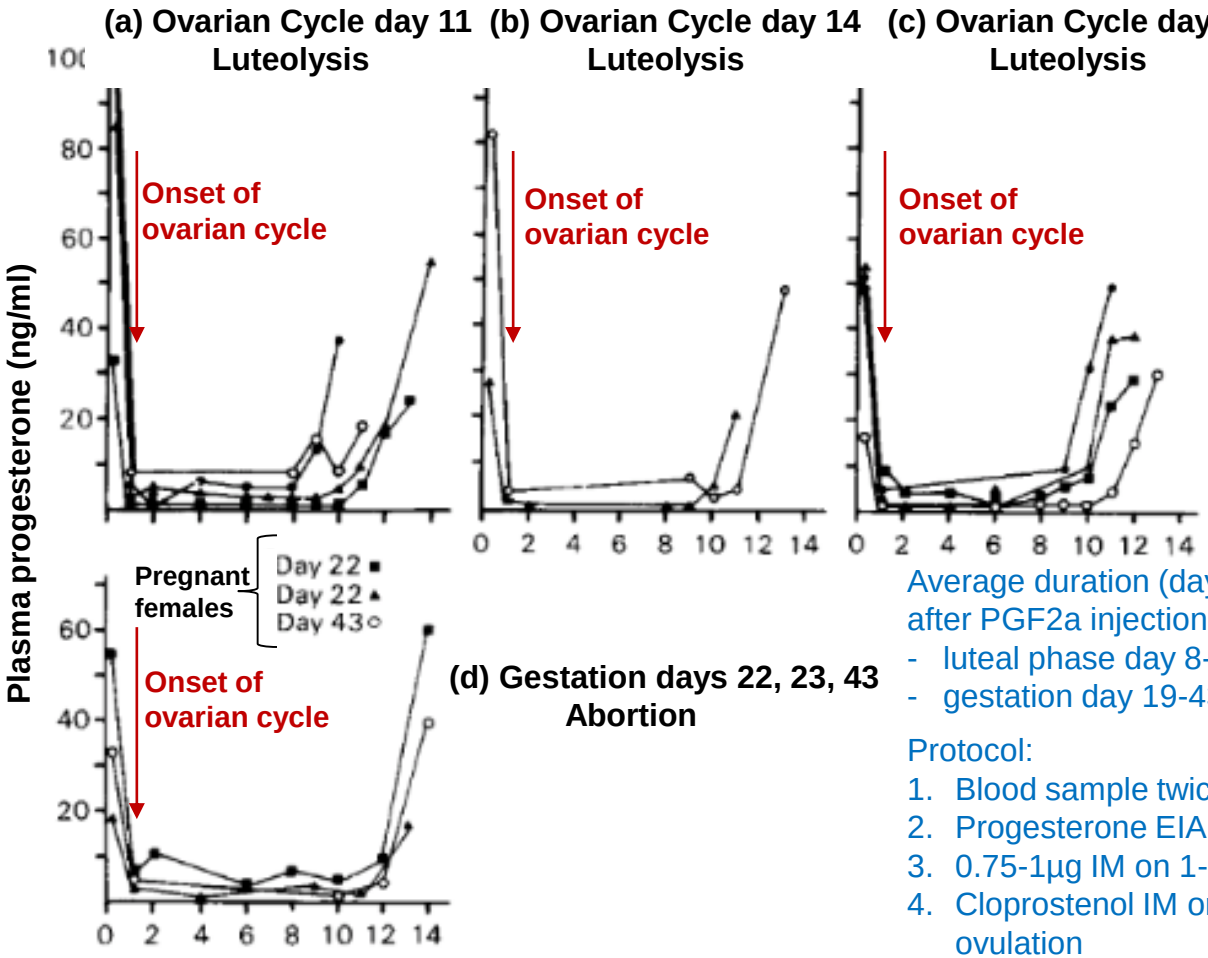
1. reliably induce rapid demise of the corpora lutea (CLs), luteolysis,
2. thus removing progesterone (P_4) and estradiol (E_2) negative feedback on CG and FSH,
3. initiating the follicular phase of the next ovarian cycle

Methods to induce luteolysis:

1. **Prostaglandin F2alpha analogue** (cloprostenol, 0.75-1µg IM)
   Mechanism: acts on **ovarian luteal cells** in CLs to engage intra-cellular inhibition of CG-driven intra-cellular signaling essential for luteal cell survival and P_4 production and release
2. **Gonadotropin-releasing hormone antagonist** (antide, 1 mg/kg SC)
   Mechanism: acts on **anterior pituitary gonadotropes** to block hypothalamic GnRH stimulation of CG release, depriving CLs of essential gonadotropin support, inhibiting P_4 production and release

Summers et al. (1985) J Reprod Fert 73:133-138.
Fraser et al. (1995) J Endocrinol. 144:201-208.
Webley et al. (2010) Gen Comp Endocrinol. 166:436-442.

Individual Examples of Cloprostenol (Prostaglandin F2alpha analogue) Injection (Day 0) Inducing (a-c) Luteolysis and (d) Abortion, Followed by Onset of Ovarian Cycle (Day 1)



Average duration (days) of follicular phase after PGF2a injection at:

- luteal phase day 8-17, 10.7 ± 0.3 (n=36)
- gestation day 19-43, 10.9 ± 0.5 (n=7)

Protocol:

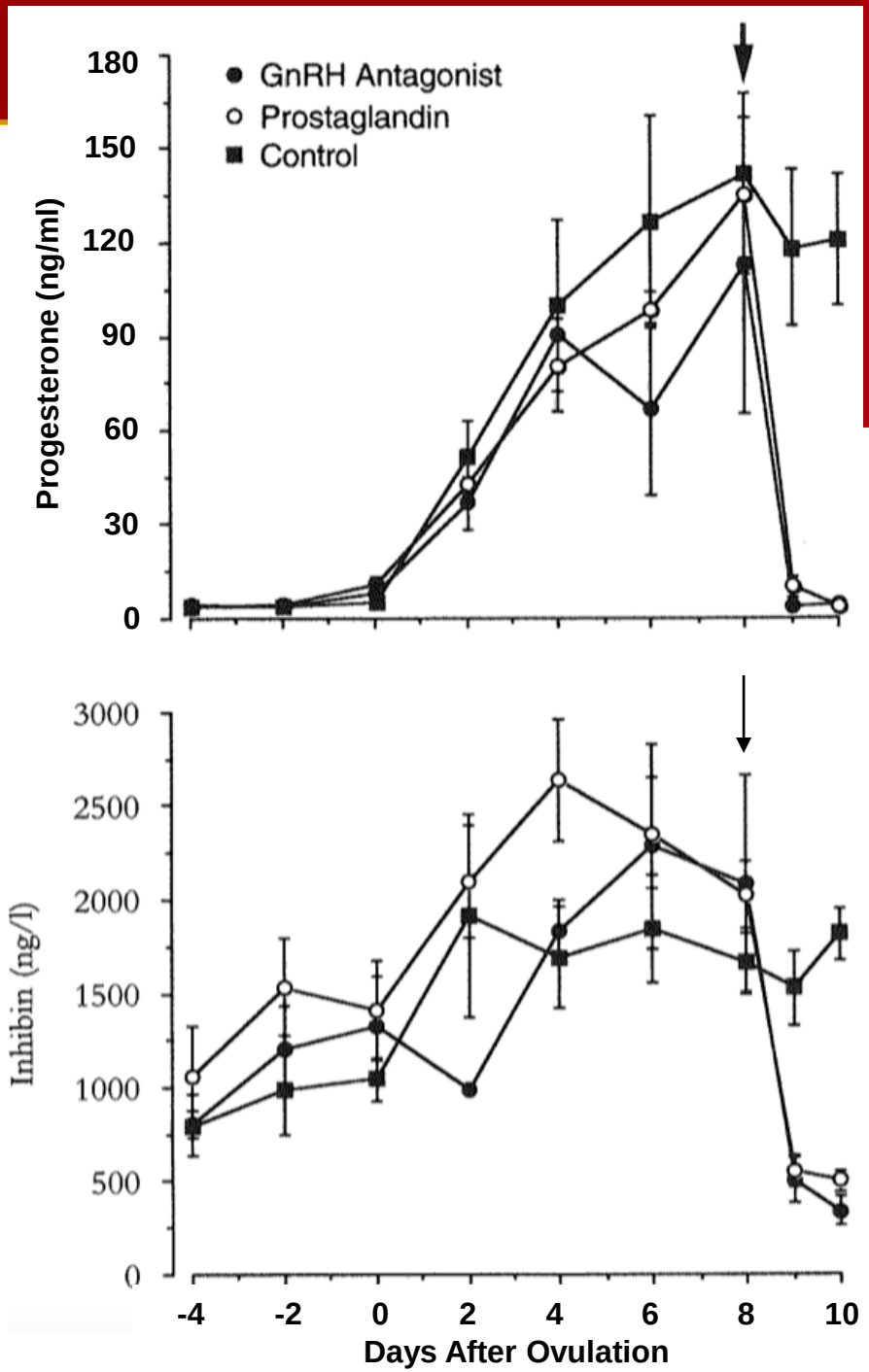
1. Blood sample twice weekly, M/Th, T/F
2. Progesterone EIA results same/next day
3. 0.75-1µg IM on 1-2 consecutive days
4. Cloprostenol IM on days 11-40 after ovulation

Days after Prostaglandin F2alpha (PGF2a) analogue* injection

*** PGF2a analogue = cloprostenol 0.75-1 µg**

Reliability ~ 90%

Summers et al. (1985) J Reprod Fert 73:133-138.
Kraynak et al. (2017) Am J Physiol Endocrinol Metab. 313: E507–E514.



Injection at arrow of: (1) Gonadotropin-Releasing Hormone Antagonist, or (2) Prostaglandin F2alpha Analogue. Both Induce Luteolysis Followed by Onset of Ovarian Cycle

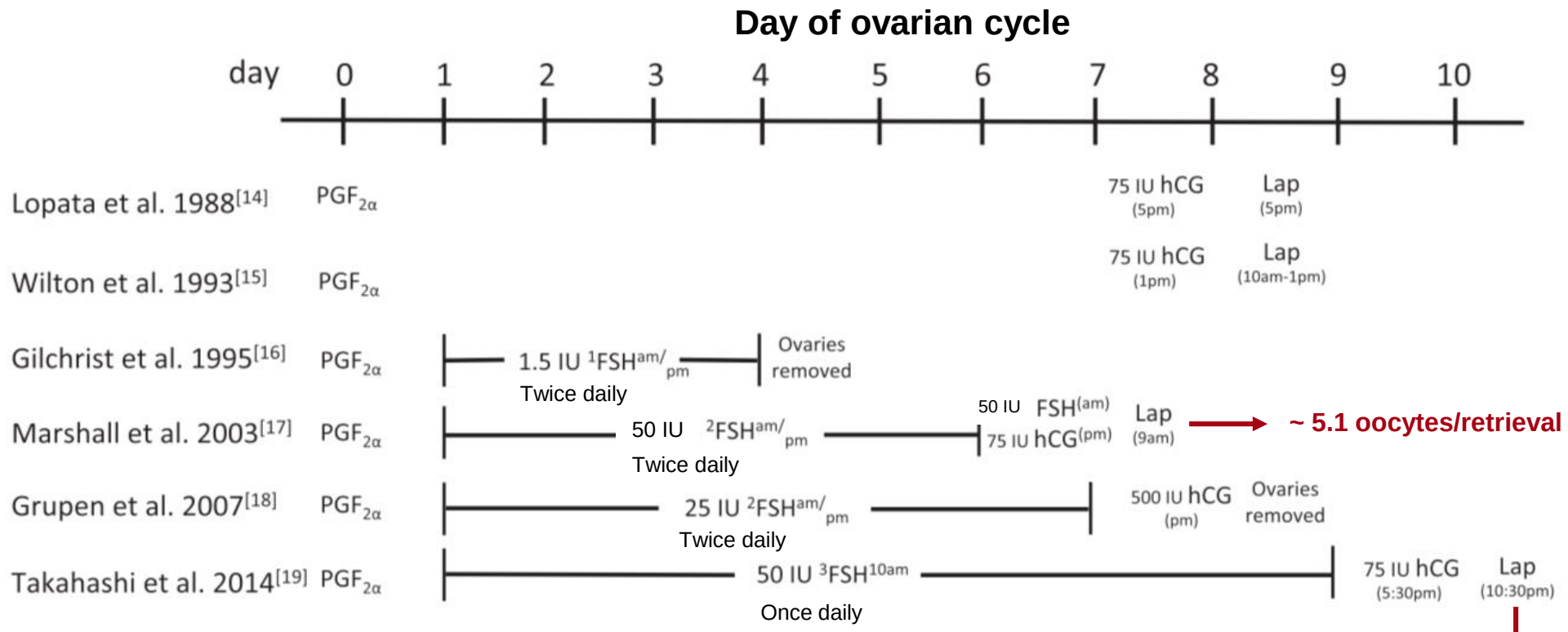
Both treatments induce similar luteolytic cellular changes in the marmoset CL:

- apoptosis
  - nuclear degeneration/condensation
- necrosis
  - dissolution of nuclear membranes
  - increased cytoplasmic volume

GnRH antagonist = antide, 1mg/kg, SC

Webley et al. (1991) J Endocrinol. 128:465-473
Fraser et al. (1995) J Endocrinol. 144:201-208.

Current Marmoset Ovarian Stimulation Protocols Employ PGF2a Analogue Induced Luteolysis (Day 0) Followed by Recombinant Human (rh) FSH Injections and Ovulation Inducing hCG Injection



FSH injection protocol	IU per kg per day	IU per kg Total	
Marmoset	250	1375	
Rhesus macaque[a]	7.5	60	~ 12.9 oocytes/retrieval
Human ("mild")[b]	2.4	19.5	~ 8.3 oocytes/retrieval

[1]FSH = Fertinorm HP75, Serono
[2]FSH = Gonal-f, Serono
[3]FSH = Folyrmon-P, Fuji Pharma

[a] *Jensen et al. (2005) Contraception 71:68-73.*
[b] *Alper and Fauser (2017) Reprod Biomed Online 34:345-353.*

Kropp et al. (2017) Biol Reprod. 96:277-287.

Female Marmoset Reproductive Cycles and How to Control Them

Summary and Conclusions

1. Adult female marmosets exhibit primate-like ovarian cycles of ~28-30 days
   - lengthy luteal phases may reflect CG, and not LH, support of the CLs

2. Female marmosets exhibit typical New World primate endocrine specializations that parallel Old World primate endocrine function, including humans, but are not identical

3. Marmosets exhibit two FSH peaks associated with ovarian follicle growth and development in the follicular phase, in contrast to a single FSH peak in humans

4. Marmoset ovarian cycles can have particularly lengthy durations of the luteal phase requiring protocols for timed cycle onset:
   - prostaglandin F2alpha analogue, cloprostenol, induces luteolysis
   - gonadotropin-releasing hormone antagonist, indirectly results in luteolysis

5. Relatively high rhFSH doses required for marmoset ovarian stimulation protocols may reflect naturally occurring requirement for high FSH levels to support multiple follicle ovulations and accommodate New World primate endocrine specializations, such as higher steroid hormone production

Ovarian Cycle Control Critical for Reliable Production of Marmoset Oocytes and Embryos for Gene-Editing and Birth of Offspring with Desired Phenotype

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