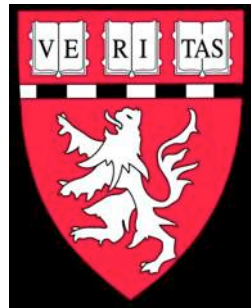




## **Gene Therapy using AAV9-Delivery of an MIS Transgene Inhibits Estrus in Female Cats.**

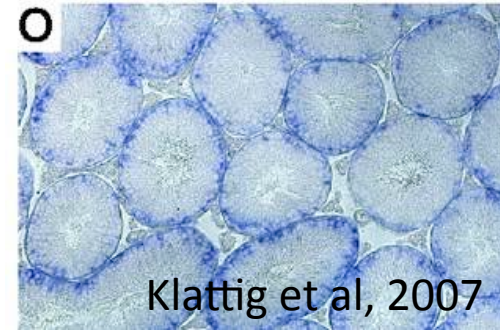
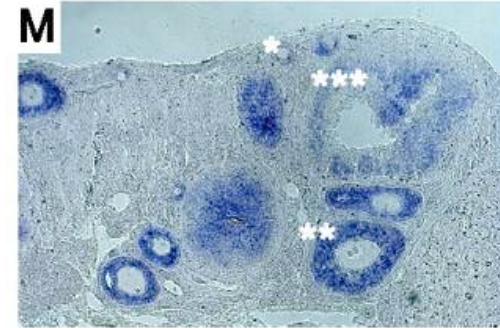


David Pépin, PhD  
Pediatric Surgical Research Laboratories  
Massachusetts General Hospital

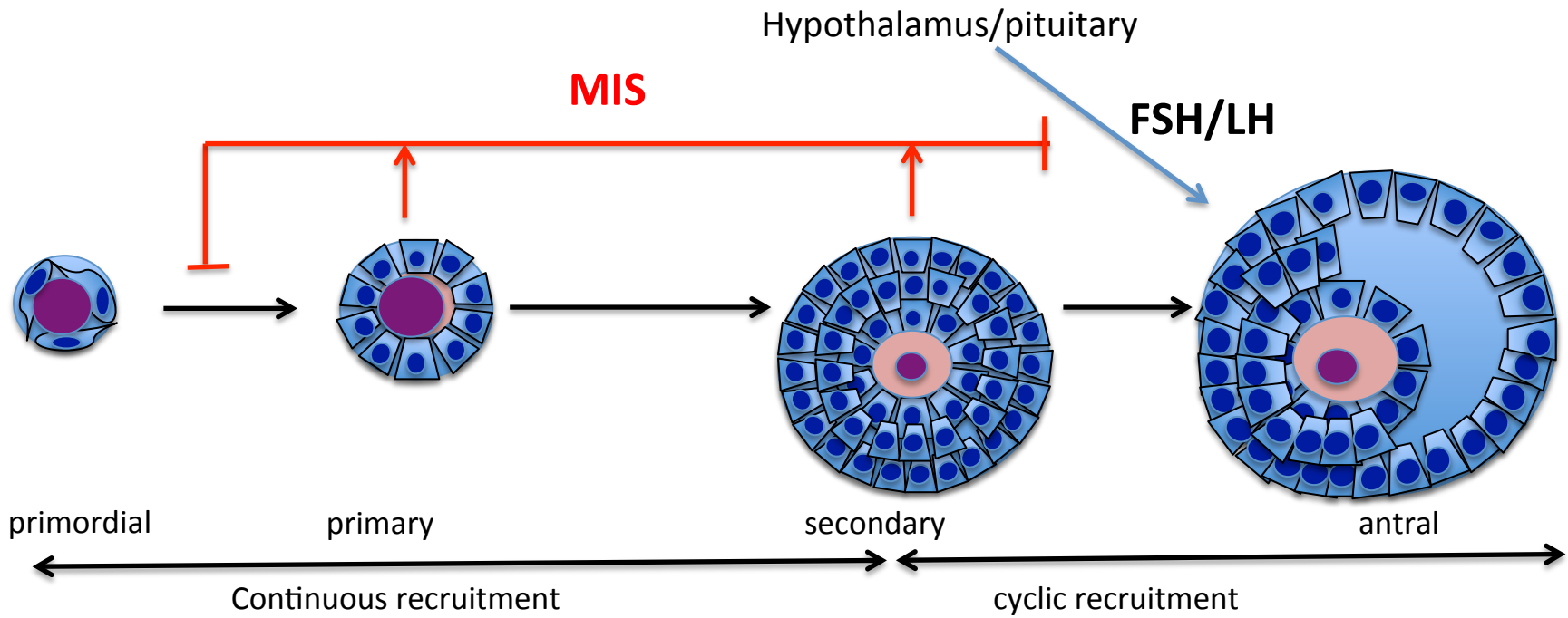


# AMH/MIS

*Amhr2*

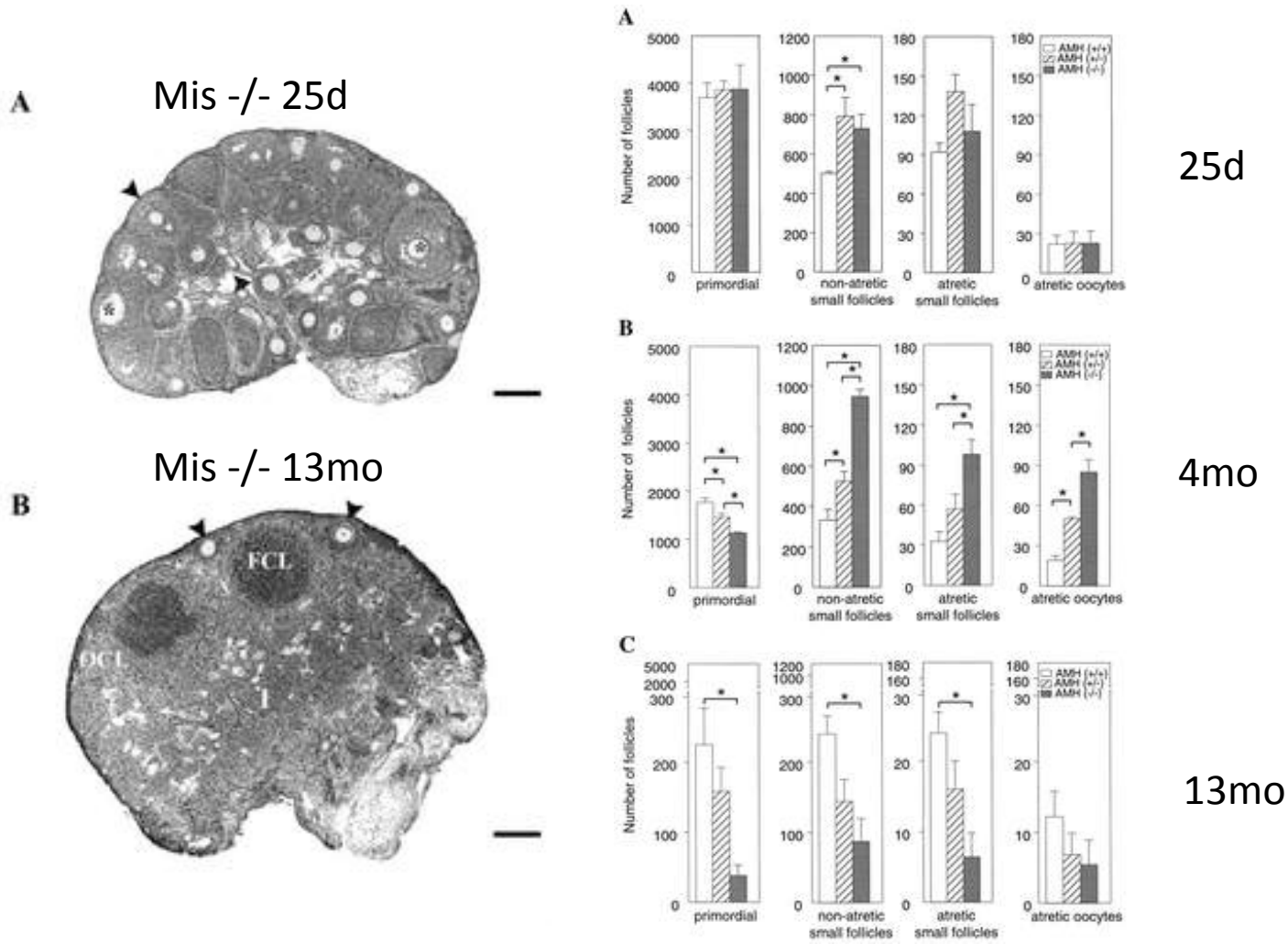


- MIS is produced by granulosa cells of growing follicles.
- MISR2 is expressed in granulosa cells.
- Blood MIS levels are representative of the growing follicle pool, which is proportional to the ovarian reserve.
- Circulating MIS levels measure the ovarian “age”.



# Control of Primordial Follicle Recruitment by Anti-Müllerian Hormone in the Mouse Ovary

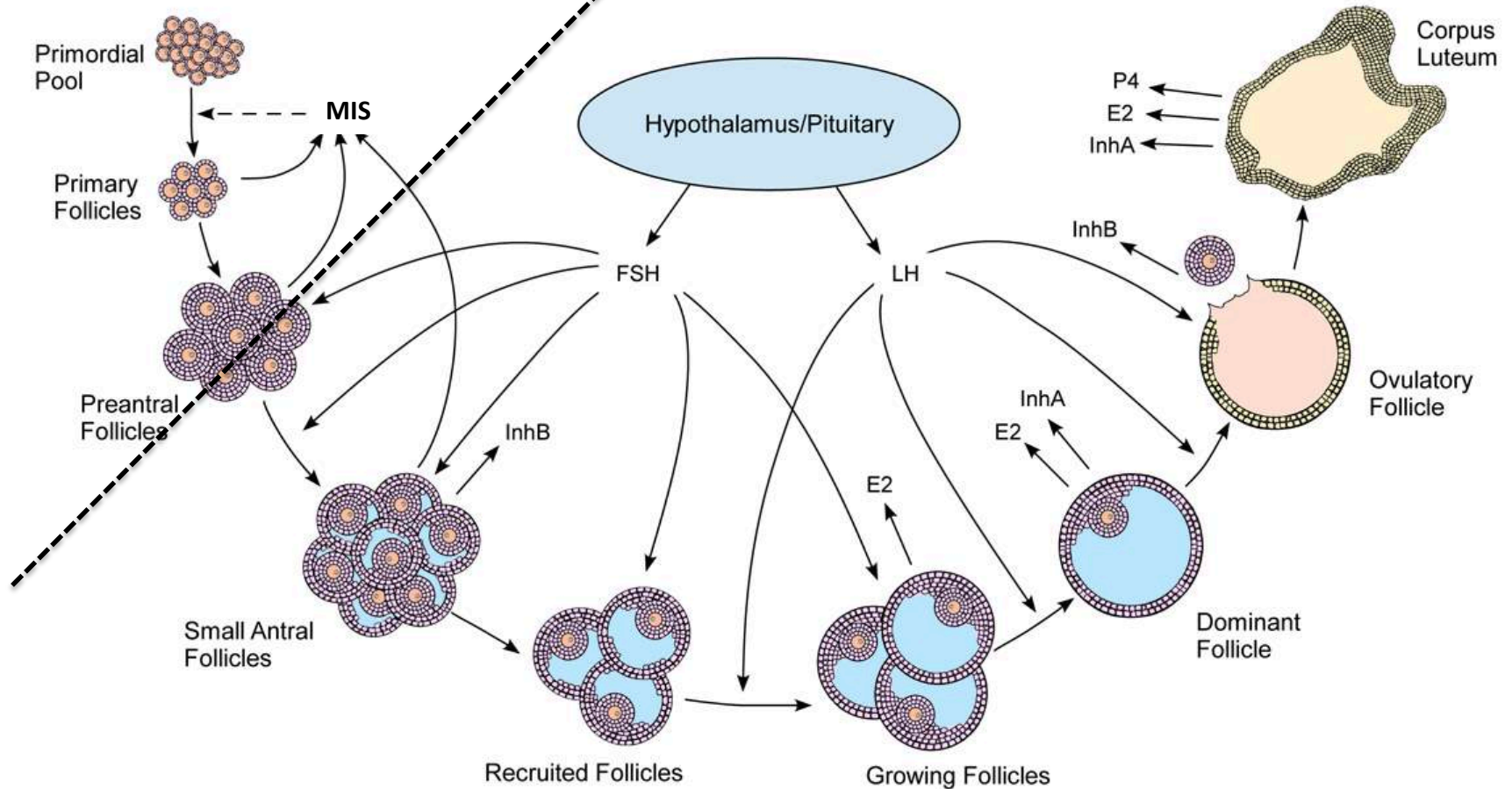
Alexandra L. L. Durlinger, Piet Kramer, Bas Karels, Frank H. de Jong, Jan Th. J. Uilenbroek, J. Anton Grootegoed, and Axel P. N. Themmen



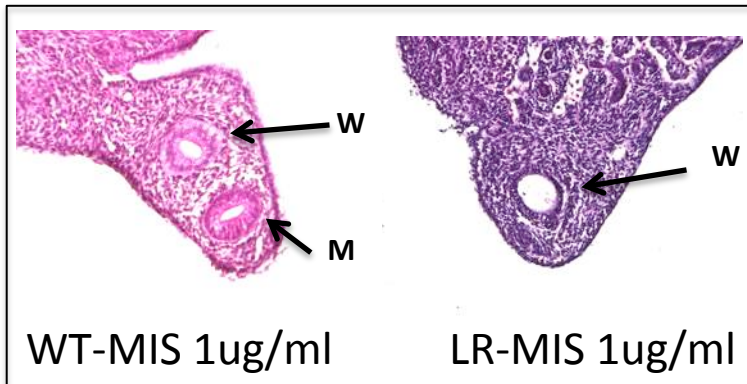
# MIS maintains negative feedback on primordial follicle recruitment

Gonadotropin/steroid independent  
(continuous)

Gonadotropin/steroid dependent  
(cyclic)

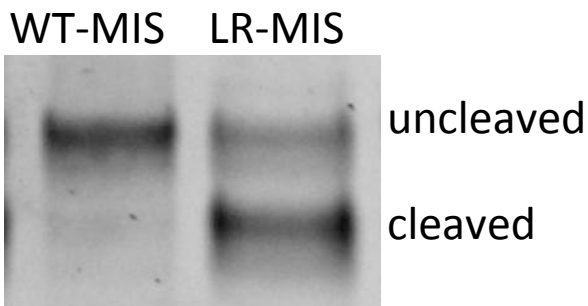


# Developing an AAV9 human “LR”-MIS vector

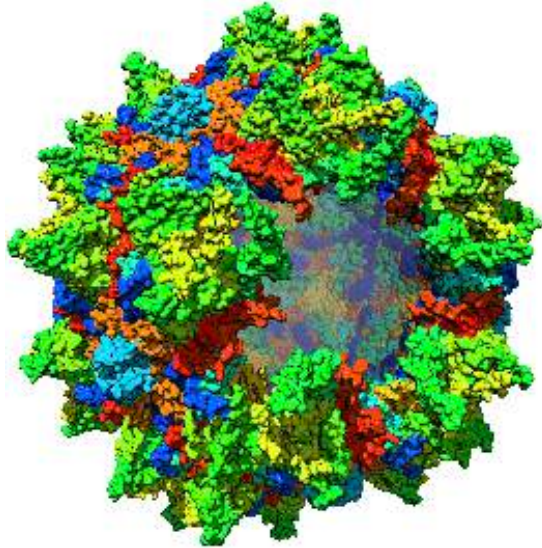


## Enhanced protein:

- Modified Leader sequence (albumin)
- **Higher expression**
- Enhanced cleavage site (Q425R)
- **Higher potency**

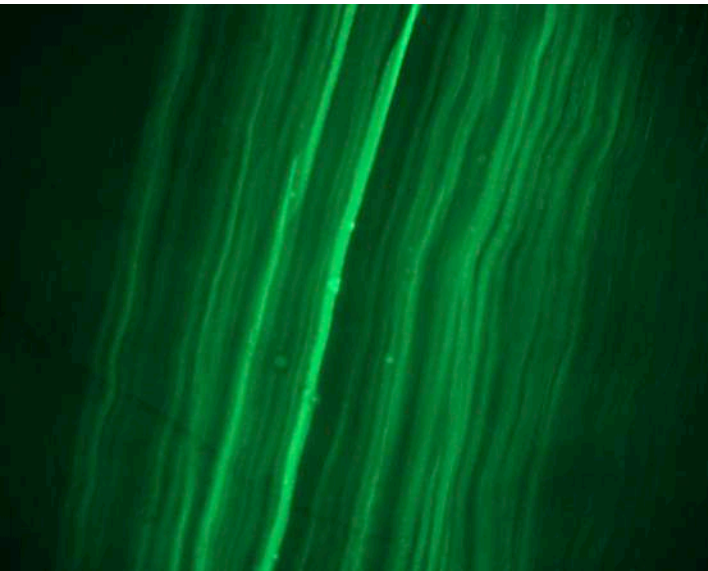


# Tropism of AAV9 delivered IP

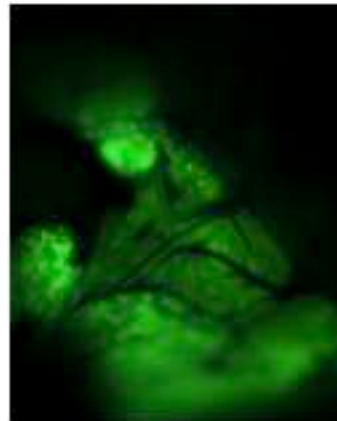


- AAV9 infects muscles
- High expression
- Expression stable for 9 years in primates
- Does not infect ovary/testis

Leg muscle



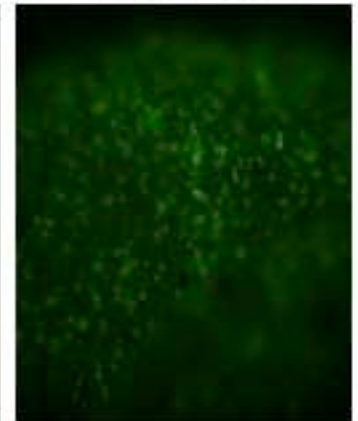
Pancreas



Liver



Body wall



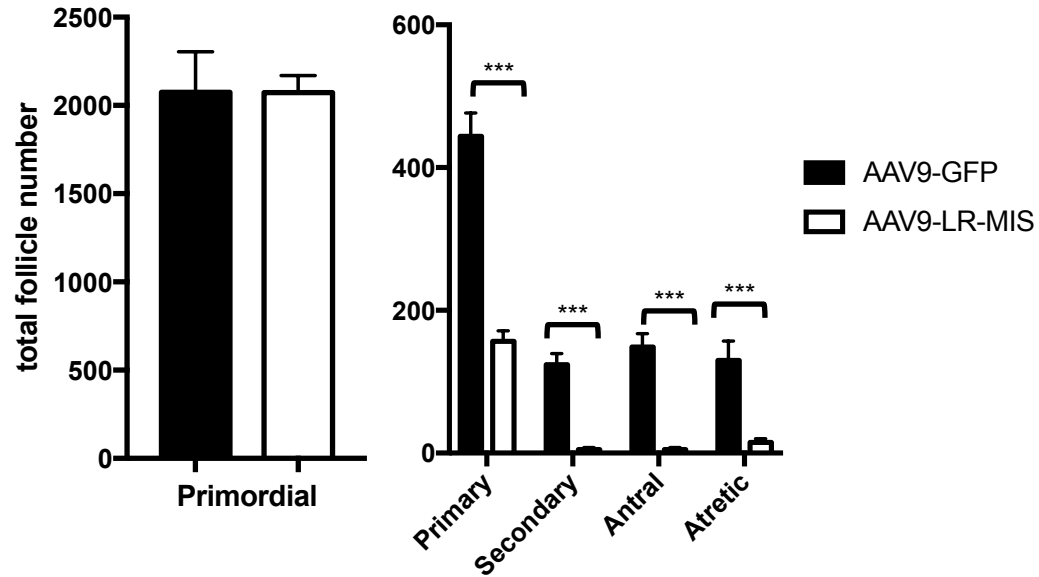
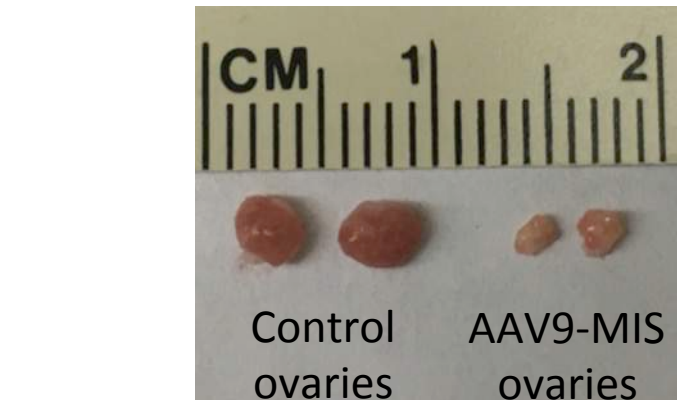
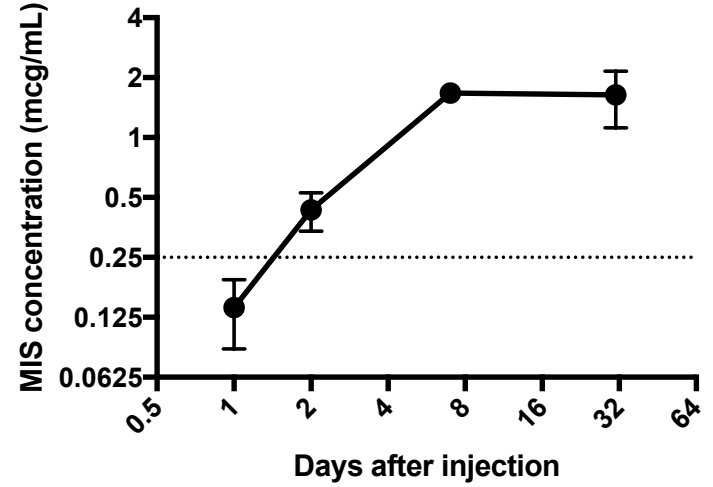
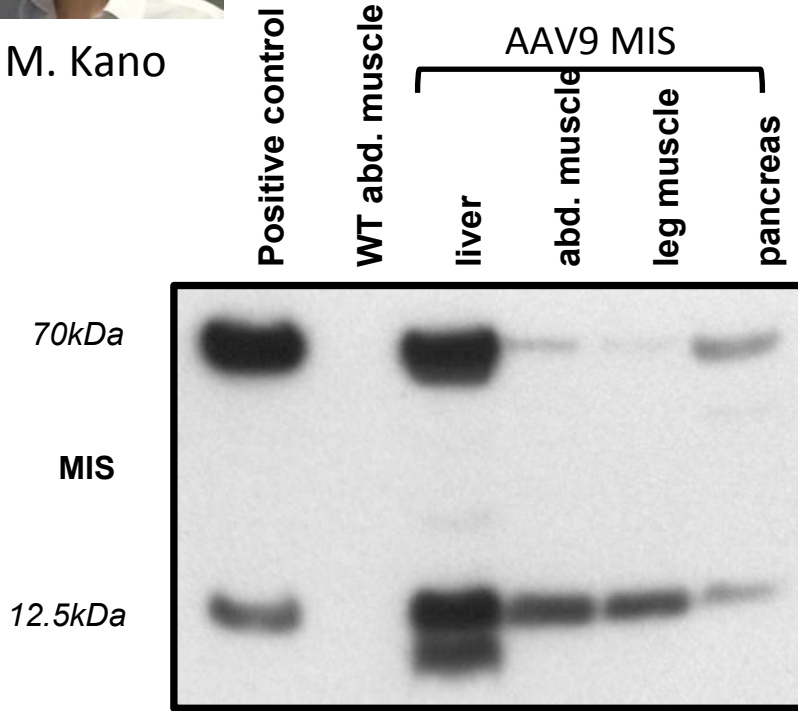
AAV9-GFP

1 year post-infection

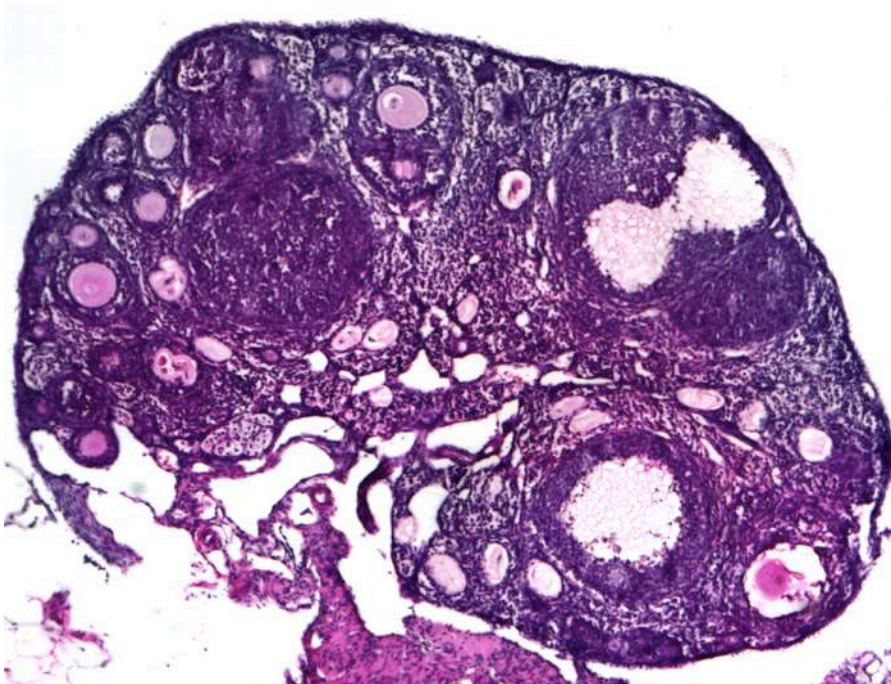
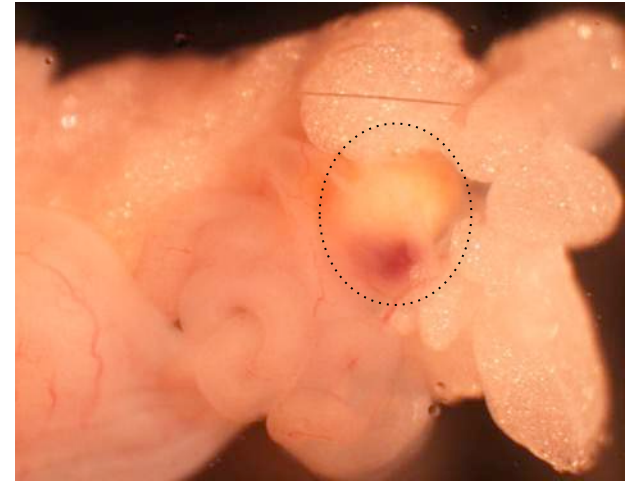
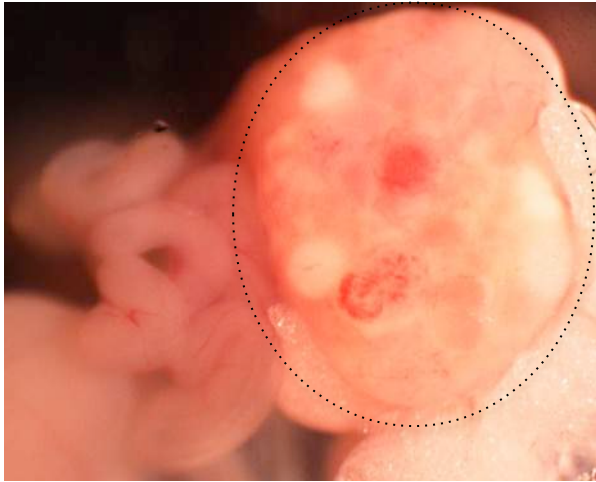


M. Kano

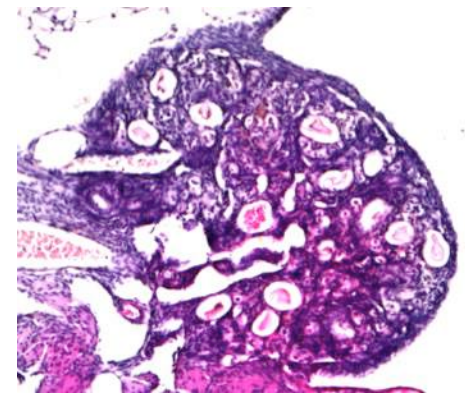
# AAV9-MIS (3E11 particles/mouse) results in complete follicular block



# MIS as a contraceptive agent



AAV-GFP

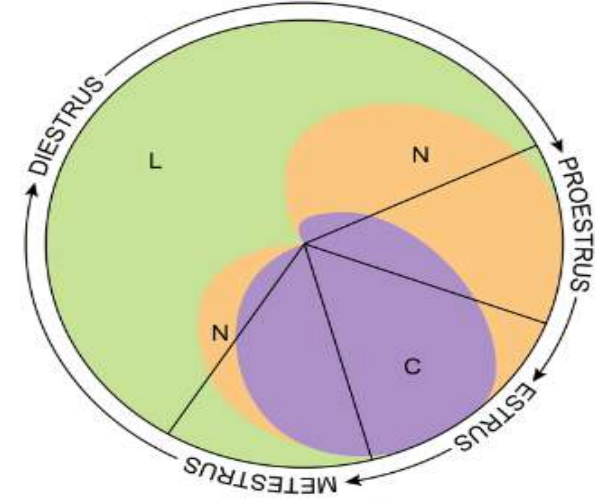


AAV-LRMIS



# Vaginal cycling

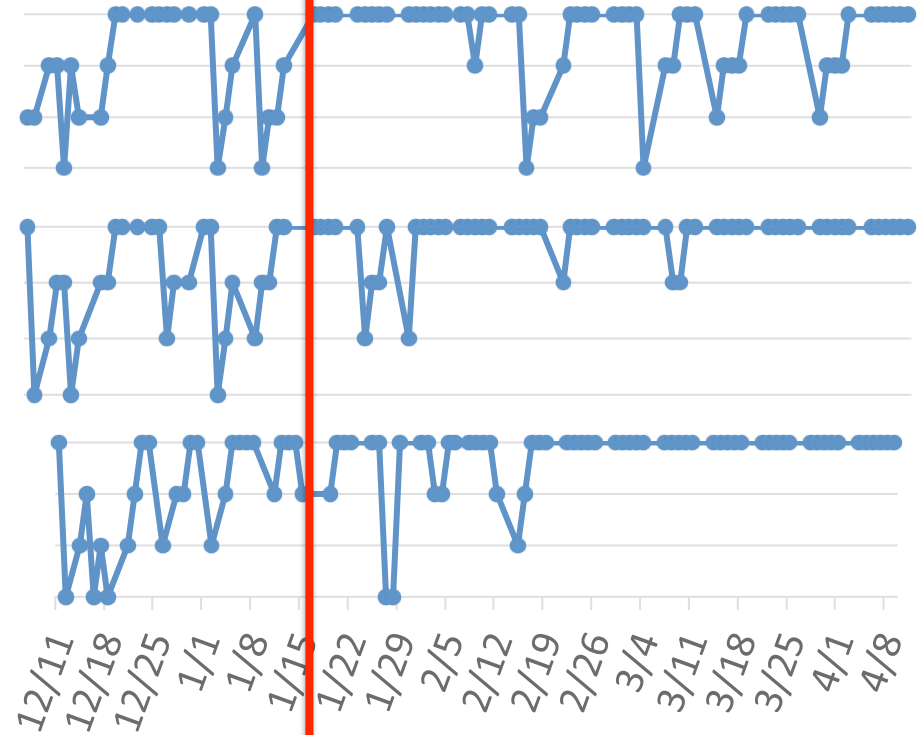
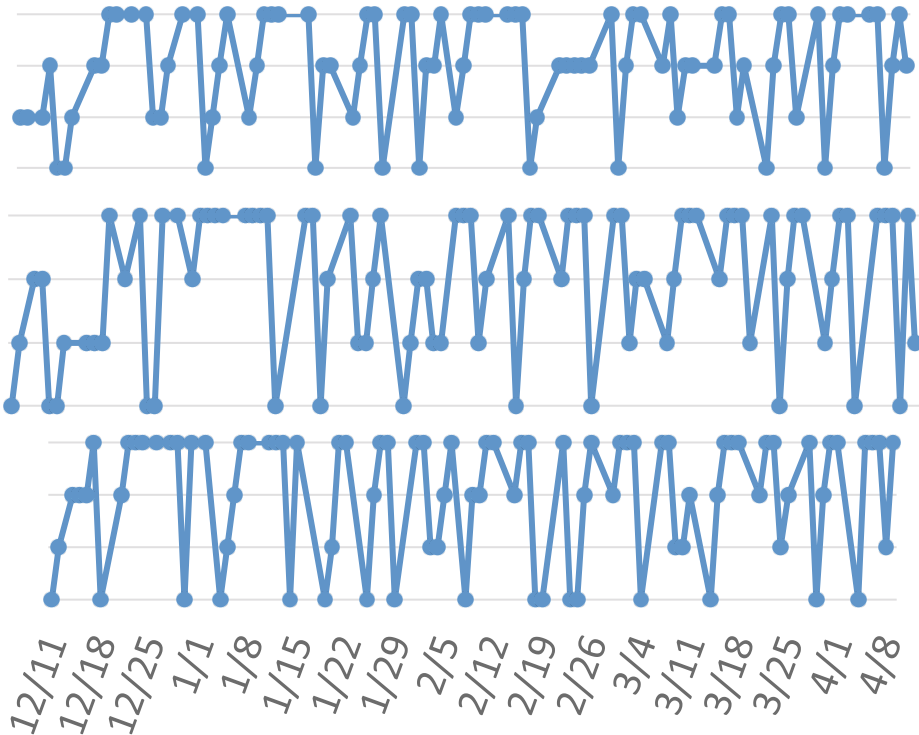
estrus 4  
 proestrus 3  
 diestrus 2  
 metestrus 1



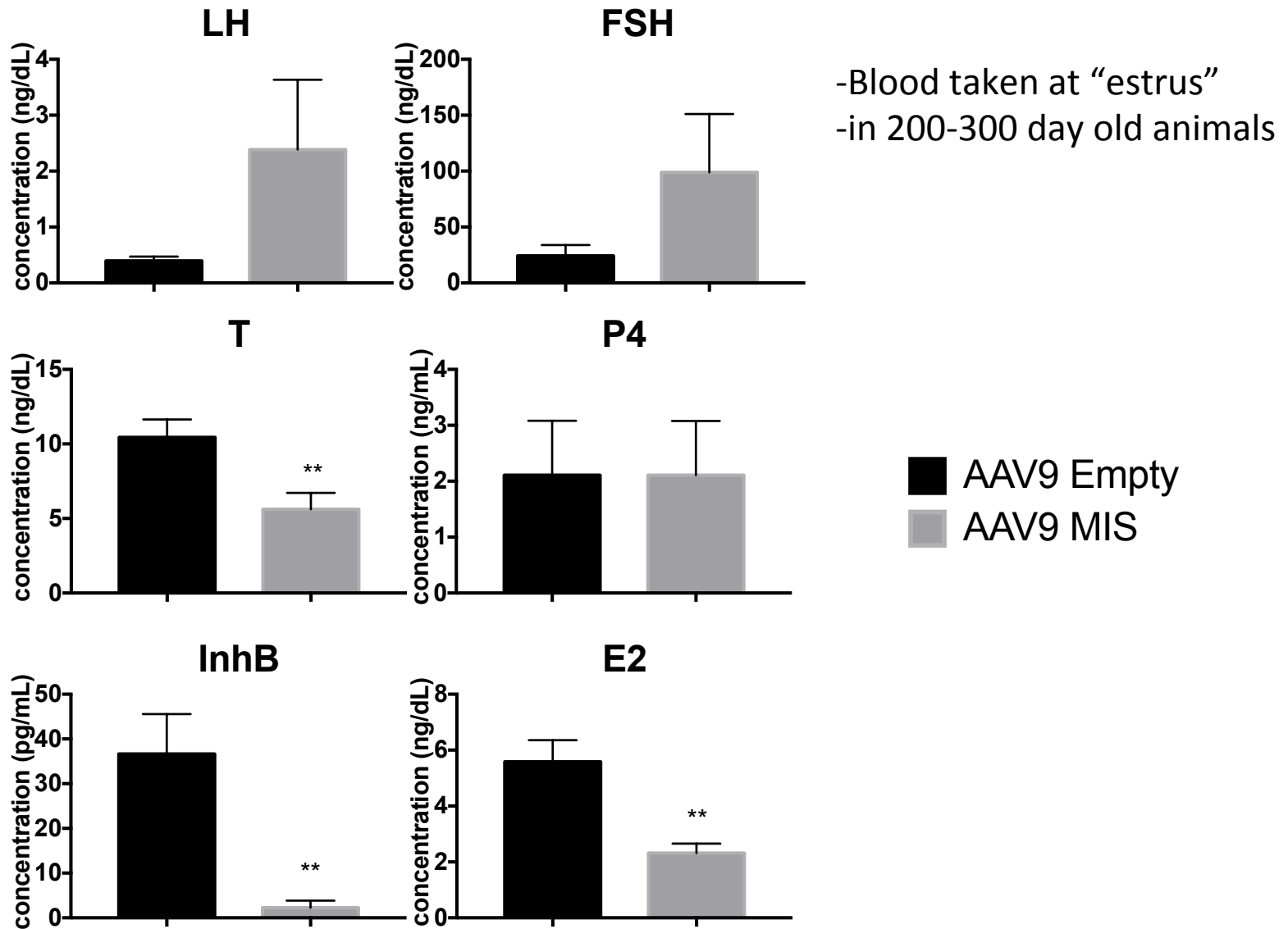
Leukocytes  
 Nucleated Epithelial Cells  
 Cornified Epithelial Cells

AAV9 Empty

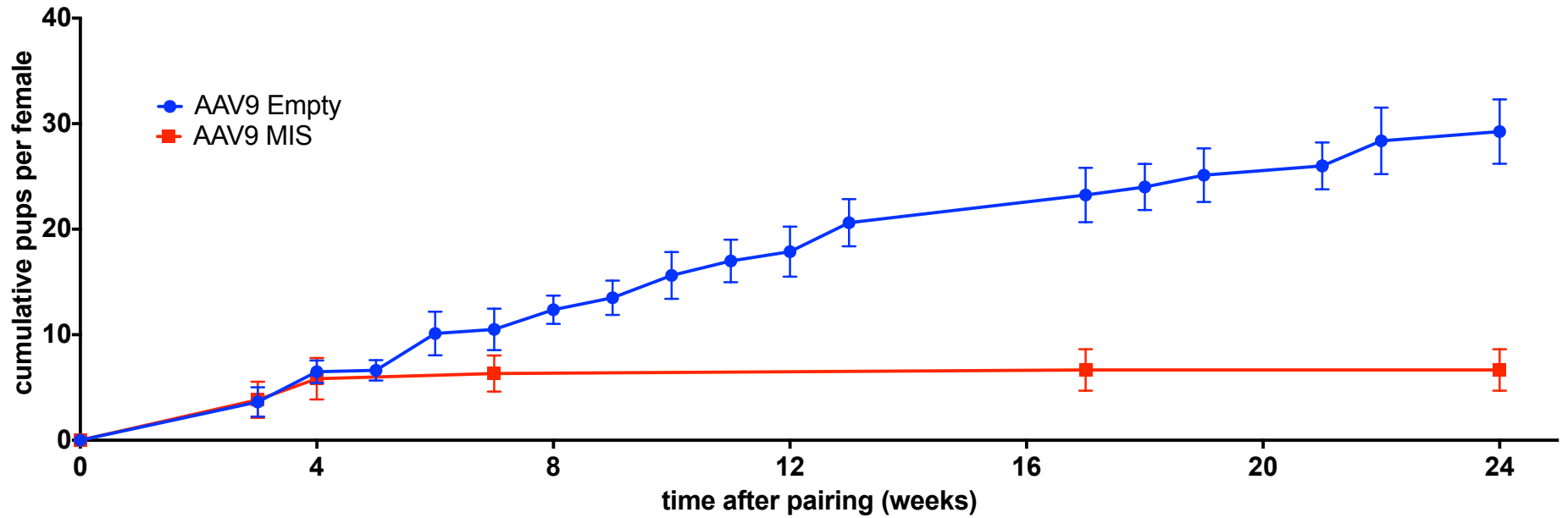
AAV9 LR-MIS



# Hormonal profile of AAV9 treated animals



# Vectored contraception in mice using AAV9-MIS (N=10 pairs per group)



4 wk delay

Complete contraception

# Cat MIS

```

cat  AACCTGTCGGACCCCGCGGCGCAGGAGCGCTGCTCAACGGCGGCGACGAGCCGCTGCTG
dog  AACCTGTCGGACCCCGCGACACAGGAGCGCTGCT---GGGCGGCGAGGAGCCCTGCTG
***** * *****

```

```

cat  CTGCTTCT-----
dog  CTGCTGCTGCCACCCCGCAGGCGCGGGCCCGGGCCCGCGCGCCCGCCCGCGCG
***** **

```

```

cat  -----
dog  TCCGCGCCCTGGGCGCGGGCCTCGCCCTGCGCGTGGCCGCGAGCTGCGGGCCGCGGCC

```

```

cat  -----GCTGCCACCCGCCACNNNNNNN-----
dog  GCGGAGCTCCGCGGGCTCCGCGGGCTGCCCGCCCGCGCGCGCTGCTGGAGCGCCTG
***** ***** *

```

```

cat  -----
dog  CTGCGCCTCTGCCCGGGGCTCGGGGGGCTCGGGGGGCTCGGGGGACCCGCTGCGCGCG

```

```

cat  -----
dog  CTGCTGCTGCTCAAGGCGTTGCAGGGCCTGCGCGCCGAGTGGCGCGGGCGGAGCGGGGC

```

```

cat  -----
dog  GGGCCCCCGGGCGCAGCGCAGCGGGGGCGGGGGCGGCCGACGGGCCCTGCGCGCTG

```

```

cat  -----
dog  CGCGAGCTGAGCGTGGACCTGCGCGCCGAGCGCTCCGTGCTCATCCCCGAGACCTACCAG

```

```

cat  ---NNAACTGCCAGGGCGCGTGC GGCTGGCCGAGTCCGACCGCAACCCGCGCTACGGC
dog  GCCAACAACAGGGCGCGTGC GGGTGGCCGAGTCCGACCGCAACCCGCGCTACGGC
*****

```

Cat MIS missing sequence  
Very high GC content

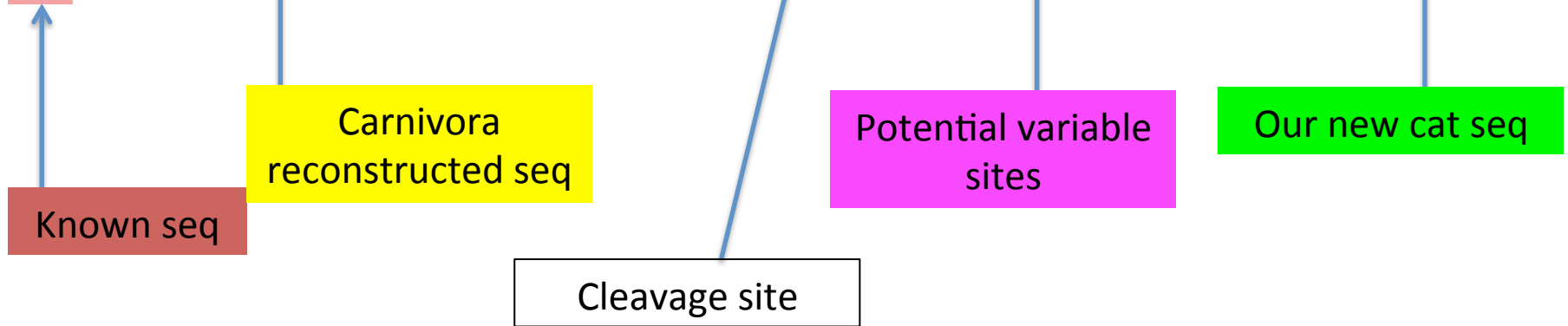


cat-MIS 0.05854  
 dog-MIS 0.06589  
 Odobenus 0.04445  
 Mustela 0.07665  
 Leptonychotes 0.04259  
 Ailuropoda 0.10263

# Reconstructed cat MIS

composite protein with enhanced homology (575AA)

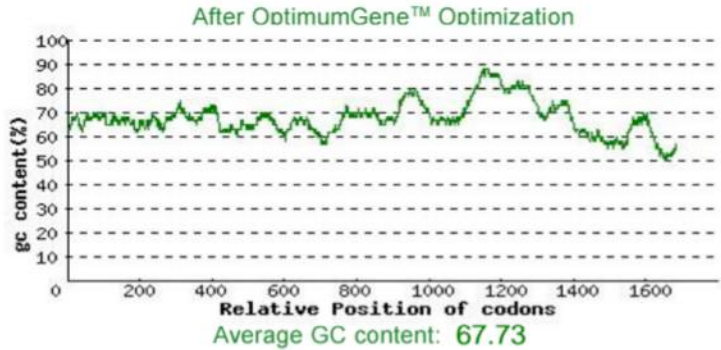
```
MPGLLSPPALVLSVMGALLMAGDPGEEVSSTPALPGGPATGTGGLIFHPDWDWQPPGSPQ
DPLCLVTLDRGGNGSGSPLRVVGALRGYEHAFLEAVRRARWGP HGLATFGVCTPRDRQAA
PFSLRQLQAWLGEPGGRRLVVLHLEEV TWEPTPSLKFQEP PP GGAGPLELAMLVLYPGPG
PEVTVTGAGLPGTQSLCQSRDTRYLVLAVDHPEGAWRSPGLTTLTQPRRDGAPLSTAQLO
ELLFGPDPRCFTRMTPALLLLPGPAPAPLPARGLLDQVPLPPRPSQEQAPEEPRSSADP
FLETLTRLVRALRGPPAQASPARLALDPGALAGFPQGLVNLSDPAAQERLLNGGDEPLLL
LLPPPTAAAGPPAPPPRPASAPWAAGLALRVA AELRAAAAELRGLPGLPPATAPLLERLLALCP
GGSGGSGGSDPLRALLLLKALQGLRAEWRGRERGGPPRAORSAGAGAADGPCALRELSVDLRAERSVLIIDETYQAN
NCQGACGWPOSDENPRYGNHVLLLLKMQARGAALAR PCCVPTAYAGKLLISLSEERISAHHVPNMVATEC
GCR
```



Potential variable sites differ between cat and other carnivora. May be sequencing errors.



# AAV9-fcMIS pilot trial in cats

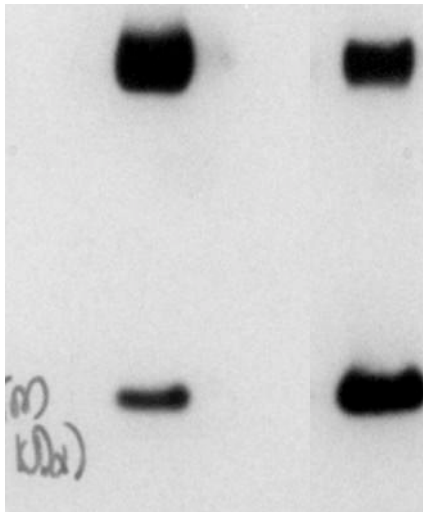


CHO

---

GFP    fcMIS    EMPTY    LR11 +VE CTRL

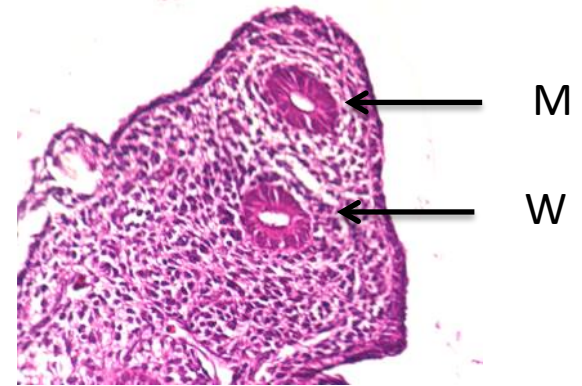
HOLO



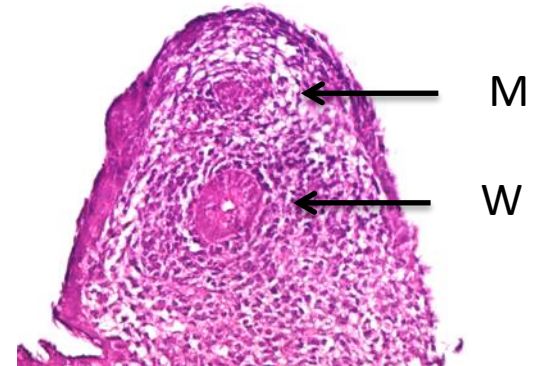
C-TERM



CHO-GFP  
Conditioned  
media



CHO-fcMIS  
Conditioned  
media



# AAV9 Intra-muscular injections induces muscles to express fcMIS

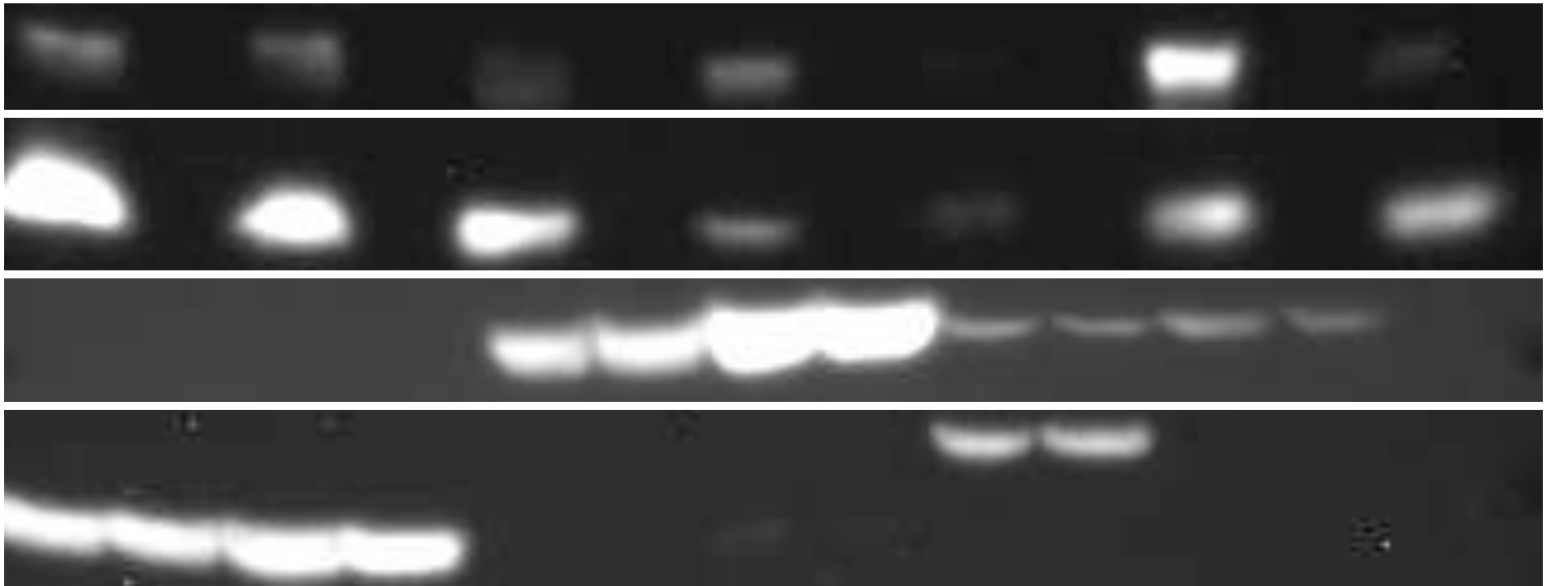
AAV9-fcMIS leg muscle  
No treatment leg muscle  
AAV9-fcMIS abd. muscle  
No treatment abd. muscle  
AAV9-fcMIS kidney  
No treatment kidney  
AAV9-fcMIS spleen  
No treatment spleen  
AAV9-fcMIS pancreas  
No treatment pancreas  
AAV9-fcMIS liver  
No treatment liver  
LR-MIS

Holo-MIS

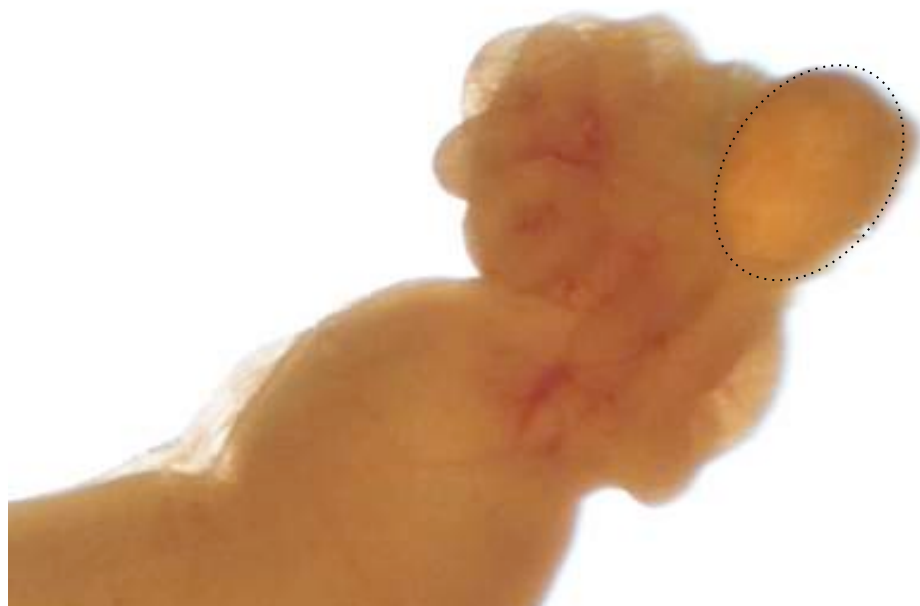
C-terminus

beta actin

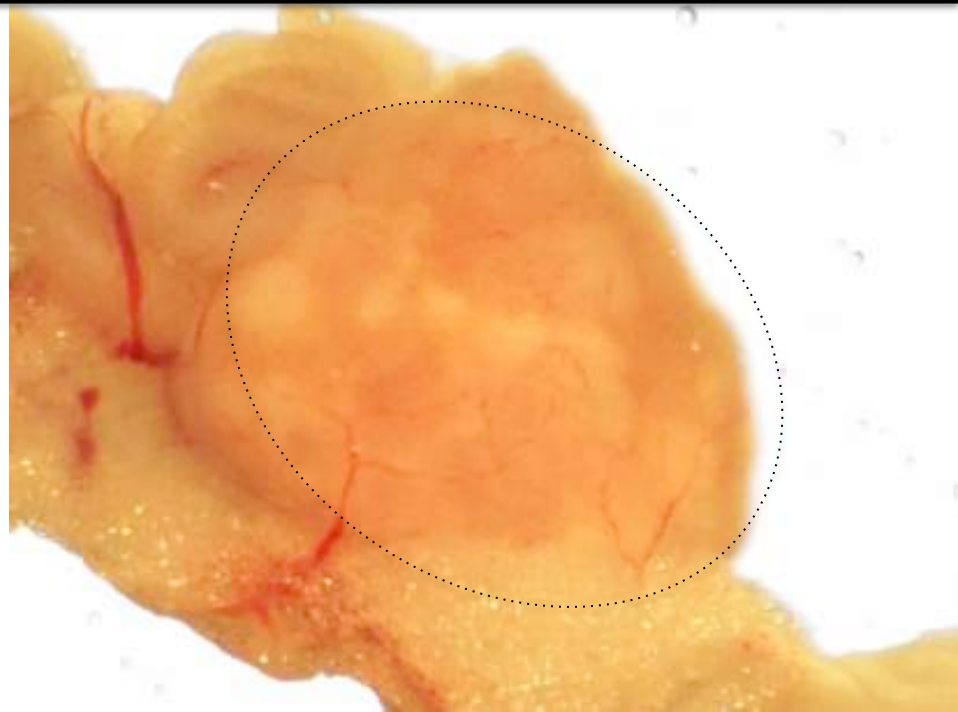
GAPDH



AAV9-fcMIS (50d)



Control 50d





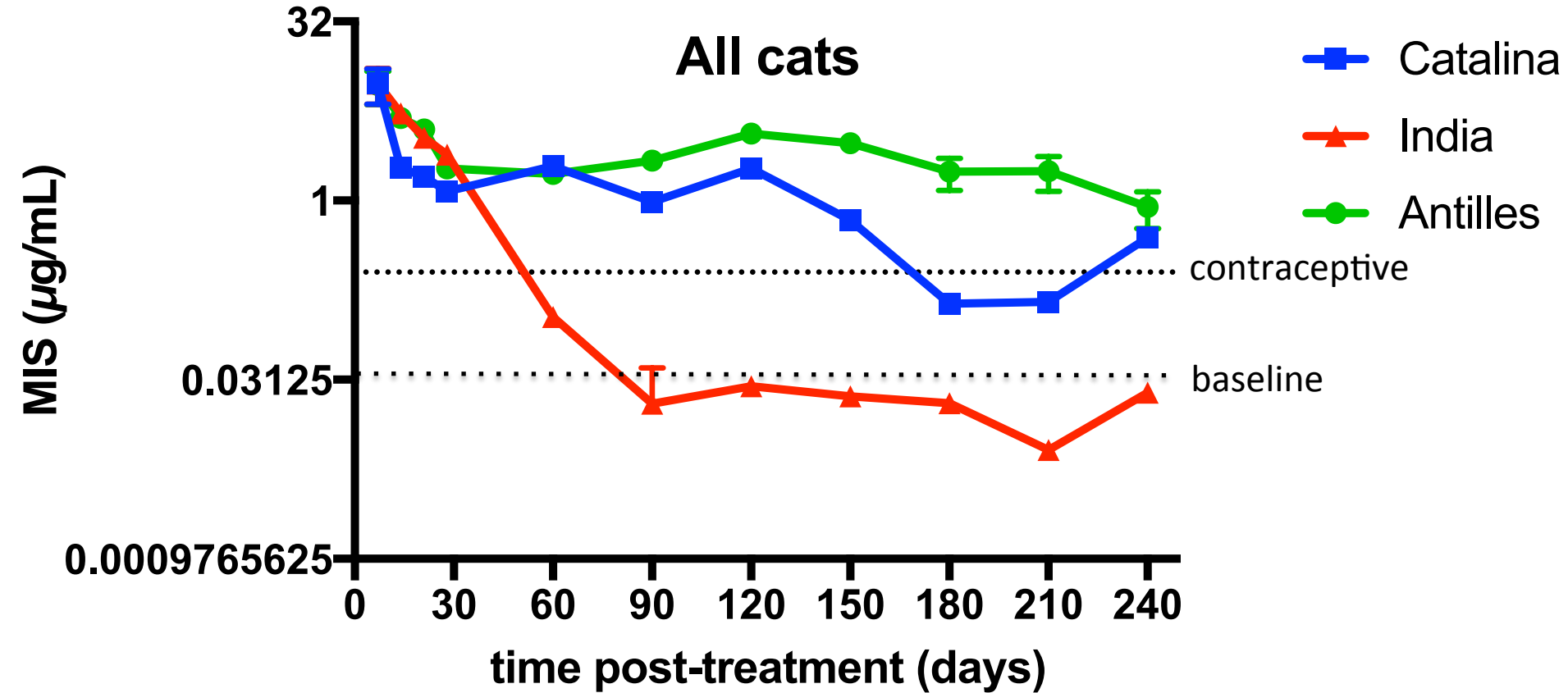


Bill Swanson

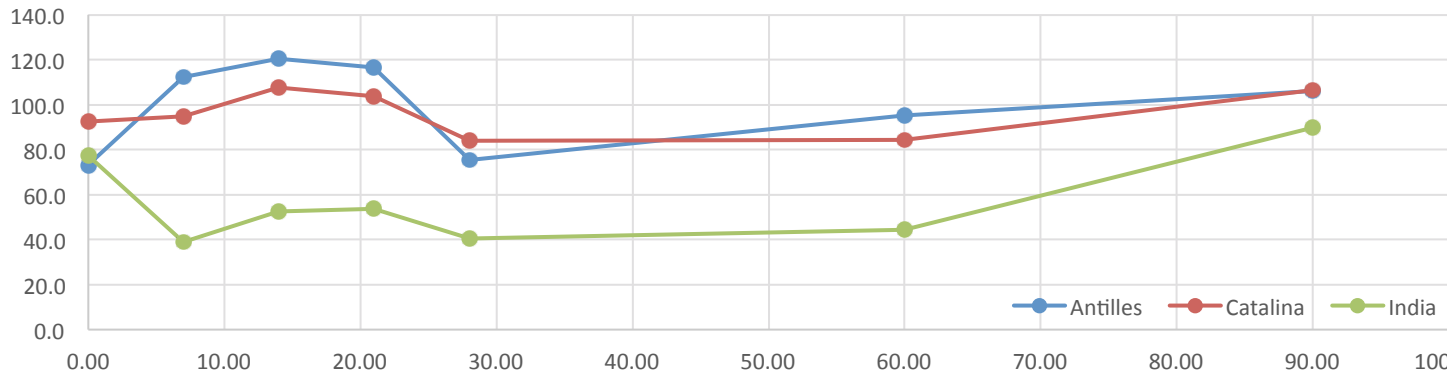
## Pilot trial design:

- 3 females ~3kg
- minimal dose of  $1.5E13$  particles/cat
- intramuscular 1,5ml each shoulder muscle.
- monitoring of health
- monitoring of cycling by fecal steroids
- monitoring of MIS levels

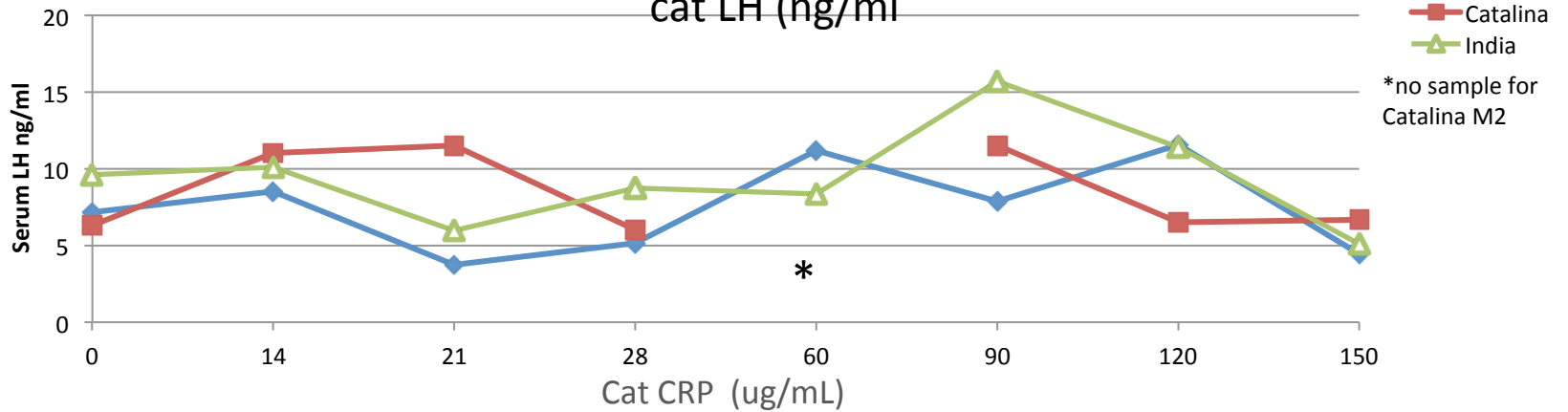
# Circulating MIS levels



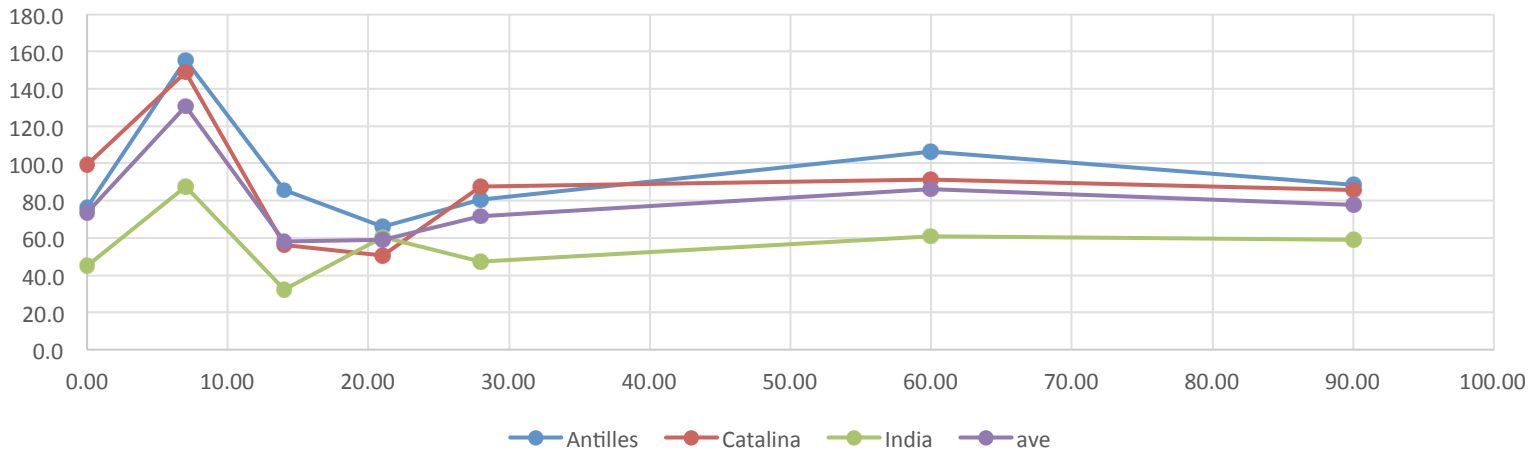
Cat INHB (pg/mL)



cat LH (ng/ml)

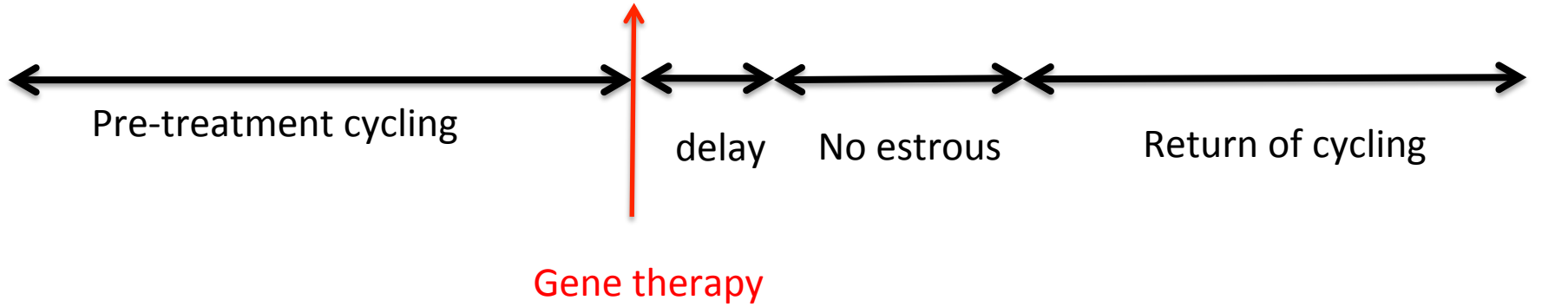
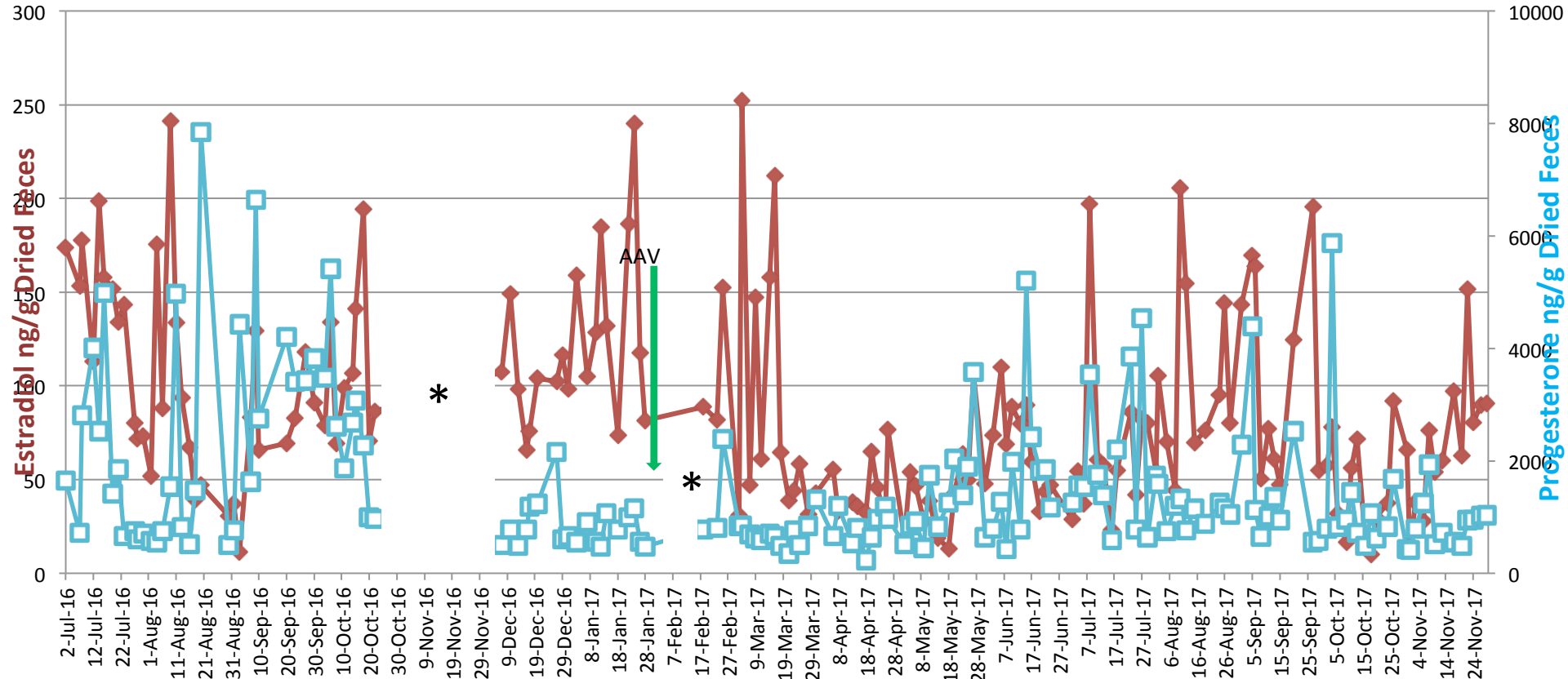


Cat CRP (ug/mL)

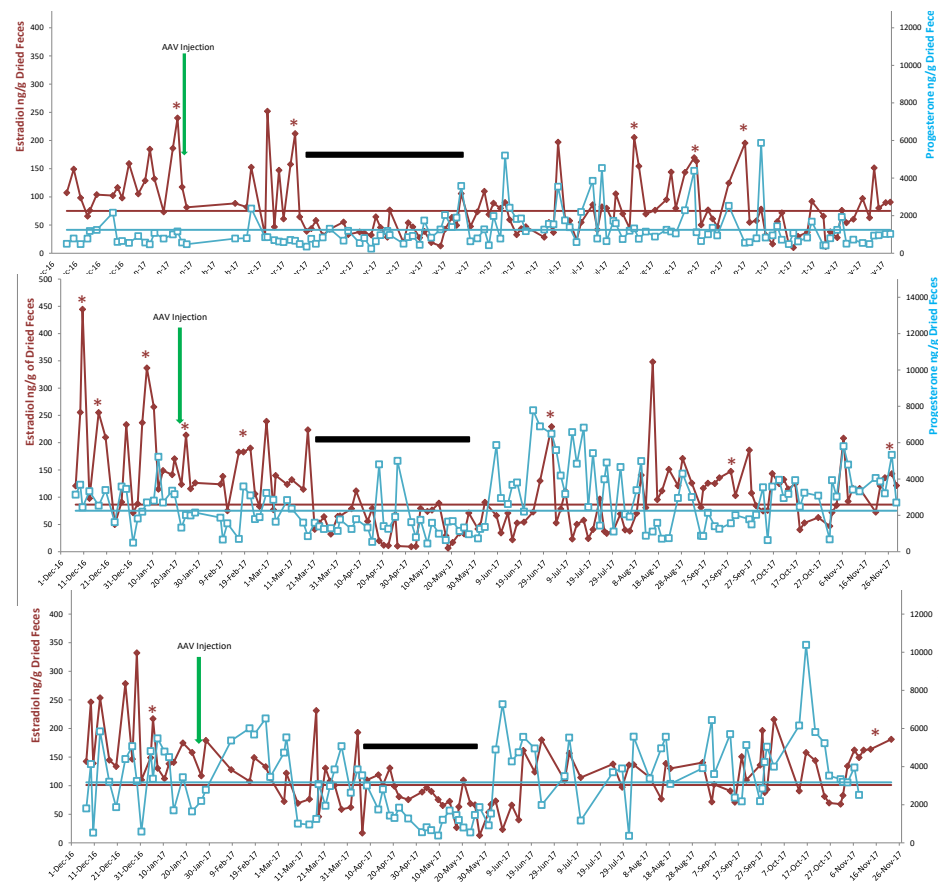


# Antilles - MIS Study 2016-2017

\* No samples



# Transient suppression of cycling in 3 female cats



	E2-Pre	E2-Sup	E2-Post	P4-Pre	P4-Sup	P4-Post	Days suppressed	Interval between injection and day 1 of suppression
Antilles	111.7	40.3	75.5	1908.7	890.4	1420.0	60.0	50.0
Catalina	116.6	48.3	95.8	2184.5	1566.8	3168.5	64.0	52.0
India	114.3	78.5	116.7	3286.6	1863.4	3903.6	51.0	66.0
Mean	114.2	55.7	96.0	2460.0	1440.2	2830.7	58.3	56.0
SE	1.4	11.6	11.9	421.0	287.9	736.6	3.8	5.0

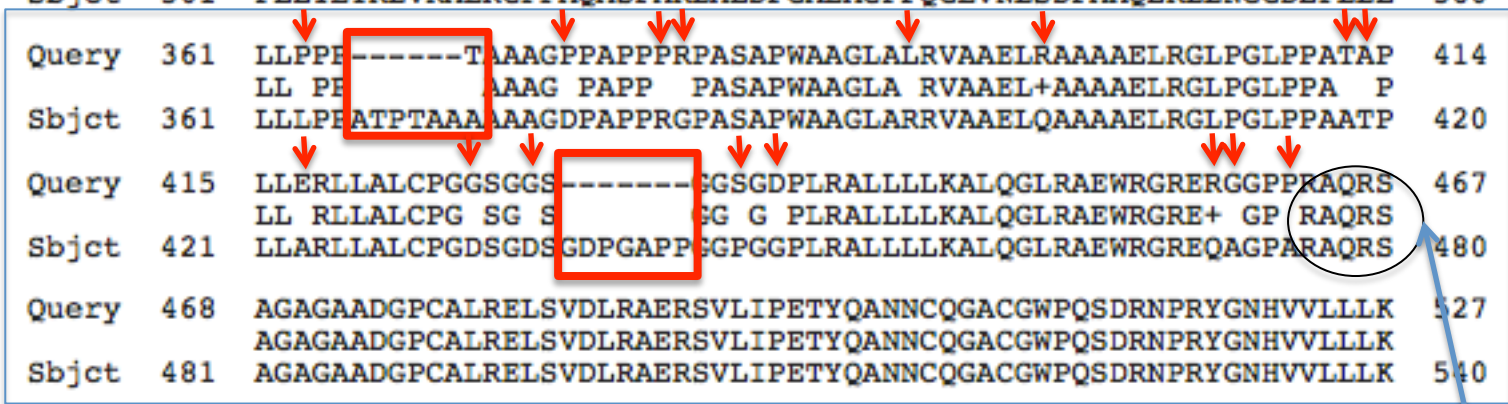
P=0.007

P=0.049

Score	Expect	Method	Identities	Positives	Gaps
1049 bits(2712)	0.0	Compositional matrix adjust.	558/588(95%)	560/588(95%)	13/588(2%)

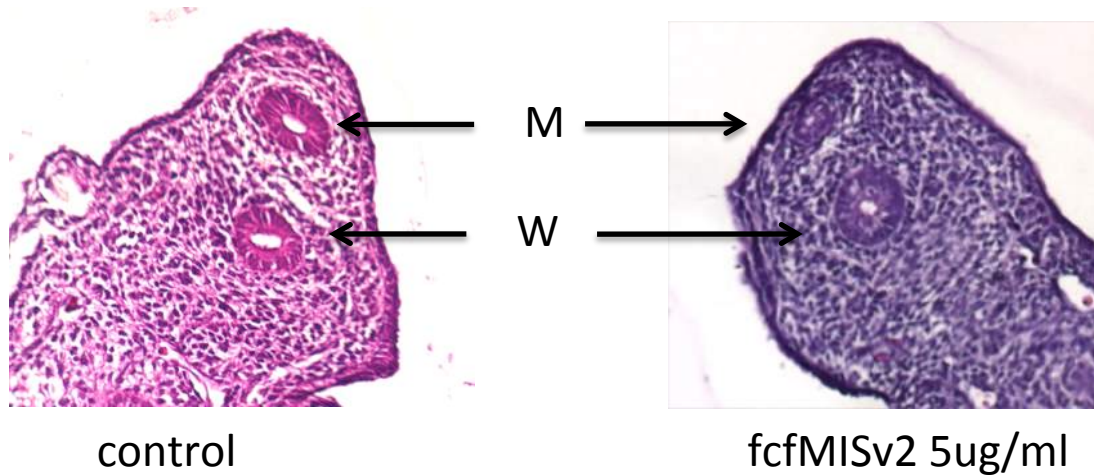
fcMIS	1	MPGLLSPPALVLSVMGALLMAGDPGEEVSSTPALPGGPATGTGGLIFHPDWDWQPPGSPQ	60
Consensus	1	MPGLLSPPALVLSVMGALLMAGDPGEEVSSTPALPGGPATGTGGLIFHPDWDWQPPGSPQ	60
Cat 9.0	1	MPGLLSPPALVLSVMGALLMAGDPGEEVSSTPALPGGPATGTGGLIFHPDWDWQPPGSPQ	60
Query	61	DPLCLVTLDRGGNGSGSPLRVVGALRGYEHAFLEAVRRARWGP HGLATFGVCTPRDRQAA	120
Sbjct	61	DPLCLVTLDRGGNGSGSPLRVVGALRGYEHAFLEAVRRARWGP HGLATFGVCTPRDRQAA	120
Query	121	PFSLRQLQAWLGEPGRRLLVVLHLEEV TWEPTPSLKFQEP PPGAGPLELAMLVLYPGPG	180
Sbjct	121	PFSLRQLQAWLGEPGRRLLVVLHLEEV TWEPTPSLKFQEP PPGAGPLELAMLVLYPGPG	180
Query	181	PEVTVTGAGLPGTQSLCQSRDTRYLVLAVDHPEGAWRSPGLTTLQPRRDGAPLSTAQLQ	240
Sbjct	181	PEVTVTGAGLPGTQSLCQSRDTRYLVLAVDHPEGAWRSPGLTTLQPRRDGAPLSTAQLQ	240
Query	241	ELLFGDPDPRCFTRMTPALLLLPGPAPAPLPARGLLDQVPLPPRPSQEQAPEEPRSSADP	300
Sbjct	241	ELLFGDPDPRCFTRMTPALLLLPGPAPAPLPARGLLDQVPLPPRPSQEQAPEEPRSSADP	300
Query	301	FLETLTRLVLRALRGPPAQASPARLALDPGALAGFPQGLVNLS DPAAQERLLNGGDEPLL	360
Sbjct	301	FLETLTRLVLRALRGPPAQASPARLALDPGALAGFPQGLVNLS DPAAQERLLNGGDEPLL	360
Query	361	LLPPE-----TAAAGPPAPPPRPASAPWAAGLALRVA AELRAAAAELRGLPGLPPATAP	414
Sbjct	361	LLPPE-----TAAAG PAPP PASAPWAAGLA RVA AEL+AAAAELRGLPGLPPA P	420
Query	415	LLERLLALCPGGSGGS-----SGSGDPLRALLLLKALQGLRAEWRGRERGGPPRAQRS	467
Sbjct	421	LLARLLALCPGDSGDSGDPGAPP SG G PLRALLLLKALQGLRAEWRGRE+ GP RAQRS	480
Query	468	AGAGAADGPCALRELSVDLRAERSVLIPE TYQANNCQGACGWPQSDRNPRYGNHVVLLK	527
Sbjct	481	AGAGAADGPCALRELSVDLRAERSVLIPE TYQANNCQGACGWPQSDRNPRYGNHVVLLK	540
Query	528	MQARGAALARPPCCVPTAYAGKLLISLSEERISAHHVPNMVATECGCR	575
Sbjct	541	MQARGAALARPPCCVPTAYAGKLLISLSEERISAHHVPNMVATECGCR	588

30AA substitutions



Cleavage site

# New AAV9-fcMISv2 Second generation vector



## Pilot trial design:

- 9 females ~3kg
- Dose of 0/1.5/3 E13 particles/cat
- intramuscular 1.5ml each shoulder muscle.
- monitoring of health (General, CRP)
- monitoring of cycling by fecal steroids
- monitoring of MIS levels
- measure other hormonal activity (INHA/B, FSH, LH)
- mating trials

# Thank you



P.K. Donahoe



G. Gao

## Ped. Surg. Res. Labs.

- Motohiro Kano
- Duygu Saatcioglu
- Jennifer Hsu
- Lihua Zhang
- Nicholas Nagykerly
- Rahgav Mohan
- Rana Suliman
- Caitlyn Sacha
- Li Yi
- Nobuhiro Takahashi

## Collaborators:

Guangping Gao  
Bill Swanson  
Lindsey Vansandt



W. Swanson



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