Neuropeptides linking the control of appetite with reproductive function in domestic animals

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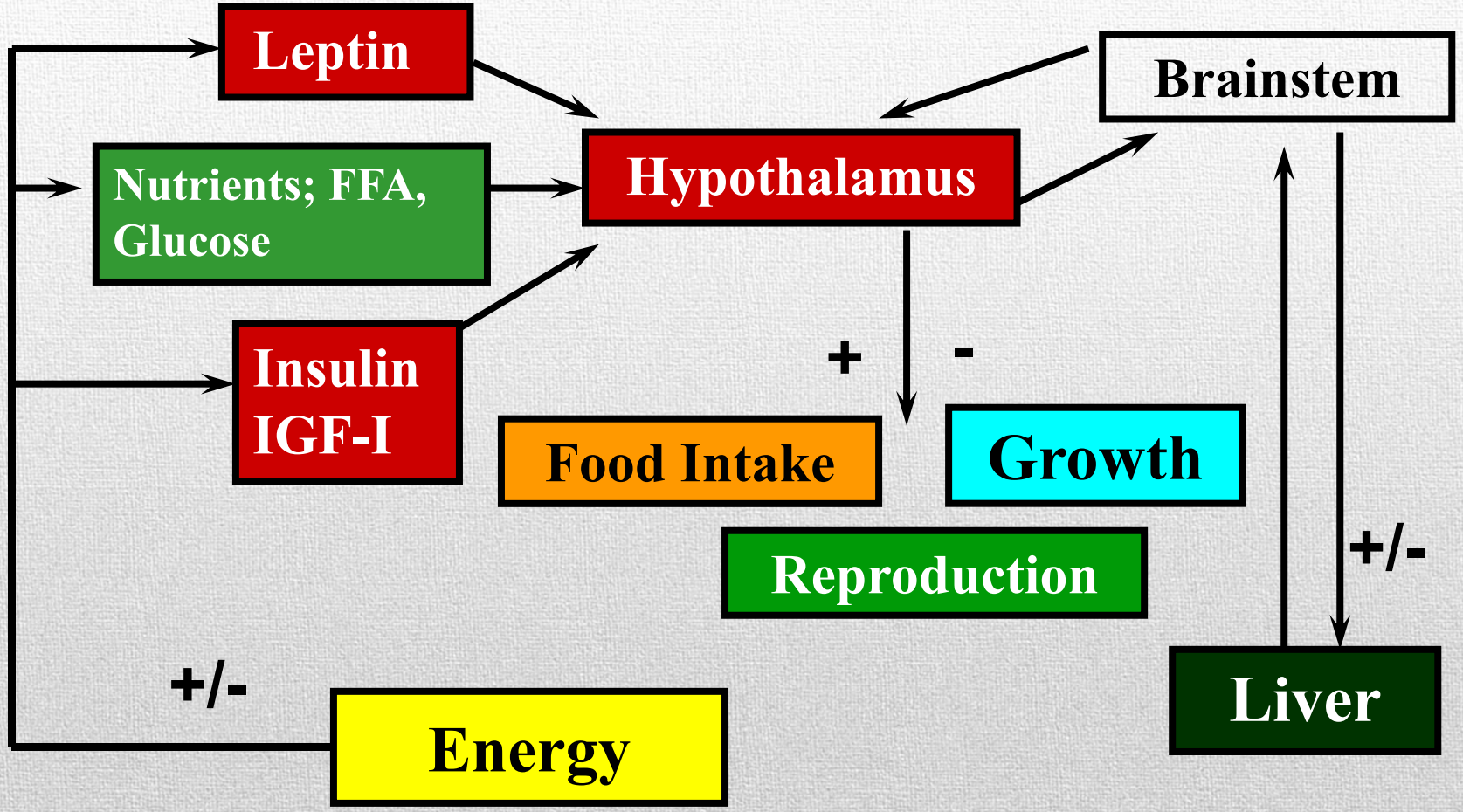
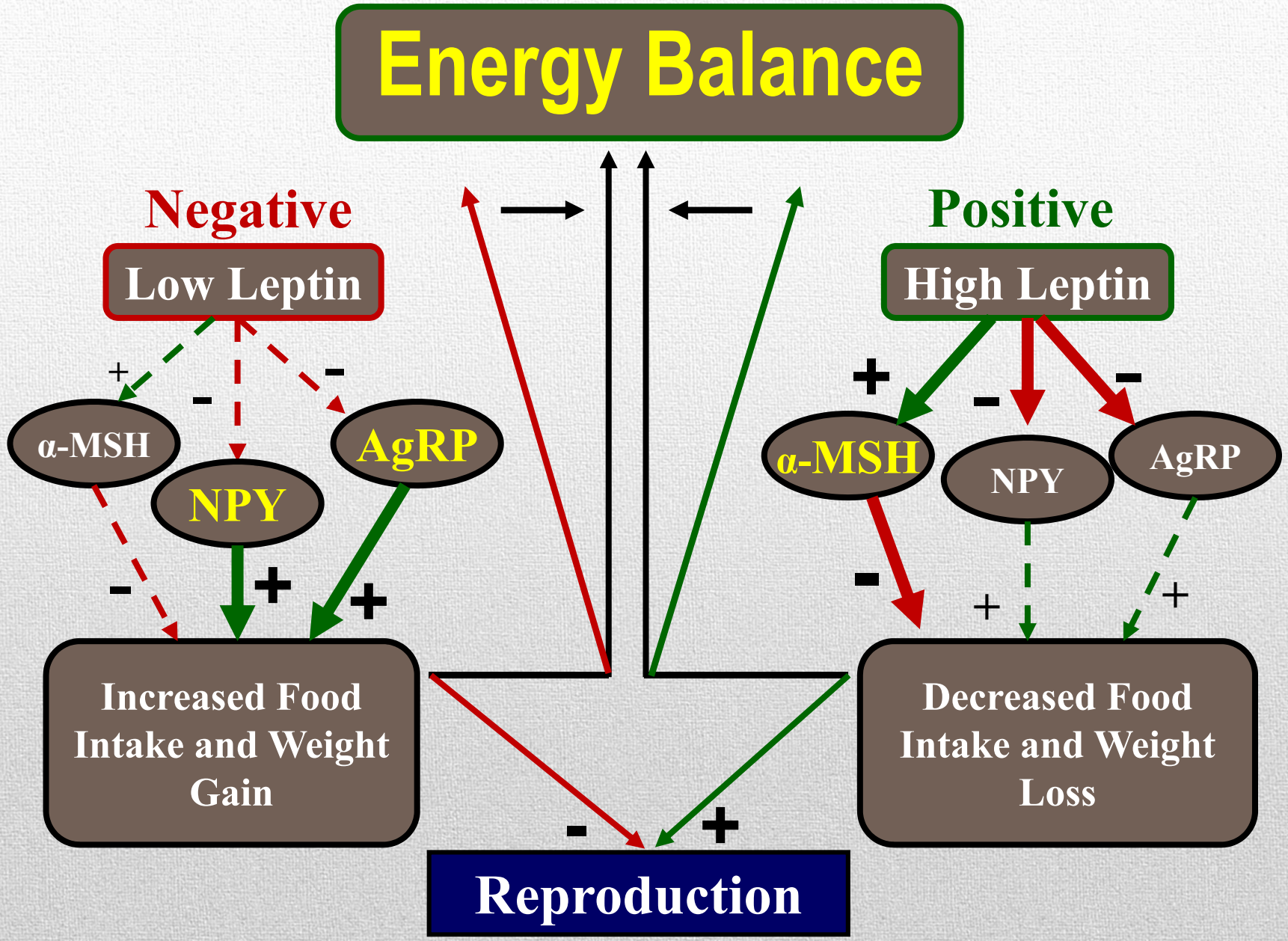
EAAP, Copenhagen Denmark; August 25, 2014

Common pathways of FI, growth & Reproduction



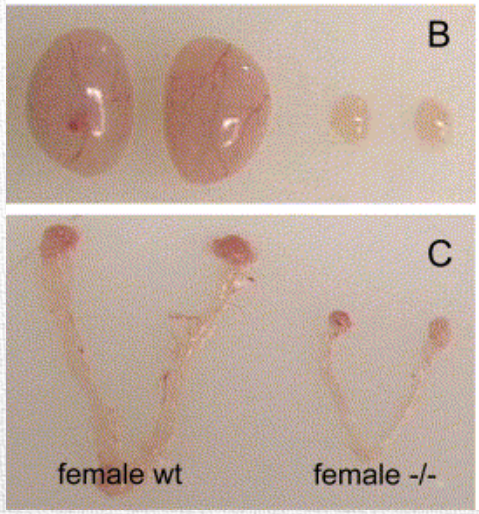
Interaction of appetite with reproduction



Barb et al., 2008 Reprod. Dom. Anim.

Kisspeptin and reproductive function

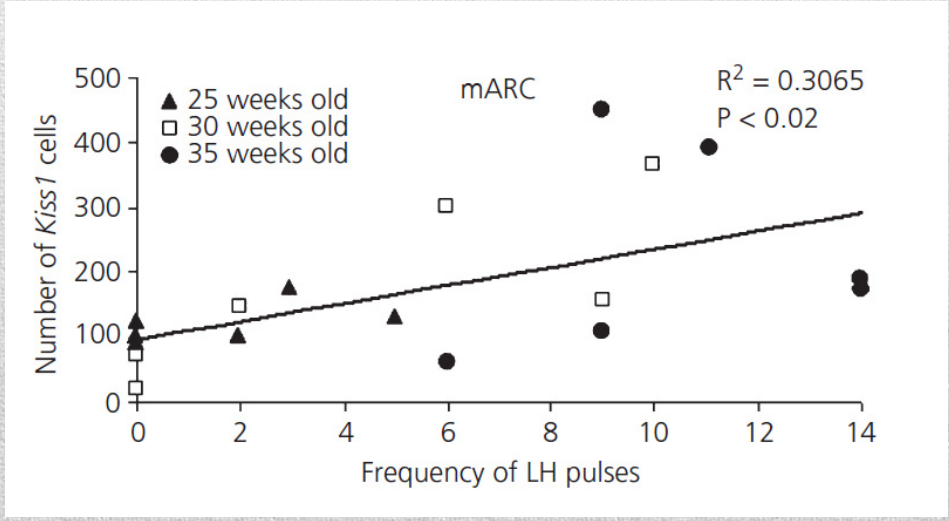
GPR54 KO



Funes et al., 2003

Mutations in Kiss or GPR54 lead to idopathic hypogonadotropic hypogonadism.

Expression of Kiss is developmentally regulated and increased expression corresponds to increased frequency of LH pulses.

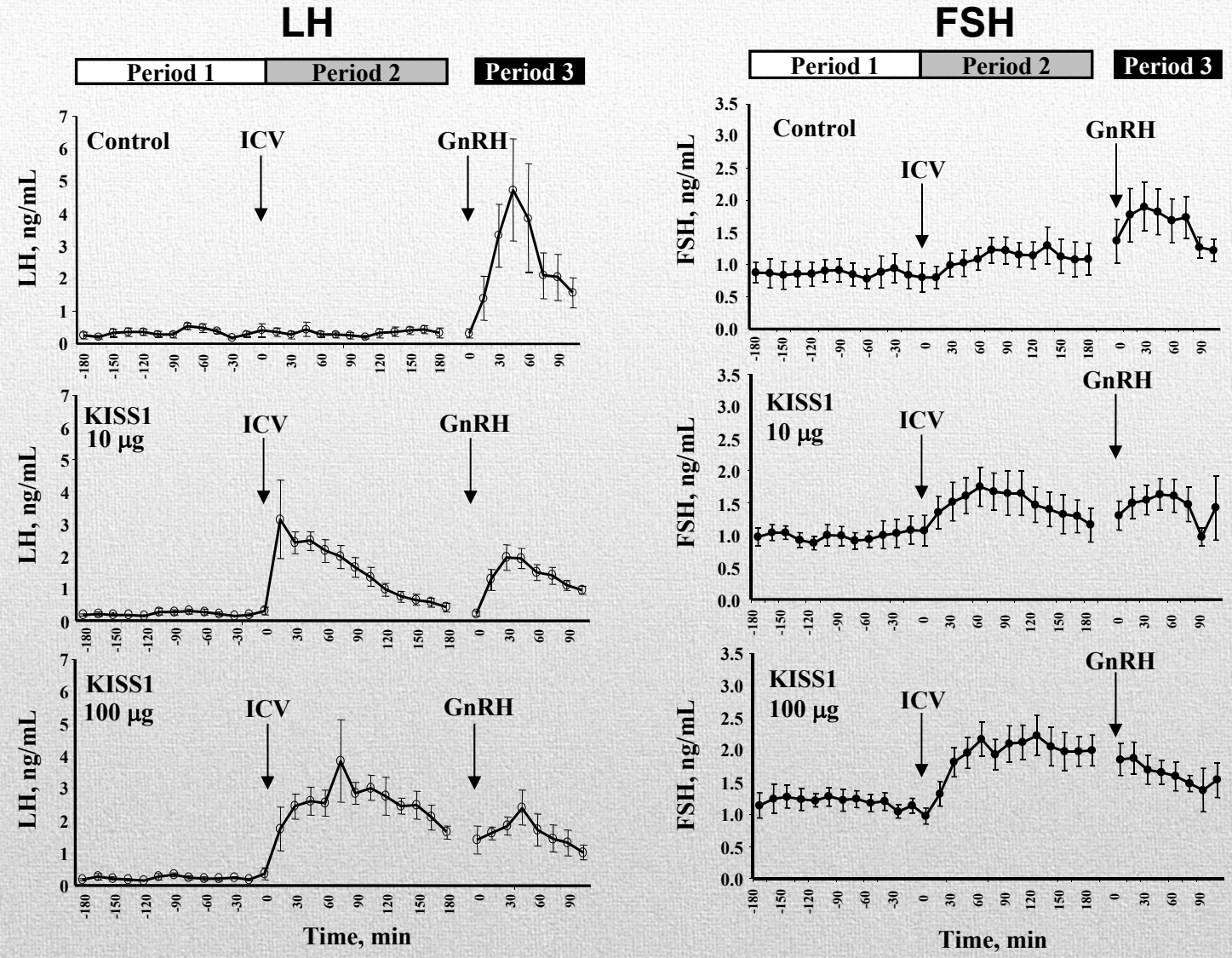


Redmond et al., 2011

Kiss treatment restored LH secretion in underfed animals.

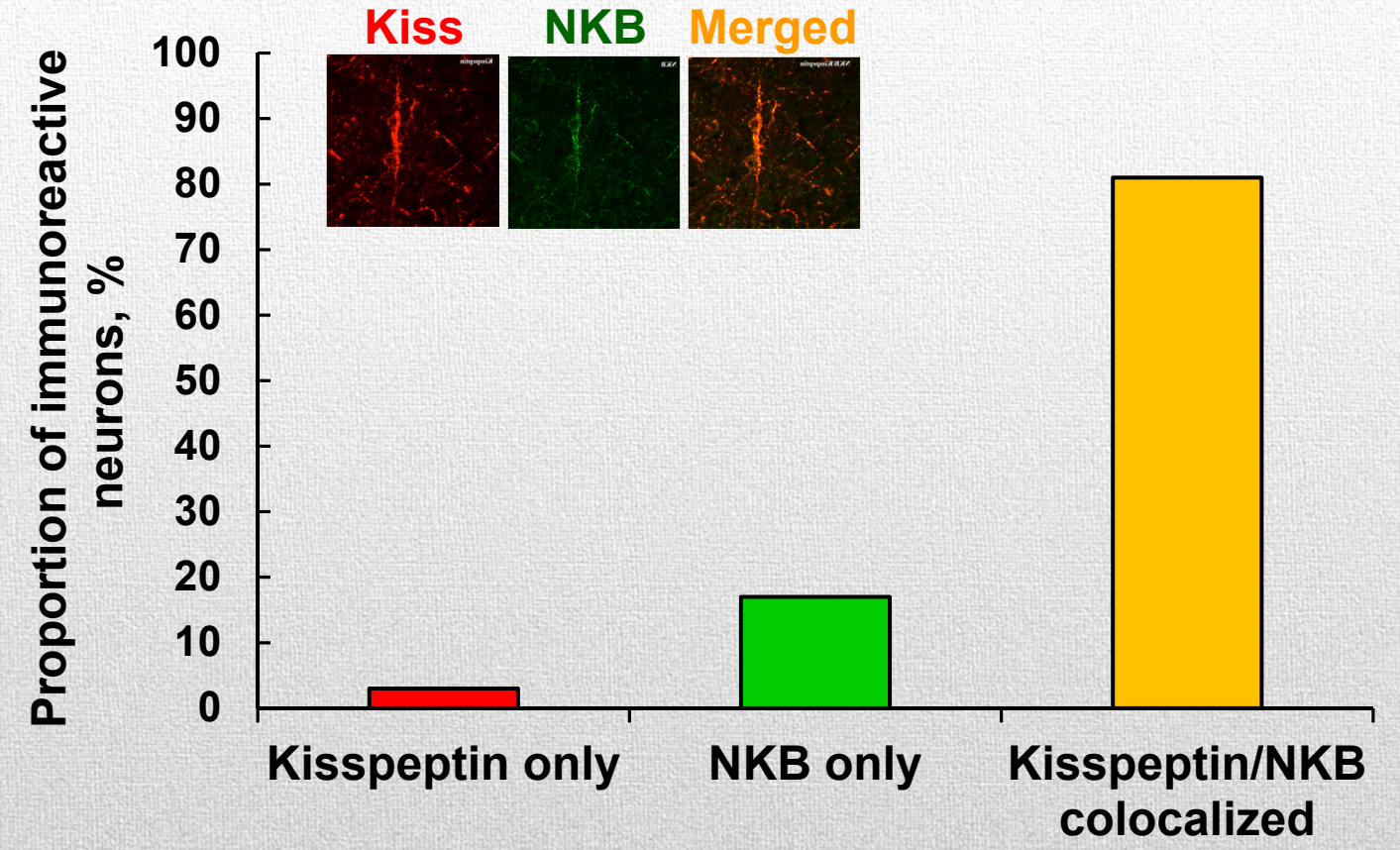
Nutrient restriction or perturbations in metabolism associated with decreased Kiss expression.

Kisspeptin stimulates LH and FSH secretion in prepubertal gilts

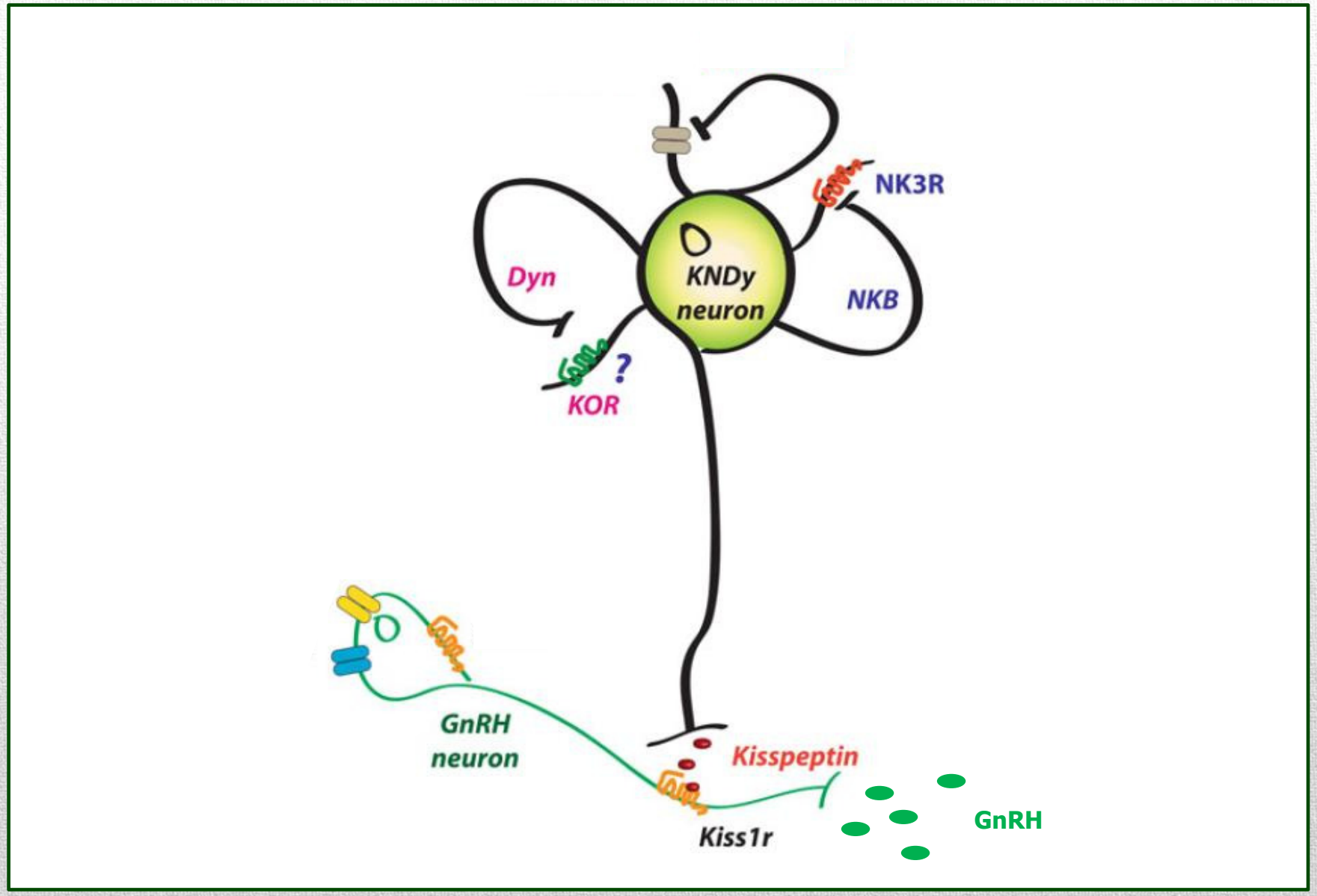


Lents et al., 2008, Reproduction

Kisspeptin and neurokinin B (NKB) coexpressed in the ARC of gilts

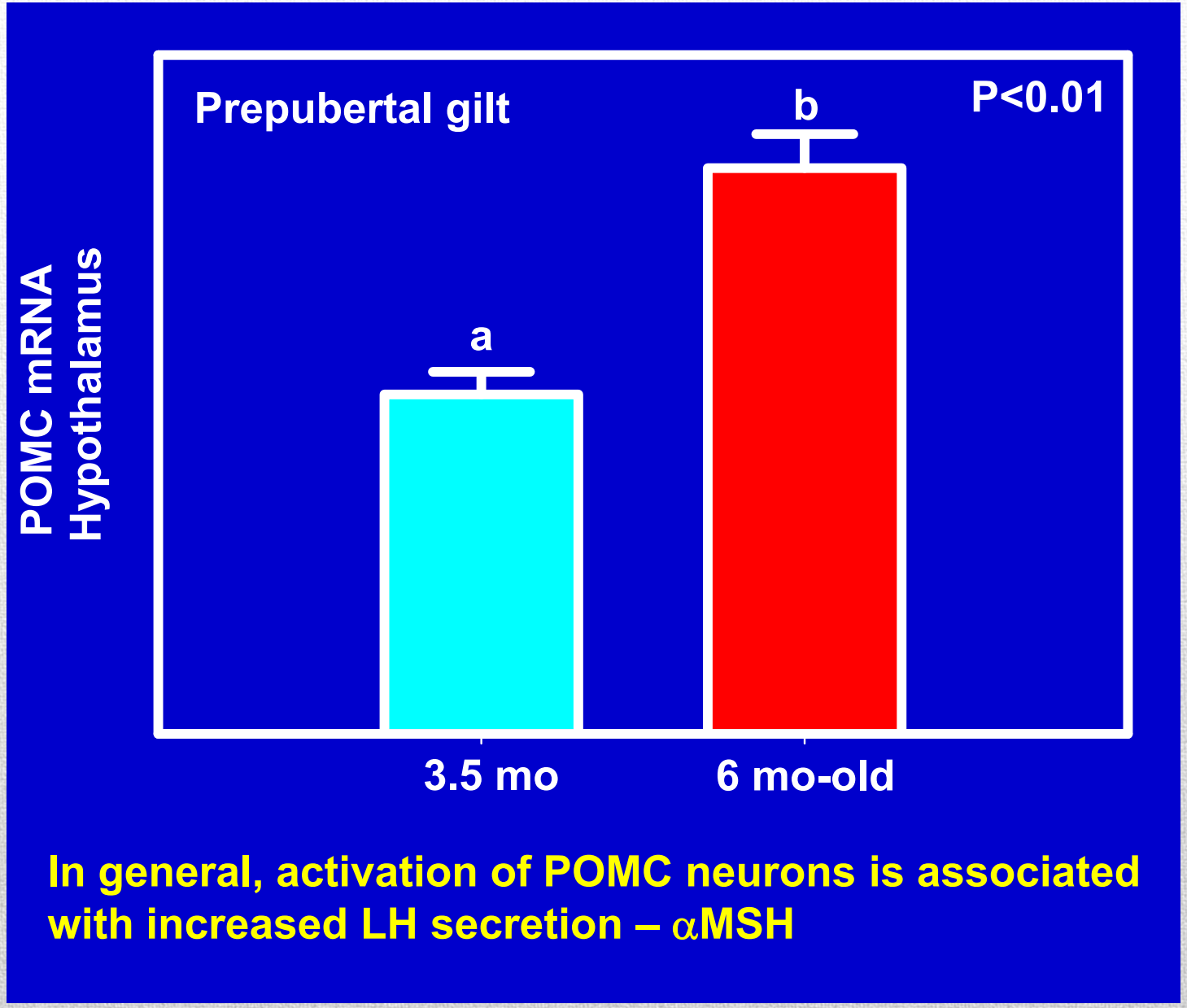


KNDy neurons



Goodman et al, 2013; Navarro et al, 2013

Expression of POMC increases with age in the gilt

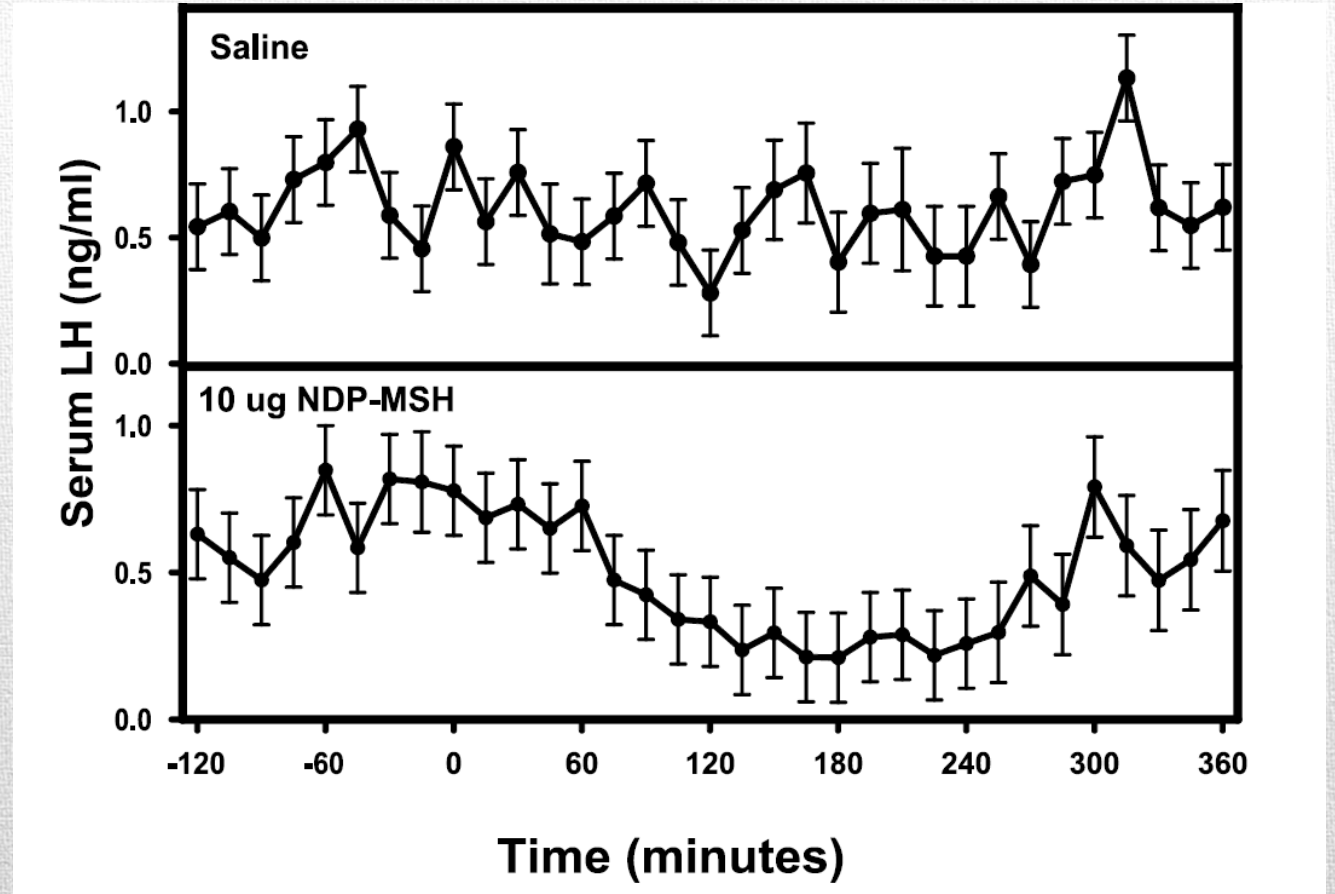


In general, activation of POMC neurons is associated with increased LH secretion – αMSH

Lin et al. Biol. Reprod. 2001

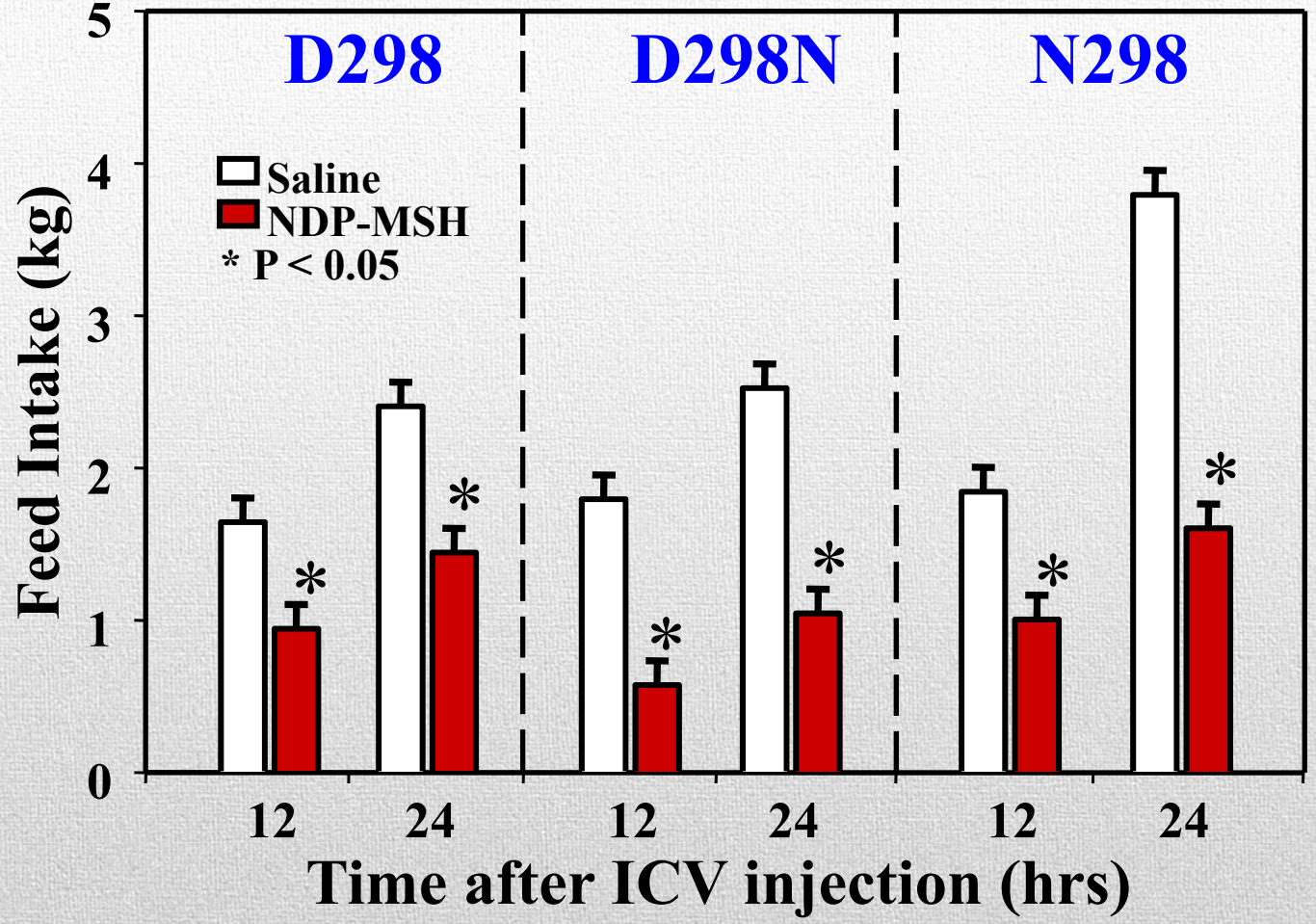
Effects of αMSH on LH secretion in gilts

Time 0 = ICV treatment



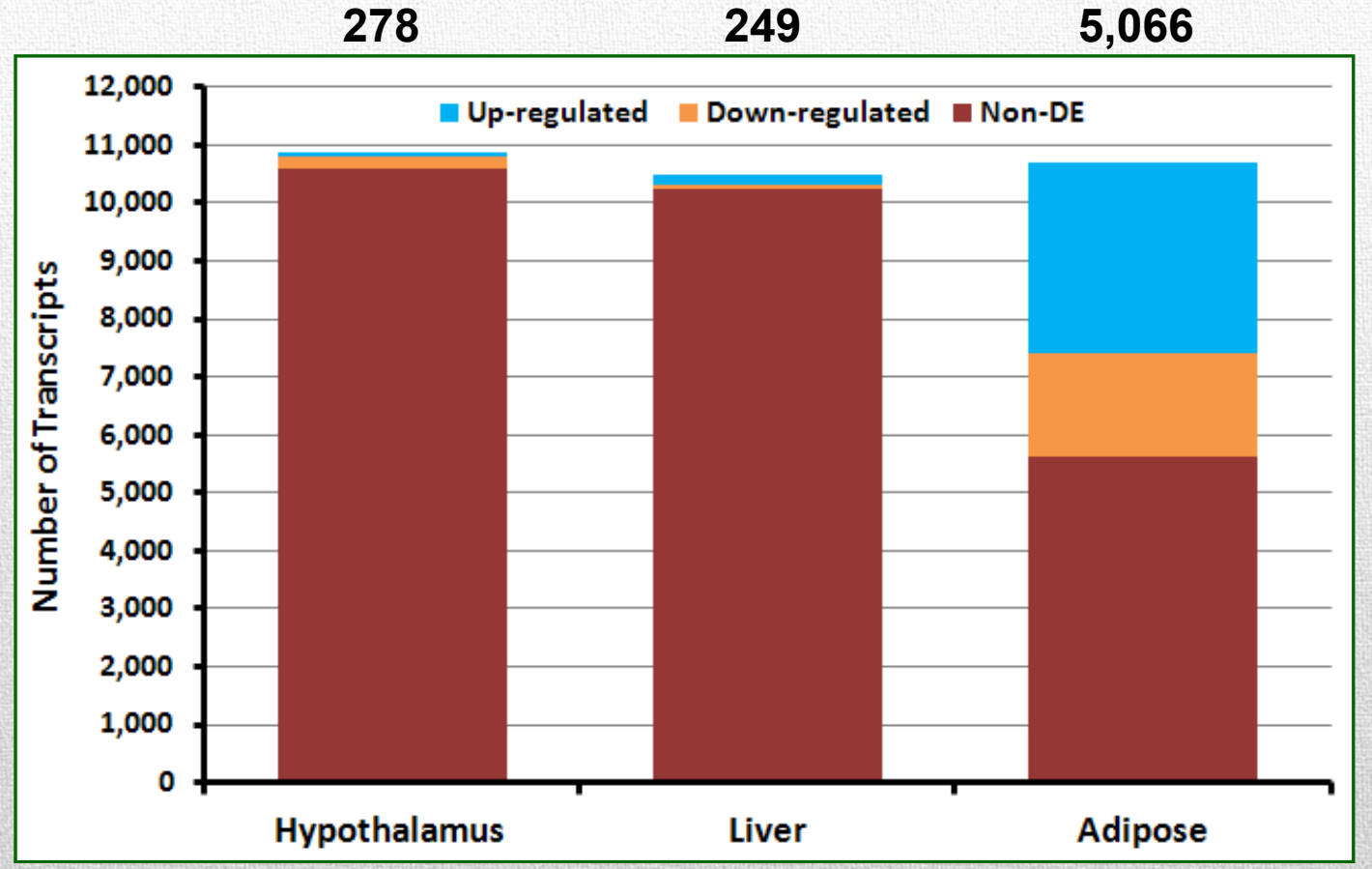
Barb et al., 2004 J. Endocrinol.

Cumulative food intake of pigs after ICV injection of NDP-MSH



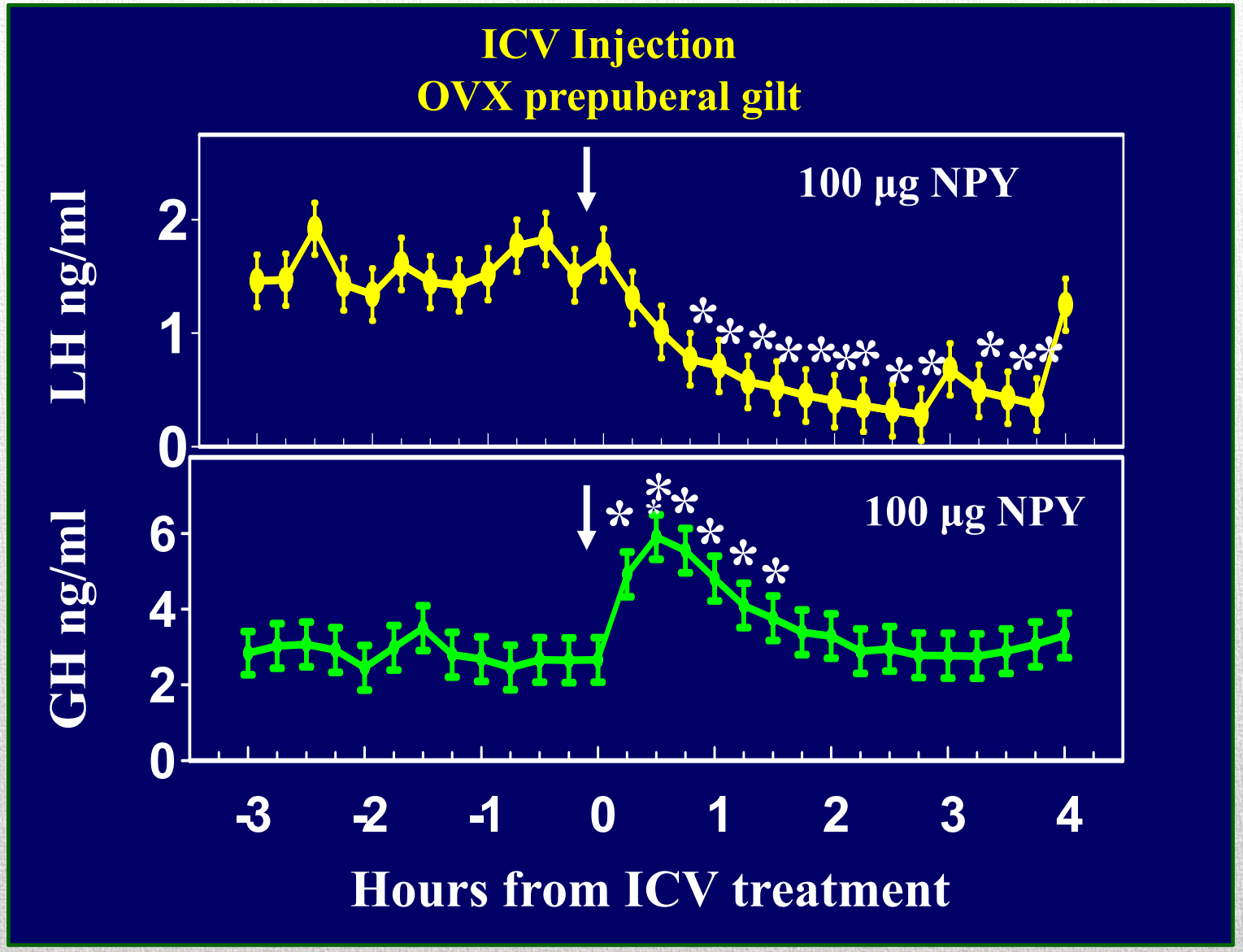
Barb et al., 2010 Physiol. Genomics

Summary of differentially expressed (DE) genes (q<0.07) in hypothalamus, liver and adipose tissues of pigs 24 hr after ICV injection of NDP-MSH



Fat = reduced leptin mRNA
Hypothalamus = increased NPY mRNA

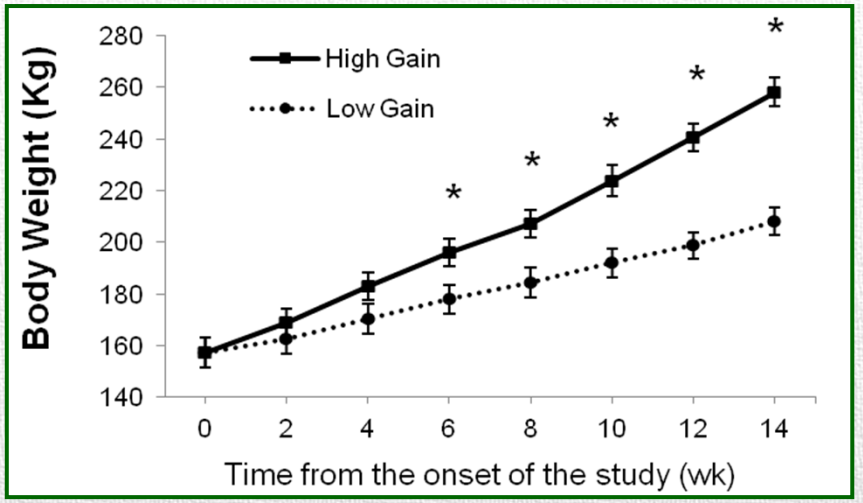
NPY inhibits LH secretion in OVX prepubertal gilts

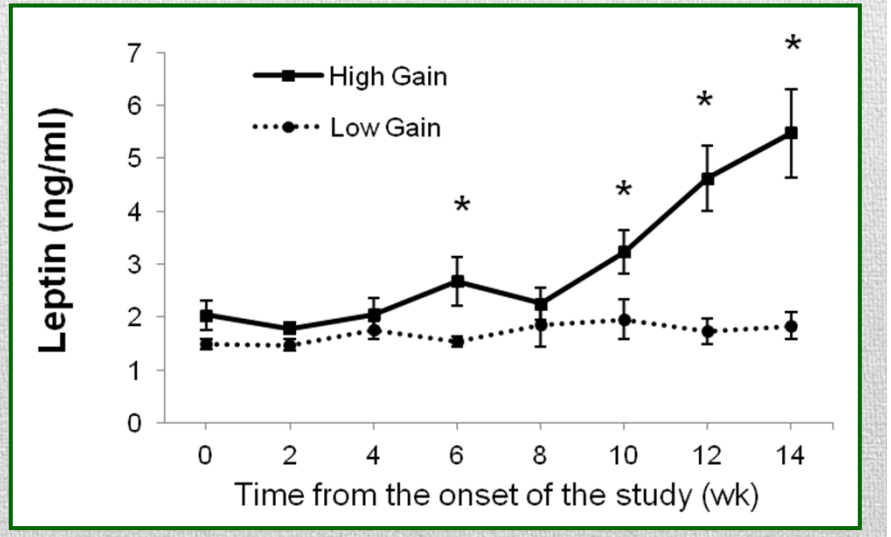


Barb et al., Reprod. 2006

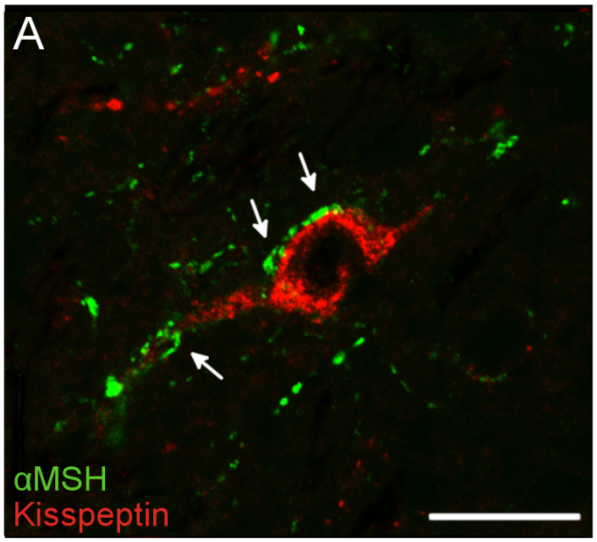
Nutrition and neuroendocrine function of heifers

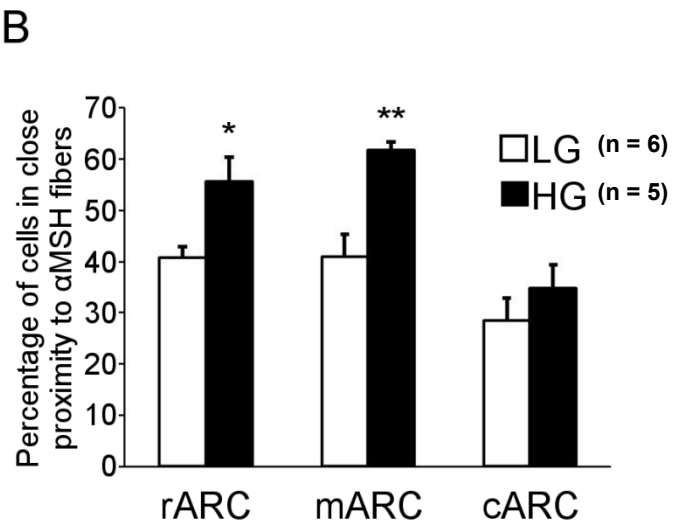
- **Early weaned heifers**
- **Diet: high concentrate**
  - **Low gain (n=12): 0.5 kg/d**
  - **High gain (n=12): 1.0 kg/d**
- **Slaughtered at 9 mo of age**

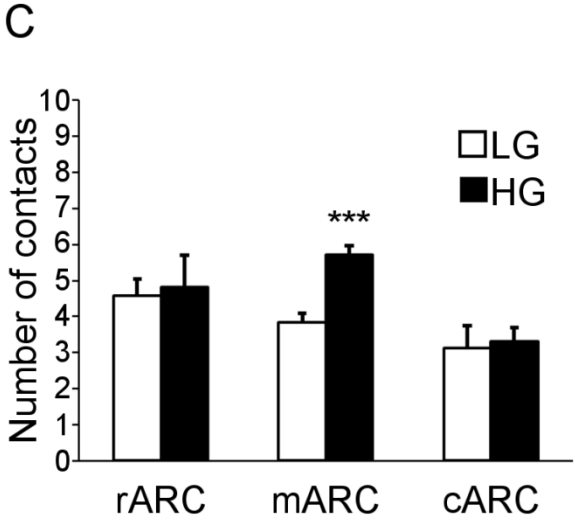


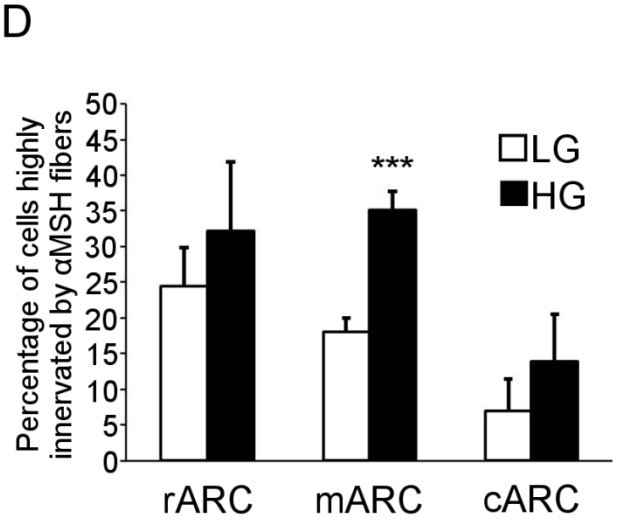


Nutrition and neuroendocrine function of heifers







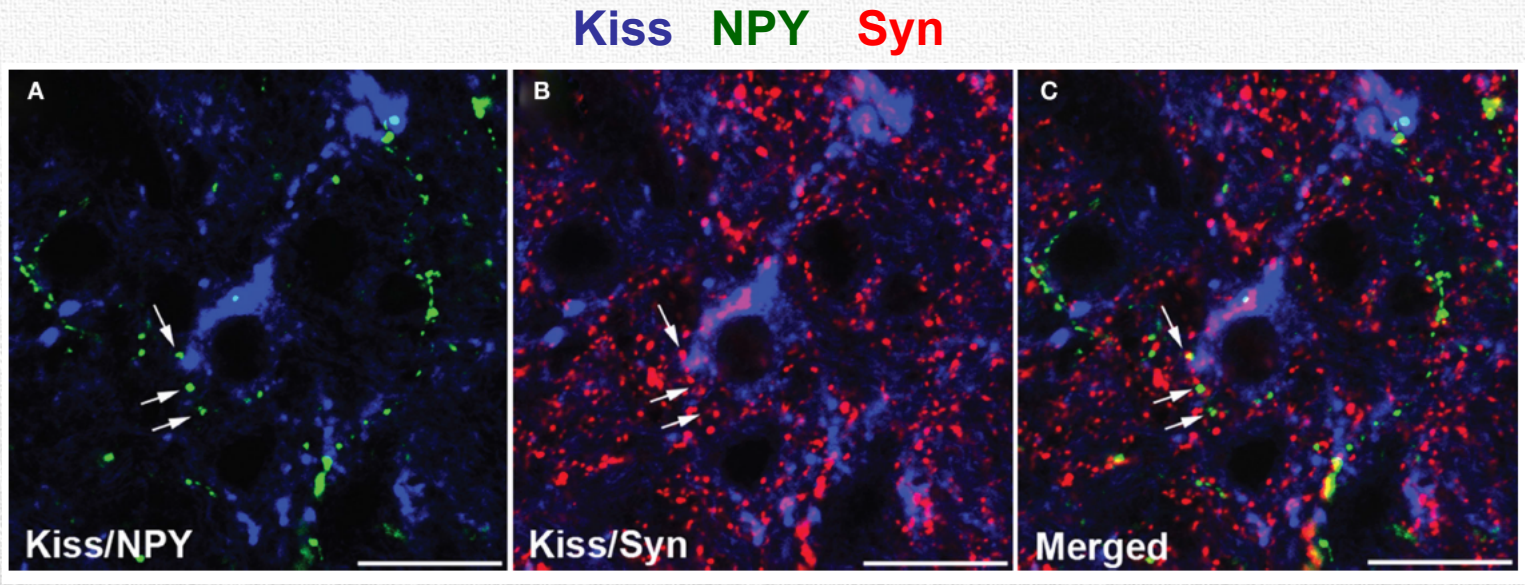


***P* ≤ 0.05

****P* ≤ 0.01

*****P* ≤ 0.001

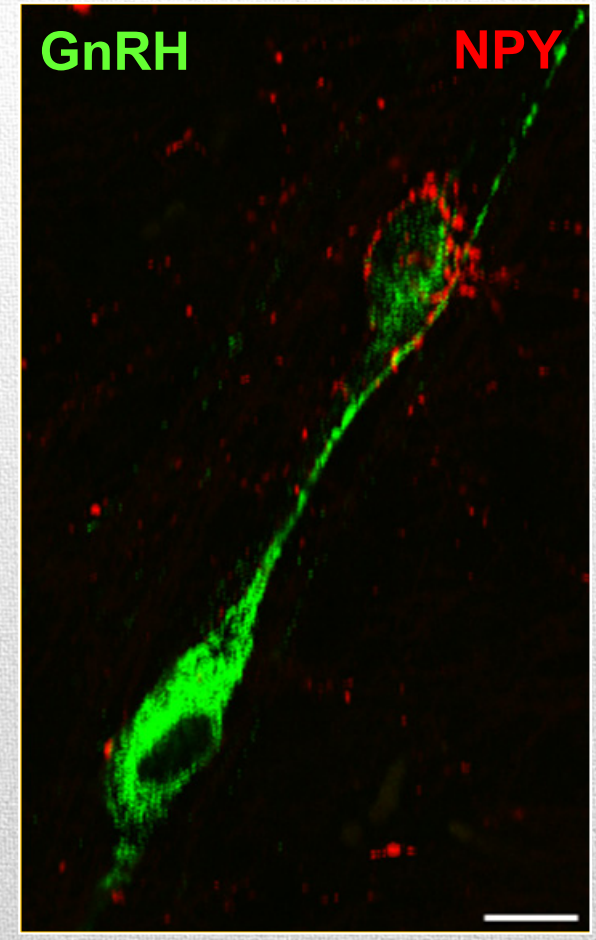
Nutrition and neuroendocrine function of heifers

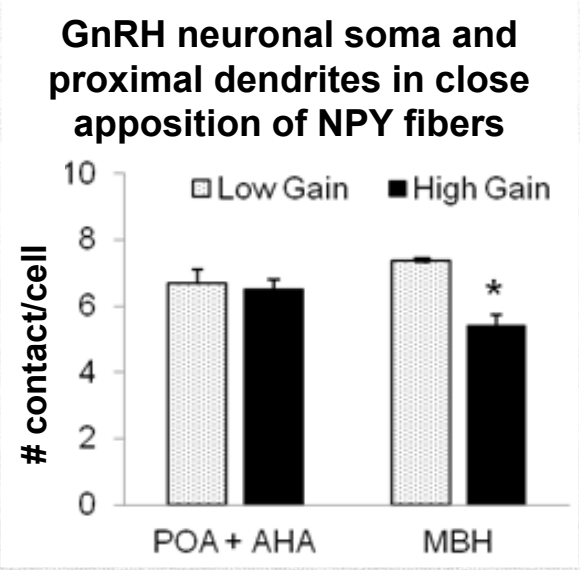


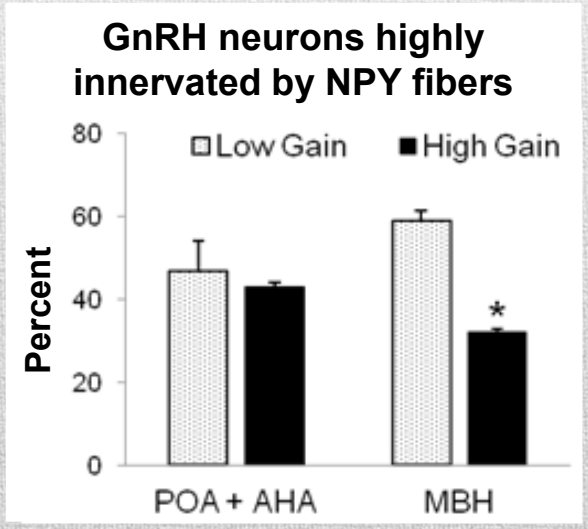
- NPY axons contacting Kiss cells are evident
- Much fewer than NPY to GnRH contacts

Amstalden et al. 2014, Front. Endocrinol.

Nutrition and neuroendocrine function of heifers

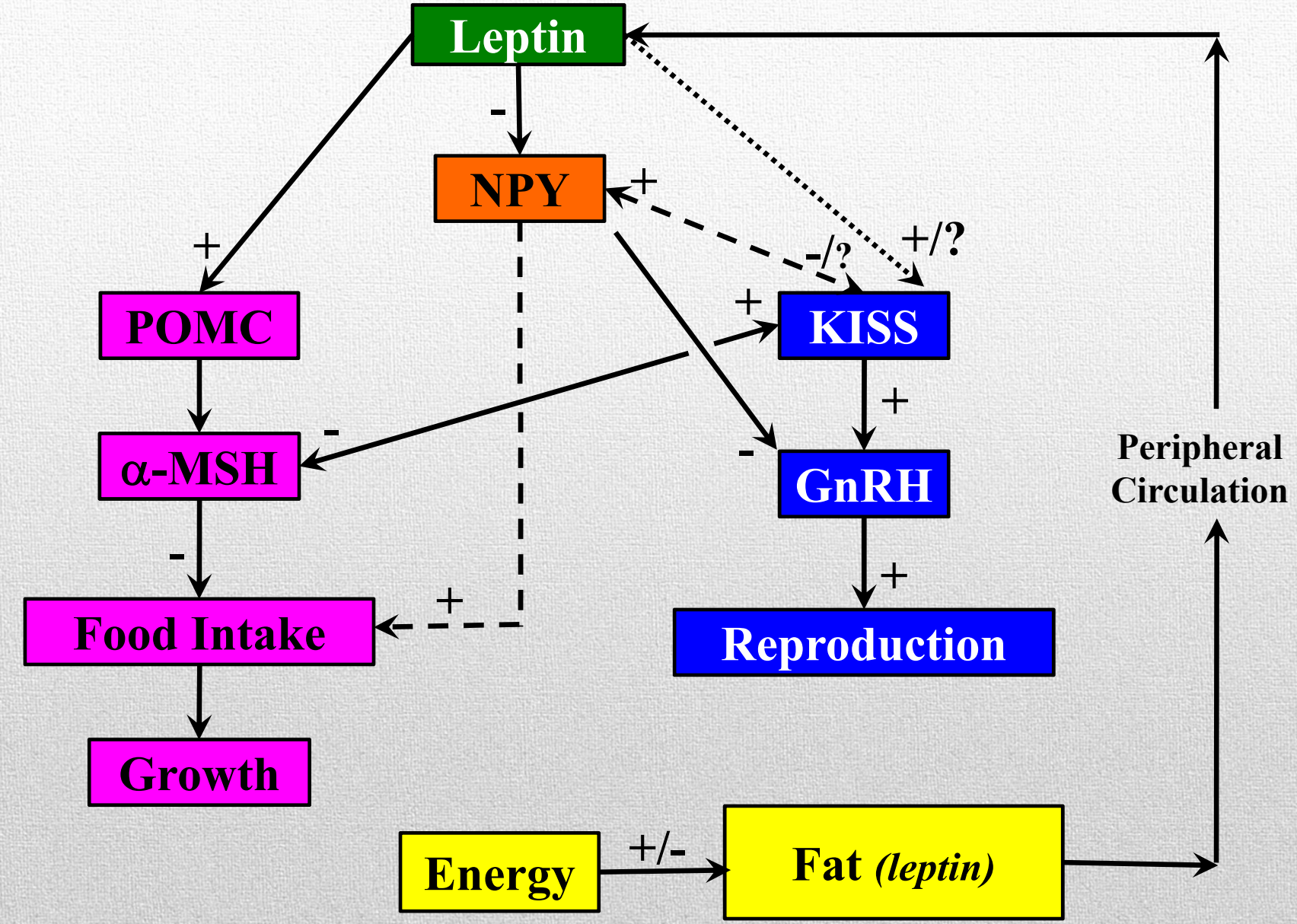






Alves et al. 2014

Hypothalamus



Adapted from Hausman et al., 2012, Biochimie

Gonadotropin-inhibitory hormone

- Gonadotropin-inhibitory hormone (GnIH)
  - Hypothalamic neuropeptide first identified in Japanese quail
  - Inhibits the gonadotropic axis of birds
- Suppresses gonadal development and sexual behavior
- GnIH receptor expression in the pituitary of chicken decreases with sexual maturation
- Seasonal control of reproductive function

Mammalian GnIH homolog; RFamide-related peptides

- Gonadotropin-inhibitory hormone (GnIH)
  - 3 peptides in birds
    - GnIH-RP-1, GnIH, GnIH-RP-2
- RFamide-related peptide (RFRP)
  - Mammalian homolog of GnIH
  - 2 peptides; RFRP-1 and RFRP-3
- Functions in mammals
  - Nociception, stress, prolactin secretion, energy balance, and reproduction

Prepro-proteins encoding RFamide-related peptides

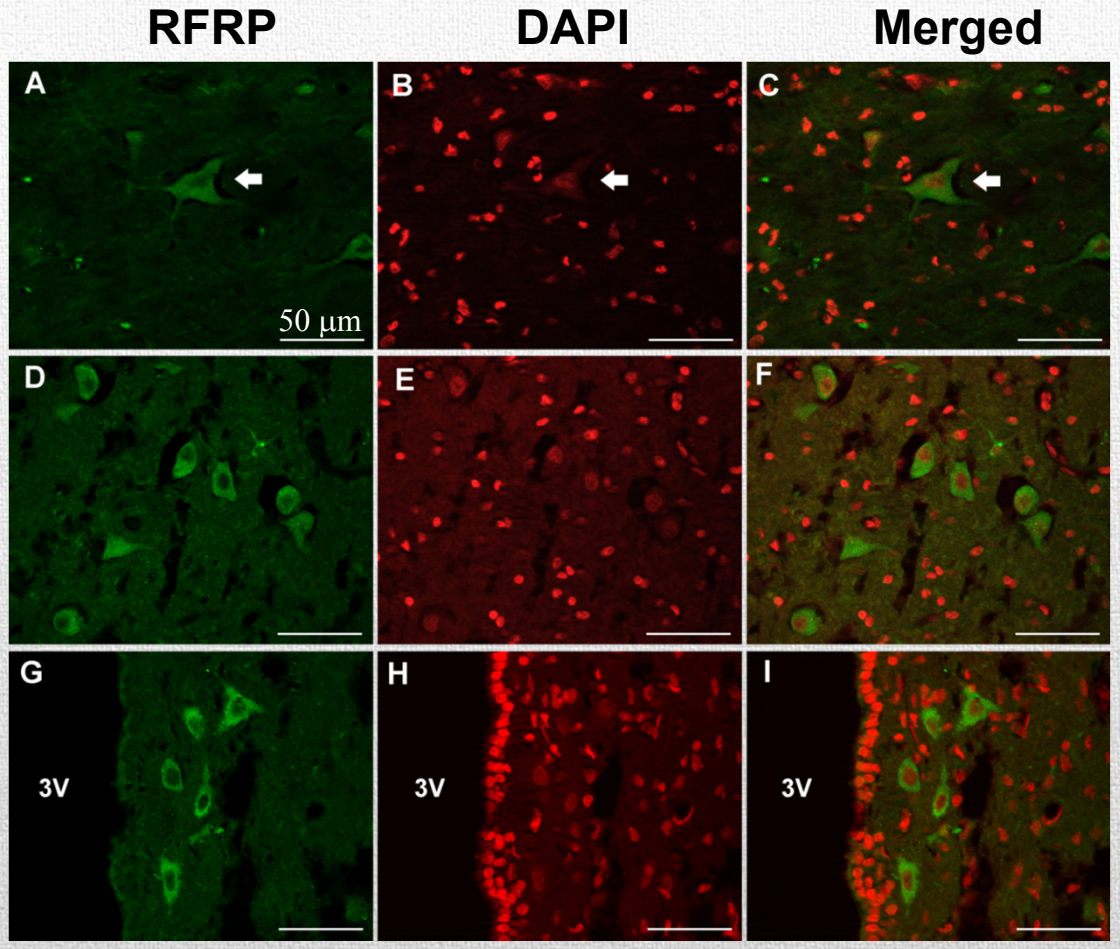
```
Sheep   MEIISLKRFILLMLATSSLLTSNIFCTDESRIPSLYSKKNYDKYSEPRGDLGWEKERSLT   60
Cow     MEIISLKRFILLMLATSSLLTSNIFCTDESRMPNLYSKKNYDKYSEPRGDLGWEKERSLT   60
Pig     ------------------LTSNIFCTDELVLSSLHSKKNYDTYSEPRRDSKWEKQRSLN   41
Human   MEIISSKLFILLTLATSSLLTSNIFCADELVMSNLHSKENYDKYSEPRGYPKGE--RSLN   58
Rat     MEIISSKRFILLTLATSSFLTSNTLCSDELMMPHFHSKEGYGKYYQLRGIPKGVKERSVT   60
                  * ***** ****  * **       **  *  *   *         **

Sheep   FEEVKDWGPK--IKMNTPAVNKMPPSAANLPLRFGRNMEEERSTRVMAHLPLRLGKNRED  118
Cow     FEEVKDWAPK--IKMNKPVVNKMPPSAANLPLRFGRNMEEERSTRAMAHLPLRLGKNRED  118
Pig     FEELKDWGPKNVIKMSTPVVNKMPPSAANLPLRFGRTKEEESSPGATAGLPLRFGRNTED  118
Human   FEELKDWGPKNVIKMSTPAVNKMPHSFANLPLRFGRNVQEERSAGATANLPLRSGRNMEV  120
Rat     FQELKDWGAKKDIKMSPAPANKVPHSAANLPLRFGRNIEDRRSPRARA--------NMEA  112
        * * ***  *  ***     ** * * *********      *    *         * *

Sheep   SLSRRVPNLPQRFGRTIAAKSITKTLSNLLQQSMHSPSTNGLLYSMTCRPQEIQNPGQKN  180
Cow     SLSRWVPNLPQRFGRTTTAKSITKTLSNLLQQSMHSPSTNGLLYSMACQPQEIQNPGQKN  180
Pig     SMSRPVPNLPQRFGRTI-ARSITKALGALLQQSTRSPSAKGLLYSITR-----------  165
Human   SLVRRVPNLPQRFGRTTTAKSVCRMLSDLCQGSMHSPCANDLFYSMTCQHQEIQNPDQKQ  180
Rat     GTMSHFPSLPQRFGRTT-ARRITKTLAGLPQKSLHSLASSELLYAMTRQHQEIQSPGQEQ  171
              * ********  *      *  * * *  *     * *      ****
```

RFRP-1	* = amino acids completely conserved
RFRP-2	Blue letters = basic residues (potential for cleavage)
RFRP-3	Red letters = cleavage site with amide-donor

RFRP immunoreactivity in the hypothalamus: prepubertal gilt



3V = third ventricle

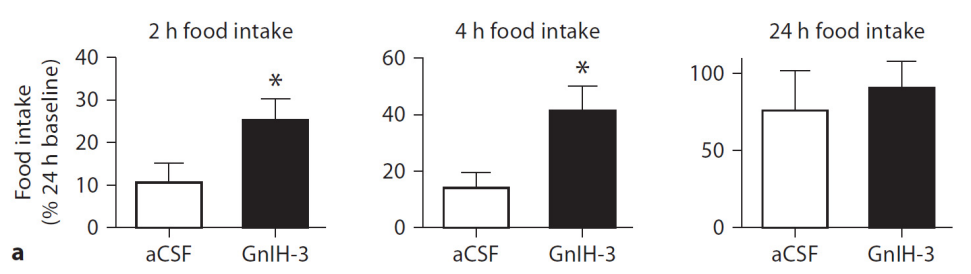
Mammalian GnIH homolog; RFamide-related peptides

- **RFRP Immunoreactive fibers in the ewe**
  - **Neurosecretory zone of the median eminence** Clarke et al., 2006
    - **Potential to regulate pituitary function**
  - **Preoptic area, septum, and diagonal band of Broca** Smith et al., 2008
    - **Potential to regulate GnRH secretion**

Projections of RFRP neurons to appetite regulating cells in the ovine hypothalamus

Cell type	Region	% contacts
POMC	Arcuate nucleus	60
NPY	Arcuate nucleus	44
Orexin	LHA	21
Orexin	DMH	61
MCH	LHA	22
CRH	PVN	33
Oxytocin	PVN	29
GnRH	Preoptic area	63

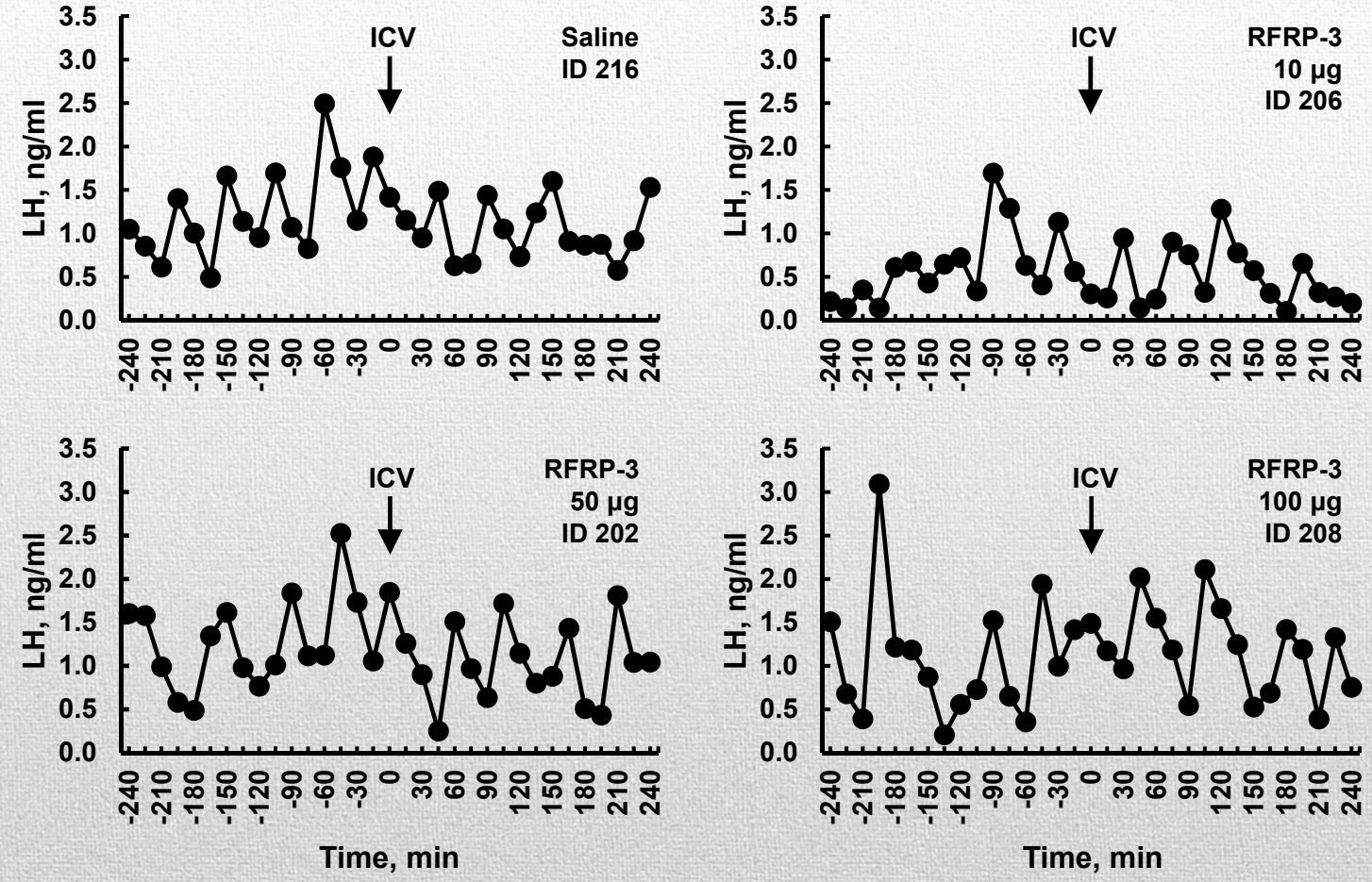
Clarke, 2014



Clarke et al., 2012

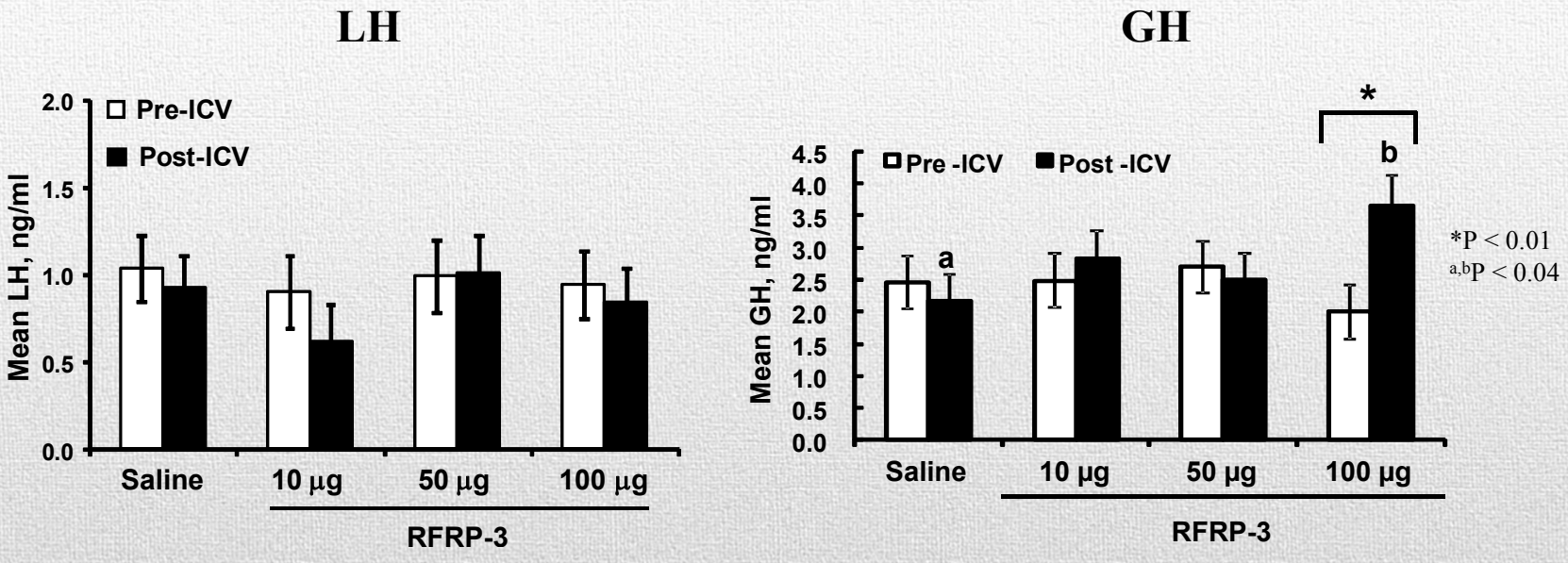
- **Implicates RFRP in the nutritional regulation of reproduction.**

ICV injection of RFRP3 to pre-pubertal ovariectomized gilts



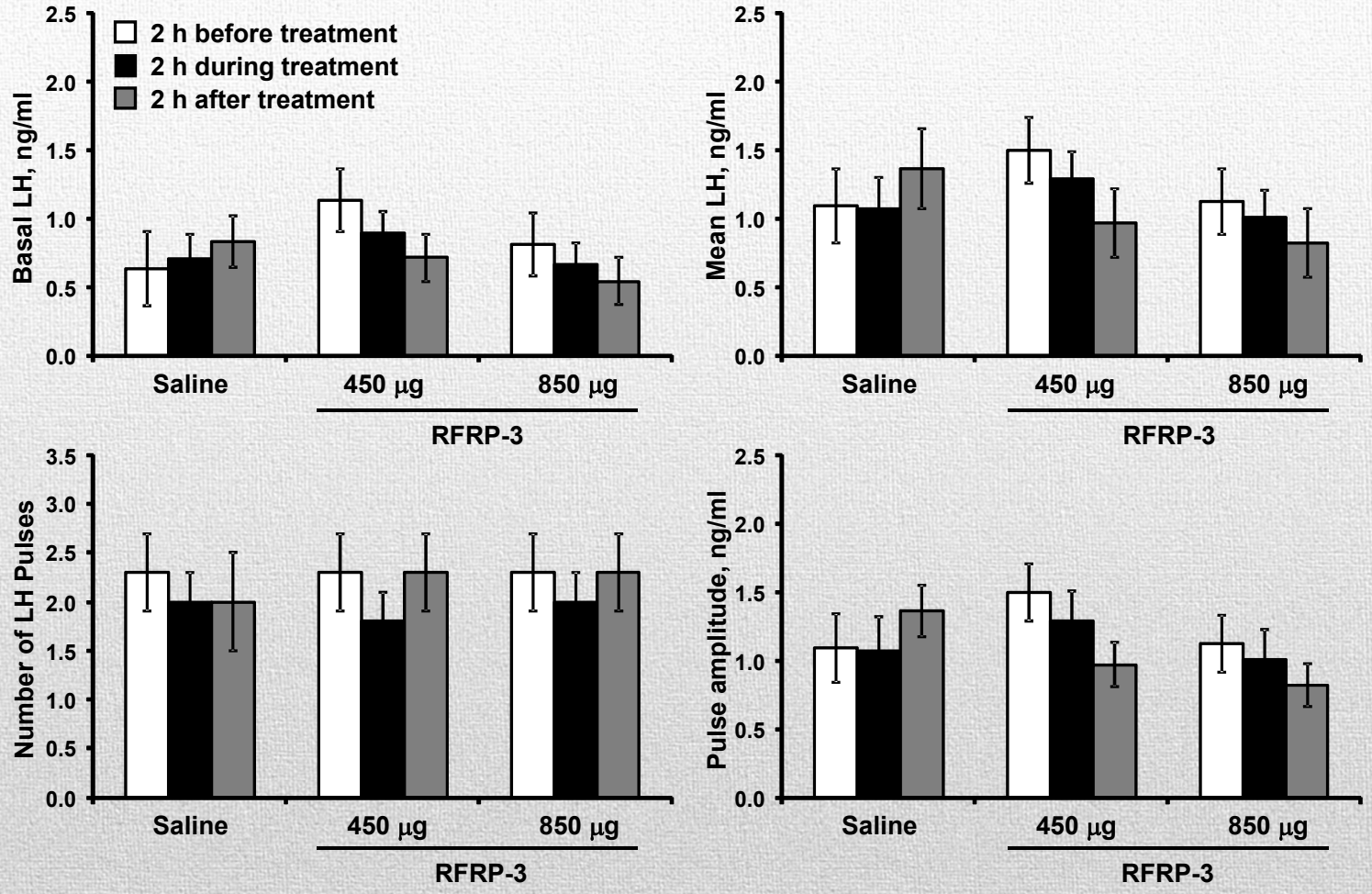
Heidorn et al., Nat. ASAS 2010

Central (ICV) injection of RFRP3 to pre-pubertal ovariectomized gilts



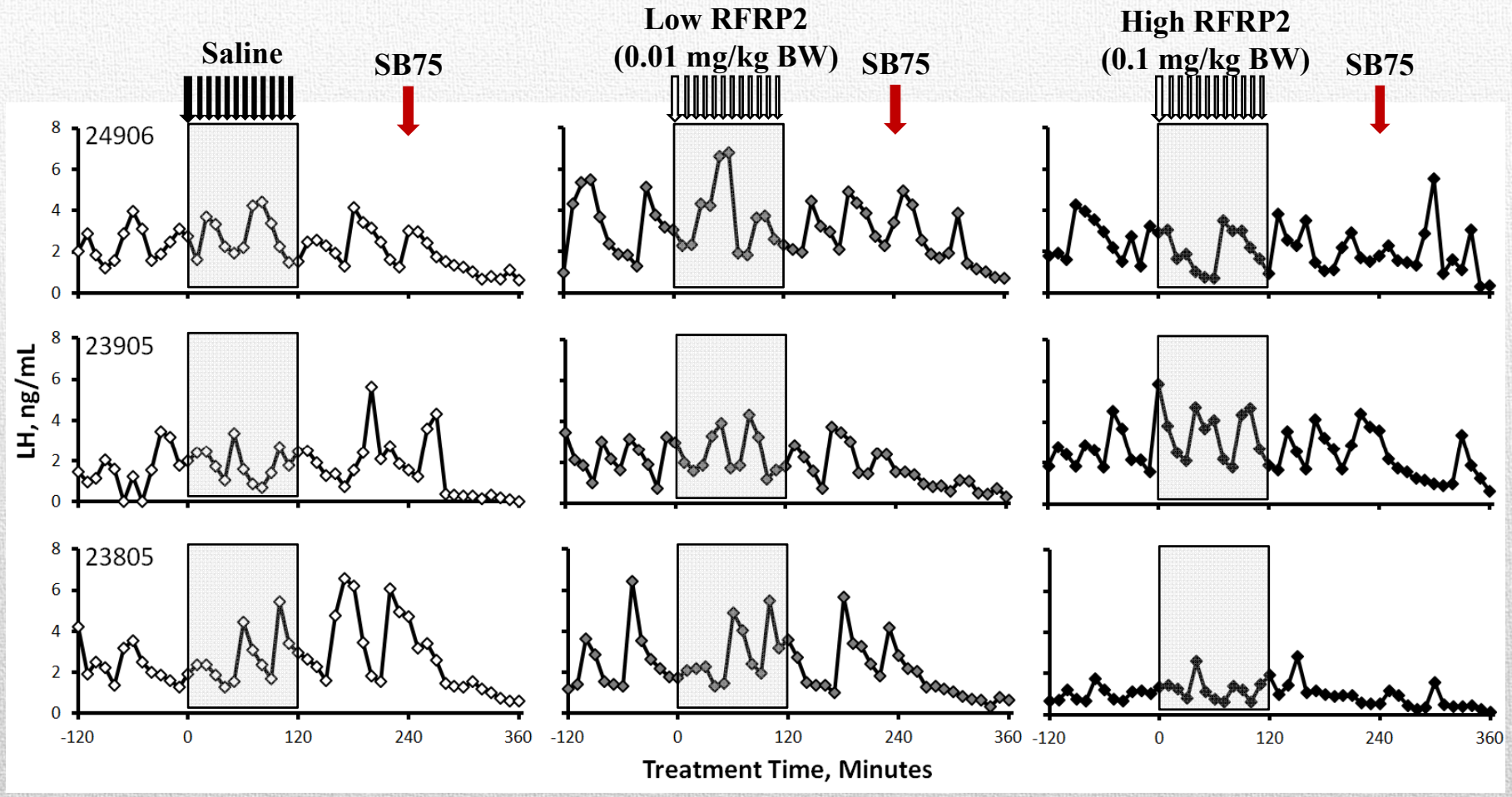
Heidorn et al., Nat. ASAS 2010

Peripheral (IV) injection of RFRP3 to pre-pubertal ovariectomized gilts



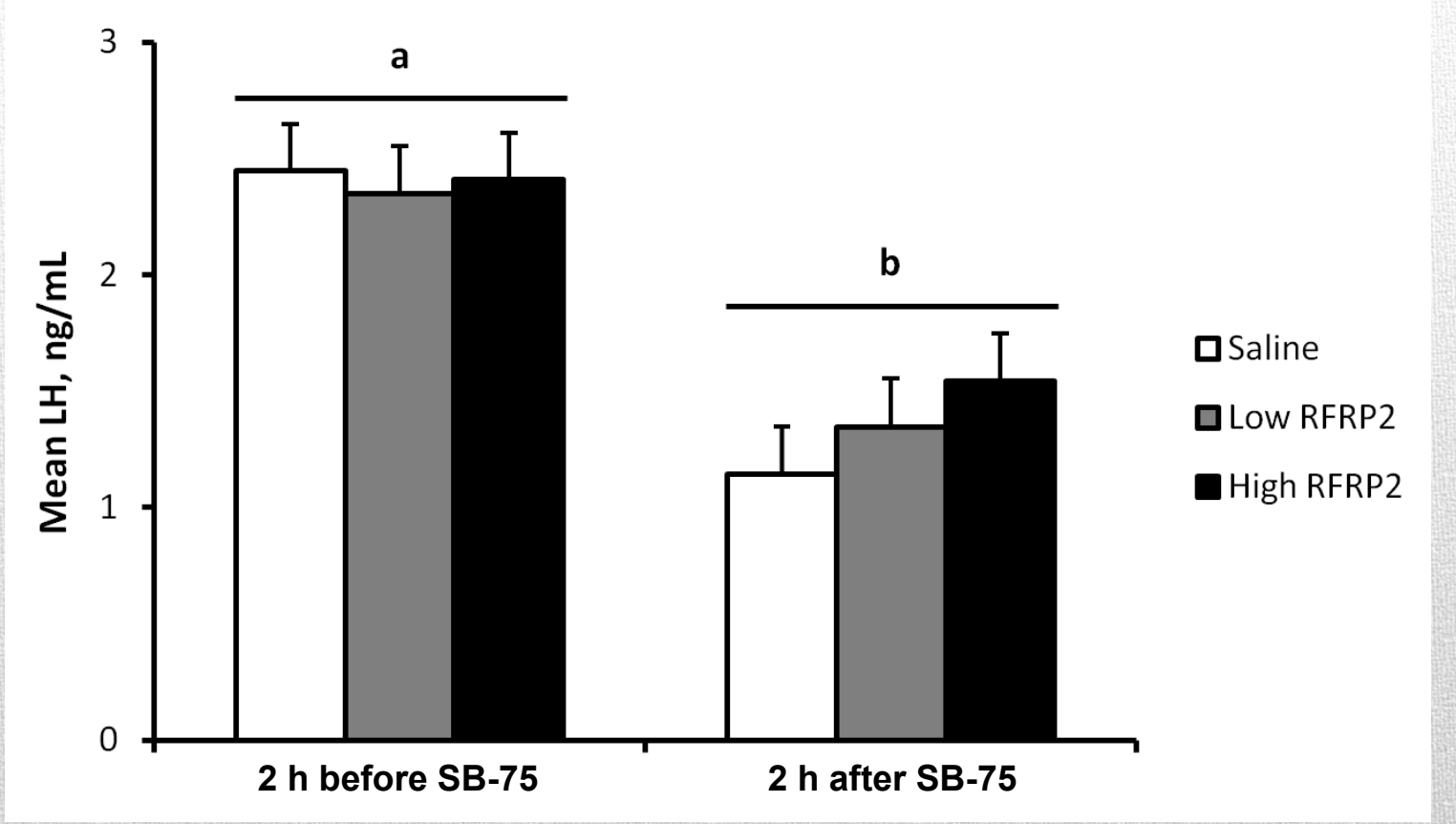
Heidorn et al., Nat. ASAS 2010

RFRP-2 does not affect LH secretion in pre-pubertal ovariectomized gilts

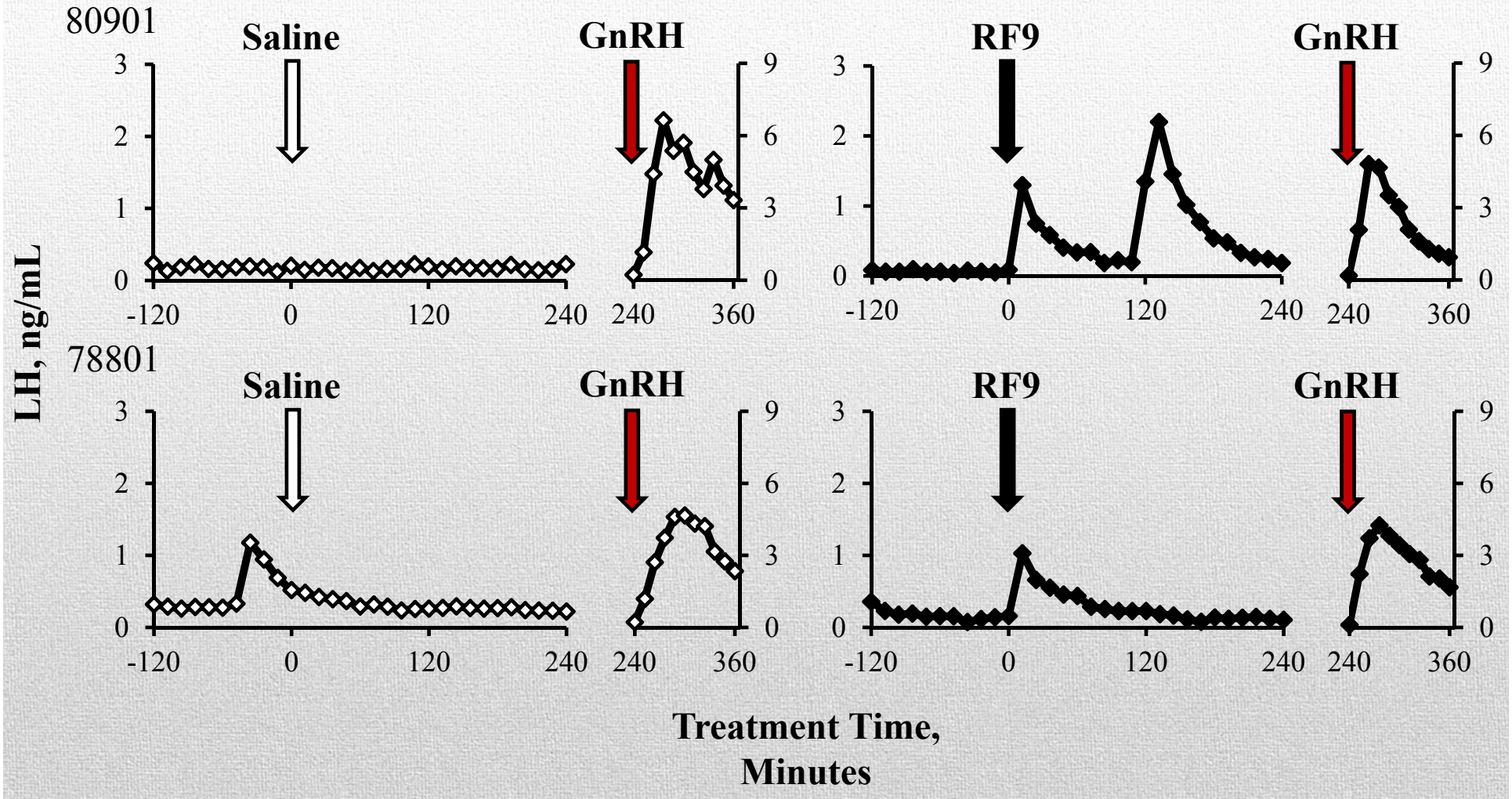


Thorson et al., Endo 2014

GnRHR antagonist suppressed LH secretion in pre-pubertal ovariectomized gilts



Intravenous Injection of RF9 & GnRH
Pre-pubertal Ovary Intact Gilts



0.5 mg RF9/kg BW; 100 µg GnRH

GWAS for Age at Puberty in Gilts

224 5-SNP windows accounting for 59.3% of genetic variance
15 previously identified with puberty failure in gilts (Nonneman et al., 2013)
7 in common with Tart et al., 2013 for age at puberty in gilts
6 in common with GWAS for age at menarche in humans.

	SNP Window, bp							
Chr	Start	End	Rank	Genetic variance %	Bootstrap P-value	Candidate gene	Function	Reference
12	14,910,939	15,006,310	1	9.7332	0.001	GH1	Somatic growth	
7	75,121,635	75,460,294	2	7.1362	0.003	PRKD1	BMI Puberty, swine	Graff et al., Hum Mol Gen. 2013 Nonneman et al., Anim. Gen. 2013
9	142,232,369	142,338,853	4	1.4283	0.004	SMYD2	Puberty, swine	Tart et al., Anim. Gen. 2013
1	121,626,075	121,978,687	21	0.4196	0.006	RORA	Menarche	Demerath et al., Hum. Mol. Gen. 2013
17	64,555,868	64,680,839	46	0.2313	0.012	BMP7	Ovarian function	
3	21,596,232	22,794,763	70	0.1765	0.006	PAQP8	Puberty, swine	Nonneman et al., Anim. Gen. 2013
15	65,197,162	65,381,481	80	0.1593	0.021	HS6ST1	IHH	Tornberg et al, PNAS 2011
2	25,692,286	25,797,751	98	0.1280	0.053	PIK3R1	Menarche	Demerath et al., 2013
1	183,533,664	183,678,408	176	0.0849	0.058	IQCH	Menarche	Elks et al., Nat. Gen. 2010
1	151,203,878	153,593,790	185	0.0828	0.044	IGF1R	Puberty, cattle	Fortes et al., Anim. Gen. 2013
2	58,719,232	59,243,471	193	0.0806	0.030	CRTC1	Menarche Puberty, pigs	Elks et al., Nat Gen 2010 Tart et al., Anim. Gen. 2013
10	9,289,183	9,488,500	197	0.0785	0.025	ESRRG	Puberty, cattle	Fortes et al., Anim. Gen. 2013
8	71,847,930	72,567,179	203	0.0769	0.029	NPFFR2	GnIH receptor	
5	78,429,610	78,537,181	208	0.0759	0.035	NELL2	Glutamatergic neurons	Ha et al. Neuroendo 2008

Output from GENSEL (http://bigs.ansci.iastate.edu)

- Neuroanatomical and in vitro data support a role for RFRP-3 in suppressing activity of the gonadotropic axis of mammals.
  - Both centrally and at the pituitary gland

- Effects of RFRP-3 on LH secretion in vivo have been inconsistent (rodents, sheep, cattle, pigs, horses).
  - Suppressive effect
  - No effect
  - Stimulatory effect

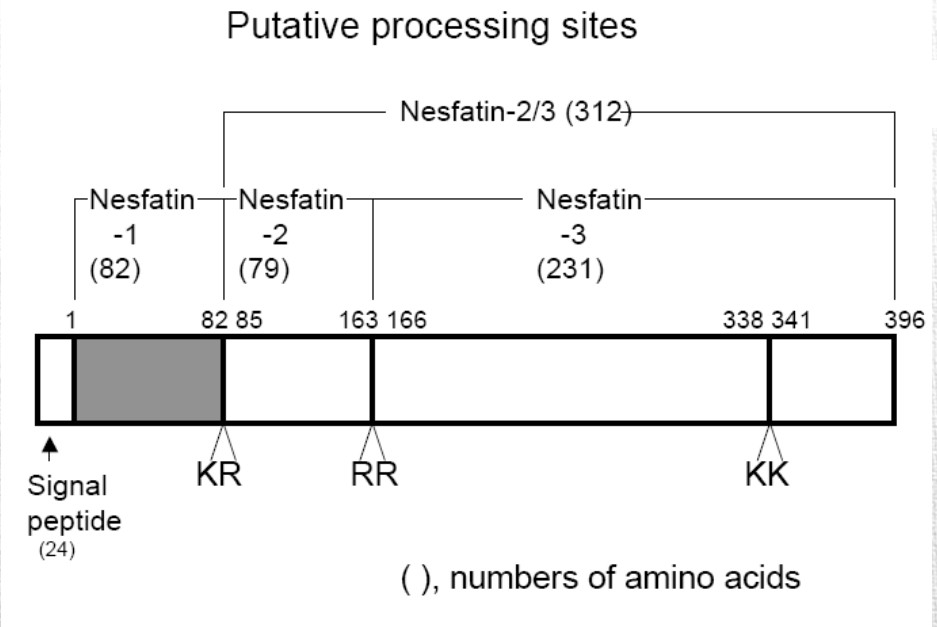
- The LH stimulatory action of NPFFR2 antagonist and the presence of NPFFR2 was candidate gene in GWAS are suggestive of a role of RFRP peptides in onset of puberty in the gilt.

Nesfatin-1: Novel adipokine linking fat and reproduction

Nesfatin-1 is a product of the NUCB2 gene

Expressed (gene & protein) in adipose tissue and hypothalamus
- negative energy balance

Produced and secreted by adipose tissue and 3T3L1 cells
- $PPAR_{\gamma}$ stimulated gene
- crosses blood-brain-barrier
- neg. correlated to BMI



Suppresses food intake and BW in the rat

ICV injection of antisense-morpholino oligonucleotides suppressed LH secretion and delayed puberty in female rats

Genomic association of NUCB2 with production traits

No association of NUCB2 gene with adiposity traits of pigs (barrows and gilts; n = 1,145) at 22 wk of age.

BW and ADG of pigs at 22 wk of age were modestly associated with SNP in the porcine NUCB2 gene.

Positive relationship of BW and body fatness with age a puberty in gilts.

Table 4
Association of SNPs in the porcine nucleobindin 2 gene with age (n = 740) and BW (n = 439) at puberty in Yorkshire-Landrace-Duroc gilts.

Trait[a]	Marker	Positive allele	Negative allele	Estimated effect	*P* value
Pubertal age (d)	57807_199	A	G	0.2857	0.67
	57813_217	C	T	0.1347	0.79
	57813_91	C	T	0.1696	0.70
Pubertal BW (kg)	57807_199	A	G	0.3062	0.73
	57813_217	C	T	1.7441	0.01
	57813_91	T	C	1.4070	0.02

[a] Mean ± SD pubertal age = 196.7 ± 0.6 d; mean ± SD pubertal BW = 128.4 ± 0.9 kg.

Lents et al., 2013 Dom. Anim. Endocrinol.

cDNA and predicted peptide sequence of porcine nesfatin-1 and identity to the human and rat orthologs.

```
  1   M  K  W  R  T  I  L  L  Q  Y  W  F  F  L  V  T  Y  V  F  T
  1   ATGAAGTGGAGGACCATCCTTCTACAATATTGGTTTTTCTTGGTTACATATGTATTTACT

 21   A  L  E  A  V  P  I  D  I  D  K  T  K  V  K  N  T  Q  P  V
 61   GCTCTTGAAGCTGTGCCTATAGACATAGACAAAACAAAAGTAAAAAATACTCAGCCTGTG

 41   D  S  A  K  I  E  P  P  D  T  G  L  Y  Y  D  E  Y  L  K  Q
121   GACAGTGCGAAGATAGAACCACCAGATACTGGACTTTATTATGATGAATACCTCAAGCAA

 61   V  I  D  V  L  E  T  D  N  H  F  R  E  K  L  Q  K  A  D  I
181   GTGATTGATGTGCTGGAAACAGATAATCATTTCAGAGAAAAACTACAGAAAGCAGACATA

 81   E  E  I  K  S  G  R  L  S  R  E  L  D  L  V  S  H  H  V  R
241   GAGGAAATAAAGAGTGGAAGATTAAGCAGAGAGCTTGATTTAGTAAGTCACCATGTGAGG

101   T  K  L  D  E  L  K  R  Q  E  V  A  R  L  R
301   ACAAAACTTGATGAACTGAAAAGGCAAGAAGTGGCAAGGTTACGAA
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Human     1   VPIDIDKTKVQNIHPVESAKIEPPDTGLYYDEYLKQVIDVLETDKHFREKLQKADIEEIKSGRLSKELDLVSHHVRTKLDEL  82
Porcine   1   VPIDIDKTKVKNTQPVDSAKIEPPDTGLYYDEYLKQVIDVLETDNHFREKLQKADIEEIKSGRLSRELDLVSHHVRTKLDEL  82
rat       1   VPIDVDKTKVHNVEPVESARIEPPDTGLYYDEYLKQVIEVLETDPHFREKLQKADIEEIRSGRLSQELDLVSHKVRTRLDEL  82
              .... ..... .  .. .. ................... ..... ............. ..... ....... ..  ....
```
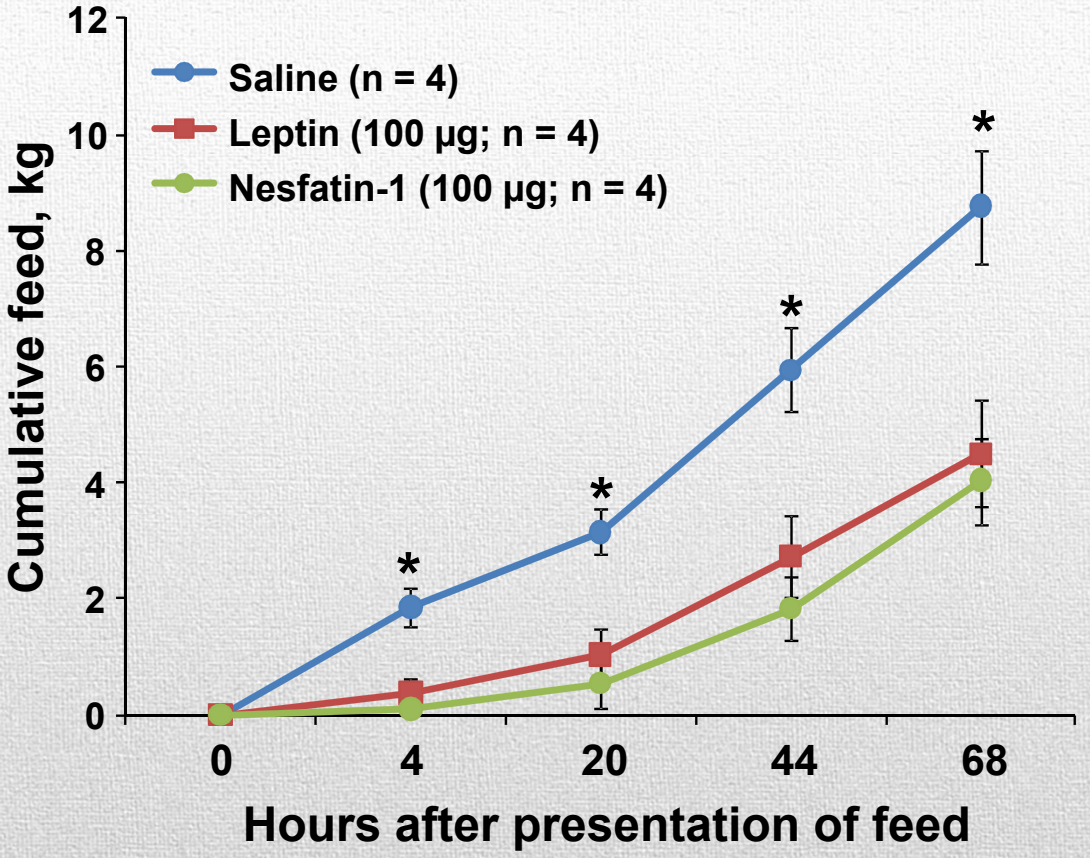

Identity: Human = 92% Rat = 85%

Lents et al., Dom. Anim. Endocrinol. 2013

ICV injection of Nesfatin-1 suppresses feed intake in prepubertal gilts (treatment x time, $P < 0.001$).

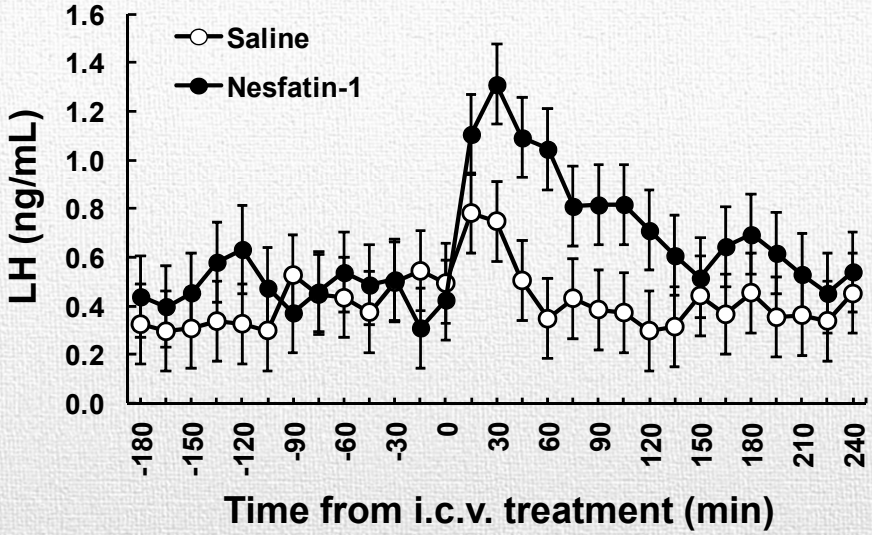


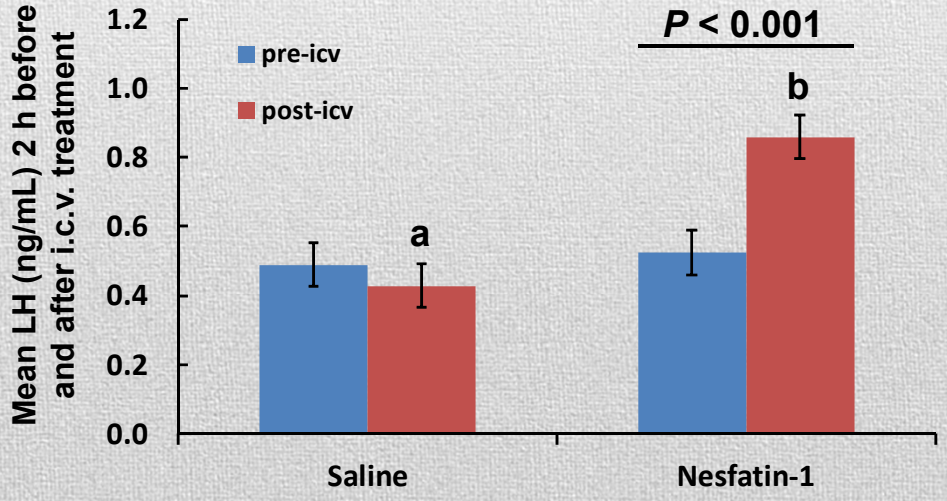
Corresponds to 8, 24, 48, 72 h after ICV treatment.

*Saline different from all other treatments, $P < 0.001$

Lents et al., 2013 Dom. Anim. Endocrinol.

LH in serum of gilts after ICV injection of Nesfatin-1 (treatment x time, $P < 0.01$).





Lents et al., 2013 Dom. Anim. Endocrinol.

Summary

Nesfatin-1 identity highly conserved in the pig.

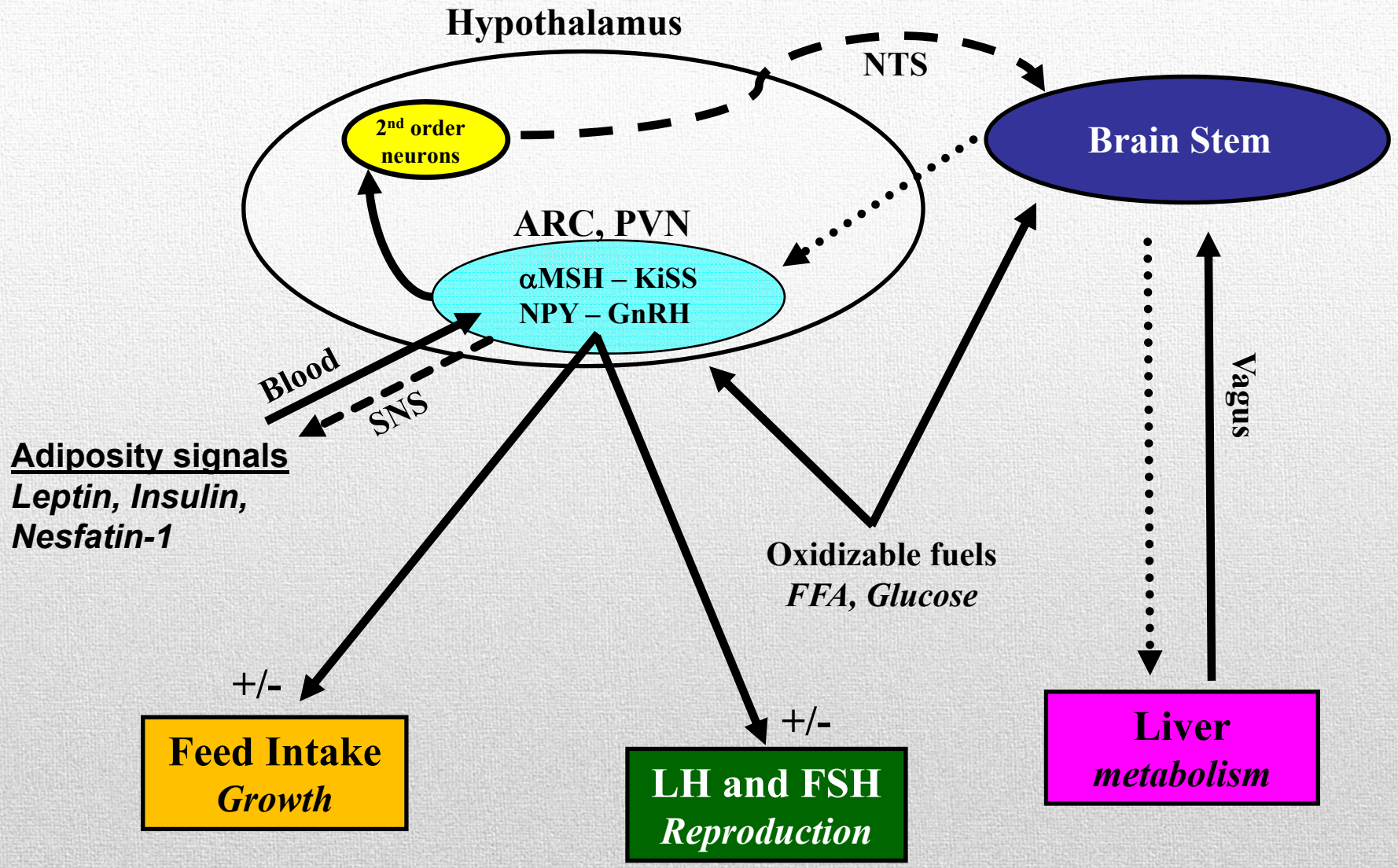
Expression of Nesfatin in pigs:

- **Adipose tissue, depot dependent** (Lents et al., 2013).
- **Hypothalamus and brain stem** (Gaige et al., 2013).

ICV injection of nesfatin-1 in prepubertal gilts:

- **Suppressed feed intake**
- **Increased LH secretion**

Functional data supports the genomic association of NUCB2 with BW traits.


Hypothalamus
NTS
2nd order neurons
Brain Stem
ARC, PVN
αMSH – KiSS
NPY – GnRH
Blood
SNS
Vagus
Adiposity signals
Leptin, Insulin,
Nesfatin-1
Oxidizable fuels
FFA, Glucose
+/-
+/-
Feed Intake
Growth
LH and FSH
Reproduction
Liver
metabolism

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Regulation of appetite by neuropeptids

Neuropeptide	Feeding Effect	Reg. by Leptin
* NPY	↑	↓
Orexin	↑	↑ ↓
MCH	↑	↓
Galanin	↑	↓
AGRP	↑	↓
**α-MSH/POMC*	↓	↑ ↓
CRH/Urocortin	↓	↑ ↓
CART	↓	↑
Neurotensin 1	↓	↑