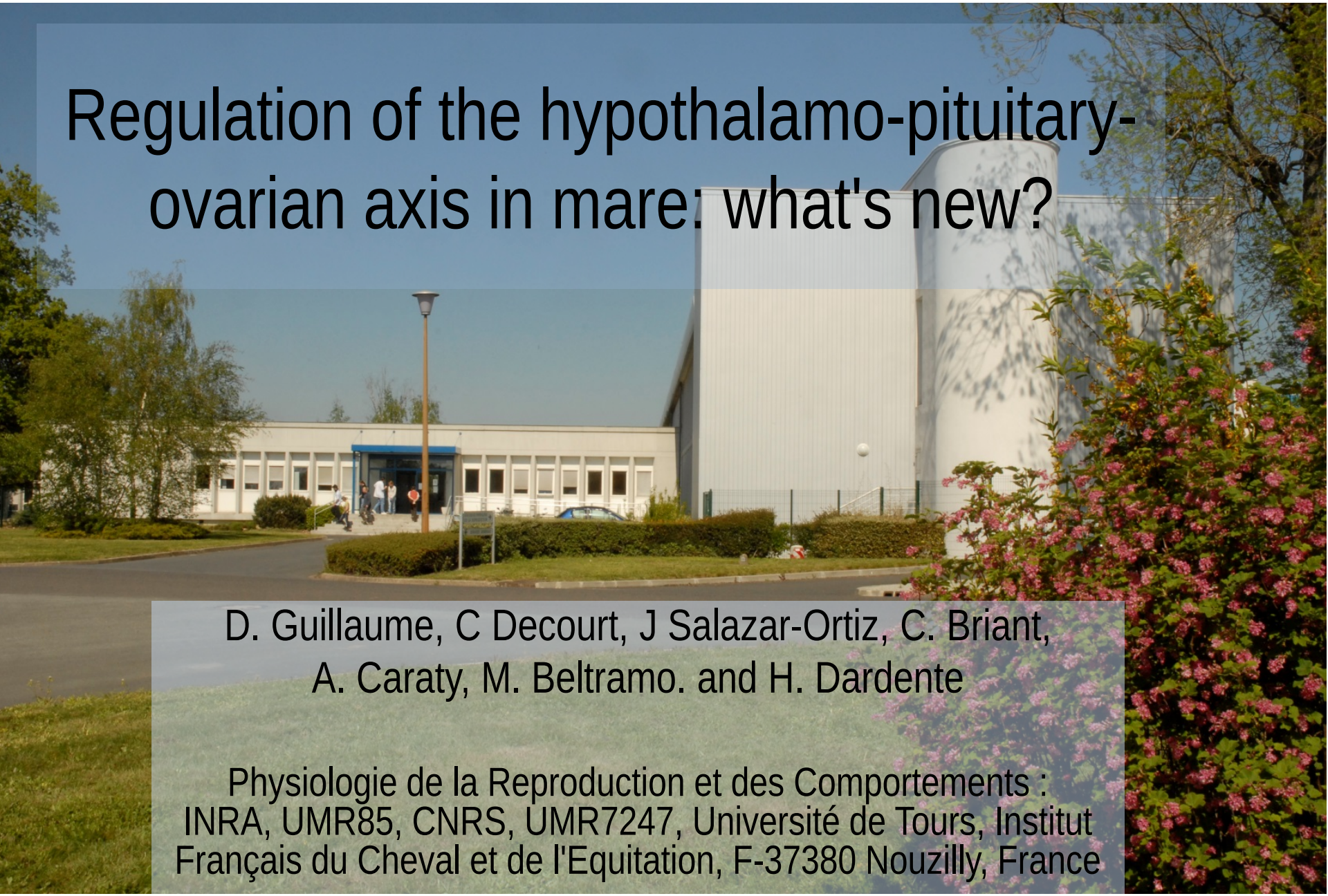
Regulation of the hypothalamo-pituitary-ovarian axis in mare: what's new?



D. Guillaume, C Decourt, J Salazar-Ortiz, C. Briant, A. Caraty, M. Beltramo. and H. Dardente

Physiologie de la Reproduction et des Comportements : INRA, UMR85, CNRS, UMR7247, Université de Tours, Institut Français du Cheval et de l'Equitation, F-37380 Nouzilly, France

Welsh pony mares

Environmental Factors

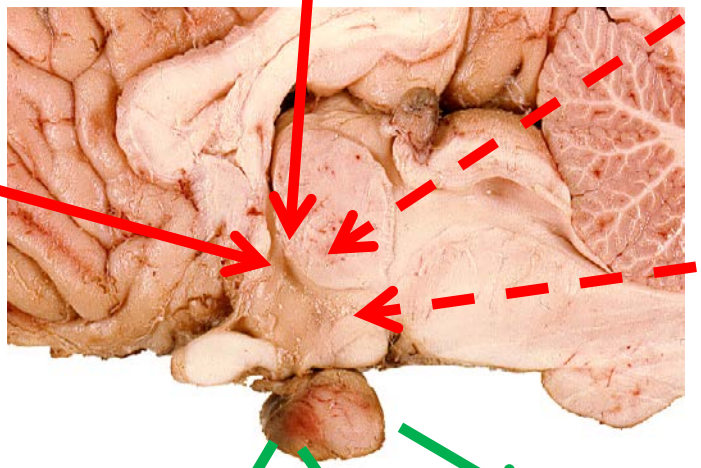
Central control of ovulation

Photoperiod / melatonin

Feed intake / Metabolism / Leptin or other adipokins

Stress / Welfare-Illness

Social relations/ Pheromones



Interacting factors

LH

FSH

Other pituitary hormones

Ovaries

1°) one example of interaction : Photoperiod / Nutrition
2°) hypothetical neuroendocrine mechanism
3°) Where can we pharmacologically act to synchronize ovulation and insemination ?

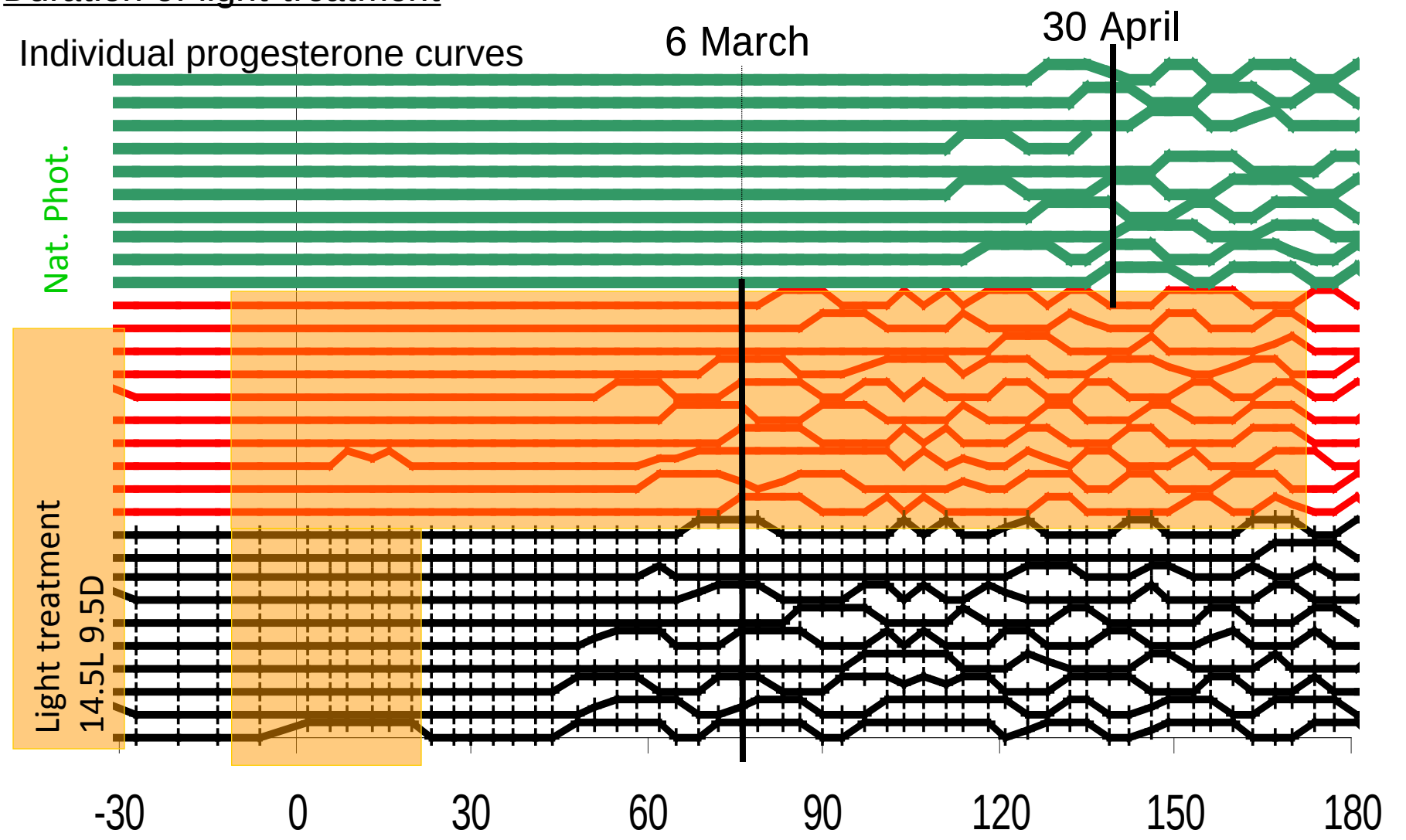
3-1) Gonadotropin level
3-2) GnRH agonist / antagonist
3-3) Kisspeptin Stimulation
3-4) Metabolism level /photostimulation

With focus on results of our research unit:
Physiology of Reproduction and Behaviours

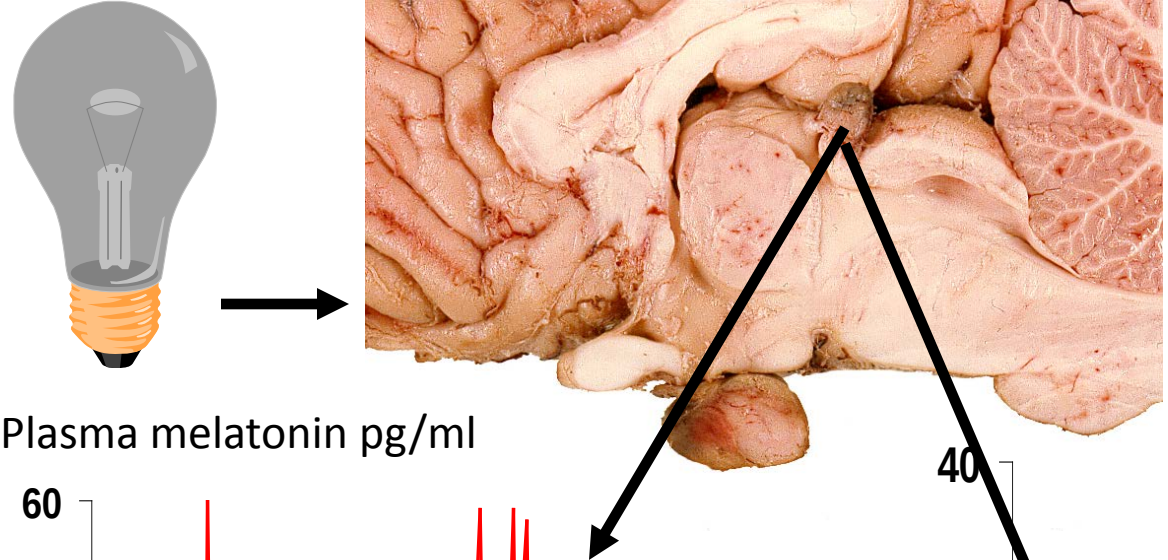
1°) one example of interaction : Photoperiod / Nutrition

Photostimulation and Winter anoestrus

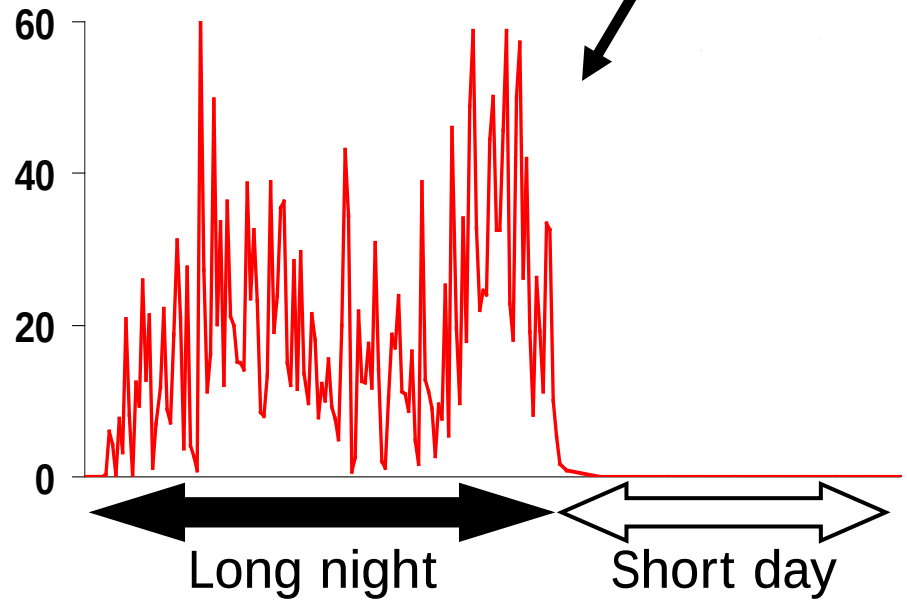
Duration of light treatment

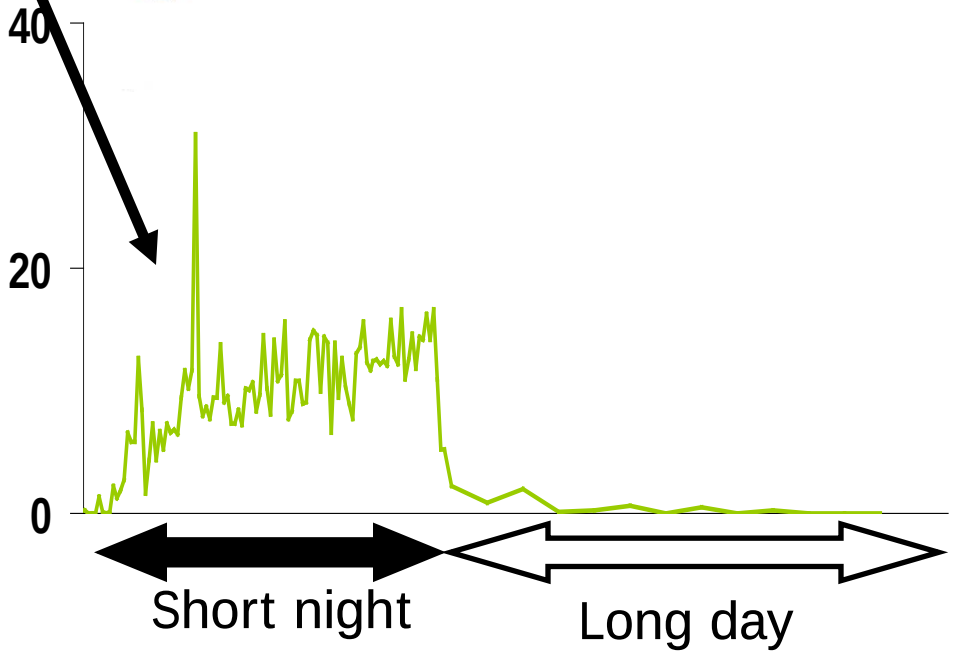


Guillaume et al 2000. J. R. F. supplement 56, 205-216.



Plasma melatonin pg/ml





GUILLAUME *et al*, American J. of Physiology 268; R1236-1241.

Melatonin is the neurohormone which acts on reproduction by its length of secretion.

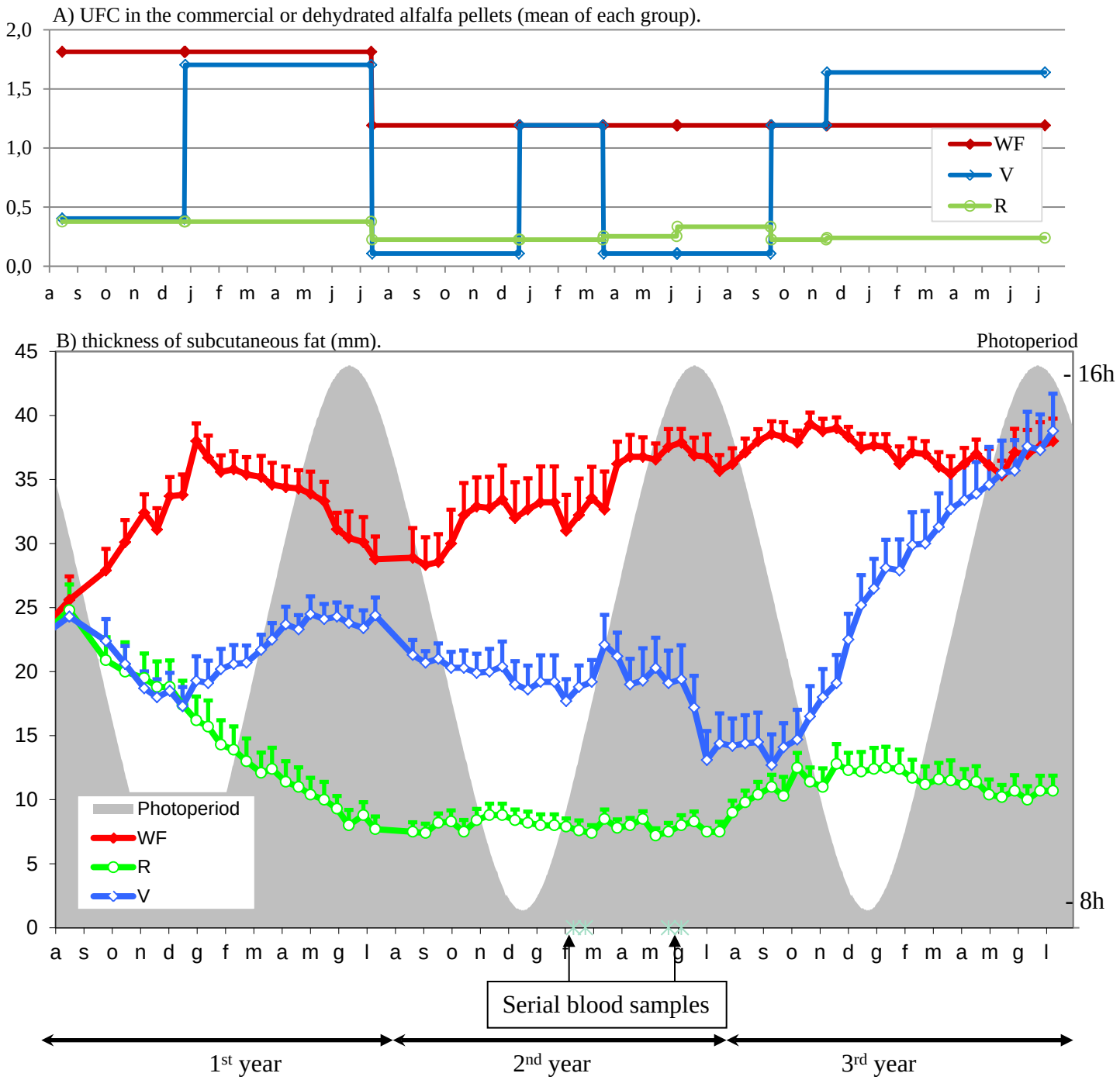
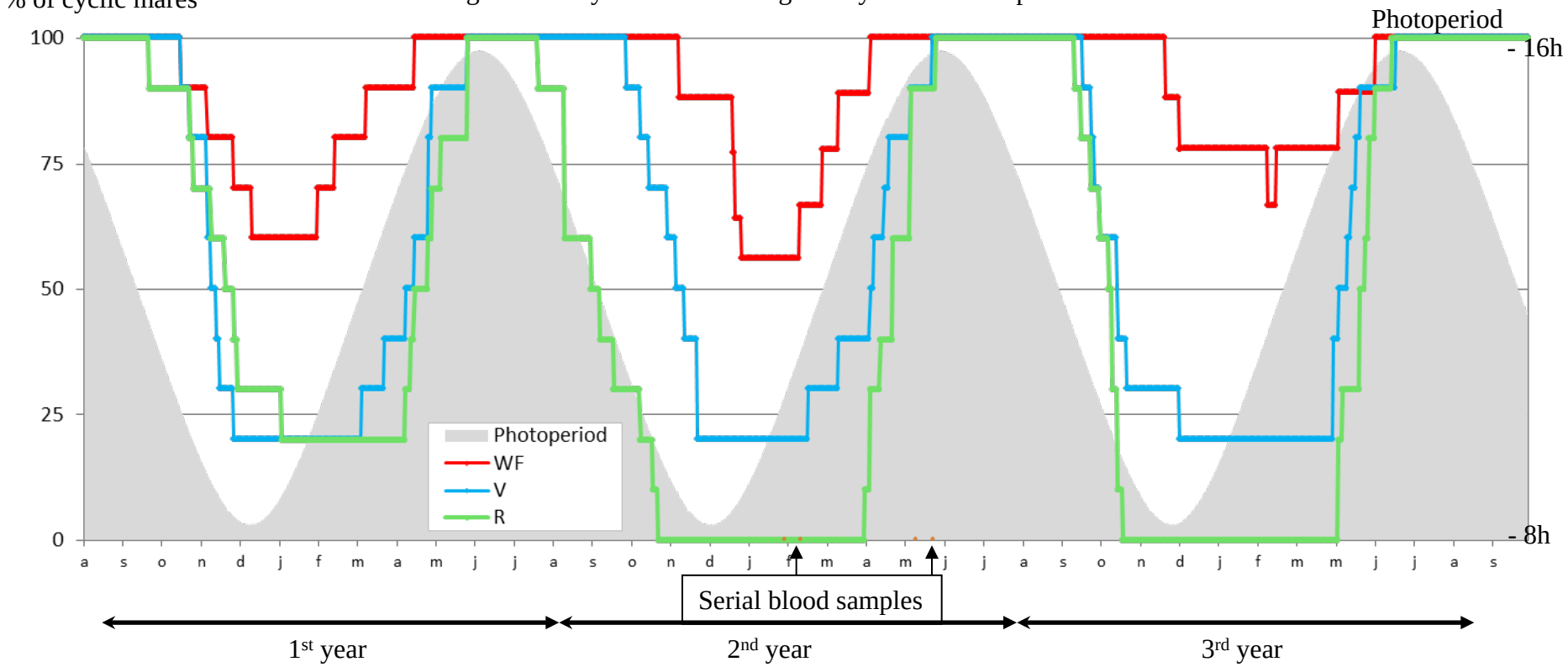

A) UFC in the commercial or dehydrated alfalfa pellets (mean of each group).
2,0
1,5
1,0
0,5
0,0
WF
V
R
a s o n d j f m a m j j a s o n d j f m a m j j a s o n d j f m a m j j
B) thickness of subcutaneous fat (mm).
Photoperiod
16h
8h
45
40
35
30
25
20
15
10
5
0
Photoperiod
WF
R
V
a s o n d g f m a m g l a s o n d g f m a m g l a s o n d g f m a m g l
Serial blood samples
1st year
2nd year
3rd year

Fig. 2: % of cyclic mares during the 3 years of the experiment



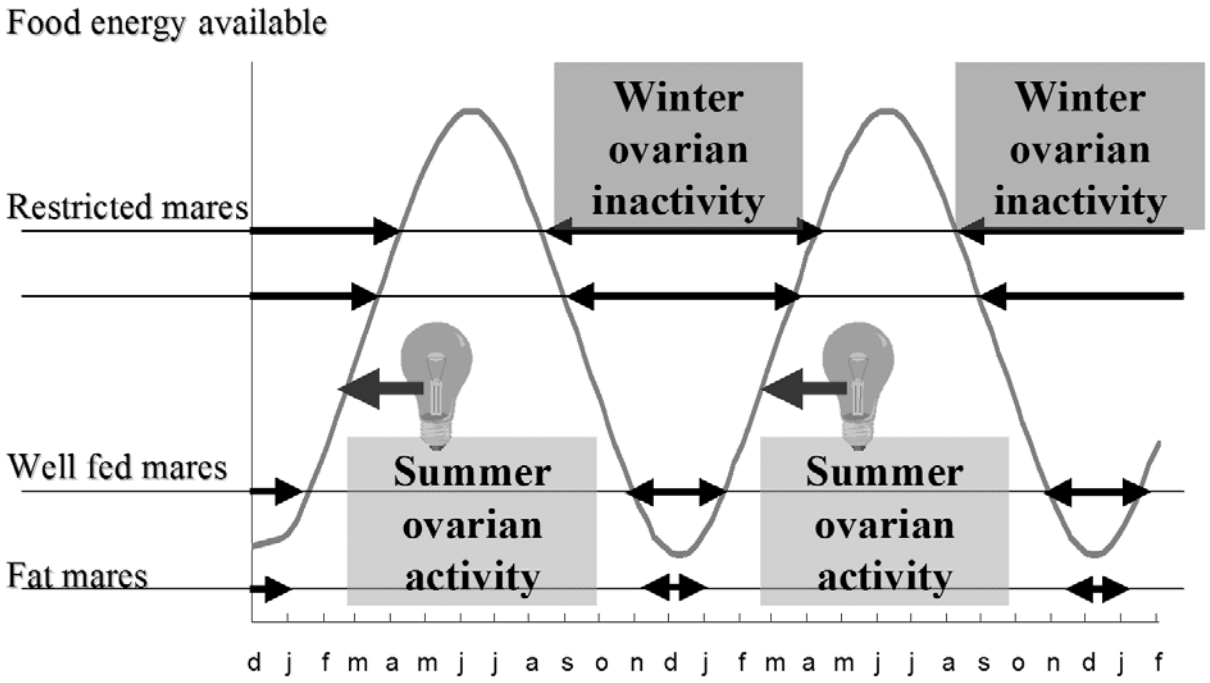
Correlations between leptin mean levels during the two 24h periods and duration of the previous or following winter ovarian inactivity. (n=29).

A calendar		Ovarian inactivity 2nd winter	Ovarian inactivity 3rd winter
R	End	15 May ± 6	21 May ± 4
V	End	2 May ± 10	13 May ± 6

Duration Of	2nd ovulatory winter inactivity	3rd ovulatory winter inactivity
Leptin	-0.67 P<0.0001	-0.73 P<0.0001

Salazar-Ortiz et al: Reproductive biology & endocrinology 2011

Annual rhythm of reproduction: interaction of nutrition and photoperiod



2°) hypothetical neuroendocrine mechanism:

Is the kisspeptin system the key?

In 2003 discovery of kisspeptin and Kp receptor Kiss1r/GPR54
(de Roux et al 2003, Seminara et al 2003)

Kisspeptin = 143 amino acid → last 10 C-term aa sufficient for 100% efficiency

In horses

kisspetin (eKP10) differs for 1 aa in second position Arg instead of Asn
Decourt et al submitted

Kiss1r or GPR54
A mistake : the sequence was absent from the genomic database EquCab2.
Now eKiss1r is identified : 380 aa, high similarity with other mammals
Decourt et al submitted

kisspeptin immunoreactive fibres exist in the mare hypothalamus
In the POA at the level of the SCN : low density
In the anterior periventricular area: higher density
In the ARC numerous fibres
In the ME numerous fibres

Exemple : ARC arcuate nucleus;

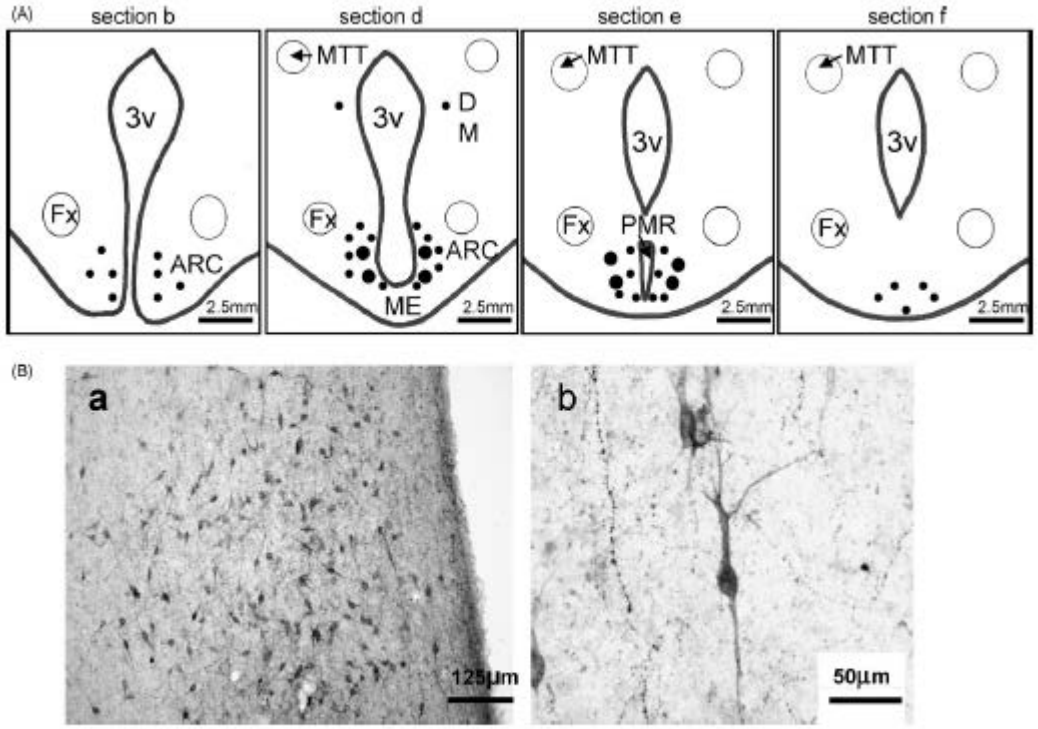


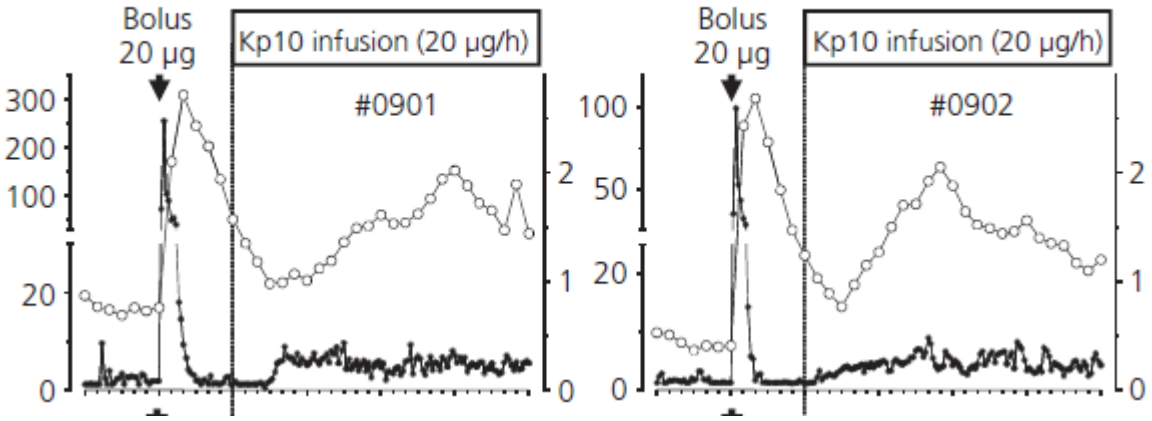
Fig. 2. (A) Diagrammatic presentation of localization and density of kisspeptin immunoreactive cell bodies in sections (b), (d), (e), and (f) (from Fig. 1) of the ARC of the mare 1. Small dot, <5 neurons; big dot, 50 neurons. (B) Photomicrographs of kisspeptin immunoreactive cell bodies taken on the section (d) above of the ARC of the mare 1, at two different magnifications showing (Ba) the density of neurons in the ARC and (Bb) a bipolar neuron in the ARC. ARC, arcuate nucleus; DM, dorsomedial nucleus; Fx, fornix; ME, median eminence; MTT, mammillothalamic tract; PMR, premamillary recessus; 3V, third ventricle.

Decourt et al. Journal of Chemical Neuroanatomy 2008

The kisspeptin system is present also in horses.

Effect of Kisspeptin injections in ewes

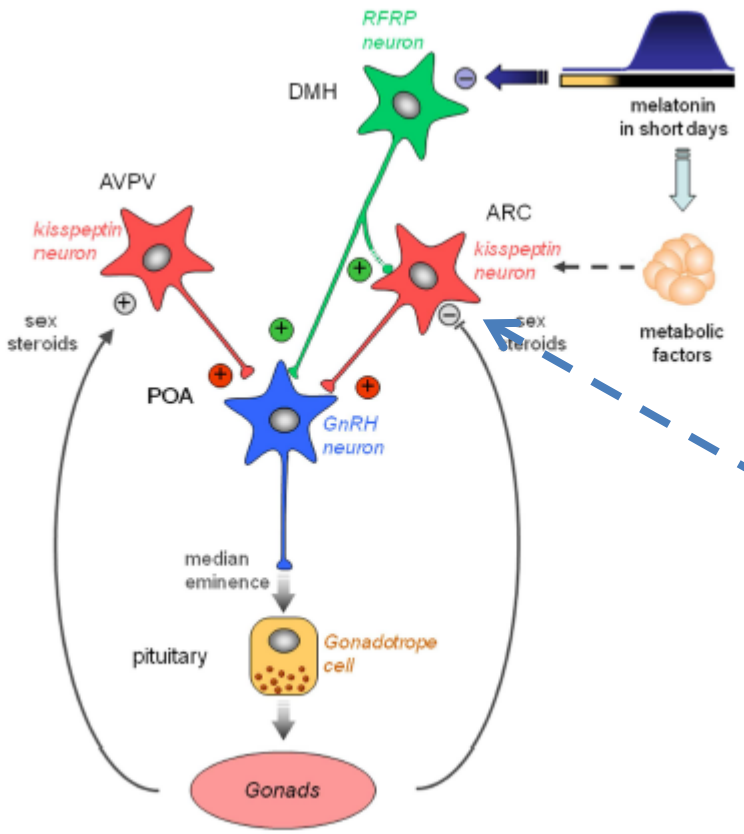
Effect of i.v. administration of Kp10 on hypophyseal portal blood concentrations of gonadotrophin-releasing hormone (GnRH) (closed circle) and peripheral concentration of luteinising hormone (LH) (open circle).



Kisspeptide → GnRH → LH and FSH → ovulation

Caraty et al: Journal of Neuroendocrinology 2013

2°) hypothetical neuroendocrine mechanism



Pheromones (in ewes)
(De Bond et al PlosOne 2013)

POA= preoptic area
AVPV= anteroventral periventricular nuclei
ARC = arcuate nuclei
DMH= dorsomedial hypothalamus
RFRP = RF-amide related peptide

(RFRP-3, the mammalian ortholog of GnIH)

Scheme for male Syrian Hamster
Adapted from Simonneaux *et al*
Frontiers in Neuroscience 2013

The closer the artificial insemination to ovulation time, the better the fertility.

Also for embryo transfer or ovum pick-up

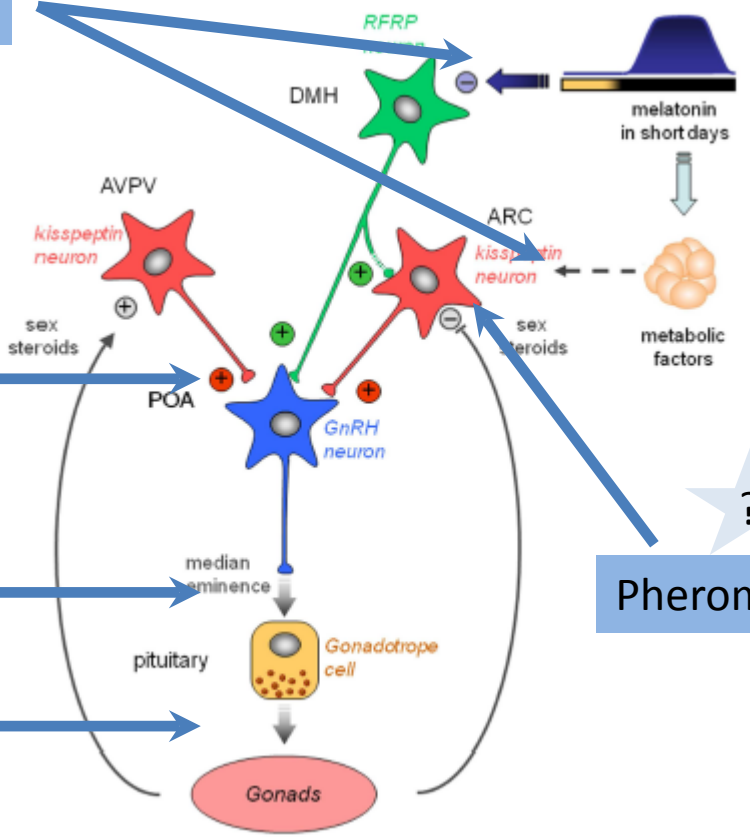
Artificial induction of ovulation is needed.

4 Melatonin and/or leptin control

3 Kisspeptin injection

2 GnRH agonist or antagonist injection

1 Gonadotropin injection



? Pheromones

1 Gonadotropin injection

Crude equine gonadotrophin extract (CEG)

Lapin and Ginther J Anim Sci. 1977

Duchamp et al J Reprod Fert 1987

hCG

Palmer and Jousset: J Reprod Fertil Suppl. 1975

Recombinant LH

Legardinier et al: Glycobiology 2005

Meyers-Brown et al: Animal Reproduction Science 2011

2 GnRH agonist or antagonist injection

Repeated IV

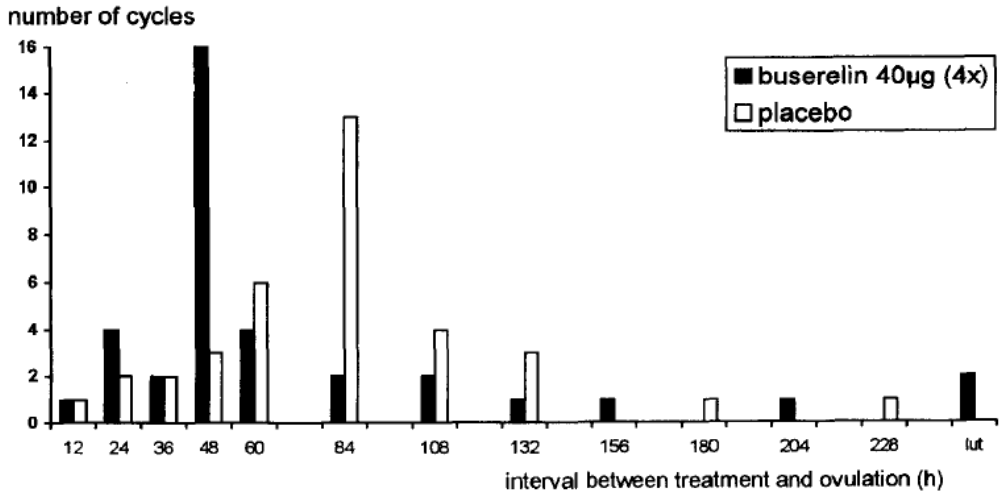


Figure 3. Experiment 1: Interval (h) between start of treatment and observation of ovulation. Treatment consisted of 4 doses of buserelin (40 µg, iv) at 12-h intervals (buserelin 40µg (4x)), or 4 doses of saline solution (NaCl 0.9%, iv) at 12-h intervals (placebo). lut = luteinization without ovulation.

Barrier-Battut *et al* Theriogenology 2001

1 subcutaneous injection of 6 ml at 1.05 mg/ml

Levy and Duchamp: Reprod Domest Animal 2007.

subcutaneous infusion with microosmotic pump (300 to 2700µg/days) = induction of 1 cycle in anoestrus

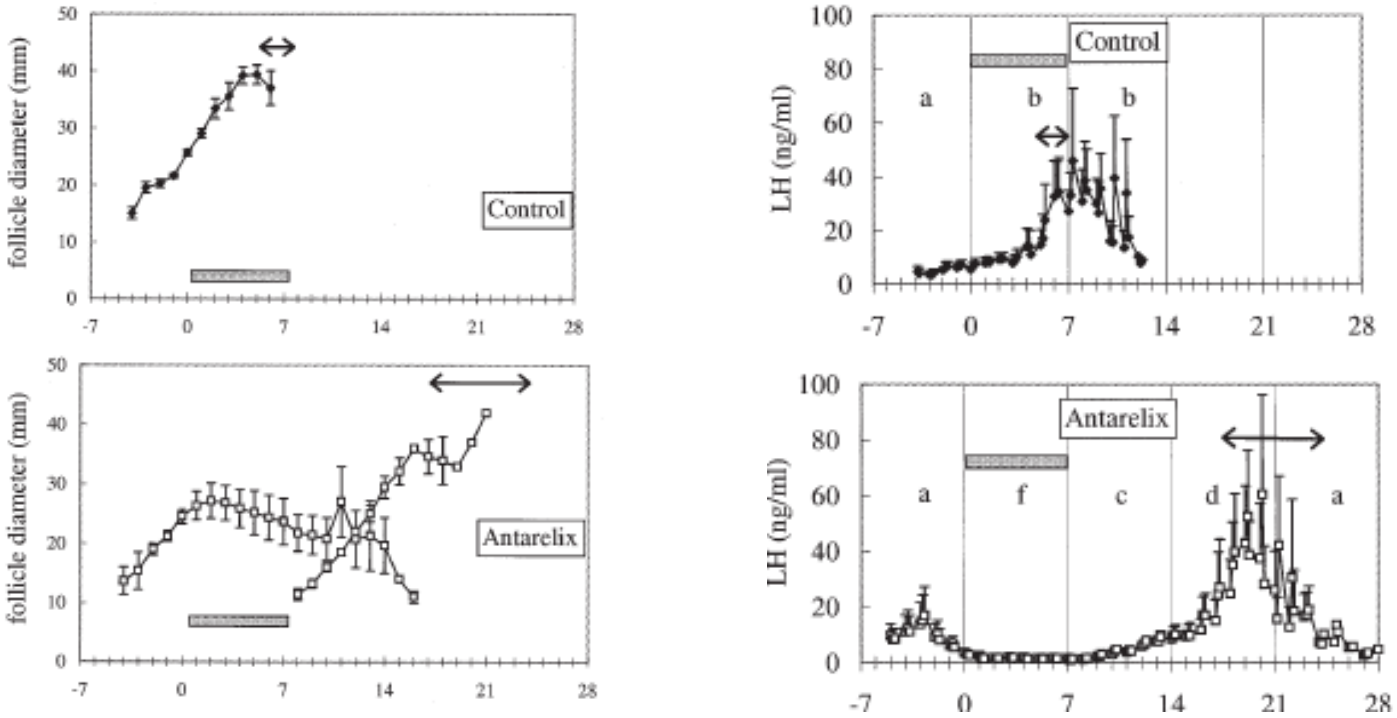
Quellier et al Societé Française d'étude de la Fertilité 1987.

Continuous infusion of GnRH induces GnRH receptor desensitization or downregulation in ewes but not in mares

Porter et al J Reprod Fert 1997

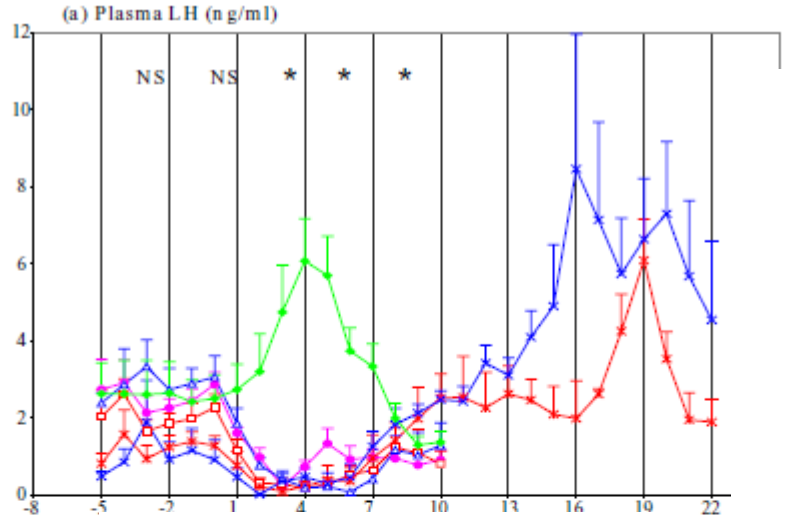
But Deslorelin® does it !

2 GnRH agonist or antagonist injection

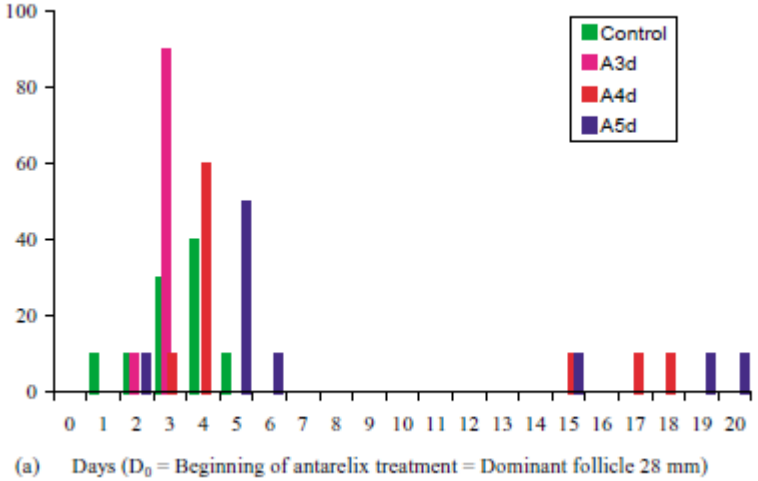


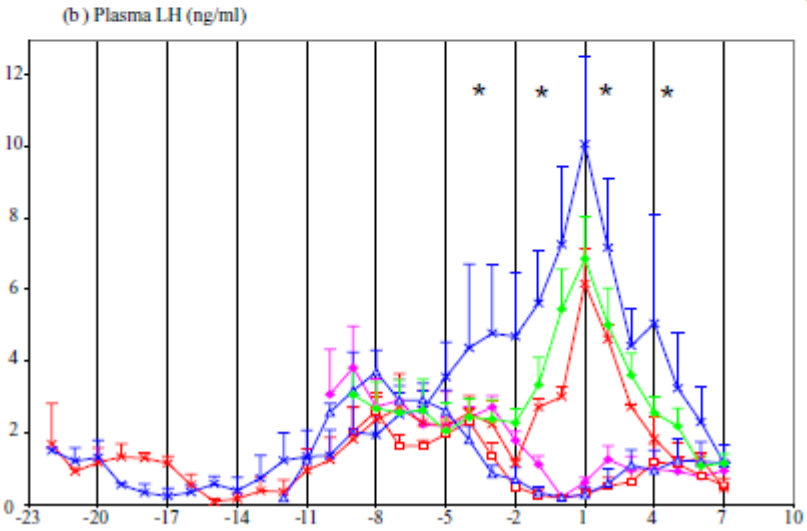
Days /beginning of the treatement = Largest follicle > 22 mm

Guillaume et al : reprod Nutri. Dev. 2002



D0 beginning of antarelix = Follicule >28





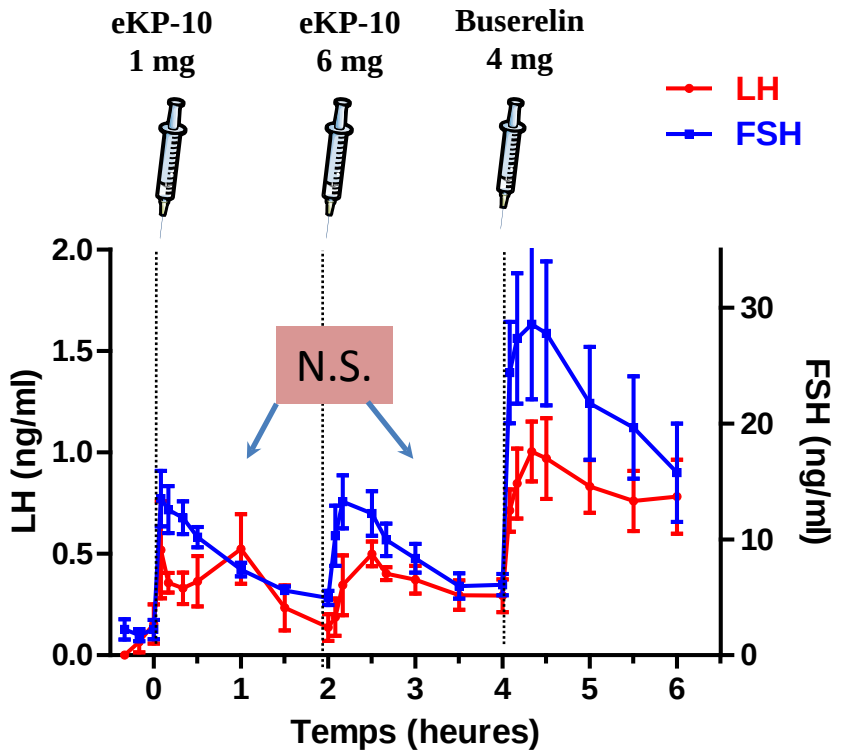
D0 = ovulation

Briant et al: Domestic Animal Endocrinology 2004

2 Kisspeptin injection

In Mares, during winter ovarian inactivity

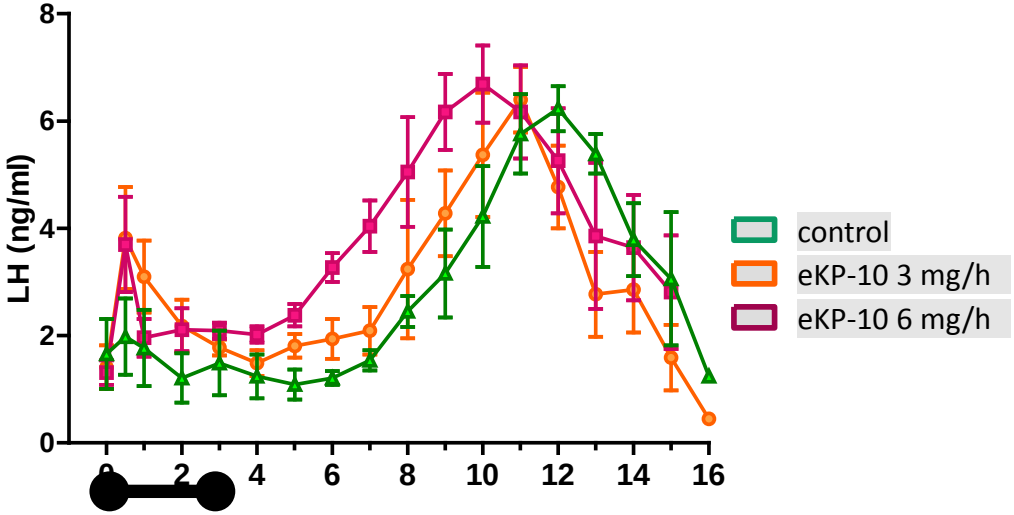
Effect of a single acute injection of eKp10 was tested in 4 anoestrus mares



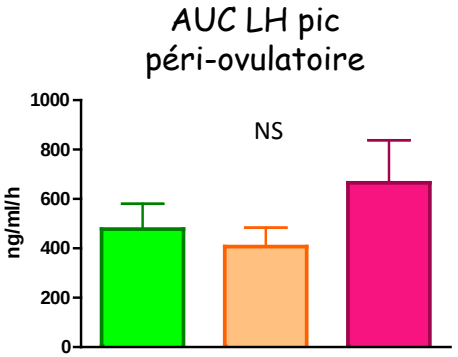
➡ Increased the LH and FSH secretion

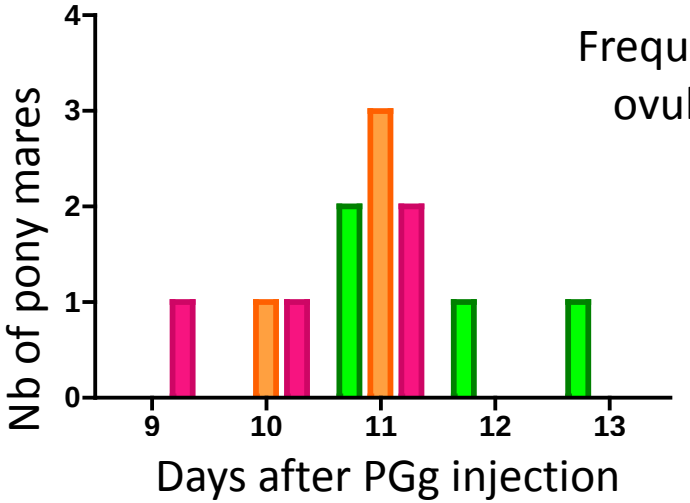
In Mares, during breeding season

Effects of mid-term perfusion of eKp10 during the early follicular phase on LH plasma concentrations



72h period of perfusion





Frequency of ovulation

Conclusion in mares :

Systematic low effect on LH or FSH secretion

No effect to trigger the ovulation

Conclusion on the use of kisspeptin in mares:

Confirmation of Magee et al previous results

Systematic low effect on LH or FSH secretion

No effect to trigger the ovulation

Hypotheses on this failure to use of kisspeptin in mares:

1°) Important plasmatic clearance

2°) Poorly crosses the blood brain barrier and just acts through the median eminence

3°) Systematic desensitization or downregulation of Kiss receptor on GnRH neurons

Solution

Development of better Kiss1r agonists?

4 Melatonin leptin and pheromones control

melatonin:
Active immunization against melatonin fail to act on the first ovulation.
Researches on antagonists are in progress

Leptin and other adiponectins:
Are poorly investigated in equine.

The mobilization of fat tissues and the modification of leptin secretion with clenbuterol not act on the first ovulation.

McManus and Fitzgerald : Animal Reproduction Science 2003

Pheromones :
open the vial and the mare ovulates ... a dream

Neuroendocrine regulations are poorly studied in Equine compared to other domestic species.
As a consequence knowledge of the horses' peculiarities on this topic is very limited.
Therefore, how can we understand and serve our favorite animal model…and dearest friend ?


END