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**
Photostimulation and Winter anoestrus

Duration of light treatment

Individual progesterone curves

14.5L 9.5D

Nat. Phot.

6 March

30 April

Numbers of days after January the 1st


1°) one example of interaction: Photoperiod / Nutrition
Melatonin is the neurohormone which acts on reproduction by its length of secretion.

GUILLAUME et al, American J. of Physiology 268; R1236-1241.
A) UFC in the commercial or dehydrated alfalfa pellets (mean of each group).

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

<table>
<thead>
<tr>
<th>A calendar</th>
<th>Ovarian inactivity 2nd winter</th>
<th>Ovarian inactivity 3rd winter</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>End</td>
<td>15 May ± 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21 May ± 4</td>
</tr>
<tr>
<td>V</td>
<td>End</td>
<td>2 May ± 10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13 May ± 6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Duration Of</th>
<th>2nd ovulatory winter inactivity</th>
<th>3rd ovulatory winter inactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin</td>
<td>-0.67</td>
<td>-0.73</td>
</tr>
<tr>
<td></td>
<td>P&lt;0.0001</td>
<td>P&lt;0.0001</td>
</tr>
</tbody>
</table>

Salazar-Ortiz et al: Reproductive biology & endocrinology 2011
Annual rhythm of reproduction: interaction of nutrition and photoperiod

Food energy available

Restricted mares

Well fed mares

Fat mares

Winter ovarian inactivity

Summer ovarian activity

Winter ovarian inactivity
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;

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).

Caraty et al: Journal of Neuroendocrinology 2013
2°) hypothetical neuroendocrine mechanism

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

Pheromones (in ewes)
(De Bond et al PlosOne 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.
1. Gonadotropin injection
2. GnRH agonist or antagonist injection
3. Kisspeptin injection
4. Melatonin and/or leptin control
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
GnRH agonist or antagonist injection

Repeated IV

1 subcutaneous injection of 6 ml at 1.05 mg/ml

Repetitive IV

1 subcutaneous injection of 6 ml at 1.05 mg/ml


subcutaneous infusion with microosmotic pump (300 to 2700μg/days)

= induction of 1 cycle in anoestrus


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!
Days /beginning of the treatment = Largest follicle > 22 mm

D0 beginning of antarelix = Follicule >28

D0 = ovulation

Briant et al: Domestic Animal Endocrinology 2004
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?
Melatonin, leptin, and pheromones control ovulation.

**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?