GnRH vaccination in mares & stallions
Immunological potentially reversible gonadectomy

Jean-François Bruyas

Menu
Mecanism ?
Tools available ?
Measured Effects (duration)?
- behaviour
- hormonal secretion
- cyclicity
- spermatogenesis
Reversibility ?
spermatogenesis, cyclicity, fertility
Practical uses ?

GnRH vaccination
Mecanism ?

GnRH = "music conductor"

GnRH = Gonadotropin Releasing Hormone
FSH = Follicle Stimulating Hormone
LH = Luteinising Hormone
T = Testosterone
E = Estrogens

In males

In females too

Scientists' old dream

How to create an immunizing GnRH (10 aa)?

- Immunity adjuvants
- Large local reactions
- Huge challenge
- many attempts & studies

Immunization against-GnRH

GnRH
GnRH Analog
GnRH

Antibodies anti-GnRH
Binding Protein

Immune stimulation of GnRH receptors in pituitary gland by GnRH

Immunogenic

Antibodies

Immunity adjuvants

Reversible effects
Immunization against GnRH

3 different "vaccines" for 3 different species

- Binding Protein
- Immunity adjuvants
- Excipient
- Immunological Dosage ≠ for each species

☞ Immunization with lowest side-effects

GnRH vaccination

Tools available?

3 different "vaccines" for 3 different species

- Binding protein
- Immunity adjuvant ≠ for each species
- Excipient
- Immunological Dosage ≠

Equity® Oestrous Control vaccine for horses

☞ anoestrus in mares

200 µg Immunizing particles/mL
2 x 1ml IM injections 4 weeks apart
1 dose 1mL into a syringe ready to use

Registered in New Zealand, Australia, Brasil, Mexico, Turkey...

400 µg Immunizing particles/mL
2 x 1ml SC injections 3 or 6 or 8 weeks apart
flasks of 50 or 100 mL

➘ sexual and aggressive behaviour of bulls

Anoestrous in cows

Equity® Oestrous Control vaccine for horses

Registered in many countries:
- Improvac®, Improvest®, Innosure®, Vivax®
- 150 µg Immunizing particles/mL
- 2 x 2 ml SC injections 4 weeks apart
  ➞ "boar taint"
  ➞ sexual and aggressive behaviour in pigs

European Marketing Authorisation
- Improvac®

☞ alternative way to surgical castration

Registered in many countries:
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- 150 µg Immunizing particles/mL
- 2 x 2 ml SC injections 4 weeks apart
  ➞ "boar taint"
  ➞ sexual and aggressive behaviour in pigs

European Marketing Authorisation
- Improvac®

☞ alternative way to surgical castration

Vascincel®

Registered only in Spain

> 150 µg Immunizing particles/mL
4 x 2 ml SC injections 4 - 12 - 12 weeks apart

☞ alternative way to surgical ovariecotmy

☞ prevent the onset of puberty, heats and pregnancy before slaughter ("Iberian Pork" > 10 months)
GnRH vaccination

Measured Effects (duration)?
- behaviour
- hormonal secretion
- cyclicity
- spermatogenesis

Reversibility?
spermatogenesis, cyclicity, fertility

Mare “vaccine” (Equity®) (200 µg/dose)
Card et al., 2007: 2 injections [4 weeks] vs controls
n = 61: cycle / ovulation stopped in 4 weeks (> 2nd inj)
Elhay et al., 2007: 2 injections [4 weeks] vs controls
n = 24: cycle / ovulation stopped in 2 to 4 weeks (> 2nd inj)

Porcine “vaccine” (Improvac®)
(300 µg / porcine dose = 2 mL)
Botha et al., 2007: 2 injections [4 weeks] vs controls
≈ (April)
n = 24: cycle / ovulation stopped in 2 to 4 weeks (> 2nd inj)

Mare “vaccine” (Equity®) (200 µg/dose)
Botha et al., 2010 – (Schulman et al., 2013): 2 injections 2mL [5 weeks]
≈ (end of April)
n = 51: cycle / ovulation stopped in 5 weeks

Reversibility = fertility?
Card et al., 2007: 2 injections [4 weeks] vs controls (July 2004)
n = 61:
54/51 cycling at the next breeding season (2005)
(46/51) 75% PB vs 21/21 controls
60/61 cycling at the 2nd breeding season
post-vaccination (2006)
(55/61) 90% PO+ vs 21/21 controls
(52/55) 95% live foal rate vs 19/21 controls

Robinson & McKinnon, 2006: clinical cases
10 Thoroughbred broodmares (4xxx) with failure to cycle
( 1 breeding season to 3 breeding seasons)
at least 2 to 4 Equity® injections during racing careers.
Feed-backs from the field: practitioners ☞ same cases
- after how many booster injections?
- performed at which age?
- totally irreversible or long duration effect?
☞ wait and follow during 2 to 3 breeding seasons
☞ no other thing to do
GnRH vaccination

Measured Effects (duration)?
- behaviour
- hormonal secretion
- Arteritis Virus semen excretion
- spermatogenesis

Reversibility?
- spermatogenesis

Few publications
+ 1 own study

Materials & methods

From a stable of 170 Lusitanian horses
(90 stallions 80 geldings) used for historical spectacles.
10 non breeding stallions immunised and followed
along the study (4 to 15 years old)
(indication : behaviour → difficulties to manage)
+ 3 control stallions non immunised
⇒ histological observation
Immunisation : 1mL Improvac® IM 2 x 28 days apart

Protocol

28 days
10 stallions
⇒ histological analysis of testes
28 days
3 months
14 days
28 days
60 days
2 months

Blood samples: level of antibodies
Urine: steroids concentration
Scrotal measurement
Surgical castration
⇒ histological analysis of testes

Results and discussion

Macroscopic evaluation

< 1st injection

Burundi
Danubio
Bal Ibi

5 months 10 days > 2nd injection

Burundi
Danubio
Bal Ibi

< 1st injection

Relampago
Espadarte
Soberbo

5 months 10 days > 2nd injection

Relampago
Espadarte
Soberbo
% decrease: (Min Size - original size)/original size: 27% to 48% (mean: 38%)

Evolution of [steroids] in urine

Stanolone

Evolution of the Scrotal Width (SW)

Results and discussion

Evolution of GnRH antibodies levels (ELISA)

Results and discussion

Evolution of [steroids] in urine

Results and discussion

Steroidomics / steroid profiling (biostatistiques)

Comparison with large population of geldings, mares, and stallions

Results and discussion

Effect on behaviour

9/10: difficulties to manage sexual behaviour

Exception — the one without immune response
**Behaviour**

2 injections 4 or 5 weeks apart (n=4 (Equity®) ; n=18 (Improvac®) 2mL) (Burger et al, 2006)

- **Indications**
  - 19/22 sexual behaviour ➔ difficulties to manage + 17/19 (~88%)
  - 18/22 high distraction level ➔ 13/18 (~72%) distraction
  - 7/22 aggressiveness ➔ 7/7 (~100%) aggressiveness

- **Effects : owners evaluation**
  - + 17/19 (~88%) libido
  - + 13/15 (~87%) distraction
  - + 7/7 (~100%) aggressiveness

**Individual variability**

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**EVA viral excretion in semen**

2 injections 4-5 weeks apart (n=10 (Equity®) ; n=1 (Improvac®) ) (2 inj. 1ml [5 weeks apart] Plasmatic concentrations of testosteron & estradiol lower ➔ 7th mo > vaccine max 18 mois Prohaska, 2011

- + 9/9 ➔ EVA viral excretion in semen 2 injections 4-5 weeks apart (n=10 (Equity®) ; n=1 (Improvac®) ) (Burger et al, 2006 ; Ferry et al., 2008)
- ➔ 0 excrétion (10/11) ➔ EVA viral excretion in semen (10/11) ➔ 0 excrétion (10/11) Failure for 1/11?

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**spermatogenesis**

3 (Equity®) injections (4 then 6 weeks apart) (n=3 (Equity®)) (Burger et al, 2006, Janett et al, 2006)

- ➔ spermatogenesis (5/5)
  - N° SPZ, % Motile SPZ, % abnormal SPZ (Individual variability)

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**Results and discussion**

**Histology**

Immune horse Castrated 6 mo > 1st injection Control stallion

- Atrophy of seminal tubes (~ 80%)
- (~ No germ cells/seminal tubes)
- (~ No germ cells/Sertoli cells)
Results and discussion

Histology

Jonhsen's score

<table>
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<tr>
<th>Control 1</th>
<th>Control 2</th>
<th>Control 3</th>
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<tr>
<td>Mean 72%</td>
<td>Mean 6.5%</td>
<td>Non-reversible</td>
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</table>

Distribution of Jonhsen’s score in
Jonhsen’s score, controls
- N° mature spermatogenesis, spermatozoa, spermatocytes, & spermatogoniae
Controls: None scores < 7; [7 to 10]
Immunized: All scores > 3; [4 to 10]

Histology

Results and discussion

0
10
20
30
40
50
60
70
1
2
3
4
5
6
7
8
9
10

Mean 72%

Mean 6.5%

Distribution of Jonhsen’s score in
Jonhsen’s score, immunized horses

Individual Variability

Libido

5/6: OK > 6 to 30 weeks > last injection
1/6: Libido > 40 weeks > last injection

Spermatogenesis

5/10: OK > 6-8 months > last injection
4/10: oligo-asthenospermia or 0 libido during at least 1 year
1/10: Plasmatic levels of testosterone > 1 year (Burger et al., 2006, Janett et al., 2009)

GnRH vaccination

Practical uses?

- Precautions
- Rules, regulations
- Availability and price

Local reactions at point of injection

2 injections (Improvac®) [4 weeks]

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<th>1st injection</th>
<th>2 weeks</th>
<th>10 days</th>
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<td>Dog 4</td>
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Side-effects

- Horse “vaccine” (Equity®) (200 µg/doe)
  - Elhay et al., 2007: 2 injections [4 weeks]
    - 1st injection 8/24 - 2nd injection 1/24 (0 in 7 days)
  - Own study: 2 injections [4 weeks]
    - 1st injection 2/5 - 3rd injection 1/5 (0 in 7 days)

Porcine “vaccine” (Improvac®)

- Imoden et al., 2007: 2 injections [4 weeks] (300 µg/isode)
  - 1st injection 8/9 hyperthermia - 4/9 local swelling
  - 2nd injection 7/9 hyperthermia - 3/9 opacity
  - 7/9 local swelling - pain - 7/9 neck stiffness (0 in 3-5 days)

½ porcine dose is enough
Where should the injection be done?

1. Pectorals
2. Gluteal muscles
3. Neck

Intramuscularly (subcutaneously)

Safety directions

Accidental self-injection (+ needle stick injection)
- same effects in men and women on gonad functions
- huge local inflammation

TAKE CARE

For injections in porcine and bovine:
- safety device (needle guard + security screens) MUST be used

Availability:

In Europe: only Improvac®

From May 2017 (12 bottles of 20 mL) (~200 €)
packaging available:
- 10 bottles of 20 mL
- 4 bottles of 125 mL
500 porcine doses ↔ 1000 horse injections (~750 €)

In Australia: Improvac®
(~100 €/dose (1mL))

Conclusion

Improvac® 2x 28 days apart
- reversible immunization against GnRH
- stop of secretion of steroids
- hypoplasia of seminal tubes, stop of Sperm Production
- stop of ovulation and oestrous cycle in mares

BUT variability among stallions and mares
- intensity of effects
- duration of effects (3 to >7 months or more)

In non predictable cases (15 to 30%?)
- failure to resume

Take home message:

scrotal evolution
Effects of boosters
Evolution of GnRH antibodies levels (ELISA)

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<th>Time (in months)</th>
<th>Booster</th>
<th>Improvement or not</th>
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Take home message: Clinical implications
- Anestrus in maiden broodmare
  - small ovaries without follicular growth
- New stallion
  - poor libido/poor semen quality

0 test → GnRH vaccinated? reversible status or not?

Future
Which effects of booster injections?
- Always reversible or not?

How to evaluate the reversibility in field?

GnRH vaccination
Which indications?
- Reversible gonadectomy
  - to improve management/training/performance of some athletic stallions or mares
  - sexual (aggressive) behaviour
  - “fatigability – laziness” of stallions
  - (endurance – cross…)
- Alternative to surgical castration/ovariectomy
  - surgical, anaesthesia risks...
  - price
  - (old horses, donkeys…)
- price
  - (working equids in some countries, stallions, mares with low commercial values…)