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# Immunization against GnRH in adult stallions: Effects on semen characteristics, behaviour and shedding of equine arteritis virus

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## 101. Introduction

There are numerous indications for suppressing reproductive endocrine function in horses, the most frequent being the inhibition of undesirable sexual behaviour. Immunization against GnRH offers a viable alternative to surgical or hormonal castration by circumventing surgical risk, frequent drug administration and residue problems (Stout and Colenbrander, 2004; Stout, 2005). Furthermore, it may prove to be a therapeutic alternative for the treatment of equine arteritis virus (EAV) shedder stallions (Burger et al., 2004), as persistence of EAV in the accessory sex glands has been shown to be androgen dependent. Earlier studies examining GnRH vaccination in stallions have reported varied effects and do not allow a clear conclusion to be drawn. In the present study, 3 experiments were carried out to examine the effect of anti-GnRH vaccination on semen characteristics, libido and shedding of equine arteritis virus in adult stallions.

## 2. Materials and methods

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### *Experiment 1:*

The experiment was carried out using 9 healthy Franches-Montagnes and Warmblood stallions (9-15 years) of proven fertility. The stallions were randomly allocated to a test group (6 stallions) and a control group (3 stallions). Ejaculates of all 9 stallions were collected and examined weekly for 52 weeks. After the first semen collection, animals in the test group were immunized with 200 µg of a GnRH-conjugate (Equity®; CSL, Australia) intramuscularly in the neck. Control animals received saline solution. The treatment was repeated 4 and 12 weeks later. Side effects to the vaccination were recorded. Simultaneously to weekly semen collection, libido was evaluated (time elapsing between entering the stallion barn and erection and ejaculation, respectively) and the number of mounts before ejaculation recorded. After ejaculation, scrotal width was measured using scrotal calipers. The volume, concentration, total sperm count and motility of the ejaculates were assessed and a morphological examination carried out. Blood samples were collected, always at the same time on the day of semen collection, and plasma testosterone concentration and GnRH antibody titre determined.

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### *Experiment 2:*

The effect of GnRH vaccination on libido, sexual behaviour and degree of distraction was evaluated in a retrospective questionnaire based field study, carried out with 22 stallions (7.4 ± 4.3 years old) of various breeds, vaccinated twice at an interval of 28 to 35 days. 18 stallions were vaccinated with 400 µg of a GnRH-conjugate (Improvac®; CSL, Australia) intramuscularly and 4 stallions with Equity®.

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### *Experiment 3:*

Four permanent EAV-shedding stallions aged 12 to 17 years were vaccinated twice 4 weeks apart with either Improvac® (1 stallion) or Equity® (3 stallions). Semen was collected and virus content (cell culture and RT-PCR assay) was determined monthly for 6 months and then individually up to 15 months after first immunization. Additionally, 12 to 17 months after the first vaccination, two EAV seronegative mares were bred and serologically tested 4 weeks later.

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## 553. Results

### Experiment 1:

Scrotal size, total sperm number, percentage of normal sperm and motility of all treated stallions continuously decreased after vaccination. Lowest mean motility values were recorded 606 months after the first immunization (Fig. 1). All the above parameters tended towards prevaccination levels 10 to 12 months after begin of the study. The decrease in libido was also significant but varied individually occurring between 13 and 18 weeks after the first vaccination and lasting between 10 to 32 weeks. While the decrease in one stallion was only slight, the remaining five showed gelding like behaviour. At the end of the experiment, 5 stallions regained their original libido but 1 stallion remained suppressed such that it was not possible to obtain an ejaculate until the end of the study.

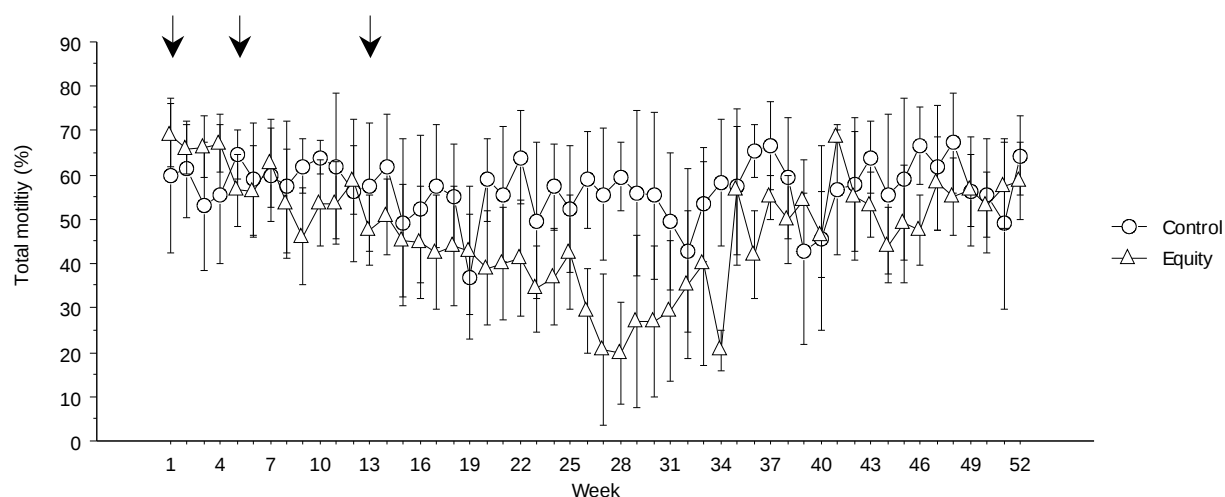


Fig.1. Mean ( $\pm$  SEM) total motility in vaccinated and non vaccinated stallions.

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Mean plasma testosterone concentrations in vaccinated stallions started to decrease significantly after the first injection and remained below 0.7 nmol/L plasma for 10 months (Fig. 2). By the end of the experiment, 3 stallions showed prevaccination levels ( $> 1$  nmol/L plasma) but 3 remained at a basal level.

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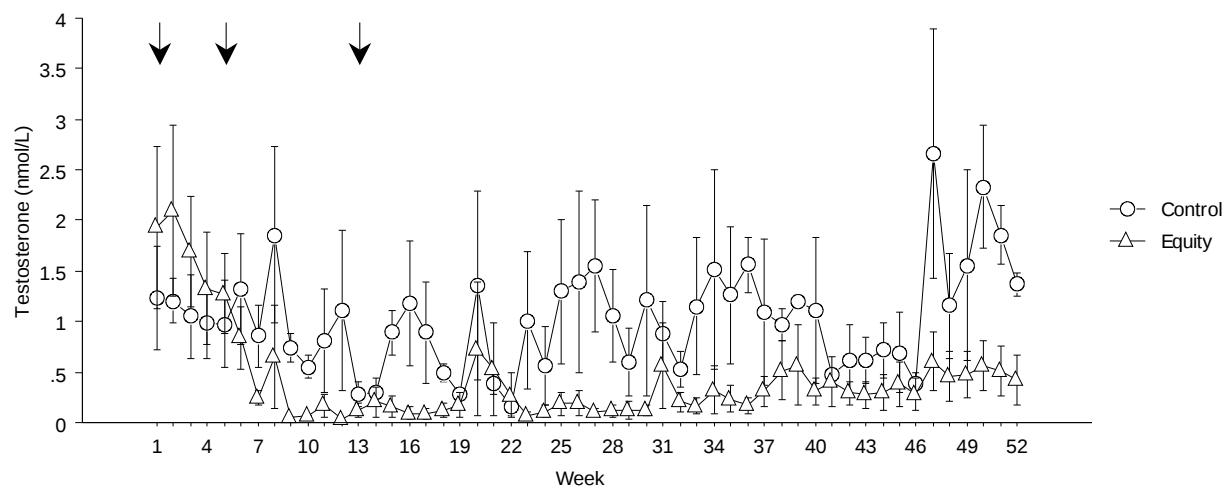


Fig. 2. Mean ( $\pm$  SEM) plasma testosterone concentrations in vaccinated and non vaccinated stallions.

80 Individual GnRH antibody titers peaked 2 months after the first immunization and remained high until the end of the study. Reactions to injection in the vaccinated group were few: 13 of the 18 vaccinations were without any side effects, the remainder showed mild to painful swellings at the injection site. No increases in body temperature ( $>38^{\circ}\text{C}$ ) or other complications were observed.

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#### *Experiment 2:*

Among the 19 stallions with a libido problem for the owner, 17 responded with the desired decrease in libido. Among the 15 stallions with a high distraction level, the owners reported less distraction in 13 stallions. Aggressiveness towards humans and/or other horses was 90 markedly reduced in all 7 horses reported to be aggressive.

#### *Experiment 3:*

EAV could no longer be isolated from the semen of all four vaccinated stallions 4 - 6 months after the first immunization and from all subsequent semen collections. One stallion died for 95 reasons not related to the study 6½ months after the first vaccination. The non-shedding, non-carrier status of the 3 remaining stallions was additionally confirmed by failure of each of the two mares to show seroconversion 28 days after breeding.

## 4. Discussion

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The efficacy of GnRH vaccination appears to depend to a great extent on the type of vaccine used. Contrary to previous studies (Clement et al., 2005), the use of both Improvac® and Equity® has led to satisfactory results. More than 80% of the stallions in experiment 1 and 2 showed clear loss of libido, the duration of which varied greatly among single individuals.

105 Similar observations regarding libido have also been reported after surgical castration of breeding stallions. This suggests that GnRH vaccination is a useful tool for temporary management of undesirable sexual behaviour in stallions.

Evaluation of semen characteristics in experiment 1 showed that although semen quality deteriorated, semen production was never entirely suppressed. At the end of the experiment, 110 all semen parameters were comparable to original values. The number and interval of booster vaccinations required for an optimal effect need further investigation. Failure of 3 stallions to reach plasma testosterone concentrations  $>1$  nmol/L in our study may be due to the third vaccination.

The GnRH antibody titres do not appear to be a useful indicator of immunization effect, either 115 on behaviour or on semen characteristics. 5 stallions showed a full return to reproductive function despite persistent antibody titres. The remaining stallion was surgically castrated 3 months after the study ended and histological examination of the testes revealed normal spermatogenesis.

All four treated EAV-shedding stallions were converted to a non-shedder status. In contrast to 120 findings after short term treatment (35-38 days) with a GnRH antagonist (Fortier et al., 2002), no stallion in our study reverted to a shedding status. After failure of the two test mares to seroconvert, the stallions resumed breeding activity and showed normal fertility. For instance, stallion P (17 years at vaccination in July 2004, EAV shedder since 4 years) had pregnancy rates per cycle of 30% (n=30 cycles) in 2004 and of 37% (n=38 cycles) in 2005, respectively, 125 before and after vaccination. This study demonstrates that vaccination against GnRH could represent a promising method for successfully treating EAV-shedding breeding stallions.

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