Immunocastration in stallions: Effects on spermatogenesis and behaviour

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Summary

Eight experienced stallions have been vaccinated against GnRH and compared to six control ones. Four of eight stallions responded to immunocastration with low levels of testosterone. They had decreased testes size, sperm concentration, total number of spermatozoa and % of motile spermatozoa compared to controls. However, sexual behaviour remained unchanged. Except for one stallion, reversibility occurred between 5 and 9 months after the last booster.

Introduction

Pharmacological manipulation of reproduction activity using GnRH vaccines, agonists or antagonists has been reviewed recently (Stout and Colenbrander, 2004). The aim of this study was to develop an effective and reversible method to suppress spermatogenesis and sexual behaviour in adult, breeding stallions. This method was based on active immunization against GnRH.

Material and Methods

Fourteen adult (12.2±3.2 years old and 580±62 kg per BW), fertile and experienced stallions were studied. Eight stallions received the vaccine (1 mg GnRH equivalent of the tandem peptide OVA conjugate in 2 ml CoVaccine adjuvant) in the pectoral muscles four times (22 Sept; 3 Nov 2000; 16 Feb; 15 May 2001). Six control stallions received adjuvant.

The stallions were monitored for local and systemic adverse reactions.

The stallions were checked at six periods, each period having 5 daily collections: (1) before the 1st injection, (2) 4 and (3) 8 weeks after the 2nd one, (4) 1,5 and (5) 5 months after the last one and for the 4 “good responders to vaccine” (6) 9 months after the last injection.

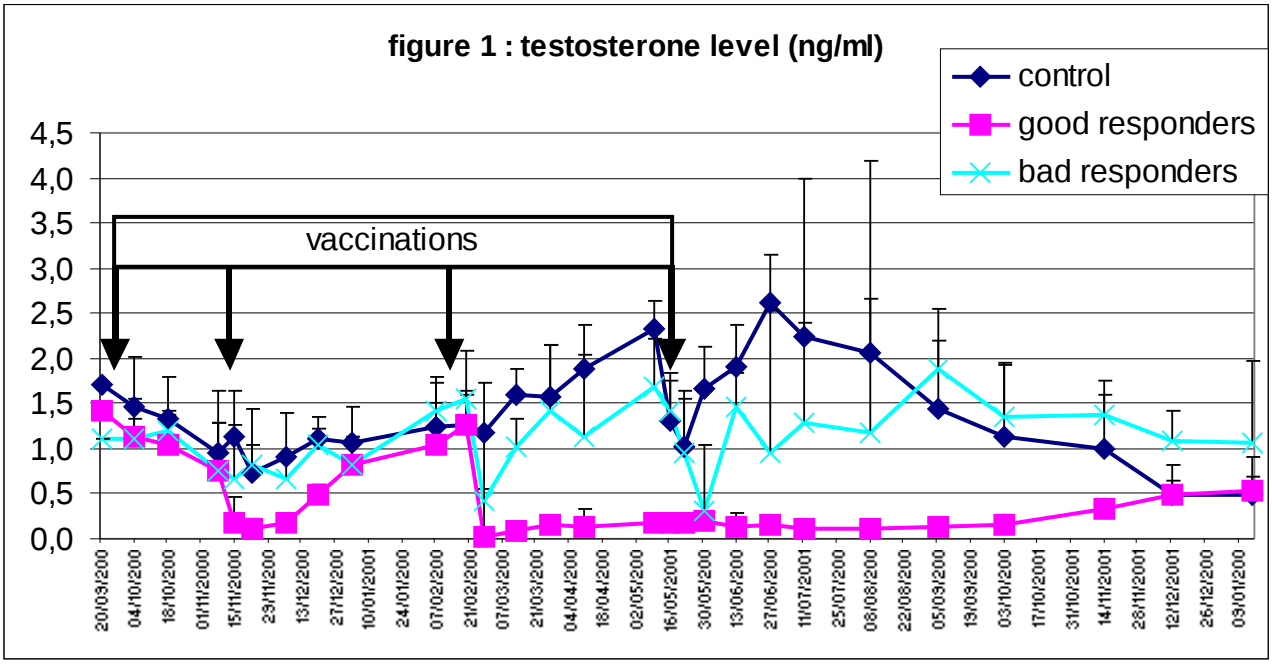
- Semen evaluation: the last 2 ejaculates during each period were analyzed for total volume, sperm concentration, total sperm number, % motile spermatozoa after dilution, after 24 and 48 hours survival at 4°C, % live spermatozoa and morphology.
- Testicle measurements: the right and left testicles width were evaluated twice using ultrasonography. The sum of the right and left values was calculated.
- Sexual behavioral parameters: the erection time, the number of mounts before ejaculation, the ejaculation time, the refractory time (= time between first ejaculation to second erection) were recorded during the last 3 collections.

Blood samples were collected weekly during the experiment. Testosterone levels have been evaluated weekly during two weeks after vaccination, every two weeks until the 8th week and then every month. Concentration of antiGnRH antibodies were assayed monthly until April, 2001.

Results

Apparent side effects were similar in both groups: mild to large edema were observed 3 days after 42/56 injections; an increased body temperature (>38°C) occurred 3 hours after 11/56

injections; one stallion had severe reaction just after the 3rd vaccination (loss of balance during 5 mn).



Vaccination efficiency

Vaccinated stallions had lower testosterone level during 1 month after the 2nd injection and 3 months after the 3rd and the 4th ones ($P<0.001$). More precisely, low testosterone levels were observed in only 4 of the 8 treated stallions (considered as good responders) and not in the others ones (bad responders) (Figure 1).

GnRH antibody titers were higher in good responders stallions than in bad responders group. For example, good responders had 6 times higher antibody titers than bad responders 1-2 months after the 3rd vaccination. There was no antibody in the control group.

Seminal characteristics and sexual behavior

There was no difference for seminal characteristics between the groups (control, good, bad responders) before (period 1) and after the first 2 vaccinations (periods 2 and 3).
However, the good responders stallions had decreased testes width (period 5), sperm concentration (period 4), total sperm number (periods 4, 5), % motile spermatozoa (period 5), % motile spermatozoa after 24 and 48h storage (periods 4, 5), % live spermatozoa (periods 4, 5) and similar % normal spermatozoa compared to control stallions.

All the sexual behavior parameters (erection time, ejaculation time, refractory times and number of mounts) remained similar between groups.

Recovery of the seminal characteristics

To observe the sperm recovery time, the 4 good responders have been checked 9 months after the last vaccination. Sperm parameters were compared to those of the control stallions at the same season during the previous year (period 3). Total testes width, sperm concentration, total sperm number and % abnormal spermatozoa were similar between the comparisons.
However, the % motile spermatozoa after dilution, after 24 or 48 hours survival and % live spermatozoa were lower in the good responders group. This absence of recovery is due to one stallion that has not yet recovered in the 9 months in contrast to the 3 others stallions, which presented normal sperm.

Discussion

The vaccination induced side effects, mainly local edema and mild fever. Similar vaccines induced same effects in the study of Van der Meer et al. (2001) and only transient fever in the study of Turkstra et al. (2005).

A good immune response was observed in 4/8 stallions and 3 vaccinations were necessary. In both Dutch studies, the immune response seemed better: 4/4 and 4/5 pony stallions were immunized after 2 vaccinations.

As observed in the Dutch studies, good responders stallions showed a decline in sperm production and testes size. However, their libido remained unchanged. Turkstra et al. (2005) showed a poor libido in only 2/8 ponies. This difference can be explained by their young age, the seasonality of the breed or their absence of previous sexual experience. As castration generally didn't resolve problem behavior in experienced stallions, the absence of effect of immunocastration on sexual behavior was predictable.

The effects of immunocastration were detectable 5 months after the last booster in 4/4 good responders stallions and even 9 months in one of them. Reversibility seems variable between stallions and needs more study.

One of the good responders stallions was arteritis shedder. It was checked negative 6 weeks after the first vaccination, but positive 2, 6, 9 and 14 months after the last vaccination. It has been suggested that AVE shedding in some stallions could be stopped by chemical castration (Fortier et al., 2002). Our results confirm a partial efficiency of castration on AVE shedding.

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