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Reversibility of the effects of GnRH-vaccination used to suppress reproductive function in mares

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Summary

Reasons for performing study: Active immunisation against gonadotrophin-releasing hormone (GnRH) provides a reversible method for control of oestrous behaviour and fertility in mares. Previous reports failed to demonstrate the interval to resumption of cyclic ovarian activity after GnRH-vaccination.
Hypothesis: Administration of the GnRH-vaccine Improvac in a large group of mares of various ages will result in effective, reliably reversible suppression of ovarian activity within a 2 year period.
Methods: The mares, subdivided into 3 age categories, were vaccinated twice (with a 35 day interval) using 400 μg Improvac and monitored via blood samples until Day 720 after initial vaccination for serum progesterone concentration determination by radioimmune assay and anti-GnRH antibody titre by enzyme immunoassay. Samples were collected until individuals resumed cyclic ovarian activity.
Results: All mares showed suppression of cyclic ovarian activity by clinical examination and serum progesterone concentration (SPC) ≤1 nmol/l by Day 70 and 92.2% resumed cyclic activity by SPC at Day 720 with a mean interval = 417.8 days (s.d. ± 23.9; range 232–488 days, median 344 days). A significant age effect (P = 0.028) on the interval, but not on GnRH-antibody titre response, was observed between the youngest (≤4 years) and oldest (≥11 years) categories.
Conclusions: Immunising adult mares of all ages with Improvac resulted in a reversible suppression of cyclic ovarian activity in most mares. An age effect, with the youngest mares showing a longer interval to reversibility, was observed.

Keywords: horse; mare; GnRH-vaccination; anoestrus; reversibility; titres

Introduction

Suppressing reproductive function and associated oestrous behaviour in mares has been managed by several approaches. Pharmacological methods utilising progesterone or progestins and potentially reversible GnRH-agonist or -antagonist therapy and their associated shortcomings including costs, practicality and potential negative side effects are reported [1–3]. Surgical ovariectomy is generally considered to be undesirable, due to its associated risks and irreversible loss of breeding potential [4].

Various immunocontraceptive methods have been used to control fertility in mares. Although porcine zona pellucida (pZP) vaccine is effective, it neither prevents mares from cycling nor suppresses oestrus-related behaviours [5]. Gonadotrophin-releasing hormone (GnRH) vaccines utilise a modified form of GnRH hormone conjugated to a foreign protein administered to stimulate the production of anti-GnRH antibodies. These antibodies neutralise endogenous GnRH, preventing natural binding to receptors on the pituitary gonadotrophs, suppressing pituitary secretion of follicle stimulating hormone (FSH) and luteinising hormone (LH) [6–8]. Studies report successful but variable suppression of ovarian activity [6–13]. Age effects on response and of using serum progesterone concentration (SPC) to reliably monitor cyclic ovarian activity have been reported [8,12]. Short monitoring periods generally limit these reports. Reliable reversibility and the associated duration of anoestrus induced by vaccination have not been reported.

This study investigated the reversibility of anoestrus over a period of 2 years in a large group of mares of different ages after 2 treatments with the GnRH-vaccine Improvac[a].

Materials and methods

Experimental design

A previous controlled study reported on the efficacy, safety and interval to suppression of reproductive activity after vaccination of nonpregnant mares with Improvac[a] [12]. The current study was designed as a continuation to observe the reversibility of treatment. Mares (n = 51) aged between 3 and 17 years and either Thoroughbred or similar light-horse type, were subdivided into 3 age categories: Category 1 (≤4 years, n = 25), Category 2 (5–10 years, n = 16) and Category 3 (≥11 years, n = 10). The trial was approved by The University of Pretoria's Animal Use and Care Committee (V068/05).

GnRH immunisation

The mares were injected into the gluteal muscles with 2 ml Improvac[a] (400 μg GnRF-protein conjugate) approximately 3 months after the onset of the physiological breeding season in South Africa. All showed cyclic reproductive activity via transrectal palpation and ultrasound examination at the time of vaccination. One booster (2 ml) was administered 35 days after primary vaccination. By 70 days after primary vaccination, 100% of mares showed SPC reflecting anoestrus concentrations and ovarian activity was absent on clinical examination [12]. Resumption of ovarian activity was henceforth defined by a blood sample exceeding a baseline SPC prospectively set at ≤6 nmol/l, the reported upper threshold concentration for winter anoestrus [11].

Blood sampling

Blood samples were collected by jugular venipuncture from Day 232 after primary vaccination at 2-weekly intervals until resuming cyclic activity or failing that, until the end of the study on Day 720. The blood samples were collected in plain Vacutainer tubes[b] and left to clot. Serum was separated by centrifugation (4000 × ***g***, 10 min) on the same day before storage at -20°C until assayed for both SPC and anti-GnRH antibody titre.

Serum progesterone assay

Assays for SPC were conducted by means of radioimmune assay (RIA)[c]. Assay sensitivity was 0.06 nmol/l. The main cross-reactivity was with progesterone (100%), 5α-pregnane-3,20-dione (9.0%), 17α-hydroxyprogesterone (3.4%) and 5β-pregnane-3,20-dione (3.2%). The intra- and inter-assay coefficients of variation were 8.8, 3.6 and 2.7 and 9.7%, 3.9 and 3.9% for low, medium and high concentrations, respectively.

GnRH-antibody titre determination

The anti-GnRH titres were determined by enzyme immunoassay (EIA), using a modification of the method described by Elhay *et al*. [11]. Briefly, 96 wells

MaxiSorp MTPs[d] were coated overnight (ON) at 4°C with 10 μg/ml GnRH peptide in sodium carbonate buffer at pH 9.6. The plates were washed with phosphate-buffered saline (PBS) containing 0.05% Tween 20 and then blocked ON at 4°C with 0.3% BSA in PBS. Plates were incubated with serial dilutions (dilution factors 200, 400, 800, 1600, 3200, 6400, 12,800) of test mare serum and standards for 1 h at 37°C. The plates were washed and bound antibody was detected by incubating plates with protein G-horseradish peroxidise (HRP)[e] for 1 h at 37°C. Subsequently, plates were washed and bound protein G-HRP was visualised with tetramethylbenzidine microwell peroxidase substrate[f]. Pooled samples of high titre (high standard) at Day 70 and low titre (low standard) at Day 35 after primary vaccination and samples of unvaccinated horses (negative control) served as reference points for the unknown samples on all plates analysed.

The plates were normalised by dividing all optical densities (ODs) by the maximal OD, thereby setting the high standard in 1:200 dilution to 1.0 for each plate. Subsequently, a negative titre threshold was defined as OD of the average of the negative controls plus 2 s.d. In this regard, titres were determined as the first OD exceeding the negative titre (negative control) threshold (starting from dilution 1:12,800 and decreasing). For method validation, titres of standards and positive controls were recorded for all 26 plates. The high and low standard titres were 3200 for all 26 plates and 800 for 18 plates and 400 for the remaining 8 plates, respectively. The negative control titres were 0 for all plates.

Data analysis

Data analysis was performed using Sigma Stat[g]. A Kruskal–Wallis one way analysis of variance (ANOVA) was performed on the ranked data to assess significance of age on the variables for general linear models. A Dunn's Multiple Comparison Test for means separation was also performed with Category 1 as control. Statistical significance was defined as $P<0.05$.

Results

Interval to resumption of cyclic activity

By Day 720, 47/51 (92.2%) mares had resumed cyclic activity with a mean interval of 417.8 days (s.d. ± 23.9; range 232–488 days, median 344 days). The remaining 4 mares (all Category 1) were still in anoestrus.

Effect of age on resumption of cyclic activity and GnRH-antibody titres

The median interval in Category 1 was significantly greater than in Category 3 mares ($P = 0.028$) (Fig 1). The mean anti-GnRH-antibody titres of 6 mares randomly selected from each of Categories 1 and 3 at 70 days post primary vaccination and at 6 weeks prior to resuming cyclic activity showed no significant difference (Fig 2) although mean titres were higher at both intervals for Category 1 mares.

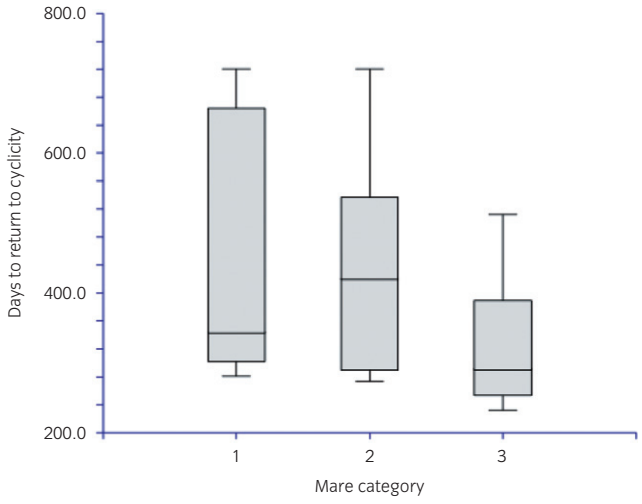

Fig 1: Box plot of days to return to cyclicity for different age categories of mares. Category 1 (≤4 years, n = 25), Category 2 (5–10 years, n = 16) and Category 3 (≥11 years, n = 10).

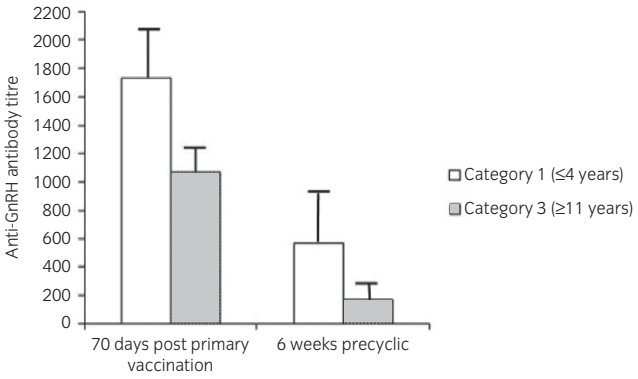

Fig 2: Mean (s.e.) gonadotrophin-releasing hormone (GnRH) antibody titres for selected mares in Categories 1 and 3 at 70 days post primary vaccination and 6 weeks prior to resumption of cyclic activity.

Discussion

In previous GnRH-vaccine studies, animal numbers were either small or monitoring discontinued before most had reversed. Dalin *et al*. [8] treated 3 mares with GnRH-bovine serum albumin-conjugate vaccine, 2 responded with reversal at 13.5 and 15 months after primary vaccination, respectively. Imboden *et al*. [10], treating 9 mares with Improvac[a], reported down-regulation of ovarian activity in all mares, but no reversibility in 4 mares within a 2-year period. Elhay *et al*. [11] reported using Equity[h] in 24 mares. No treated mare exceeded anoestrus SPC threshold levels at 20 weeks and ovarian shutdown lasted 4–23 weeks in 10/16 mares. The other 6 mares remained inactive at the end of the 29–34 weeks observation period. Killian *et al*. [13] comparing contraceptive methods, treated 15/43 mares with a single-shot GnRH-vaccine (GonaCon). The rates of contraception in these mares decreased from 94–40% between years 1–4, but time to resumption of cycling was undetermined.

The resumption of cyclic ovarian activity was used to monitor the reversibility of anoestrus induced in all mares following Improvac[a] administration. By Day 720, all but 4 mares (all Category 1) had resumed ovarian activity with a median interval of 344 days. The effect of vaccination on subsequent reproductive performance in these mares was not observed. A significant effect of mare age on reversibility was found between the oldest and youngest categories. Stout and Colenbrander [2] similarly reported 2 treatments of GnRH-tandem-dimer vaccine suppressed testosterone secretion in young sexually-mature stallions whereas older stallions generally required further boosters. In this study, although anti-GnRH-antibody titres were not significantly different between youngest and oldest mares, the mean titres were higher for the youngest and may explain their later return to cyclic activity.

In conclusion, variable reversal of anoestrus was shown in 92.2% of mares within 2 years after vaccination with Improvac[a]. Older mares showed significantly earlier resumption of cyclic ovarian activity than the youngest mares.

Conflicts of interest

No conflicts of interest have been declared.

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Manufacturers' addresses

[a]Pfizer Animal Health, Sandton, South Africa
[b]BD Vacutainer Systems, Plymouth, UK
[c]Coat-a-Count, Diagnostic Products Corp, Los Angeles, California, USA
[d]Nunc, Roskilde, Denmark
[e]Sigma-Aldrich, Steinheim, Germany
[f]KLP, Gaithersburg, USA
[g]Systat Software Inc., San Jose, California, USA
[h]Pfizer Animal Health, West Ryde, New South Wales, Australia

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