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Research article

GnRH vaccine could suppress serum progesterone level in Thai pony mares; A preliminary study

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Abstract

Colic-like discomfort during periovulatory period was one of the problems affecting athletic performance in horses. Objective of this study was to evaluate level of anti-GnRH antibody and serum progesterone in Thai pony mares after receiving GnRH vaccine. Hypothesis was that mares could produce anti-GnRH antibody, in which endogenous GnRH was neutralized, resulting in anestrus-like serum progesterone level after vaccination. Three Thai pony mares received GnRH vaccine 200 µg intramuscularly at week 0, 4 and 12. Each horse was examined for some clinical adverse reaction after each vaccination. Blood samples were collected every two weeks, starting from two weeks before first vaccination until week 16. All mares produced anti-GnRH antibody after first vaccination. Anti-GnRH antibody titers peaked at 2 weeks after each vaccination in 2 mares and at 4 weeks in one mare. Characteristic rise of progesterone level after ovulation was not observed in 2 mares from week 4-16, and in one mare from week 8-16. Anti-GnRH antibody titer was adequate to neutralize GnRH, resulting in anestrus-liked progesterone level up to 16 weeks after first vaccination with minimal clinical adverse reaction. The protocol in this study might be able to use for suppression of estrus in Thai pony mares.

Keywords: Anti-GnRH antibody, Anestrus-like, Progesterone suppression

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INTRODUCTION

Discomfort due to pain around ovulation period could influence mare in several aspects, such as undesirable behavioral changes, hyperexcitability, colic-like symptoms and reduction of athletic performance (Pryor and Tibary, 2005; Vanderwall, 2013). Suppression of estrous cycle could alleviate mares experiencing discomfort that related to normal physiology. Estrous cycle could be suppressed permanently by ovariectomy but was not suitable for prospective broodmare. Temporary estrus suppression could be performed by daily oral administration of altrenogest at 0.044 mg/kg. Care must be taken during administration since serious side effects might occur to both male and female human. Alternative method to suppress estrous cycle was immunization against gonadotropin releasing hormone (GnRH) which was most likely the best prospective agent in clinical use for temporary neuter the horses (Stout and Colenbrander, 2004). Hypothalamo-pituitary-gonadal axis could be interrupted using GnRH vaccine, in which Anti-GnRH antibody bound to endogenous GnRH. Absent of GnRH indicated no stimulation for releasing of follicle stimulating hormone (FSH) and luteinizing hormone (LH), resulting in suppression of ovarian activities and estrous cycle.

There are two commercial GnRH vaccines that has been used in horses. Equity® (Zoetis, Australia) has been licensed for horses with recommendation of 200 µg intramuscularly, two times at 4 weeks apart. In previous study (Elhay et al., 2007), mares immunized with 200 µg (Equity TM®) on day 0 and 28 could produce anti-GnRH antibody, concurrent with the decline of serum 17β-oestradiol level. The estrus-related behaviors were reduced which lasted a minimum of 3 months. Visible swelling at the injection site was observed after first vaccination, but there was no swelling in any mares after booster. The other GnRH vaccine, Improvac® (Zoetis, Australia) for suppression of boar taint in male pigs, is available in Thailand. In both products, Improvac® and Equity® has similar amount of active ingredient. Study using GnRH vaccine (Improvac®) in mares at 400 µg at week 0 and 4 resulted in absence of ovarian activity within 4 weeks after second injection (Imboden et al., 2006). Several adverse effects were observed up to 88%. The other study (Botha et al., 2008) using GnRH vaccine (Improvac®) at 400 µg at day 0 and 35 resulted in absence of ovarian activities in all 55 treatment mares at day 70. There was no swelling at injecting site after first vaccination, although 6 mares showed sign of swelling, while 2 mares exhibited swelling with lameness after second vaccination. It seemed that GnRH vaccine (Improvac®) has potential to suppress ovarian function in mares, but occurrence of adverse effects might drawback the use in clinical setting. More tissue reaction might due to increase in amount of antigen and adjuvant in the studies using Improvac® compared to Equity®. Further study for answering this suspicion might enhance the use of GnRH vaccine as a choice to suppress estrous cycle for mares in Thailand.

The aim of this study was to evaluate the effect of giving 200 µg GnRH vaccine at various times on level of plasma anti-GnRH antibody, serum progesterone and occurrence of clinical adverse effects. Hypothesis was that the treatment could induce adequate antibody titer to suppress GnRH, which in theory should suppress ovarian function, resulting in suppression of serum progesterone level.

MATERIALS and METHODS

Animals and GnRH vaccination

This study was approved by Animal Care and Use Committee, Faculty of Veterinary Medicine, Chiang Mai University (R10/2552). Three pony mares were participated (Mare 1, 2 and 3). Their ages were 15, 12 and 6 years old with body weights of 242, 301 and 293 kg, respectively. The week of first vaccination was indicated as week 0. Each pony received 200 µg GnRH vaccine (Improvac®, Zoetis, Australia) intramuscularly on week 0, 4 and 12. The injection site was indicated at 10 cm in front of scapular and in the middle between proximal and distal border of the neck. First and third injection were on the left neck while second injection was on the right neck.

Blood samples collection

Twenty ml blood samples were collected using 18G needle and 20-ml syringe on week -2, 0 and every 2 weeks until week 16. Each blood sample was divided in half and added into heparin tube and plain tube. Samples were kept in ice box during transportation and were centrifuged at 2000 g for 5 min. Plasma was kept at -70° C for anti-GnRH antibody analysis, and serum was kept at -20° C for progesterone analysis. Both plasma and serum were thawed at room temperature before analysis.

Plasma anti-GnRH antibody analysis

Sample was analyzed using direct non-competitive ELISA (Somgird et al., 2016; Zamaratskaia et al., 2008). Each well in 96-well plates was coated with 100 µl of 10 mg/ml GnRH (LFRH, L7134, Sigma Chemical Co., Singapore) at 1:1,000 dilution in 1 M sodium carbonate buffer, pH 9.6 and incubated overnight at 2-8° C. All plates and reagents were equilibrated to room temperature (RT) before analysis. Plates were washed five times, blocked with a protein buffer (0.01 M Na_2HPO_4, 0.001 M KH_2PO_4, 0.05 M thimerosal, 5% casein, 0.149 M NaCl, 0.003 M KCl) and incubated for 1 hour at RT. Plates were washed five times, loaded with 100 µl each of a control (from a vaccinated animal with IMPROVAC, Zoetis, Australia), diluted plasma samples (1:200), high and low controls, and incubated for 1 hour at RT. Plates were washed five time, added with 100 µl conjugate (rabbit anti-horse gamma globulin-peroxidase, A6917, Sigma Chemical Co., Singapore) at 1:10,000 dilution in buffer and incubated for 1 hour at RT. After washing, 100 µl ABTS substrate was added and incubated for 45 min at RT. Absorbance was read at 405 nm. Antibody titers were compared to standard curve and reported as value of percent binding at 1:200 dilution.

Serum progesterone analysis

Sample was analyzed using direct, single antibody competitive enzyme immunoassay (EIA) (Munro and Stabenfeldt, 1984; Thitaram et al., 2008). In brief, the progesterone EIA utilized a monoclonal progesterone antibody (1:10,000; Quidel clone #425), horseradish peroxidase-conjugated progesterone label (1:40,000; C. Munro, University of California-Davis), and proges-

terone standards (catalog #P0130; Sigma Chemical Co., St. Louis, MO). Sensitivity of the assay was 0.03 ng/ml. The inter-assay coefficient of variation (CV) for the high and low concentration controls were less than 15%, and the intra-assay CVs were less than 10%.

Clinical adverse effects

Each horse was examined before each vaccination and once a day for 2 days after each vaccination for clinical adverse effects, including body temperature, swelling and pain at injection site and ability to move the neck. Swelling and pain was scored as followed; 0: no swelling and no pain; 1: swelling less than 2 cm and no pain; 2: swelling more than 2 cm and pain. Pain was examined by hand pressing at injection site for 3 times. Mare that avoided pressure for 0-1 time was judged as no pain. Voluntary neck movement was tested by luring horse with feed to bend the neck until its mouth could reach left or right tuber coxa and pectoral area. Score was as followed; 0: mouth can reach desired point; 1: movement occurred more than 50% to desired point; 2: movement occurred less than 50% to desired point.

Data analysis

Data were evaluated using descriptive analysis for anti-GnRH antibody titer, progesterone level and occurrence of clinical adverse effects.

RESULTS

All mares produced anti-GnRH antibody after the first vaccination (Figure 1). Antibody titers in Mare 1 and 2 rose up at 2 weeks after first and second vaccination, while that of Mare 3 was at 4 weeks. The highest titers were at 2 weeks after third vaccination in all mares (Table 1). The values of anti-GnRH antibody were in Table 1. Progesterone levels in Mare 1 and 2 declined and was below 1 ng/ml during week 4-16 (Figure 1). Mare 3 had fluctuation of progesterone level which started to decline after second vaccination.

Slight fever was found in two different mares. Rectal temperature of Mare 3 was 39.4^{o} C on next day after first vaccination, while Mare 2 was 39.1^{o} C on next day after third vaccination. After received phenylbutazone, their temperature was below 39^{o} C on the following day. Injection site for Mare 3 was score as 1 after first vaccination, while all mares were score as 1 after third vaccination. Mare 1 and 2 were score as 1 for neck bending to the opposite side of injection site after first vaccination, which might due to uncomfortable sensation from stretching of injection site. Swelling and neck movement restriction was found only on the next day after vaccination.

Table 1 Highest level of anti-GnRH antibody (% Binding at 1:200 dilution) after vaccination at week 0, 4 and 12.

Subject	First vaccination		Second vaccination		Third vaccination	
	Level	Week	Level	Week	Level	Week
Mare 1	2.50	2	22.26	6	31.52	14
Mare 2	3.90	2	3.00	6	68.80	14
Mare 3	2.29	4	10.84	8	11.28	14

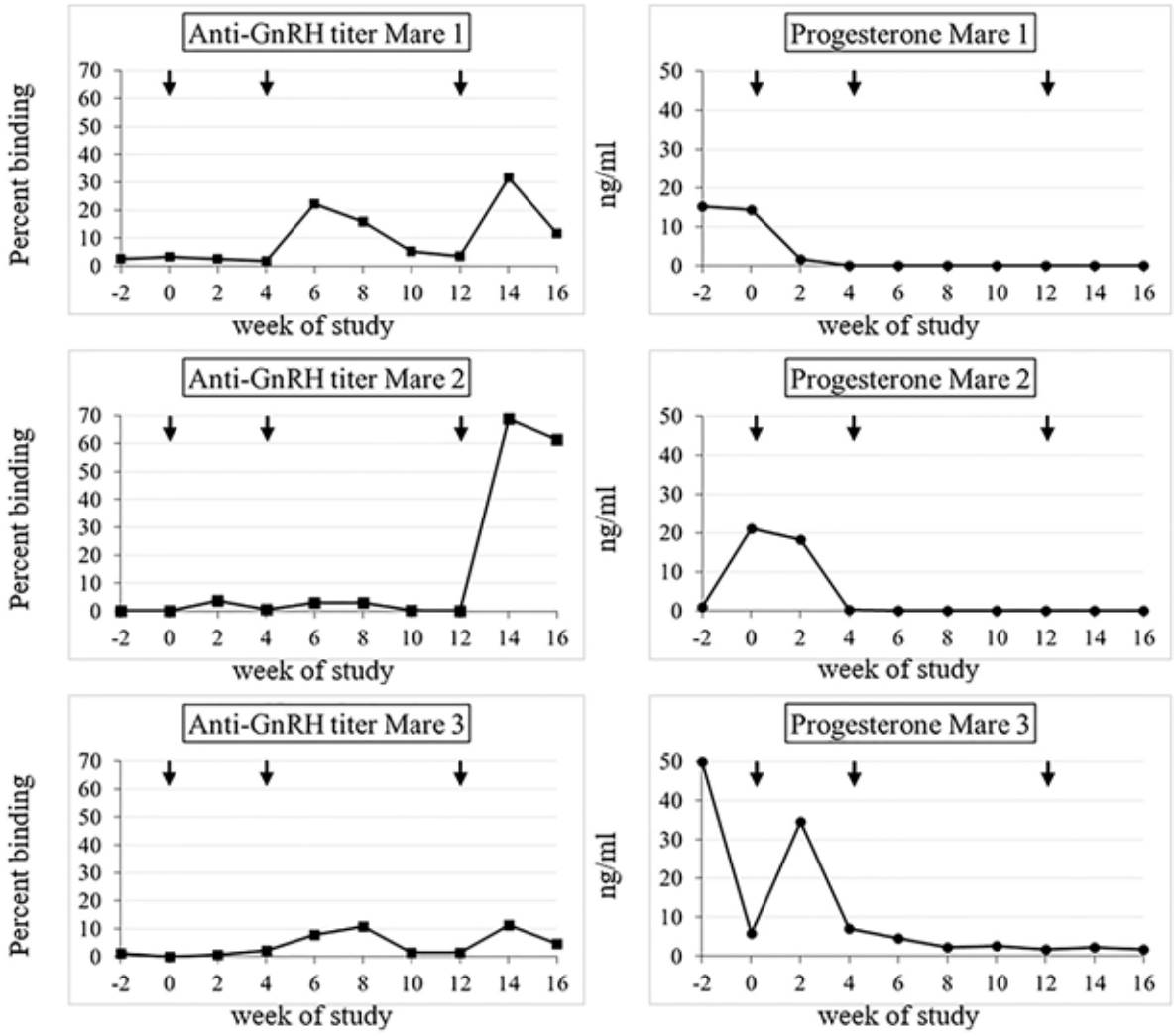

Figure 1 Anti-GnRH antibody titer and progesterone level in each mare. Vertical axis indicated value of anti-GnRH antibody (percent binding at dilution of 1:200) or value of serum progesterone (ng/ml). Horizontal axis indicated week of study, which the week of first vaccination was indicated as week 0. Arrows indicated time when each vaccine was given.

DISCUSSION

This study confirmed the efficacy of GnRH vaccine to suppress serum progesterone level in Thai pony mares, although the variations of onset of hormone suppression were observed. The highest progesterone level in Mare 3 was quite different from those of Mare 1 and 2. Several factors affected level of blood progesterone during estrous cycle in mares. Individual variation of progesterone levels occurred among mares during diestrus regardless of the numbers of ovulation (Nagy et al., 2004). Mares with double ovulation, resulting in two corpus luteum, had approximately 50% of blood progesterone level more than those with single ovulation (Valerie and Allen, 1983), which double ovulation occurred approximately 20% (Henry et al., 1982) and 35% (Nagy et al., 2004) in mares. The authors speculate that one of these factors might be a reason for high progesterone level finding in Mare 3. Although double ovulation condition was not confirmed for Mare 3 in this study.

Efficacy of GnRH vaccine on ovarian activity were related to level of antibody titer (Dalin et al., 2002). However, timing of vaccination during estrous cycle might also be important. Mare 1 and 2 received first vaccination when serum progesterone level was high, which mimic progesterone level in diestrus period. While Mare 3 received first vaccination when serum progesterone level was low, which mimic progesterone level close to estrus period. GnRH was released 2 pulses per hour during estrus, and reduced to be 2 pulses per day during diestrus (Satue and Gardon, 2013). Anti-GnRH titer of Mare 1 and 2 might be high enough to bind to amount of GnRH during diestrus, which in theory would inhibit FSH releasing and folliculogenesis, and also inhibit LH releasing and ovulation, resulting in absent of corpus luteum formation and low progesterone level during week 4-16. Anti-GnRH titer in Mare 3 might not sufficient to bind large amount of endogenous GnRH during estrus, which in theory the remaining GnRH could enhance FSH and LH secretion, resulting in high progesterone level during week 2-4. After the second vaccination, Mare 3 had higher Anti-GnRH titer than Anti-GnRH titer after the first vaccination, which might be adequate to neutralize GnRH, resulting in decline of progesterone level from week 8 thereafter. Therefore, progesterone suppression might take longer time in mare receiving GnRH vaccine during estrus period. This was corresponded with previous study (Imboden et al., 2006) that cessation time for ovarian activity was longer in mares vaccinated during estrus period.

The adverse effects in this study were minimal, compared to previous study (Imboden et al., 2006), which induced several adverse effects, such as swelling and pain at the injection site, stiffness of the neck, and fever. Some horses' temperature reached 39.8° C and duration of swelling and pain were 4-5 days. In other study (Botha et al., 2008), 55 mares treated first time with GnRH vaccine at gluteus muscle did not have injection site reaction, although there were 6 mares with visible swelling and 2 mares with swelling accompanied by lameness after booster vaccination. Study using GnRH vaccine at 200 µg (Elhay et al., 2007) resulted in tissue swelling in 8 from 24 horses after first vaccination and in one horse after second vaccination. Duration of swelling was around one week. In our study, two different mares

had slight fever after different times of vaccination, although the mares in our study had lower fever compared to previous study (Imboden et al., 2006). In our study, none of the mare showed sign of pain at the injection site. Swelling was small and subsided by one day. It seemed that protocol in our study could reduce some clinical adverse effects compared to previous studies (Elhay et al., 2007; Imboden et al., 2006).

There was precaution for using GnRH vaccine in prospective broodmare. Ninety two percent of mares treated with GnRH vaccine at 400 µg twice resumed cyclic activity at range between 232 – 488 days. Four mares (8%) did not have resumption for at least 720 days (Schulman et al., 2013). Ovarian activity resumed around 4-23 weeks in mares receiving GnRH vaccine at 200 µg twice (Elhay et al., 2007), although 37.5% of mares did not have folliculogenesis up to 34 weeks. This concern must be discussed with owner before prescribing GnRH vaccine to the mare.

CONCLUSION

GnRH vaccine 200 µg at week 0, 4 and 12 could induce anti-GnRH antibody production in Thai pony mares. Anti-GnRH antibody titer was adequate to neutralize GnRH, resulting in anestrus-liked progesterone level from 4-16 weeks after first vaccination. Adverse reaction was minor and may have no effect on equestrian athletic use.

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CONFLICT of INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

AUTHOR CONTRIBUTION

Siriporn Khumsap; Data collection, Data analysis, Manuscript preparation, Chatchote Thitaram; Laboratory analysis (serum progesterone), Read and approved the final manuscript. Chaleamchat Somgird; Laboratory analysis (anti-GnRH antibody), Read and approved the final manuscript.

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