Adenoviral-vectored gonadotropin releasing hormone vaccine for estrus suppression in mares

Rochelle Jensen,[a] Aime Johnson,[a] Robyn Wilborn,[a] Timothy Braden,[b]
Michelle Kutzler,[c] Scot Roberts,[d]
Kent Van Kampen,[e] Joshua Trumble,[a] Henry Baker[f]
[a]College of Veterinary Medicine, Auburn University, Auburn, AL
[b]Department of Anatomy Physiology and Pharmacology, Auburn University, Auburn, AL
[c]Department of Animal and Rangeland Sciences, Oregon State University, Corvallis, OR
[d]Altimmune Inc. Gaithersburg, MD
[e]The Van Kampen Group, Inc., Payson, UT
[f]Scott Ritchey Research Center, Auburn University, Auburn, AL

Abstract

Objective of this study was to evaluate an adenoviral-vectored gonadotropin releasing hormone vaccine's ability to temporarily suppress reproductive cyclicity in mares. Five mares were vaccinated twice, 30 days apart and 5 mares served as unvaccinated controls. Following year, 4 vaccinated mares and 2 naïve mares (adjuvant controls) were given a gonadotropin releasing hormone peptide-adjuvant vaccine containing 100 μg of gonadotropin releasing hormone. Antibody responses, ovarian follicular dynamics and serum progesterone concentrations were determined during 2 ovulatory seasons. During first year, vaccinated mares developed gonadotropin releasing hormone antibodies ($p < 0.05$), but there was no significant effect on estrous cyclicity. During second year, antibody responses increased ($p < 0.05$), accompanied by suppression of estrous cyclicity (determined by progesterone concentrations and transrectal palpation and ultrasonography). Three of 4 treated mares returned to cyclicity between 154 and 326 days, whereas the fourth mare was still in anestrus at study completion. Adjuvant control mares developed a short-lived, minimal antibody response and cycled normally. In conclusion, mares developed an immune response to homologous immunization using an adenoviral-vectored gonadotropin releasing hormone vaccine. Additional heterologous vaccination with a gonadotropin releasing hormone peptide-adjuvant vaccine demonstrated establishment of immune memory and achieved estrus suppression.

Keywords: Estrous cycle, horse, immunocontraception, interestrus interval

Introduction

During estrus, mares can become difficult to train and ride. This unpredictable behavior may endanger handlers and riders. Progesterone and other progestogens are used to suppress estrus in mares.[1-3] In United States, altrenogest is the most commonly used synthetic progestin product labeled for estrus suppression in mares.[1] Although effective for estrus suppression, clinical use of these products is limited due to high cost and need for daily administration. Other progestogens available in injectable forms can induce local swelling and muscle pain that can interfere with training and performance.[1,4] Intrauterine devices such as glass marbles prolong luteal phase in mares, but are unreliable and have a low success rate.[5-7] Hence there is a need for a reliable, cost-effective, and practical method for temporary suppression of cyclicity in mares.

Blocking gonadotropin releasing hormone (GnRH) receptor binding is effective in controlling reproduction and sex-related behavior. Due to its high degree of homology among mammals and its control of both male and female gonadal function, a method that suppresses GnRH can be used in many species in both sexes.[8-12] Synthetic agonists and antagonists suppressed reproductive function.[8,13-17] However, field use of GnRH agonists (such as deslorelin) for estrus suppression in mares resulted in variable response rates that are short-lived, requiring expensive repeated or continuous administration.[18-21]

AntiGnRH antibodies inhibit binding of GnRH to its receptor, preventing receptor activation and release of gonadotropins from anterior pituitary. This induces infertility and prevents reproductive behavior in many species.[11] One of the greatest challenges in formulating a GnRH vaccine that can induce a physiologically effective immune response is limited antigenicity of GnRH. Gonadotropin releasing hormone is weakly immunogenic, due to its small size (10 peptides) and recognition as 'self' by the immune system.[22]

GnRH peptide-adjuvant vaccine formulations effectively suppressed ovarian function and cyclicity in mares.[1,23-28] These vaccines caused a rapid increase in antiGnRH antibodies, reduced circulating progesterone and suppressed follicular activity.[23,26,27] Equity™ (Zoetis, Australia) is a commercial, protein-based, antiGnRH vaccine labeled for use in horses and licensed in Australia, but not in United States. Initial and booster doses of Equity™ (200 μg of GnRH in each dose) given 30 days apart resulted in estrus suppression from 3 months to > 2 years.[25-29] In addition to high variability in interestrus interval, there was a high incidence of adverse effects. Some effects were mild (e.g. local injection site swelling) or more severe, to include transient fever.[27,29] These adverse reactions limited field use of GnRH peptide-adjuvant vaccines in high-performance mares. Mares that suffer skin swelling, neck pain, or fever may not perform optimally.

Viruses engineered to express antigenic proteins can induce an immune response and improve vaccine safety and efficacy. For example, the human adenovirus variant 5 (Ad5) vector induced potent humoral and cell-mediated immune responses, had intrinsic adjuvant properties, induced innate immunity, had effective memory response, provided a natural presentation of immunogens and had broad host tropism.[30,31] Nonreplicating Ad5-vectored vaccines are adaptable for induction of protective immunity and did not vary significantly as a function of either antigen expressed or species immunized.[30,32-35] Prime-boost immunizations can be given with unmatched delivery vectors (heterologous vaccination) and in many cases, were more effective than homologous prime-boost delivery modality.[36]

Objective of this study was to determine if an adenovirus vector engineered to express GnRH antigen (Ad-GnRH) could induce antiGnRH antibodies in mares following homologous prime-boost vaccinations, sufficient to suppress cyclicity. It was hypothesized that this vaccine is antigenic with no adverse reactions in immunized mares. Second objective was to utilize a heterologous vaccination strategy in which administration of a subtherapeutic dose of a protein-antigen based GnRH (P-GnRH) vaccine could be used to determine if homologous prime-boost vaccination of mares with Ad-GnRH elicited immune memory. Prime-boost immunizations can be given with unmatched delivery vectors (heterologous vaccination) and in many cases, were more effective than homologous prime-boost delivery modality.[36] The P-GnRH dose used was less than total prime-boost dose recommended by the manufacturer (2 x 1.0 ml injections each containing 200 μg of GnRH, given 4 weeks apart (Equity™, Zoetis).[29] It was hypothesized that vaccination of mares using a protein-antigen construct containing 100 μg (0.5 ml) of GnRH elicits sufficient GnRH antibody production to suppress estrous cycle only in mares primed with Ad-GnRH.

Materials and methods

Experimental design

Twelve, nonpregnant light-horse cycling mares (14 - 23 years) that belonged to Auburn University Equine Reproduction Center teaching herd were used. Mares were housed by groups in large pens and fed free-choice Bermuda hay supplemented with grain. Study was conducted over 2 consecutive ovulatory seasons, starting May 2015. During first season, 5 mares were randomly assigned to control (Ad-GnRH control) and 5 mares to treatment (Ad-GnRH) groups. Treatment consisted of 2 intramuscular injections of Ad-GnRH vaccine. Each TR mare received 1 ml dose containing 4.64×10^{10} infectious units (IFU) into the left cervical musculature, followed by a booster after 30 days. Vaccine effects were measured for 12 months. The Ad-GnRH control mares were monitored for cyclicity and seasonality.

Because antibody response to this immunization schedule was ineffective to suppress cyclicity, a heterologous vaccination strategy was utilized during the subsequent ovulatory season.[37] Following spring, 4 of 5 Ad-GnRH mares were given a single 100 μg (0.5 ml) intramuscular injection of a P-GnRH vaccine. To determine effects of a single administration of this vaccine at this low dose, 2 unvaccinated mares were given similar single dose (100 μg; P-GnRH control). Effects of P-GnRH vaccination were measured for 12 months. Data collection for this project ended 23 months after initial vaccination with Ad-GnRH.

Vaccine

The Ad-GnRH vaccine vector consisted of a thoroughly characterized E1/E3 deleted Ad5 human adenovirus produced for use in animal immunization.[30,31,38,39] This vector expressed a nucleotide sequence consisting of a tissue plasminogen activator (tPA) leader sequence followed by multimers of GnRH (EHWSYGLRPG) linked to the leukotoxin A1 gene of *Pasteurella haemolyti*ca (LKT) and T-helper epitopes. The Ad5 vector vaccine was synthesized by GenScript (Piscataway, NJ), and cloned into the Ad5 vector to generate Adenovirus 5-*Pasteurella haemolytica*-gonadotropin releasing hormone (AdLKTGnRH) vaccine, as described.[40] Adenovirus 5-*Pasteurella haemolytica*-gonadotropin releasing hormone vaccine virus was propagated on HEK293 cells, purified on a cesium chloride gradient, sterilized using 0.22 µm filtration and stored at -80ºC in a formulation buffer.[41] Viral titer was determined by Adeno-XTM rapid titer kit (BD Biosciences, Palo Alto, CA) on HEK293 cells. Correct structure of the antigen was verified by DNA sequencing (Genewiz, Germantown, MD). Each 1 ml dose of vaccine contained 4.64 x 10^{10} IFU of vector.

Assays

AntiGnRH antibodies and serum progesterone concentrations were monitored throughout the study. A blood sample was collected by jugular venipuncture from each mare immediately prior to initial vaccination with Ad-GnRH vaccine, then monthly thereafter. Serum was separated, aliquoted, and frozen (-80ºC) until analyzed.

Serum antiGnRH antibody was determined with ^{125}I-GnRH (L8008, Sigma-Aldrich, St Louis, MO) using a radioimmunoprecipitation technique. Samples were assayed in duplicate using 100 µl of ^{125}I-GnRH added to 100 µl of test serum diluted 1:100 in 200 µl buffer. After overnight incubation at 4ºC, 100 µl of bovine IgG (250 µg) was added (Sigma-Aldrich) and bound ^{125}I-GnRH-Ab complexes precipitated from unbound hormone by adding 500 µl of a 24% solution of polyethylene glycol (Carbowax™ PEG 8000, P156-500, Thermo Fisher Scientific, Waltham, MA). Reaction tubes were centrifuged at 1400 x g for 15 minutes and radioactivity in the precipitate was measured using a gamma counter (Packard, Cobra ll, Ramsey, MN). Nonspecific binding (NSB) of ^{125}I-GnRH was determined from the mean of duplicate tubes in which diluted serum was replaced by PBS/BSA buffer. Mean NSB was subtracted from individual sample measurements. Serum antiGnRH antibody bound to ^{125}I-GnRH was expressed as a percentage of a known internal standard positive control of rabbit antiGnRH antisera used in this laboratory. Internal standard negative control serum was also of rabbit origin.

Serum progesterone concentrations were determined using validated chemiluminescence immunoassay (Immulite® Diagnostic Products Corporation, Los Angeles, CA). Lower detection limit was 0.2 ng/ml and inter- and intra-assay CVs were < 10%.[27]

Ovarian and uterine activity

Transrectal palpation and ultrasonography (5 MHz linear-array transducer, MicroMaxx, Sonosite Inc, Bothell, WA) of reproductive tracts were performed. Presence of corpus luteum (CL) and nature of uterine edema (none, slight, moderate, or heavy) were recorded. Diameter of largest follicle on each ovary was measured. During ovulatory season, ultrasonography was performed twice weekly (Months 1 - 7 during Year 1 and Months 13 - 19 during Year 2). During nonovulatory season, ultrasonography was performed once weekly Months 8 - 12 during Year 1 and Months 13 - 20 during Year 2).

Interestrus interval

Interestrus interval (IEI) was measured from ovulation of 1 or more dominant estrus follicles to ovulation of the succeeding estrus dominant follicle/s. Diestrus was determined based on ultrasonographic evidence of a CL and serum progesterone concentration > 2 ng/ml and anestrus based on no or minimal uterine edema observed concurrent with no or minimal ovarian follicle growth, no ultrasonographic evidence of a CL and serum progesterone concentration < 1 ng/ml.[23,28] Suppression of cyclicity was defined as presence of anestrus during the breeding season. A return to cyclicity was defined by a period of estrus, growth and

ovulation of $\geq$ 1 dominant follicles and subsequent establishment of a CL that maintained serum progesterone concentrations > 1 ng/ml for ~ 7 - 18 days.

Vaccine-induced adverse effects

Vaccine-induced adverse effects were monitored by twice-daily physical examination and inspection of injection site for 3 consecutive days, or until vaccine-induced adverse reaction resolved, whichever was the longest. For the first 3 days after vaccination, mares continued to be monitored by twice daily observations only (inspection of injection site and overall demeanor). All animal procedures were approved by the Institutional Animal Care and Use Committee, Office of Animal Resources, Auburn University.

Data analyses

Data were analyzed using Minitab®18 Statistical Software (State College, PA) and summarized using Microsoft Excel (1808, Santa Rosa, CA). Presence of antiGnRH antibodies over both ovulatory seasons was analyzed using ANOVA and was measured based on an upper 99% confidence interval of 3.32%. Interestrus interval was examined using Wilcoxon rank sum test. Significance was set at $p < 0.05$.

Results

AntiGnRH antibody

All mares were seronegative for antiGnRH antibody prior to first vaccination (Month 1). Effects of both Ad-GnRH and P-GnRH vaccination are summarized in Figure 1. Three of 5 Ad-GnRH mares responded to the initial Ad-GnRH vaccination with antiGnRH antibody production by Day 30, on the day of Ad-GnRH booster. All Ad-GnRH mares produced antiGnRH antibody following the Ad-GnRH booster vaccination. Maximum antibody response for within Ad-GnRH mares varied but were within the range of 3.59 to 36.01% relative to the internal standard. These responses occurred at different time points for individual mares but ranged from July (Month 2) to November (Month 6). Duration of continued antibody production also varied between individual mares and ranged from 2 to 11 months after initial Ad-GnRH vaccination.

All Ad-GnRH mares responded to P-GnRH vaccination with production of antiGnRH antibody, with no response from Ad-GnRH control mares. There was an effect of P-GnRH vaccination ($F [1,106] = 147.17$, $p < 0.05$). Maximum individual antiGnRH antibody production following P-GnRH vaccination within Ad-GnRH mares ranged from 35.54 to 89.72%. Maximum antibody production for individual mares occurred at various time points, but occurred from July (Month 14) to February (Month 21). AntiGnRH antibody was still measurable in all Ad-GnRH mares at study completion (24 months). AntiGnRH antibody was not measurable in sera collected from P-GnRH control mares prior to P-GnRH vaccination. Maximum antiGnRH production following P-GnRH vaccination for the 2 P-GnRH control mares was 7.72 and 6.45%, which occurred during June (Month 13) and July (Month 14), respectively.

Interestrus interval

Following homologous prime and boost Ad-GnRH vaccinations, 4 of the 5 Ad-GnRH mares displayed normal IEI (mean IEI: 23 ± 2 days), not different from Ad-GnRH control mares (mean IEI: 22 ± 2 days) ($p > 0.05$). One Ad-GnRH mare (mare #5) experienced 2 prolonged luteal phases (70 and 91 days, respectively). Data for this mare were excluded for statistical analysis of IEI because interestrus intervals were > 33 days, 1.5 times the interquartile range above the third quartile of all data.

Immediately prior to P-GnRH vaccination, 3 of the 4 remaining treatment mares displayed normal IEIs (25 ± 4 days). The same mare that had experienced prolonged luteal activity during the first ovulatory season (Ad-GnRH mare #5) also experienced prolonged luteal activity that extended into the second ovulatory season, but data for this mare were also not considered for analysis of IEI prior to P-GnRH booster vaccination.
By 30 days post P-GnRH vaccination, cyclicity was suppressed in all treatment mares. Three of 4 Ad-GnRH mares returned to cyclicity between 154 and 326 days following vaccination. Mare Ad-GnRH #5 was still in anestrus at study completion. Serum progesterone concentrations and antiGnRH antibody responses were not

evaluated after study completion, but continuation of twice-weekly transrectal ultrasonographic examinations revealed that anestrus persisted until 1088 days from the time that the mare received the P-GnRH vaccination. Two P-GnRH control mares exhibited normal IEI (27 ± 3 days).

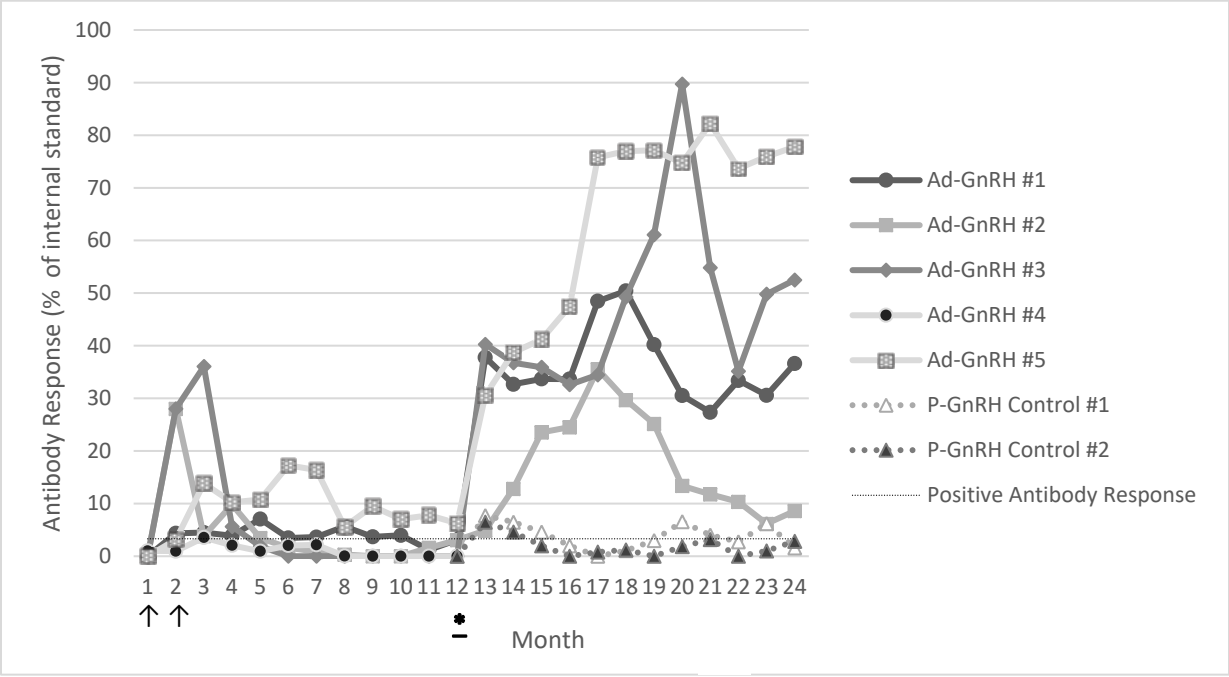

Figure 1. AntiGnRH antibody responses of individual mares following homologous Ad-GnRH initial and booster vaccination and P-GnRH vaccination. Time of initial and booster Ad-GnRH vaccination (↑) and the P-GnRH vaccination (*). Data for Ad-GnRH control mares not shown. Ad-GnRH mare #4 was removed from the study prior to P-GnRH vaccination, due to a chronic injury requiring euthanasia.

Progesterone concentrations

Following initial and booster Ad-GnRH vaccinations, serum progesterone concentrations for 4 of the 5 Ad-GnRH mares (Figure 2), all of which experienced normal IEI, reflected normal cyclicity. Based on once-monthly progesterone samples, there was minimal effect of seasonality on cyclicity. During Months 7 and 9, only 2 of the 5 mares had baseline serum progesterone concentrations were also consistent with anestrus, based on transrectal ultrasonographic examinations. During Month 8, 4 of 5 treatment mares had baseline serum progesterone concentrations.

By 30 days after P-GnRH vaccination, serum progesterone concentrations of all treatment mares had declined to baseline (< 2 ng/ml). Serum progesterone concentrations for the P-GnRH control mares reflected normal cyclicity throughout the study period.

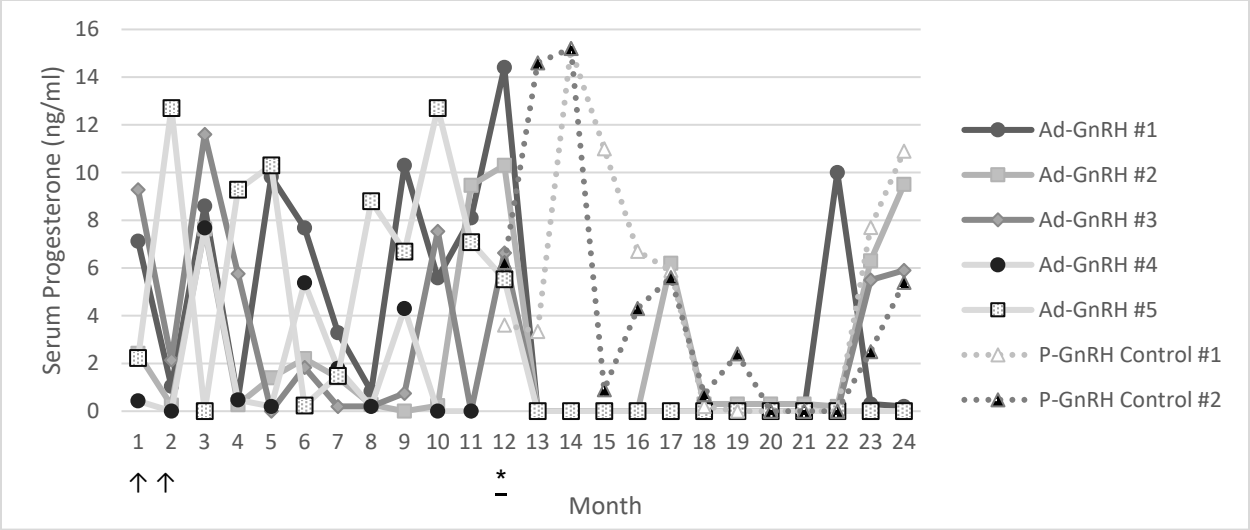

Figure 2. Progesterone concentrations for individual treatment mares (Ad-GnRH) following homologous prime and boost Ad-GnRH vaccination (arrows). Progesterone concentrations for both Ad-GnRH and P-GnRH control mares (P-GnRH control) following administration of the P-GnRH vaccine (*) during the subsequent ovulatory season (Month 12).

Adverse vaccine reactions

There were no systemic vaccine reactions observed after any vaccination. There were no vaccine site reactions observed following the initial Ad-GnRH vaccination. One day following Ad-GnRH booster vaccination, 3 of 5 treatment mares developed a nonpainful 1 - 3 cm raised nodule at the injection site that resolved within 3 days without treatment. Following P-GnRH vaccination of Ad-GnRH mares, 2 of 4 mares developed a small (< 2 cm) raised, nonpainful nodule that resolved without treatment within 3 days. P-GnRH control mares did not experience any adverse, vaccine-associated reactions.

Discussion

Two immunizations 30 days apart with an Ad-GnRH vaccine stimulated production of anti-GnRH antibody in all treated mares, but estrous cyclicity was not suppressed. The antiGnRH antibody response that occurred in Ad-GnRH treatment mares following P-GnRH vaccination effectively suppressed cyclicity. It is already known that P-GnRH vaccination at the labeled dose results in antiGnRH antibody production and suppression of cyclicity.[29] At the dose used in the current study (25% of labeled initial dose), transient low-level anti-GnRH antibodies were produced in control mares, which had no effect on cyclicity. This confirms that the dose used in the current study was able to elicit immune memory induced by Ad-GnRH priming. Vaccine-mediated immune responses require not only an antigen-specific increase in antibodies, but also production of antibodies with high avidity and persistence of vaccine antibodies and/or generation of immune memory cells capable of rapid and effective expansion upon subsequent exposure.[42]

Duration of estrus suppression was highly variable between mares, ranging from 154 days to > 35 months, consistent with other studies evaluating effects and duration of GnRH immunization in mares.[25,27,28] Duration of effect of the same GnRH peptide-adjuvant vaccine utilized for a heterologous vaccination strategy (similar to current study) was from 6 weeks to > 2 years.[29] Cyclicity was suppressed up to 2 years or more with youngest mares (≤ 4 years) experienced a longer interval from vaccination to returned cyclicity when compared to older mares (≥ 11 years), with no change in the titer.[25] All mares used in the current study were ≥ 14 years of age. Further research on Ad-GnRH vaccination of mares warrants incorporation of evaluation of age effect on both period of anestrus following vaccination, and GnRH antibody response.

Heterologous vaccination strategy including administration of a P-GnRH vaccination resulted in temporary estrus suppression in 3 of 4 treated mares, whereas Mare Ad-GnRH #5 was still anestrus at study completion.

Evaluation of duration of estrus suppression cannot be discussed without consideration of reproductive seasonality of mares. Mares naturally enter anestrus during the fall and winter. At a latitude of 38° North, the mean date of anestrus onset in young mares (< 5 years of age) is November 6th (range October to January), with an average duration of anestrus being 176 ± 30 days.[43] Mature mares at this same latitude (> 10 years of age) generally continue to exhibit normal cyclicity at the time that younger mares become seasonally anestrus, with the mean time of onset of anestrus occurring during the first week of January. As a result, mature mares experience a relatively shorter duration of anestrus (75 ± 18 days). All mares used in this experiment were mature (> 10 years of age); therefore, induction of estrus suppression was attributed to vaccine effects and not seasonality. Additionally, all control mares continued to cycle at the time of year that treatment mares first experienced a cessation in reproductive cyclicity. The highest magnitude of antibody response was measured from treatment mares during the interval when mares may become seasonally anestrus, and a return to cyclicity was observed at a time of the season that mares would be expected to return to cyclicity as a result of seasonality, in the absence of GnRH immunization. While this makes objective measure of duration of vaccine effect challenging, all treatment mares remained anestrus as a direct result of vaccine-induced effects for least 154 ± 3 days. In a clinical setting, this interval spans the majority and in some mares the entire, ovulatory season.

One of the major challenges to GnRH immunization is to overcome the phenomenon of tolerance to the GnRH antigen. A variety of carrier proteins have been used successfully to overcome tolerance and enhance induction of immunity against GnRH.[9,12,23,26] The Ad-GnRH vaccine used in this study contained the carrier protein bacterial leukotoxin (Leukotoxin A1 gene *Pasteurella haemolytica*). Similar carrier protein used in the study was used in an earlier vaccine.[45] This vaccine consisted of 8 tandem repeats of GnRH fused to each terminus of a 52 Kda fragment of leukotoxin A (*Pasteurella haemolytica*). Cats and mice immunized with an Ad5 vector and protein vaccines expressing multiple copies of the GnRH antigen ligated to this leukotoxin carrier peptide experienced an antibody response that inhibited gonadal development.[45] Vaccine used in this study had the same structure as that used safely in cats and mice. Estrus suppression was not achieved by 2 immunizations with the Ad-GnRH vaccine in this study. There was an apparent induction of immune memory. This is evidenced by the fact that the anti-GnRH antibody response in treatment mares was significantly different from that in P-GnRH control mare and was therefore a result of Ad-GnRH priming. In addition to the construct of vaccine carrier, dose of an adenoviral vector can influence consistency and strength of the immune response to an expressed antigen.[46] Because there was an observed antiGnRH antibody response to Ad-GnRH vaccination that did not effectively suppress cyclicity, it is possible that the Ad5 vector dose used may have been inappropriate to elicit enough antiGnRH antibodies to inhibit GnRH activity. It has been shown that antibody production must reach a threshold to impede GnRH activity.[23,44] Immunogenicity of an Ad5 vector encoding mycobacterial antigens using a heterologous prime-boost vaccination regime was evaluated in cattle.[46] Cattle were primed with live attenuated *Mycobacterium bovis* vaccine (Bacillus Calmette–Guérin vaccine) and Ad5 vectored vaccine served as the heterologous vaccine. An optimum dose of 2.0×10^9 IFU of the Ad5 vector given by the intradermal route conferred the most consistent and strongest immune response compared to lower doses.[46] Infectious unit dose used to vaccinate mares in this experiment was based on testing in laboratory mice using the accepted biopharmacological calculation-based method of determining body surface area. Based on this calculation, the dose required was anywhere from 4.64×10^{10} - 2×10^{11} IFU. Additional studies in mares are required to determine an optimal dose or route of administration that would provide an enhanced immune response to 2 immunizations with an Ad-GnRH vaccine.

All mares experienced minimal side effects following vaccination with Ad-GnRH and again following vaccination with P-GnRH. Minor reaction occurred at the injection site following Ad-GnRH and P-GnRH vaccinations. Following second Ad-GnRH vaccination and P-GnRH vaccination, 3 of 5 and 2 of 4 mares, respectively, developed a small (< 1 cm) injection site nodule that resolved within 3 days without treatment. This finding was interesting, due to reported high incidence of reactions following vaccination with other protein-based GnRH vaccines. Reported effects include raised nodules, swelling and pain at the injection site,[29] stiffness of the neck, pyrexia, and apathy.[23] These effects are often due to adjuvants. Development of a viral vectored vaccine therefore has promise for immunizing mares against GnRH without a need for adjuvants. Side effects observed in this study were not severe and would not be expected to interfere with athletic

performance. Based on previous work, adenoviral vectors seemed to be able to prime and boost B cell responses more effectively than other vectors.[47] This may be due to prolonged, high-level antigen expression following adenoviral vector vaccination, which favors B cell priming. Since modification of cyclicity was a primary objective of this study, a nonreplicating Ad-GnRH vaccine was a primary choice for suppression of cyclicity. Results of our study provided "proof of concept" that mares can mount an immune response and establish immune memory following Ad-GnRH prime-boost vaccination. Moreover, this immune response was enhanced with a P-GnRH vaccination administered at a fraction of labeled dose.

Conclusion

Homologous Ad-GnRH prime-boost vaccination using a replication-defective adenovirus vector encoding multimers of GnRH, bacterial leukotoxin and T-helper epitopes induced antiGnRH antibody in vaccinated mares. The dose, construct and/or frequency used in this study did not result in suppression of cyclicity. Despite this, mares developed immune memory that led to suppression of cyclicity when P-GnRH was administered 12 months later. Vaccinated mares experienced minimal, clinically insignificant local effects following vaccination with either Ad-GnRH or P-GnRH. These results provided a promising gateway for further studies with larger numbers and to develop a cost-effective and safe Ad-GnRH vaccine that can be used for reversible suppression of estrous cycle in mares.

Conflict of interest

Scot Roberts is an employee of Altimmune Inc. and has received company stock.

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