Available online at www.sciencedirect.com
ScienceDirect
Theriogenology 68 (2007) 67–75
Theriogenology
www.theriojournal.com

Continuous administration of low-dose GnRH in mares I. Control of persistent anovulation during the ovulatory season

G.L. Williams [a,b,*], M. Amstalden [a,b], G.P. Blodgett [c], J.E. Ward [d], D.A. Unnerstall [e], K.S. Quirk [f]

[a] *Animal Reproduction Laboratory, Texas A&M University Agricultural Research Station, Beeville, TX 78102, USA*
[b] *Center for Animal Biotechnology and Genomics, Department of Animal Science, Texas A&M University, College Station, TX 77843, USA*
[c] *6666 Ranch, P.O. Box 130, Guthrie, TX 79236, USA*
[d] *319 Houston, Nacogdoches, TX 75961, USA*
[e] *401 McCormick Road, Oak Point, TX 75068, USA*
[f] *Q6 Ranch, 16707 FM 2115, Salado, TX 76571, USA*

Received 23 October 2006; accepted 18 March 2007

Abstract

Three experiments were conducted during the operational breeding season to confirm that continuous, subcutaneous infusion of low-dose GnRH would not disrupt established estrous cycles (Experiment 1), and test the hypotheses that a similar treatment would stimulate secretion of LH and induce development of ovulatory follicles in persistently anovulatory mares (Experiments 2 and 3). Treatment with GnRH (5 μg/h) increased ($P < 0.001$) serum P4 during the luteal phase (7.7 ± 0.5 versus 6.4 ± 0.5 ng/mL), tended to increase serum LH (2.6 ± 0.27 versus 1.9 ± 0.25 ng/mL), and did not modify interovulatory intervals. In Experiment 2, GnRH treatment (2.5–5 μg/h) of persistently anovulatory mares increased ($P < 0.001$) serum LH compared to controls (0.5 ± 0.08 versus 0.1 ± 0.03 ng/mL), with all GnRH-treated and no Control mares ovulating. Mares exhibiting Delayed Recrudescence ($n = 29$) or Lactational Anovulation ($n = 18$), were assigned randomly in Experiment 3 to receive either (1) GnRH/GnRH ($n = 23$); 2.5 μg GnRH/h for 14 d (Period I) and 5 μg/h during the subsequent 28 d (Periods II and III); or (2) Control/GnRH ($n = 24$); no treatment during Period I (control period) and GnRH treatments as in 1 during Periods II and III. Percentage of mares ovulating and pregnant during Period I was greater ($P < 0.05$) for GnRH-treated than Control mares. Thereafter, cumulative ovulation frequency (85%), pregnancy (72%) and cycles/conception (1.3 ± 0.2) were similar between groups; however, interval to conception was reduced ($P < 0.01$) by 10.3 d in GnRH/GnRH compared to Control/GnRH.

Keywords: Lactational anovulation; GnRH; Mares; Estrous cycle; Idiopathic

1. Introduction

Equine seasonality is characterized in 85% or more of the standard horse mare population by a cessation of ovarian cyclicity between October and April in the northern hemisphere [1–3]. With 1 January serving as the universal birth date of foals in over 45% of current breed registries, seasonal anovulation remains a major managerial and economic issue in horse breeding [2,3].

The use of artificial illumination to extend day length to 15 to 16 h/d, beginning in mid-December [3,4], can hasten the onset of the breeding season in a majority of mares to as early as 1 March. However, some mares exhibit states of anovulation that persist well into both

* Corresponding author at: Animal Reproduction Laboratory, Texas A&M University Agricultural Research Station, 3507 Hwy 59E, Beeville, TX 78102-8571, USA. Tel.: +1 361 358 6390; fax: +1 361 358 4930.
E-mail address: glwilliams@tamu.edu (G.L. Williams).

0093-691X/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2007.03.024

the operational and natural breeding seasons, with or without light supplementation. These include early-foaling mares that have reverted to a seasonal-anovulatory state during lactation (lactational anovulation), and non-pregnant fillies or mares exhibiting delays in spring transition (delayed recrudescence). However, other cases emerge that are difficult to ascribe to the influence of photoperiod.

The efficacies and limitations associated with pulsatile and continuous infusions of GnRH and synthetic GnRH agonists (GnRHa) to hasten first ovulation in seasonally anovulatory mares and mares in early spring transition have also been reported [5–10]. Treatments have been highly variable in their ability to successfully and consistently establish ovulatory cycles [7] and, with only a few exceptions, have involved very limited numbers of mares [5,6,8]. Moreover, little attention has been paid to the responsiveness of mares that remain anovulatory after the start of the operational breeding season. In addition, while many of these studies have demonstrated a relative resistance of the equine pituitary to desensitization by the practical approach of continuous GnRH infusion [11,12], very large doses (20–100 μg/h) of the native molecule [5,6] and GnRHa [7,9,13] have produced evidence of desensitization. Therefore, objectives of the current studies were to: (1) confirm a lack of pituitary desensitization to continuous, subcutaneous infusion of native, low-dose GnRH by demonstrating its failure to disrupt established estrous cycles; (2) test the hypothesis that a similar treatment would induce development of preovulatory follicles and ovulation in mares exhibiting persistent states of anovulation during the breeding season.

2. Materials and methods

Studies reported herein were approved by the Institutional Agricultural Animal Care and Use Committee of the Texas A&M University System and the Hospital Review Board, College of Veterinary Medicine, Texas A&M University.

2.1. Experiment 1: effects of continuous treatment with GnRH on estrous cycle characteristics during the breeding season

2.1.1. Treatment description

Five normally cycling horse mares were assigned alternately to be administered GnRH (5 μg/h) or saline, each through two ovulatory cycles, in a switchback design. During Period 1, three mares received saline (control) and two received GnRH. During Period 2, each mare was switched to the other treatment. The dose of GnRH was selected because: (1) it was the largest dose chosen for testing in subsequent anovulatory mare experiments; (2) continuous administration of a dose as low as 2 μg/h has been shown previously to induce development of a preovulatory (35 mm) follicle in anovulatory mares during early spring transition [5]; (3) the development of therapeutic strategies for treating anovulation using native GnRH is not likely to be successful unless costs associated with its use can be kept to a minimum and methodologies made practical (i.e., continuous, subcutaneous treatment versus pulsatile administration).

Mares were teased individually with an intact stallion and signs of estrus recorded daily. Teasing scores were: (1) breaks down, urinates, (2) winking, intense interest in stallion, (3) some interest in stallion, (4) passive and (5) rejects stallion. Ovarian structures were monitored daily by transrectal ultrasonography (Dynamic Imaging, Concept/MCV; dual 5/7.5 MHz linear-array transducer; Livingston, UK). Treatments were applied in random order, each through two complete ovulatory cycles (total of four cycles), beginning mid-cycle and ending on Day 10 after the fourth ovulation. Initiation of treatments began by administering each mare an im injection (2 mL) of 10 mg $PGF_{2\alpha}$ (Pfizer, New York, NY, USA) on Day 12 after ovulation and instituting GnRH or saline treatments. Treatments were delivered via subcutaneous Alzet osmotic minipumps (Model 2004; Durect Corporation, Cupertino, CA, USA). Pumps were equilibrated in 0.9% physiological saline at 37 °C for 40 h and disinfected using a chlorhexidine gluconate solution (Vedco, St. Joseph, MO, USA) before implantation. Gonadotropin-releasing hormone (Bachem, Torrance, CA, USA) was delivered in 0.9% physiological saline, with control pumps containing saline only. Pumps were inserted sc at the base of the neck using aseptic technique and 3 mL lidocaine HCl (Vedco, St. Joseph, MO, USA) as a local anesthetic. A blunt surgical instrument was utilized to create a subcutaneous pocket ventral to the incision to accommodate the pump. Pumps had a mean volume of 243 μL and a mean pumping rate of 0.55 μL/h. The concentration of GnRH in each pump used during Period I was 9.1 μg/μL, thus delivering GnRH at 5.0 μg/h for 14 d. Pumps were replaced every 14 d, with re-insertion at different locations on alternating sides of the neck. After each mare had completed two ovulatory cycles with the starting treatment (either GnRH or saline), $PGF_{2\alpha}$ was administered again on Day 12 after the second ovulation, followed by the next treatment.