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Kisspeptin stimulates LH secretion but not ovulation in mares during vernal transition

Briony M. McGrath [a], Christopher J. Scott [a], Peter C. Wynn [b], Jaymie Loy [b], Scott T. Norman [b,*]

[a] School of Biomedical Sciences, Charles Sturt University, Wagga Wagga, NSW, Australia
[b] School of Animal and Veterinary Sciences, Charles Sturt University, Wagga Wagga, NSW, Australia

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ABSTRACT

Managing the return to regular cyclicity after the winter anestrous period in the mare has been a challenge for the equine breeding industry. Specifically, efforts have been made to shift or shorten the vernal transition period and to have it followed by a predictable first ovulation at the commencement of the breeding season. Intravenous administration of kisspeptin is known to stimulate an LH response in both reproductively active and inactive mares. This study examined the effects of a constant rate infusion (CRI) of kisspeptin on mares during vernal transition. Mares were given a 30 hours infusion of kisspeptin at a low and high rate (66 nmol [88 μg] and 100 nmol [130 μg] per hour, respectively) or saline, and the LH and follicular response tracked. Plasma samples were collected every 15 minutes for the first 6 hours to determine if there is an acute effect of kisspeptin infusion on LH secretion. Plasma samples were then collected every 3 hours for a total of 72 hours to examine the ability of kisspeptin to stimulate an LH surge. A CRI of kisspeptin increased LH secretion in these mares but was not able to stimulate an LH surge. To examine the effect of kisspeptin on ovarian activity, follicular measurements were collected ultrasonographically until ovulation occurred or the follicles regressed. CRI of kisspeptin at these rates was unable to induce ovulation earlier than controls.

1. Introduction

In the mare, vernal transition is the period between the nonbreeding and breeding seasons. This period lasts between 6 and 8 weeks during spring and is characterized by displays of estrus that are not coordinated with ovulation. In the Australian horse-breeding industry, there is a desire for early conception for several reasons. First, due to the standardized horses' birthday (first August), competition breeds aim for early foals to maximize athletic development in age-category competition. Second, in warmer climates, breeders prefer foals to be born early in the season rather than having neonates exposed to the sometimes searing heat of midsummer to late-summer. Third, owners may be concerned that mares could progressively foal later in the season as they age, eventually missing a season, or having foals born during periods of high temperature or poor feed availability. Current programs such as light manipulation, treatment with dopamine antagonists, GnRH agonists or eFSH are expensive, in addition to being time and labor intensive [1]. A single short-term treatment, similar to protocols that are successful in inducing ovulation in cycling mares would be invaluable, especially when faced with a limited supply of semen, or clients that desire early conceptions.

In 2003, kisspeptin emerged as a major regulator of the hypothalamic-pituitary-gonadal (HPG) axis and its role in regulating LH has been well characterized in a number of species [2,3]. In all studied species, kisspeptin acts primarily through regulation of GnRH secretion and a

* Corresponding author. Tel.: +612 6933 2088; fax: +612 6933 2991.
E-mail address: snorman@csu.edu.au (S.T. Norman).

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minor effect directly on gonadotropes. Kisspeptin action includes relaying the feedback actions of sex-steroid hormones [4–7].

Mares, unlike other domestic species such as sheep and cattle, have a prolonged LH surge. This begins at the onset of estrus and ends approximately 24 hours postovulation [8]. The neural and ovarian mechanisms that control ovulation in the mare remain poorly understood, and the potential for kisspeptin to induce LH surges in mares has been an area of interest for several years. Unfortunately, the effect of kisspeptin treatment in mares remains unclear, and these varied results are possibly due to the differing conditions under which kisspeptin administration has been examined [9–12]. The present understanding is that high concentrations of kisspeptin delivered to the mare as bolus injections, or in short- to long-term infusions, stimulate an immediate LH response at all stages of the estrous cycle and during anestrus [11]. However, this response is short-lasting [9,12], and has not been able to induce reliable ovulation, with the exception of pony mares receiving extraordinarily high doses of kisspeptin and exhibiting very specific–follicle conditions [13]. Notably, equine studies have used proportionally higher concentrations of kisspeptin when compared with that required to stimulate ovulation in seasonally anestrous ewes [14], and this may have resulted in receptor desensitization. A constant rate infusion (CRI) of a lower total kisspeptin dose may avoid potential receptor desensitization and thus allow an extended LH response.

In addition, mares in vernal transition have anovulatory follicular waves and develop steroidogenically incompetent follicles, which display varied follicular growth patterns [15]. Research in the anestrous ewe found that an initial gonadotropin response to kisspeptin treatment stimulated ovarian follicular maturation and hence estrogen production, which resulted in the production of an endogenous LH surge, and hence ovulation [14]. This suggests the possibility that kisspeptin administration in the mare during vernal transition may be able to stimulate the switch in follicles from being steroidogenically incompetent to competent leading to induction of an endogenous GnRH and/or LH surge and subsequent ovulation.

This study was designed to investigate whether a prolonged CRI of kisspeptin, at low concentrations, would stimulate a sustained LH response. In addition, the unique follicular environment of mares during vernal transition prompted testing of the question; could kisspeptin stimulate predictable ovulation from vernal transition follicles? To explore these questions, the following hypotheses were tested:

Prolonged, low-concentration CRI of kisspeptin to mares during vernal transition will stimulate (1) sustained LH secretion and (2) follicular development leading to induced ovulation.

2. Material and methods

2.1. Animals

Animals were managed in accordance with the Code of Practice for the Care and Use of Animals for Scientific Purposes, outlined by the National Health and Medical Research Council of Australia (2004) and following the approval by the Charles Sturt University Animal Care and Ethics Committee. Standardbred mares that ranged in age from 3 to 15 years old were brought into Charles Sturt University Equine Centre (Wagga Wagga, New South Wales, Australia) when required, during late winter (Southern Hemisphere; mid-August,). Although parity of the older mares was unknown, none of the mares had been bred within 18 months before the commencement of the trial. Mares had body condition scores from 2 to 5 using a 1–5 scale, where 1 was considered underweight and 5 considered obese [16]. All 16 mares met our criteria for vernal transition, including: no ultrasonographically visible corpora lutea on either ovary after 2 examinations, a week apart; each ovary having several follicles; and little or no endometrial edema.

2.2. Effect of a CRI of kisspeptin delivered to mares during vernal transition

2.2.1. Effect on LH profile

This project consisted of 2 trials during the Southern hemisphere vernal transition period (August/September) leading into the 2010 and 2012 breeding seasons. For the 4-day experimental period, mares were individually housed in 4 × 4-m box stalls with *ad libitum* access to water. Mares received fresh lucerne hay twice daily for the duration of the study, with the exception of mare 8, which was at risk for laminitis and so was provided with oaten hay.

With access to infusion pumps being a limiting factor, both trials were run in 2 cohorts, 3 days apart. Each cohort consisted of 2 treatments and 2 control mares. Mares were paired as treated or control animals, based on transrectal palpation, ultrasonographic evaluation of the ovaries showing similar stages of follicular development, and similar body condition scores. All mares were evaluated within 48 hours before commencement of the study to allow these group allocations to be made. After administration of lignocaine hydrochloride, bilateral jugular venous catheters (BD Angiocath, Australia) were placed on the morning of the commencement of the CRI. An infusion pump (T34 syringe driven pump, Caesera Medical Electronics Ltd., Germany) was attached to one side, whereas blood samples were obtained *via* the other catheter. The pumps were housed in pockets sewn into the side of horse neck rugs allowing the horses to move around freely without interfering with the infusion.

For both studies, human kisspeptin-10 protein (Phoenix Pharmaceuticals) was diluted in normal saline and infused over a 30 hours period. Control mares ($n = 7$) received an equivalent volume of vehicle (normal saline) infused over the 30 hours period of the study (15 mL at 0.5 mL/h).

Due to concerns that previous studies using kisspeptin in mares used high doses that may have resulted in receptor downregulation, we adopted a lower dose, on the basis of rates known to induce ovulation in acyclic ewes [14] and adjusted for the weight of mares. Similarly, in light of the wide range in length of infusion in previous studies of kisspeptin in mares, we likewise adopted the model of the sheep