Original Research

Preovulatory Follicle Dynamics, and Ovulatory and Endometrial Responses to Different Doses of hCG and Prediction of Ovulation in Mares

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A R T I C L E   I N F O

A B S T R A C T

A more precise characterization of the effects of human chorionic gonadotropin (hCG) treatment on the preovulatory follicle is needed to improve reproductive efficiency in broodmare management. The objectives of this study were (1) to study the effects of different hCG doses within and among three consecutive estrous cycles on ovulatory response, preovulatory follicle dynamics, and endometrial echotexture; (2) to study the temporal relationship among changes in preovulatory follicle diameter, endometrial echotexture, and estradiol levels; and (3) to analyze a system for prediction of ovulation. In experiment 1, mares were treated with 2,500 IU hCG, 1,500 IU hCG, 500 IU hCG, or saline (controls) and examined by ultrasonography every 6 hours across three consecutive preovulatory periods (n = 135). Similar treatment groups were utilized in experiment 2 (n = 177 preovulatory periods) with examinations performed every six, 12, or 24 hours to test the effectiveness of a prediction of ovulation system. Effective hCG doses (2,500 and 1,500 IU) led to cessation of growth rate and reduction of the preovulatory follicle diameter concurrently with a reduction in systemic estradiol concentration and endometrial echotexture score. A system of prediction of ovulation, based on preovulatory follicle ultrasonographic signs of impending ovulation, was highly accurate for the diagnosis of positive and negative outcomes in both hCG-treated and control groups. However, the effectiveness of this prediction system was reduced due to the false-negative diagnoses that were a reflection of a lack of sensitivity for the detection of nonovulation.

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1. Introduction

Human chorionic gonadotropin (hCG) is a large, two-chained glycoprotein hormone and is currently one of the most cost effective and frequently used ovulation-induction agents in the horse industry [1,2]. Besides hCG, other ovulation-induction agents such as GnRH, deslorelin,

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buserelin, histrelin, recombinant equine LH, and PGF analogues have been used and investigated in horses [2–9]. The economic benefits of induction of ovulation and an accurate system for prediction of ovulation are numerous in a wide range of aspects of assisted reproductive management.

Although a large number of studies have looked at the use of hCG in mares, several questions are still unanswered. For example, the results of retrospective analyses of clinical records to study efficacy, pregnancy rates, ovulation rates, and other parameters such as effect of season and age of mare [1,7] are potentially confounded by other factors (e.g., mare management, hormone treatments). An important clinical aspect of hCG use is the dose and timing of administration. The dose used and the preovulatory follicle diameter when the treatment is administered are not well standardized in many studies. Reported doses of hCG used in the literature range from 750 IU to 5,000 IU, with a 2,500 IU dose being the most common [2,10–12]. Furthermore, studies have shown that in a significant proportion of mares, it is not possible to accurately predict the time of ovulation [13,14]. For example, ovulation-induction agents result in ovulation in most (60–90%) mares within a predictable time (e.g., 24–48 hours); however, 10–40% of mares do not respond in a timely fashion and presumably ovulate at a time unrelated to the treatment [15]. The effect of repeated use of hCG [1,7] and production of hCG antibodies [16–18] within a breeding season on the effectiveness of inducing ovulation has generated conflicting results. Some studies have shown reduced efficacy when hCG has been used repeatedly [7,17,19–21], whereas others [1,16] have reported no significant effect. Therefore, the effect of season, age, and hCG antibody formation as well as other factors such as the effect of the time of day [22–24] and the size of the preovulatory follicle when hCG is administered [20,25] warrants more investigation utilizing different hCG doses to understand the effect of different and repeated hCG doses on the ovulation response.

High-resolution ultrasonographic machines with B-mode (gray scale) and power-, color-, and spectral-Doppler modes have been used to extensively study quantitative and nonquantitative preovulatory follicle characteristics to predict impending ovulation or anovulation (e.g., atresia, hemorrhagic anovulatory follicle [HAF]) [26–35]. During estrus, the uterine echotexture detected through ultrasonography usually reaches a maximum 2–4 days before ovulation and then declines [20,27,36,37] and is thought to be associated with the prevailing circulating levels of estradiol and progesterone [37,38]. A reduction in growth rate of the preovulatory follicle, estradiol systemic concentration, and endometrial echotexture before ovulation has been reported but not compared in hCG-treated mares [36,39,40]. An association between declining uterine echotexture at 24 hours post-hCG treatment and the likelihood of ovulation within 24–48 hours suggests the potential usefulness of uterine echotexture in the prediction of ovulation [20,36]. A better understanding of the effect of hCG on the relationship among preovulatory follicle diameter, uterine echotexture, and circulating concentration of estradiol is needed.

The objectives of the present experiment in mares were (1) to compare the ability of different doses of hCG to induce ovulation on three consecutive estrous cycles during the breeding season; (2) to study ovarian follicular growth within and among estrous cycles after different hCG treatments; (3) to study the effects of the mare's age and season on the interval to ovulation after hCG treatment; (4) to assess the influence of different doses of hCG on multiple ovulations and HAFs; (5) to study the temporal relationship among changes in preovulatory follicle diameter, endometrial echotexture, and estradiol levels in mares treated with hCG; and (6) to evaluate the accuracy and effectiveness of a subjective system for prediction of ovulation using B-mode ultrasonographic signs of the preovulatory follicle. The hypothesis tested was that hCG treatment induces a cessation in growth rate of the preovulatory follicle concurrently with a reduction in systemic estradiol concentration and endometrial echotexture score.

2. Materials and Methods

In this study, two experiments were conducted. Experiment 1 was designed to evaluate preovulatory follicle dynamics, and ovulatory and endometrial response to different doses of hCG. In experiment 2, the accuracy and effectiveness of a prediction of ovulation system using ultrasonographic preovulatory follicle characteristics were evaluated using different examination time intervals: every 6 hours (part 1), 12 hours (part 2), or 24 hours (part 3). All procedures on animals and management in experiments 1 and 2—part 1 were approved by the Research Ethics Committee of the Federal University of Viçosa, Brazil. All animals in the experiment 2—parts 2 and 3 were handled in accordance with the United States Department of Agricultural Guide for Care and Use of Agricultural Animals in Research.

In experiments 1 and 2—part 1, nonlactating, estrous cycling, crossbred Breton mares (n = 45) between 3 and 15 years of age, weighing 350–600 kg, and in good body condition were studied during three consecutive estrous cycles (n = 135) from October to March during the ovulatory season in Brazil (latitude, 21° S). In experiment 2—parts 2 and 3, nonlactating, estrous cycling, large pony mares (n = 52) of mixed breeds, between 6 and 20 years of age, weighing 250–400 kg, and in good body condition were studied from May to July during the ovulatory season in the United States of America (latitude, 43° N).

2.1. Experiment 1. Follicle Dynamics, and Ovulatory and Endometrial Response

2.1.1. Ultrasonography and Groups

Transrectal ultrasound scanning of the ovaries and uterus was performed once daily starting 13–15 days after ovulation until a growing, 28-mm follicle was detected at the beginning of estrus. Thereafter, mares were scanned every 12 hours until treated with hCG (Ovusyn, Syntex S.A., Argentina) or saline when the diameter of the largest follicle first reached ≥35 mm (hour 0 = treatment). At hour 0, mares were randomly assigned to one of four groups (n = 9 per group) to receive the following intravenous treatments: 2,500 IU hCG, 1,500 IU hCG, 500 IU hCG, or saline (control).