Journal of Equine Veterinary Science 35 (2015) 591–597

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Original Research

Characterization of Luteal Blood Flow and Secretion of Progesterone in Mares Treated With Human Chorionic Gonadotropin for Ovulation Induction or During Early Diestrus

Rafael Mide Romano [a], Jair Camargo Ferreira [a,b,*], Heloisa de Siqueira Canesin [a], Yatta Linhares Boakari [a], Fernanda Saules Ignácio [a], Luiz Fernando Novaes Filho [a], Donald L. Thompson Jr. [b], Cezinande de Meira [a]

[a] Department of Animal Reproduction and Veterinary Radiology, School of Veterinary Medicine and Animal Science, UNESP, Botucatu, SP, Brazil
[b] School of Animal Sciences, Louisiana State University Agricultural Center, Baton Rouge, LA

ARTICLE INFO

Article history:
Received 24 December 2014
Received in revised form 25 February 2015
Accepted 25 March 2015
Available online 2 April 2015

Keywords:
Corpus luteum
Progesterone
Diestrus
Blood flow
Doppler ultrasonography

ABSTRACT

Human chorionic gonadotropin (hCG) has been used to induce ovulation and as a luteotrophic agent in cattle. However, the effect of hCG therapy on the functional status of the equine corpus luteum (CL) is unclear. This study aimed to characterize the hemodynamic and secretory function of early CL of mares treated with different doses of hCG at distinct stages of the estrous cycle. Mares were assigned to nine experimental groups (n = 6 mares/group) according to dose of hCG and time of treatment. A single injection of one of three different doses of hCG (750, 1,500, or 2,500 IU) was performed in one of three distinct stages of the estrous cycle: preovulatory follicle ≥35 mm, day of ovulation (D0), or 48 hours after ovulation (D2). In addition, a control group treated with NaCl 0.9% was included in the study. The end points evaluated daily from D0 to D8 were area of the CL, luteal vascularity, number of colored pixels and total pixel intensity, and concentrations of plasma progesterone (P4). No effect ($P > .1$) of dose or time of treatment was observed for any end point, within each day. Luteal area did not differ throughout the days ($P > .1$), whereas Doppler parameters and concentrations of plasma P4 presented a progressive increase ($P < .05$) after ovulation in all groups. Secretory function and luteal hemodynamic were not affected ($P > .1$) by hCG dose and time of treatment. In conclusion, hCG therapy during estrus or early diestrus, at the doses tested, did not improve P4 secretion or luteal blood flow.

1. Introduction

The corpus luteum (CL) is a transient endocrine gland responsible for synthesis of progesterone (P4). Physiological functionality of this dynamic structure requires a prompt development of an extensive vascular network [1] that needs intense and progressive luteal angiogenesis during early diestrus [2].

Color Doppler ultrasonography is a noninvasive real-time pulse-wave technique currently used for transrectal study of hemodynamics of the reproductive system in large animals [1]. Considering the extensive luteal angiogenesis observed during early diestrus, Doppler ultrasonography has proven to be an efficient real-time method for in vivo evaluation of structural and functional status of the CL in mares [3].

* Corresponding author at: Jair Camargo Ferreira, Department of Animal Reproduction and Veterinary Radiology, School of Veterinary Medicine and Animal Science, UNESP, 18618-970 Botucatu, SP, Brazil.
E-mail address: jaircfvet@yahoo.com.br (J.C. Ferreira).

0737-0806/$ – see front matter © 2015 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jevs.2015.03.196

Luteinizing hormone (LH) is essential for the luteogenic process and adequate function of the CL [4]. Human chorionic gonadotropin (hCG) is a glycoprotein with LH-like biological activity and luteotrophic action [5]. In cattle, hCG has been used to trigger a long-term rise in P4 [6,7], however, usually as a result of the formation of an accessory CL [8,9]. In mares, in vivo and in vitro studies suggested a positive correlation between hCG administration, concentrations of plasma P4, and fertility [10,11]. Conversely, Urquieta et al [12] and Hendriks et al [13] reported no effect of hCG therapy during estrus and early diestrus on future CL functionality.

As maturation of luteal cells in the newly formed CL is completed approximately 3–4 days after ovulation [3], treating mares with hCG to induce ovulation may have the potential to enhance luteal function. Therefore, considering the unclear effect of hCG on equine CL functionally, the effect of hCG therapy on P4 synthesis and luteal angiogenesis must be clarified.

Therefore, we have hypothesized that hCG therapy in the presence of a preovulatory follicle or at the day of ovulation, or 2 days after ovulation, would alter P4 secretion in mares. Accordingly, the main purpose of this study was to characterize the hemodynamic and secretory function of early CL of mares treated with different doses of hCG at distinct stages of the estrous cycle. Specific goals were to determine the temporal relationship between luteal blood flow, area of the CL, and concentrations of plasma P4 of mares treated with hCG.

2. Material and Methods

2.1. Animals and Experimental Groups

Cycling mixed breed mares 4–18 years of age, weighing 250–380 kg, were used. Animal care was carried out according to the São Paulo State University Guide for Care and Use of Agricultural Animals in Research. Mares were fed grass hay, pelleted feed, and trace-mineralized salt with free access to water. Body condition score for all mares was >7 (of 14 points; [14]). Age of mares was estimated from dental characteristics as described by the American Association of Equine Practitioners Manual [15]. Mares were scanned daily for follicular development monitoring and ovulation detection using B-mode ultrasonography.

Before the beginning of the experiment, all mares were submitted to ovulation inducing treatment with 2,500 IU of hCG to identify animals with refractory responses to hCG therapy. Only mares that ovulated between 24 and 48 hours after treatment with hCG were used.

Mares were assigned to nine experimental groups (n = 6 mares/group) according to time of treatment and dose of hCG. Treatments were performed in one of three distinct stages of the estrous cycle: (a) when a dominant follicle ≥35 mm and uterine edema were observed (induction group); (b) on the day of ovulation (D0 group); or (c) 48 hours after ovulation detection (D2 group). A single IV injection of one of three different doses of hCG was used: 750, 1,500, or 2,500 IU (Vetecor 5000 U.I.; Calier S.A, Barcelona, Spain).

A control group of non-hCG–treated mares (n = 6) was included in the experimental approach. Mares from the control group were treated with a single IV injection of 2 mL of 0.9% NaCl solution when a preovulatory follicle ≥35 mm associated with uterine edema was observed. Only mares with spontaneous ovulation were used in control, D0, and D2 groups. Transrectal ultrasonography examination was done once daily for monitoring of follicular development and detection of ovulation in D0 and D2 groups. In induction group, ultrasonography examination was performed every 6 hours from the moment of hCG treatment until the observation of a CL.

2.2. Ultrasonography

Doppler ultrasonography examination was performed every day from D0 to D8. Color Doppler ultrasound (Sonoace Pico; Medison do Brasil Ltda) equipped with a linear-array multifrequency transducer (LV5-9CDn, 5–9 MHz) was used for evaluation of vascular perfusion of the CL. Brightness, contrast, and gain settings of the ultrasound unit were kept constant throughout the experiment [16].

Power Doppler function was used to display blood flow signals in the luteal tissue as previously described by Ginther et al [17]. Vascular perfusion of the CL was initially estimated subjectively by considering the percentage (0%–100%) of luteal tissue with color Doppler signals during

Table 1
Mean (±standard error of the mean) for follicle diameter (mm) on the last ultrasonography examination before ovulation detection, luteal area (mm^2) between D0 and D8, and concentration of plasma progesterone (ng/mL) on D8 in mares treated with 2,500, 1,500, or 750 IU of hCG (n = 6 mares/group).

Groups	End Point		
	Follicle Diameter	Luteal Area	Progesterone
Induction-750	35.7 ± 0.58[a] (35.0–37.8)	616.4 ± 62.6 (577.2–658.2)	12.9 ± 1.7
Induction-1500	35.8 ± 0.5[a] (35.0–39.1)	566.3 ± 45.3 (487.3–614.0)	10.6 ± 1.7
Induction-2500	35.8 ± 0.6[a] (35.0–37.6)	562.6 ± 67.3 (415.1–686.9)	11.4 ± 1.4
D0-750	44.4 ± 2.2[b] (50.0–52.9)	613.9 ± 49.8 (473.5–700.6)	14.2 ± 1.6
D0-1500	45.1 ± 2.5[b] (38.9–53.0)	627.9 ± 68.3 (571.9–672.1)	15.5 ± 2.5
D0-2500	46.4 ± 1.6[b] (41.1–51.0)	598.9 ± 71.0 (491.4–708.1)	13.8 ± 1.6
D2-750	44.1 ± 1.3[b] (41.8–49.0)	529.7 ± 53.6 (452.1–591.8)	12.2 ± 0.7
D2-1500	43.5 ± 1.0[b] (41.6–47.9)	528.5 ± 45.8 (472.6–595.1)	12.7 ± 1.3
D2-2500	45.4 ± 2.8[b] (39.4–54.1)	544.2 ± 71.5 (499,4–596.3)	13.8 ± 1.8

Abbreviation: hCG, human chorionic gonadotropin.
Treatments have been performed in the presence of a preovulatory follicle >35 mm, on the first day of corpus luteum visualization or 2 days after ovulation (induction, D0, and D2 groups, respectively).
[a,b] are different ($P < .05$) within an end point.