Theriogenology 83 (2015) 485–490

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Color Doppler provides a reliable and rapid means of monitoring luteolysis in female donkeys

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ARTICLE INFO

Article history:
Received 14 July 2014
Received in revised form 6 October 2014
Accepted 6 October 2014

Keywords:
Induced luteolysis
Corpus luteum
Donkey
Color Doppler ultrasound
Progesterone

ABSTRACT

When artificial reproduction technologies designed for use with horses are used with donkeys, success is dependent on awareness of the physiological differences between these species, yet little information is available on many aspects of donkey reproduction. The present work examines the activity of the CL in Catalonian jennies after induced luteolysis. Plasma progesterone concentration, luteal blood flow (determined by color Doppler), and CL cross-sectional area (CL-CSA; determined by B-mode ultrasound examination) were assessed after a single dose (5 mg intramuscular) of dinoprost thromethamine (DT, a PGF2α analog) on Day 10 after ovulation in two experiments. In experiment 1, a preliminary experiment, data were collected daily for 4 days after DT administration. Values for all the measured variables decreased over this period. In experiment 2, data were collected during the first 24 hours after DT administration because in experiment 1, most luteolytic activity occurred during this time. An increase in luteal blood flow was seen between 0 and 3 hours, followed by a progressive reduction, whereas the values for plasma progesterone and CL-CSA gradually decreased from 0 hours onward. In both studies, negative correlations were seen between all variables and the time of sampling. In contrast, positive correlations were seen between plasma progesterone, CL-CSA, uterine tone, and luteal blood flow. Indeed, a strong correlation was recorded between plasma progesterone and luteal blood flow ($r = 0.70$; $P < 0.0001$). In conclusion, plasma progesterone and CL-CSA both become reduced after induced luteolysis in Catalonian jennies. Unlike in mares, an increase in luteal blood flow occurs soon after induced luteolysis, rather like that seen in the cow. The luteal blood flow, as evaluated here by color Doppler, was also closely related to the plasma progesterone concentration. Color Doppler would appear therefore to offer a rapid and easy means of examining the state of luteolysis.

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1. Introduction

Little information is available on many aspects of donkey reproduction. This could reduce the success of artificial reproduction programs when techniques originally developed for use with mares are used.

The CL is a temporary endocrine gland formed from fibroblasts and the remaining cells of the ovulated follicle's granulose layer. It produces progesterone and maintains the uterus lining during early pregnancy [1,2]. When pregnancy does not occur, the CL naturally involutes in response to the release of PGF2α from the nonpregnant endometrium. This occurs from Days 13 to 16 after ovulation in the mare [3–5]. Sequential pulses of PGF2α, on average separated by 9 hours, lead to a reduced plasma

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0093-691X/$ – see front matter © 2015 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2014.10.007

progesterone concentration by 3 to 4 hours after the first pulse. Estrous is achieved when plasma progesterone drops to 1 to 2 ng/mL [5–7]. The exogenous administration of prostaglandins is commonly used to shorten the estrous cycle in domestic farm animals and has been used to control postbreeding endometritis in mares [4,8–10]. A single high-dose bolus can be administered to induce luteolysis. Natural rather than synthetic PGF2α analogs are usually used in mares and jennies and at lower doses than those needed for ruminants (5 vs. 25 mg), given the greater sensitivity of their luteal cells to prostaglandins [11–13]. However, luteolysis has been little studied in the jenny [37], and understanding CL physiology and morphology after induced luteolysis is important if reproductive technologies are to be successfully used in this species.

Luteal vascularization plays an important role in the physiology of the CL [14]. Intense angiogenesis occurs during luteinization in many species [15–17], but curiously, in the cow, increased luteal vascularization also occurs before an intense reduction of the same just before luteolysis [18,19]. Until about 10 years ago, changes in the CL during the estrus cycle, and after induced luteolysis, in mares were usually monitored by B-mode ultrasound [1,20] and by following changes in the plasma progesterone concentration [21–23]. In humans, however, color Doppler ultrasound was already being used to examine blood circulation in patients presenting with CL development problems [24]. Later, Bollwein et al. [25] showed that color Doppler monitoring offered valuable information on CL activity and vascularization in the mare and that the technique was a useful noninvasive tool for examining luteal blood flow. Color Doppler assessment of the CL in mares [26,27] and heifers [28] has been increasing over recent years, and in the latter, it has recently been shown to better assess luteal blood flow than CL cross-sectional area (CL-CSA) or plasma progesterone measurements [29,30].

In the bovine CL, the luteal blood flow increases temporarily (0.5 hours) before the fall of the plasma progesterone concentration at 1 hour after physiological [31–33] and induced luteolysis [31,34]. However, in the mare, luteal blood flow does not increase during early luteolysis, and plasma progesterone decreases before significant reductions in luteal blood flow are detected [32,35,36]. Moreover, a reduction in angiogenesis in the CL of mares has been described during the late luteal phase induced by PGF2α [15]. The literature, however, contains no information on what happens in jennies.

The aim of the present study was to examine the changes in the CL, luteal blood flow, and plasma progesterone after the administration of a single intramuscular dose of a PGF2α analog to Catalonian jennies on Day 10 after ovulation.

2. Materials and methods

The animals examined were seven clinically healthy and normally cycling Catalonian jennies (*Equus asinus*) aged 4 to 13 years. All were kept together outdoors and fed grain forage, straw, hay, and water *ad libitum*. All were monitored daily from estrus to ovulation *via* transrectal palpation and real-time ultrasound (MyLabTM30 VET; Esaote, Genoa, Italy) using a 5-MHz linear transducer. After detecting an active CL, 5 mg of dinoprost thromethamine (DT; a natural PGF2α analog; Dinolytic, Pfizer Animal Health, Belgium) was administered intramuscularly on Day 10 after ovulation to induce luteolysis.

2.1. Experiment 1: CL activity over the 4 days after induced luteolysis

Twelve active CL involving three jennies were detected. Corpus luteum activity in these jennies was examined daily by measuring, as described in the following, the CL-CSA, luteal blood flow, and plasma progesterone from Days 0 to 4 after DT administration. Corpus luteum echogenicity and uterine tone were also recorded as previously described [38].

The CL-CSA (cm^2) was determined by B-mode ultrasound analysis. Cross-sectional images of the identified CL at their maximum size were stored, and the CL-CSA was determined using the software provided with the ultrasound equipment. The color Doppler function was then activated to examine the luteal blood flow [27]. Four transverse sections were recorded at maximum color pixel density. Images were later analyzed using a computer image analyzer running analySIS 2.1 software (Soft Imaging System GmbH, Münster, Germany). The percentage area of the CL-CSA with color Doppler signals returned by the blood flow was determined as previously described [25,39].

At the same time, blood samples from the jugular vein were collected in vacutainers and centrifuged (10 minutes, × 1500*g*). Plasma was decanted and frozen at −20 °C until being assayed for progesterone using a progesterone radioimmunoassay kit (Immunotech SAS, Marseille, France).

Given the results obtained, a second experiment was performed involving closer monitoring over the first 24 hours after induced luteolyis.

2.2. Experiment 2: CL activity over the first 24 hours after induced luteolysis

Ten active CL involving four jennies were detected. Corpus luteum activity was monitored by measuring the CL-CSA, the luteal blood flow, and plasma progesterone as described in experiment 1. Readings were taken every hour from 0 to 7 hours after DT administration and then at 10, 12, and 24 hours.

2.3. Statistical analysis

The Kolmogorov–Smirnov test was used to test the normality of distribution of the results. Analysis of variance (general linear model and univariate procedure) was performed to detect differences between variables at different experimental times. When significant differences were detected, the least significant difference test was performed to examine variables that showed homogeneity of variance (plasma progesterone and CL-CSA), and the Tamhane test was used to examine those that did not (blood flow as determined by color Doppler). Multiple linear regression analysis was performed to detect relationships between variables and between variables and experimental time. When double ovulations were detected, the mean

CL-CSA and luteal blood flow (as determined by color Doppler) were calculated to test their correlation with plasma progesterone. All data were expressed as mean ± standard error of the mean. Significance was set at $P < 0.05$. All calculations were performed using IBM SPSS 19.0 for Windows (Statistical Package for the Social Sciences Inc., IBM Company, Chicago, IL, USA) software.

3. Results

All the CL examined were highly echogenic and showed a central hyperechogenic area (B mode). Color Doppler detected strong blood movement around the CL. The plasma progesterone concentration was greater than 20 ng/mL on Day 10 after ovulation (Day 0 of the experiments), indicating the presence of a functional CL. Plasma progesterone correlated positively with uterine tone ($r = 0.53$; $P < 0.05$) in both experiments. All the jennies ovulated 4 to 7 days after DT treatment.

3.1. Experiment 1: CL activity over the 4 days after induced luteolysis

Significant differences (up to $P < 0.0001$) in luteal blood flow, CL-CSA, and plasma progesterone were seen between Day 0 and the other days (Figs. 1 and 2).

A strong positive correlation was seen between luteal blood flow as determined by color Doppler and plasma progesterone ($r = 0.70$; $P < 0.0001$) and between the CL-CSA and plasma progesterone ($r = 0.62$; $P < 0.05$). Uterine tone was maximum on Day 0 and decreased over time after DT administration ($r = -0.75$; $P < 0.0001$). Positive correlations were seen between uterine tone and plasma progesterone concentration ($r = 0.37$; $P < 0.05$), luteal blood flow ($r = 0.44$; $P < 0.05$), and the CL-CSA ($r = 0.44$; $P < 0.05$). Negative correlations were recorded between the day of experiment and luteal blood flow, CL-CSA, and plasma progesterone ($P < 0.0001$; $r = -0.58$, -0.68, and -0.79, respectively).

3.2. Experiment 2: CL activity over the first 24 hours after induced luteolysis

The plasma progesterone concentration and CL-CSA decreased over time, with significant differences observed at 7 and 10 hours after DT administration, respectively. Luteal blood flow tended to increase between 0 and 3 hours after DT administration; it then progressively decreased until 24 hours, with significant differences observed between 3 and 10 hours and between 10 and 24 hours (Figs. 3 and 4; Table 1).

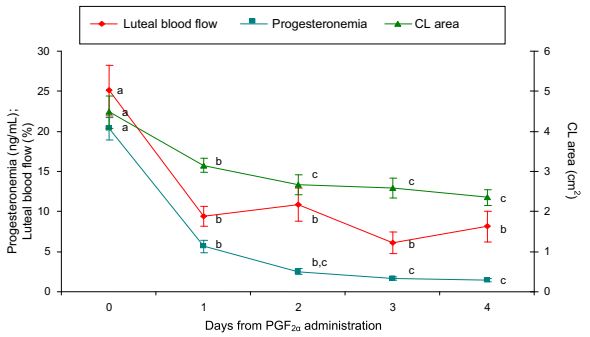

Fig. 1. Daily mean ± standard error of the mean values of progesteronemia, CL area, and luteal blood flow after PGF2α administration on Day 10 of ovulation. Means with different letters (a, b, and c) within a day are significantly different. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

A negative correlation ($r = -0.71$, $P < 0.001$) was seen between the time of sampling and plasma progesterone, luteal blood flow, and CL-CSA. Plasma progesterone showed a positive correlation with CL-CSA ($r = 0.33$; $P < 0.01$) but no correlation with luteal blood flow. However, a positive correlation between CL-CSA and luteal blood flow ($r = 0.48$; $P < 0.001$) was observed.

4. Discussion

Exogenous prostaglandins are commonly used in large farm animal reproduction programs, although gaps exist in our understanding of the luteolytic process in some species. In the present study, plasma progesterone, luteal blood flow, and CL-CSA were examined to assess the activity of the CL after induced luteolysis in the jenny. Corpus luteum echogenicity and uterine tone were also monitored.

All the CL detected showed strong echogenicity and a hyperechogenic central area [40]. In contrast, an active CL in the mare is normally characterized by a uniform echogenic pattern with a hypoechogenic central area [20].

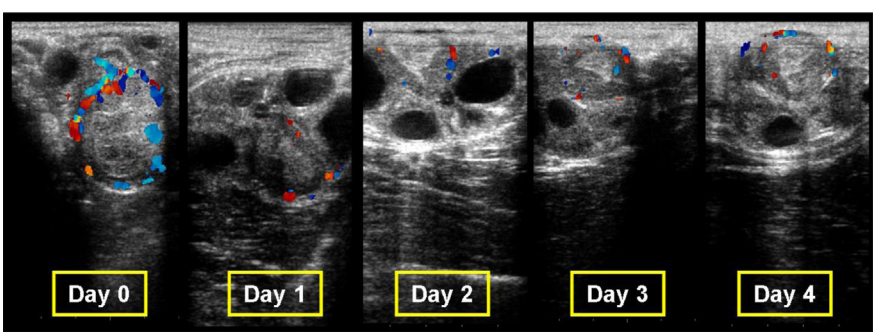

Fig. 2. Representative images of the luteal blood flow assessed by color Doppler ultrasound before PGF2α administration (Day 0) and daily over the next 4 days. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

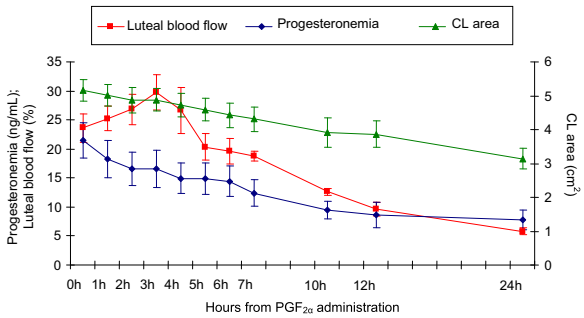

Fig. 3. Mean ± standard error of the mean values of progesteronemia, CL area, and luteal blood flow during the first 24 hours of PGF2α administration on Day 10 of ovulation. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

Echogenicity and uterine tone are generally used to predict ovulation in equine veterinary practice [38]. In the present jennies, echogenicity was found to be positively correlated with the plasma progesterone concentration, but the uterine tone tended to decrease in the presence of this hormone.

Starting from peak plasma progesterone values of greater than 20 ng/mL, reductions were seen in the present jennies as soon as 1 hour after DT administration. However, even by Day 3, they had not fallen below 2 ng/mL. Similar results have been reported for Martina Franca jennies, although lower plasma progesterone concentrations (8–10 ng/mL) were observed before PGF2α treatment on Day 3 of ovulation [37]. In the mare, however, rapid luteolytic activity occurs with plasma progesterone falling to less than 1 ng/mL by 10 hours after PGF2α treatment [41]. Similar reductions in plasma progesterone are seen by 30 minutes in the cow [31,32]. A temporary increase in plasma progesterone, along with LH, FSH, and cortisol, has been described in mares, with a peak at 10 minutes after PGF2α treatment [42]. No such behavior, however, was seen in the present jennies.

An increase in luteal blood flow appears to be a universal phenomenon in mares whether luteolysis is natural or induced [34,36,43–46]. A transient increase in luteal blood flow between 1 and 3 hours was also seen in the present jennies. In cows too, an early increase (at 0–0.5 hours) in luteal blood flow occurs after natural and induced luteolysis, remaining elevated for up to 2 hours, at which time CL vascularization begins to decrease [34,47]. Interestingly, a lack of intraluteal vascular changes in response to PGF2α treatment appears to be responsible for the refractory luteolytic action seen in cows before Day 5 after ovulation [31].

Prostaglandin synthesis from arachidonic acid and the expression of COX-2 in the late luteal phase have been reported in cows and ewes [48,49]. In mares, an increase in cortisol has been observed after the administration of a single bolus of PGF2α [42]. Thus, although more studies are needed, luteolysis should be understood as a physiological process of inflammation involving a release of prostaglandins, other hormones, and cytokines, which results in ischemia-induced necrosis of the luteal tissue [14]. As described in the cow, an increase in luteal blood flow may be triggered by vasodilatory prostaglandin I2 or prostacyclin and nitrogen oxide release from arterioles surrounding the CL; this probably also causes vasodilatation in the active CL jenny after induced luteolysis. The secretion of angiotensin II and endothelin 1 is thus stimulated from microcapillary vessels in the CL, resulting in vasoconstriction of the arterioles and, eventually, luteolysis [18,31,34,43]. Recently, Korzewa et al. [50] described cytotoxic and proapoptotic effects for PGF2α *in vitro* that caused luteal cell death in the cow. In addition, the effect of DT (also used in the present study) was found to be more similar to natural luteolysis than that induced by synthetic prostaglandins. In cows, plasma progesterone decreases by 0.5 hours after PGF2α administration, but luteal blood flow remains unchanged until 8 hours, coinciding with the initiation of structural luteolysis as reflected by a significant reduction in CL volume [31]. In mares, CL-CSA decreases while luteal tissue echogenicity increases after induced luteolysis [40]. In the present jennies, the CL-CSA decreased slowly over the first 48 hours compared with the luteal blood flow and plasma progesterone, suggesting that functional luteolysis manifests itself before structural luteolysis. However, this needs to be further studied.

In both experiments, the time of sampling was negatively correlated with all variables, with positive correlations seen between all the latter. Similar results were described when a whole estrus cycle was monitored in the ewe [2]. In addition, a highly significant positive correlation was detected between plasma progesterone and luteal blood flow, although this did not reach significance in experiment 2 because of the initial increase in luteal blood flow before the progressive reduction starting 3 hours after the DT treatment. Daily plasma progesterone levels in the

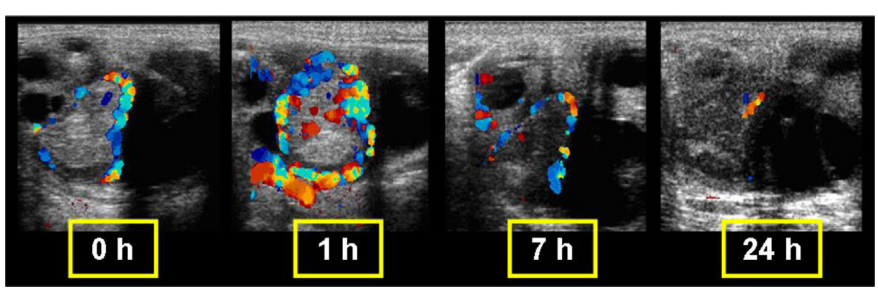

Fig. 4. Representative images of the luteal blood flow assessed by color Doppler ultrasound before (Day 0) and after 1 hour, 7 hours, and 24 hours of PGF2α administration. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

Table 1
Mean ± standard error of the mean values of progesteronemia and luteal blood flow by color Doppler and CL area during the first 24 hours of PGF2α administration.

Hours of PGF2α administration (h)	Progesteronemia (ng/mL)	Luteal blood flow (%)	CL area (cm^2)
0	21.5 ± 3.08[a]	23.65 ± 2.46[a,d]	5.16 ± 0.31[a]
1	18.26 ± 3.15[a,c]	25.27 ± 2.12[a]	5.02 ± 0.32[a,b]
2	16.52 ± 2.84[a,b]	26.82 ± 2.56[a]	4.87 ± 0.37[a,b]
3	16.55 ± 3.16[a,b]	29.70 ± 3.05[a]	4.87 ± 0.33[a,b]
4	14.96 ± 2.69[a,b]	26.71 ± 3.97[a,e]	4.72 ± 0.34[a,b]
5	14.88 ± 2.66[a,b]	20.37 ± 2.27[a,e]	4.59 ± 0.34[a,b]
6	14.45 ± 2.57[a,b]	19.56 ± 2.21[a,e]	4.42 ± 0.36[a,b]
7	12.41 ± 2.21[b,c]	18.80 ± 0.80[a,f]	4.31 ± 0.36[a,b]
10	9.44 ± 1.56[b,c]	12.62 ± 0.55[b,d,e]	3.92 ± 0.44[b,c]
12	8.66 ± 2.16[b]	9.62 ± 1.24[c,e,f]	3.86 ± 0.39[b,c]
24	7.76 ± 1.66[b]	5.80 ± 0.60[c]	3.14 ± 0.31[c]

[a–f]Different superscripts in the same column indicate significant differences.

jenny might therefore be reliably predicted by color Doppler assessment of the luteal blood flow, making it a useful noninvasive tool for rapidly evaluating CL function, as described in other species [25,28].

In conclusion, exogenous administration of DT to jennies on Day 10 of ovulation induced a progressive reduction in the plasma progesterone concentration and CL-CSA. However, plasma progesterone did not fall to less than 2 ng/mL until after Day 3 after DT administration, indicating it to have a slower luteolytic effect than that in mares and cows. The luteal blood flow increased over the first 3 hours after the induction of luteolysis, after which time, it continued to decrease as in the cow but not as in the mare. The very strong correlation seen between the luteal blood flow and the plasma progesterone concentration suggests color Doppler to be an excellent means of monitoring induced luteolysis in jennies.

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