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

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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.

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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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