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# Role of PGF2α in luteolysis based on inhibition of PGF2α synthesis in the mare

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ABSTRACT

The effects of inhibition of PGF2α synthesis on luteolysis in mares and on the incidence of prolonged luteal activity were studied in controls and in a group treated with flunixin meglumine (FM), a PGF2α inhibitor ($n = 6$/group). The FM was given every 8 hours (1.0 mg/kg) on each of Days 14.0 to 16.7. Concentration (pg/mL) of PGF2α metabolite averaged over 8 hours of hourly blood sampling at the beginning of each day, was lower in the FM group than in the controls on Day 14 after ovulation (6.7 ± 1.3 vs. 13.8 ± 2.9, $P < 0.05$), Day 15 (15.0 ± 3.9 vs. 35.2 ± 10.4, $P < 0.10$), and Day 16 (21.9 ± 5.7 vs. 54.7 ± 11.4, $P < 0.03$). Concentration (ng/mL) of progesterone (P4) was greater in the FM group than in the controls on Day 14 (10.1 ± 0.9 vs. 7.7 ± 0.9, $P < 0.08$), Day 15 (9.2 ± 1.0 vs. 4.3 ± 1.0, $P < 0.008$), and Day 16 (5.6 ± 1.6 vs. 1.2 ± 0.4, $P < 0.02$). The interval from ovulation to the beginning of a decrease in P4 and to the end of luteolysis (P4 < 1 ng/mL) was each delayed ($P < 0.03$) by ~1 day in the FM group. Intervals involving the luteal phase were long (statistical outliers, $P < 0.05$) in two mares in the FM group, indicating prolonged luteal activity. Results supported the hypotheses that (1) inhibition of PGF2α synthesis interferes with luteolysis in mares and (2) inhibition of PGF2α at the expected time of luteolysis may lead to prolonged luteal activity.



## 1. Introduction

The decrease in progesterone (P4) or regression of the CL at the end of diestrus is a pivotal event during the estrous cycle in farm species including mares (reviewed in Ref. [1–4]). A 40-year chronology of research findings on the nature of luteolysis in mares has been reported [5]. Luteal terminologies before, during, and after the end of luteolysis are preluteolysis, luteolysis, and postluteolysis, respectively, and in mares, luteolysis occurs on Days 14 to 16 (Day 0 = ovulation) [6]. The transition between preluteolysis and luteolysis is manifested within 1 hour [7] on the basis of P4 concentrations in blood samples collected hourly. The end of luteolysis and beginning of postluteolysis is commonly defined as a P4 decrease to <1 ng/mL [6].

Luteolysis is a response to the secretion of PGF2α from the endometrium in the absence of an embryo [1,2]. In mares, as in other farm species, circulating concentrations of a PGF2α metabolite (PGFM) is often used to represent changes in PGF2α concentrations [8], owing to the short half-life of PGF2α [9]. Classical approaches to demonstrating that PGF2α is the luteolysin in farm species are as follows: (1) administering PGF2α, (2) assaying circulating PGF2α concentrations, and (3) blocking PGF2α secretion. In mares (1) luteolysis occurs after administration of PGF2α [10]; (2) at the expected time of luteolysis, an increase in

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PGF occurs in the uterine vein [11] and an increase in PGFM occurs in the systemic circulation [6,12,13]; and (3) removal of the site of PGF2α secretion (uterus) results in maintenance of the CL [14,15]. Administration of an inhibitor of PGF2α biosynthesis (flunixin meglumine, FM) has been done in ruminants [16], but systemic administration has not been used as an experimental approach for the study of luteolysis in mares. However, FM in mares prevents PGF2α secretion into the uterine lumen [17]. Systemic treatment with FM has been used to inhibit prostaglandin synthesis in mares during the follicular phase to induce hemorrhagic anovulatory follicles [18] and after insemination to alter uterine contractions [19]. The FM acts on the cyclooxygenase enzyme in the endometrium [20] and thereby blocks PGF synthesis [21]. Phenylbutazone also inhibits the synthesis of PGF2α and has been used in attempts to alter CL function but without success [22].

Complete luteal regression in mares involves secretion of PGF2α in more than one pulse during the length of the luteolytic period on the basis of the P4 response to simulated PGFM pulses [23]. Therefore, PGFM pulses are an important consideration in the study of the luteolytic mechanism. Pulses of PGFM occur about every 9 hours during luteolysis and encompass 4 to 5 hours from beginning to ending nadirs [24]. During preluteolysis, PGFM concentrations remain below assay sensitivity or the identified pulses are small (e.g., 30 pg/mL peak) [6]. Pulse prominence increases considerably during luteolysis and postluteolysis (e.g., 300 pg/mL). The peak of a small (e.g., 45 pg/mL) pulse occurs at or within 1 hour of the transitional hour between preluteolysis and luteolysis and may be similar in prominence to the previous pulse during preluteolysis [6]. Oxytocin increases in synchrony with the small PGFM pulse at the hour of transition and during prominent pulses of luteolysis but does not increase during the small pulses before the transitional pulse [25]. Concentrations of P4 decrease linearly within the hours of a PGFM pulse [13,26] and between the PGFM nadirs of adjacent pulses [24]. Luteal blood flow begins to decrease during the descending portion of a PGFM pulse [26].

Prolonged luteal activity or luteal persistence occurs in mares (reviewed in Refs [1,2,27]). Persistent CL can result from impairment of uterine PGF2α secretion by severe uterine pathology [28]; this form of luteal persistence has been termed uteropathic [29]. Spontaneous [27,30] or idiopathic [29] persistent CL in mares that do not have known uterine pathology has also been reported, and the mean length of the luteal phase is ~2 months [30]. The incidence has been reported as 25% of estrous cycles [30] and as 8% to 10% but with a higher incidence (20%–35%) near the end of the ovulatory season [31]. Although the pathogenesis of idiopathic luteal persistence is unknown, an impairment in the secretion of PGF2α has been suspected [27,29,30]. Prolonged P4 production also occurs when a secondary CL forms from ovulation during diestrus, and the CL is too immature to respond to PGF2α secretion [29,30].

Inhibition of PGF2α biosynthesis has not been used in mares to study the role of PGF2α in luteolysis, and therefore FM was used in the current experiment during the expected time of luteolysis (Days 14–16). Hypotheses were as follows: (1) inhibition of PGF2α synthesis interferes with luteolysis in mares, and (2) inhibition of PGF2α at the expected time of luteolysis may lead to prolonged luteal activity. The effect of FM on circulating P4 was on the basis of concentrations when assessed daily or every 8 hours and by the number and prominence of PGFM pulses in hourly samples.

## 2. Materials and methods

### 2.1. Mares

Mares were mixed breeds of nonlactating large ponies and apparent pony-horse crosses, aged 4 to 18 years and weighed 400 to 600 kg. The mares had not been bred for at least 3 years. Abnormalities of the reproductive tract were not detected by ultrasonographic examinations [32]. The experiment was done in the summer (June). The mares were housed under natural light in an open shelter and outdoor paddock and were maintained by free access to primarily grass hay, trace-mineralized salt, and water. All mares remained healthy and in good body condition throughout the study. Mares were handled according to the United States Department of Agriculture Guide for Care and Use of Agricultural Animals in Agricultural Research and Teaching.

### 2.2. Protocol

Mares were assigned randomly to a control group and an FM-treated group ($n = 8$ mares/group). Mares in the FM-treated group were given an iv treatment of FM (1.0 mg/kg) every 8 hours, whereas the mares in the control group were not treated. The dose of FM approximated the dose of 1.1 mg/kg that was recommended by the manufacturer and was experimentally used to block the role of PGF2α in uterine contractions [19]. Treatments were given on each of Days 14, 15, and 16 at 7 am, 3 pm, and 11 pm (8-hour intervals). Days 14 to 16 are the expected days of functional regression of the CL [6].

Mares were examined daily from Day 11 until ovulation by gray-scale and color-Doppler ultrasonography for determining luteal area ($cm^2$) at the maximal cross-sectional plane, diameter of the two largest follicles in each ovary, endometrial score from 1 to 4 (minimal to maximal) as an estimate of the extent of endometrial edema [32], and percentage of CL with color-Doppler signals for blood flow of the CL [33]. The percentage of the CL with color-Doppler signals of blood flow was estimated from a scan of the entire CL. A duplex B-mode (gray scale) and color-Doppler ultrasound instrument (Aloka SSD 3500; Aloka American, Wallingford, CT, USA) equipped with a linear-array 7.5-MHz transducer was used. Blood samples were collected into heparinized vacutainer tubes from a jugular vein. Samples were taken every day from Day 11 to ovulation and every 8 hours from Day 13 at 7 am, 3 pm, and 11 pm (Days 13.0, 13.3, and 13.7, respectively) until Day 17.0 (Fig. 1). In addition to 8-hour samples, blood samples were taken each hour for 8 hours on each of Days 14, 15, and 16 beginning at 7 am. The first sample in each 8-hour set was designated hour 0 and was taken just before FM treatment.

Prolonged luteal activity was indicated if a statistical outlier occurred in the interval from ovulation to the beginning of luteolysis or from ovulation to the end of