ABSTRACT

The objective of this study was to test the efficacy of a compounded long-acting progesterone formulation (BioRelease P4 LA 150; BETPHARM, Lexington, KY) containing 150 mg progesterone/ml for pregnancy maintenance in mares after prostaglandin (PG) F2α-induced luteolysis. On day 18 of gestation, mares were randomly assigned to one of four groups (n = 7/group): (1) saline-treated control (Saline); (2) PGF2α-treated control (PGF); (3) PGF2α- and Regu-Matetreated (Regu-Mate); and (4) PGF2α- and BioRelease P4 LA 150-treated (BioRelease). On day 18, Saline mares received 1 ml sterile saline IM, whereas PGF, Regu-Mate, and BioRelease mares received 250 μg cloprostenol IM. Beginning on day 18, Regu-Mate mares received 10 ml Regu-Mate orally once daily and BioRelease mares received 10 ml BioRelease P4 LA 150 containing 150 mg/ml progesterone IM once every 7 days; treatments were continued until day 45 or until pregnancy loss occurred. Pregnancy diagnosis was performed every 3 days between days 18 and 45 (or until pregnancy loss). Pregnancy loss was defined as complete absence of a discernible embryonic vesicle as determined with transrectal ultrasonography. Pregnancy loss rates between days 18 and 45 were: Saline, 1/7; PGF, 7/7; Regu-Mate, 1/7; and BioRelease, 0/7. The pregnancy loss rate was higher (P < .01) in PGF2α-treated control mares compared with the other groups. There were no differences (P > .1) in pregnancy loss rates among the saline-treated control, Regu-Mate-treated, and BioRelease P4 LA 150-treated mares. These results indicate that intramuscular administration of BioRelease P4 LA 150 containing a total of 1.5 g progesterone every 7 days provided a sufficient level of progesterone to maintain pregnancy between days 18 and 45 of gestation in mares that lacked an endogenous source of progesterone; therefore, this long-acting formulation of progesterone appears to be an efficacious and suitable alternative to currently available progesterone formulations that require daily administration.

Keywords: Equine; Mare; Pregnancy; Exogenous progesterone; Pregnancy loss

INTRODUCTION

Despite a paucity of scientific evidence supporting its efficacy, administration of exogenous progesterone to pregnant mares in an effort to enhance maintenance of pregnancy continues to be a widespread practice.1 Although low progesterone levels caused by primary corpora luteal (CL) insufficiency has been proposed as a cause of early embryonic loss in mares, its occurrence has not been clearly documented.1 Without a specific indication (or contraindication) for its use, progesterone supplementation is often empirically performed in mares that have a history of repeated pregnancy failure when no specific factor causing pregnancy loss is identified. In pregnant mares, the fetal-placental unit begins secreting progesterone and related progestogens between days 80 and 100 of gestation,2 the levels of which become sufficient to maintain pregnancy after day 100.3,4 Because fetal-placental progesterone secretion becomes sufficient to maintain pregnancy between days 80 and 100 of gestation, exogenous progesterone supplementation is generally discontinued between days 100 and 120, because there is no physiological basis for its continued administration after that time.

Exogenous progesterone also has been used to maintain pregnancy in ovariectomized embryo recipient mares, which eliminates the need for synchronizing ovulation in donor and recipient mares4-7; however, the use of ovariec-tomized embryo recipient mares has not been widely adopted. More recently, however, exogenous progesterone has been used successfully to maintain pregnancy in intact, nonovulatory recipient mares used for oocyte transfer.8,9 The use of nonovulatory mares not only eliminates the need to synchronize the estrous cycles of oocyte donor and recipient mares, but more importantly eliminates the need to recover the recipient mare’s oocyte, which otherwise must be removed from cycling mares to preclude it from becoming fertilized when the recipient mare is
inseminated. Because nonovulating recipient mares (whether ovariectomized or intact) lack an endogenous source of progesterone, maintenance of pregnancy is entirely dependent on receiving a sufficient amount of exogenous progesterone until accessory CL form (in intact mares) or fetal-placental production of progesterone becomes sufficient to maintain pregnancy.

Currently, no commercially available formulation of progesterone is labeled for use in pregnant mares; therefore, the use of exogenous progesterone for maintenance of pregnancy is an “extra-label” use. Two preparations of progesterone commonly administered to pregnant mares are daily oral administration of the synthetic progestin altrenogest (Regu-Mate; Intervet, Inc., Millsboro, DE) or daily intramuscular administration of progesterone in oil. Although both treatments have been shown to be efficacious by their ability to maintain pregnancy in mares without an endogenous source of progesterone,

MATERIALS AND METHODS

This study was conducted in the Northern Hemisphere (lat. 47° 7’ N) using mares of mixed breeding that were 3 to 12 years old and weighed 300 to 500 kg. Experiment 1 was performed in January using seasonally anovulatory mares to determine the blood levels of progesterone produced after administration of the long-acting progesterone formulation. During the week before treatment, the reproductive tracts of the mares were examined with transrectal palpation and ultrasonography; and jugular blood samples were collected for subsequent progesterone analysis three times. Mares were considered to be seasonally anovulatory if they met the following criteria at each pretreatment examination: (1) absence of ovarian follicles >20 mm in diameter; (2) absence of a CL; (3) minimal uterine and cervical tone; and (4) blood progesterone level <0.5 ng/ml. Mares that met those criteria were randomly assigned to one of two groups (n = 5/group): (1) untreated control and (2) BioRelease P4 LA 150-treated. BioRelease P4 LA 150-treated mares received a single intramuscular injection of 10 ml (split in two separate injection sites on the same side of the neck) BioRelease P4 LA 150 containing 150 mg/ml progesterone. Jugular blood samples were collected from control and treated mares immediately before administration of hormone to treated mares, then every 8 hours for 48 hours (to monitor the acute change in progesterone level), and then once daily through day 14 posttreatment. Blood samples were allowed to clot at room temperature, after which the serum was removed and kept frozen at −20°C until progesterone was measured by radioimmunoasay. During the posttreatment blood collection period, the reproductive tracts of control and treated mares were examined twice weekly with transrectal palpation and ultrasonography to confirm continued anovulatory status.

Experiment 2 was conducted during September and October to assess the ability of the long-acting progesterone formulation to maintain pregnancy after PGF2α-induced luteolysis. The reproductive tracts of cycling mares were examined 4 times weekly with transrectal palpation and ultrasonography. Mares with an ovarian follicle ≥30 mm in diameter and prominent endometrial edema were examined every other day until ovulation was detected. Ovulation was defined as disappearance of ovarian follicles >35 mm in diameter between two successive examinations, and subsequent identification of the CL by ultrasound. The day ovulation was detected was defined as day 0.

When mares developed a follicle ≥35 mm in diameter, they were artificially inseminated with at least 500 million progressively motile spermatozoa from one fertile stallion and treated with 2,500 IU human chorionic gonadotropin (Chorulon; Intervet, Inc., Millsboro, DE) IV or 2.1 mg deslorelin acetate (Ovuplant; Fort Dodge Animal Health, Fort Dodge, IA) SQ. If ovulation had not occurred within 48 hours after insemination, mares were re inseminated with fresh semen from the same stallion. Pregnancy diagnosis was performed using transrectal ultrasonography beginning on day 12. A positive pregnancy diagnosis was defined as identification of an embryonic vesicle that changed location within the uterine lumen or increased in size between two daily examinations during days 12 to 16.

On day 18, mares were confirmed pregnant and randomly assigned to one of four groups (n = 7/group): (1) saline-treated control (Saline); (2) PGF2α-treated control (PGF); (3) PGF2α- and Regu-Mate-treated (Regu-Mate); and (4) PGF2α- and BioRelease P4 LA 150-treated (BioRelease). On day 18, Saline control mares received 1 ml sterile saline IM, whereas PGF control, Regu-Mate, and BioRelease mares received 250 μg cloprostenol (Estrumate; Bayer Corp., Shawnee Mission, KS) IM. Also beginning on day 18, Regu-Mate mares
received 10 ml Regu-Mate orally once daily and BioRelease mares received 10 ml BioRelease P4 LA 150 IM (split in two separate injection sites on the same side of the neck) once every 7 days (alternated between the right and left sides of the neck) until day 45 or until pregnancy loss occurred. Jugular blood samples were collected (and processed as described) immediately before treatments were administered on day 18, daily until day 27, and then every 3 days until day 45 (or until the time of pregnancy loss). Pregnancy diagnosis with transrectal ultrasonography was performed every 3 days between days 18 and 45 (or until the time of pregnancy loss). The following endpoints were recorded at each examination: (1) size and location of the conceptus and (2) presence or absence of an embryonic heartbeat once the embryo-proper was evident within the embryonic vesicle. Pregnancy loss was defined as complete absence of a discernible embryonic vesicle as determined with transrectal ultrasonography.

Progesterone was measured directly in unelextracted serum using an 125I-progesterone radioimmunoassay (DSL-3400; Diagnostic Systems Laboratories, Inc., Webster, TX) that was validated for equine serum. Serial dilutions of pooled equine serum were parallel to the standard curve. Addition of 0.01, 0.05, 0.2, and 0.7 ng progesterone to pooled equine serum gave a regression of amount added (x = ng) to amount recovered (y = ng) of y = 1.3x - 0.02 (R² = 0.995; P < .01). Sensitivity of the assay was 0.1 ng/ml; values below the assay sensitivity were assigned a value equal to the sensitivity. The intra- and interassay coefficients of variation were 6.4% and 8.1%, respectively.

Statistical analyses were performed with SAS (version 9.1; SAS Institute Inc., Cary, NC). Blood progesterone levels in both experiments were compared using a repeated measures ANOVA; for experiment 2, the analysis was limited to days 18 to 27, the period during which blood samples were collected daily. In experiment 2, the incidence of pregnancy loss was compared among groups using chi-square analysis and Fisher’s exact test. Differences were considered significant if P < .05.

RESULTS

Experiment 1
There was no difference (P > .1) in the pretreatment level of progesterone between untreated control and BioRelease P4 LA 150-treated seasonally anovulatory mares, whereas after treatment, progesterone levels were higher (P < .001) in BioRelease P4 LA 150-treated mares beginning at 8 hours and continuing through day 12 posttreatment (Fig. 1). The blood progesterone concentration decreased to approximately 2.0 ng/ml by day 7 posttreatment in the BioRelease P4 LA 150-treated mares, which was the treatment interval used in experiment 2.

Experiment 2
The pregnancy loss rates between days 18 and 45 were: Saline control, 1/7; PGF control, 7/7; Regu-Mate, 1/7; and BioRelease, 0/7. All seven PGF control mares lost their pregnancies by day 24 of gestation, whereas one Saline control mare and one Regu-Mate mare lost their pregnancies on days 33 and 39, respectively. The incidence of pregnancy loss was higher (P < .01) in PGF control mares compared with the other groups, and there were no differences (P > .1) in pregnancy loss rates among the Saline control, Regu-Mate, and BioRelease mares. Progesterone concentrations in the PGF control and Regu-Mate mares were lower (P < .01) than Saline control mares on day 19, and reached basal levels (<1.0 ng/ml) by day 20 (Fig. 2). Compared with Saline control mares, the progesterone concentration in the BioRelease mares tended (P < .1) to be higher on day 19; tended (P < .1) to be lower on day 23; was lower (P < .01) on days 24 and 25; and (after treatment on day 25) was higher (P < .01) on days 26 and 27 (Fig. 2). As in experiment 1, the blood progesterone concentration decreased to approximately 2.0 ng/ml by day 7 posttreatment in the BioRelease P4 LA 150-treated mares (see day 25 in Fig. 2). Overt signs of adverse injection site reactions (pain, swelling, etc.) to the BioRelease P4 LA 150 were not observed in any of the treated mares.

DISCUSSION
The results of experiment 1 demonstrated that intramuscular administration of 10 ml BioRelease P4 LA 150 containing a total of 1.5 g progesterone maintained circulating concentrations of progesterone above basal levels for 12 days posttreatment. However, the progesterone concentration in treated mares decreased to approximately 2.0 ng/ml by day 7 posttreatment, which was the treatment interval used in experiment 2. The results of experiment 2 demonstrated that administration of 10 ml BioRelease P4 LA 150 every 7 days provided a sufficient level of progesterone to maintain pregnancy between days 18 and 45 of gestation.
in mares after PGF2α-induced luteolysis on day 18. Therefore, BioRelease P4 LA 150 appears to be a suitable alternative to progesterone formulations that require daily administration for maintenance of pregnancy in mares.

As expected, administration of PGF2α on day 18 induced complete luteolysis as evidenced by the decline in progesterone to <1.0 ng/ml within 2 days of treatment in both the PGF2α-treated control mares and PGF2α- and Regu-Mate-treated groups; altrenogest does not cross-react with native progesterone, which allows accurate determination of endogenous progesterone levels during Regu-Mate treatment. Although progesterone delivered by the BioRelease P4 LA 150 masked the fall in endogenous progesterone after PGF2α treatment, the progesterone profile in the BioRelease P4 LA 150-treated mares was consistent with complete luteolysis in those mares, because both the pattern and level of progesterone were similar to those observed in the BioRelease-treated anovulatory mares in experiment 1.

A gradual decline in progesterone concentration to approximately 2.0 ng/ml in the BioRelease P4 LA 150-treated mares was clearly compatible with maintenance of pregnancy during the study period. This finding is similar to previous work using ovariolectomized mares that demonstrated early pregnancy was supported when mares received exogenous progesterone supplementation that maintained blood levels of progesterone at approximately 2.0 ng/ml; however, in those mares the blood progesterone level was determined approximately 24 hours after administration of the exogenous hormone (ie, immediately before administration of the subsequent dose of hormone), so the actual level of circulating progesterone was probably higher than 2.0 ng/ml for much of the 24-hour interval between treatments. Therefore, the absolute minimum threshold of progesterone (on a continuous basis over time) necessary for maintenance of early pregnancy in the mare remains unknown. The level is likely somewhere between 2.0 and 4.0 ng/ml, because in another study using ovariolectomized mares, mares that maintained pregnancy with exogenous hormone supplementation consistently had serum progesterone concentrations above 4.0 ng/ml. Further work will be necessary to definitively identify the threshold level of progesterone necessary for maintenance of early pregnancy in the mare.

In summary, these results indicate that intramuscular administration of 10 ml BioRelease P4 LA 150 containing 150 mg progesterone every 7 days provided sufficient levels of progesterone to maintain pregnancy between days 18 and 45 of gestation in mares without an endogenous source of progesterone; therefore, this long-acting formulation of progesterone appears to be an efficacious and suitable alternative to currently available progesterone formulations that require daily administration.

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