Effects of flunixin meglumine on postponement of ovulation in mares

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OBJECTIVE
To evaluate use of flunixin meglumine as a treatment to postpone ovulation in mares, mare fertility after flunixin meglumine treatment during estrous cycles, and effects of flunixin meglumine on function of the corpus luteum after ovulation.

ANIMALS
13 healthy mares.

PROCEDURES
A single-blinded, placebo-controlled, crossover study was conducted. Flunixin meglumine (1.1 mg/kg, IV, q 24 h) or lactated Ringer solution (placebo treatment) was administered for 2 days to mares with a dominant follicle (≥ 35 mm in diameter) and behavioral signs of estrus. Mares then were bred by artificial insemination. Number of days to ovulation from initial detection of a follicle ≥ 30 mm in diameter, uterine edema score, and pregnancy were determined by ultrasonography; the examiner was unaware of the treatment of each mare. Serum progesterone concentrations were evaluated 5 and 12 days after ovulation by use of radioimmunoassay.

RESULTS
Data were available for 45 estrus cycles of the 13 mares. Number of days to ovulation from initial detection of a follicle ≥ 30 mm was not significantly affected by administration of flunixin meglumine versus the placebo. Per-cycle pregnancy rate was not significantly different between flunixin meglumine (20/24 [83%] breedings) and the placebo (13/19 [68%] breedings). Flunixin meglumine did not significantly affect behavioral signs of estrus, uterine edema, or serum progesterone concentrations.

CONCLUSIONS AND CLINICAL RELEVANCE
Findings did not support the use of flunixin meglumine to postpone ovulation in mares. (*Am J Vet Res* 2019;80:306–310)

The onset of ovulation in mares can be advanced via pharmaceutical administration.[1–3] The use of ovulation-inducing agents such as human chorionic gonadotropin and deslorelin acetate has become commonplace. The predictability for ovulation-induction agents has aided in the rapid adoption of the use of chilled-shipped and frozen semen in horses.[4,5] However, an intervention that postpones ovulation and does not impinge fertility has not yet been described. Advancing the time of ovulation has been used to schedule mating or insemination, and postponement of ovulation may be used in a similar manner. This would be especially true for popular stallions with full breeding schedules and for times when shipments of semen may not be possible (eg, holiday weekends).

Attempts to postpone ovulation with progestogens have been unsuccessful.[6] The administration of flunixin meglumine to postpone ovulation has been applied on the basis of only empirical evidence, with the assumption that it will impact the local production of prostaglandins, particularly in the follicular wall, at the time of ovulation.[7,8] Inhibiting or delaying the production of prostaglandins may inhibit ovulation for a time. Administration of high and repeated doses of NSAIDs to mares will completely block ovulation.[9–14] The use of NSAIDS inhibits ovulation and also replicates the pathological anovulatory conditions of hemorrhagic and luteinized unruptured follicles.[15] The objectives of the study reported here were to determine whether administration of flunixin meglumine would alter the time of ovulation and to evaluate the effects of flunixin meglumine treatment on fertility. We hypothesized that administration of flunixin meglumine to mares during the preovulatory period would delay ovulation, compared with the time of ovulation after placebo treatment. Additionally, we hypothesized that flunixin meglumine administration would result in a lower overall pregnancy rate, compared with the pregnancy rate for the placebo treatment.

Methods and Materials

Animals

Thirteen university-owned mares of various breeds (Thoroughbreds, Quarter Horses, and warm-

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bloods) were used in the study. Mares were 4 to 15 years old. The study was conducted during the natural breeding season of the Northern Hemisphere of 2015 (June to September). Mares were kept at pasture during the experiment with ad libitum access to hay and water. Animal procedures were in accordance with Cornell University Institution Animal Care and Use Committee guidelines (Protocol No. 013-0095).

Ultrasonographic and clinical examinations

A placebo-controlled crossover study was conducted. Mares initially were examined every 1 to 3 days via transrectal palpation and ultrasonography[a] and then every day during estrus. Mares also were examined for behavioral signs of estrus by teasing to a stallion. Mares were considered to be in estrus if they had a follicle ≥ 30 mm in diameter, evidence of uterine edema, and behavioral signs of estrus (mare urinated, raised her tail, had winking of the vulva, or squatted and was judged to be receptive to mating). At each examination, data were recorded on size of the dominant follicle, size of the subordinate follicle, uterine edema (scale of 0 to 3, with 0 = no edema and 3 = marked edema), uterine intraluminal fluid, ovulation, and behavioral signs of estrus.

Treatments

Mares were initially randomly assigned (by drawing slips of paper from a hat) to receive an IV injection of 500 mg of flunixin meglumine[b] (1.1 mg/kg) or an equivalent volume of sterile lactated Ringer solution[c] (placebo treatment) once daily for 2 consecutive days. Each mare was intended to have 2 estruses with flunixin meglumine treatment and 2 estruses with placebo treatment in random order. Mares received flunixin meglumine or the placebo on the first day on which they had at least 1 follicle ≥ 35 mm in diameter, evidence of uterine edema, and behavioral signs of estrus in response to teasing to a stallion. The treatment was then repeated 24 hours later. Mares were bred every 48 hours after the second injection until ovulation was detected. Breeding was by artificial insemination with fresh extended semen from a Dutch Warmblood stallion of known fertility. Mares were bred with 1 billion progressively motile spermatozoa extended in a skim milk–based extender with amikacin.[d] Mares were examined every 24 hours after each insemination to determine intrauterine fluid accumulation and ovulation until the mare ovulated. Mares were examined on day 12 after ovulation to determine pregnancy. Pregnant mares were administered cloprostenol[e] (250 µg, IM). Mares that did not have a visible embryonic vesicle at day 12 after ovulation were reexamined on day 14; after nonpregnant status was confirmed, they were administered cloprostenol (250 µg, IM). Mares were reevaluated 5 to 7 days after induction of luteolysis. Examiners (CGD, JCD, and JLS) were not aware of the treatment that each mare received during the monitoring period.

Venous blood samples (20 mL/sample) were obtained 5 and 12 days after ovulation. Blood samples were allowed to clot for 30 minutes and then centrifuged at 500 × *g* for 10 minutes; serum was harvested and frozen at −80°C until analysis. Serum progesterone concentration was determined as a single batch analysis via radioimmunoassay at the New York State Animal Health Diagnostic Laboratory.

Statistical analysis

Effects of flunixin meglumine treatment on continuous outcomes (number of days to ovulation from initial detection of follicle ≥ 30 mm, follicular growth rate, maximal preovulatory follicular diameter, and serum progesterone concentrations) were examined by use of mixed-effects linear regression, with mare and month within mare included as random variables. Effect of pregnancy on serum progesterone concentration was examined in a similar manner. Continuous outcomes were transformed, when necessary, to fulfill model assumptions. Similarly, the effect of flunixin meglumine on number of ovulations was examined by use of a mixed-effects Poisson model and on endometrial edema and behavioral signs of estrus by use of a mixed-effects ordered logistic regression. Effect of flunixin meglumine treatment on pregnancy was modeled by use of mixed-effects multiple logistic regression. In addition to flunixin meglumine treat-

Table 1—Mean ± SD values for 45 estrus cycles of 13 mares treated at the time of estrus with flunixin meglumine or lactated Ringer solution (placebo treatment).

Variable	Flunixin meglumine	Placebo	*P* value
Time to ovulation (d)*	3.71 ± 0.30	3.76 ± 0.45	0.92
Initial follicular diameter (mm)	37.50 ± 0.61	38.50 ± 0.81	0.32
Follicular growth (mm/d)	1.59 ± 0.34	1.74 ± 0.31	0.75
Follicle diameter at ovulation (mm)	44.20 ± 1.21	45.80 ± 1.30	0.37
Number of ovulations	1.13 ± 0.07	1.15 ± 0.08	0.81

Values were considered significant at $P < 0.05$.
*Represents the number of days to ovulation from initial detection of a follicle ≥ 30 mm in diameter.

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ment, other variables (eg, maximal preovulatory follicular diameter and number of ovulations) were used in the model. Analyses were performed with commercial software.[f] Significance was set at values of $P < 0.05$.

Results

Data from 45 estrus cycles were available for analysis; data from 3 estrus cycles were incomplete and excluded, and 4 estrus cycles were not completed because the experiment was terminated in late summer. Complete data were available for 24 estrus cycles for the flunixin meglumine treatment and 21 estrus cycles for the placebo treatment. All mares had at least 3 estrus cycles with complete data, including at least 1 estrus cycle for the placebo treatment and 1 estrus cycle for the flunixin meglumine treatment.

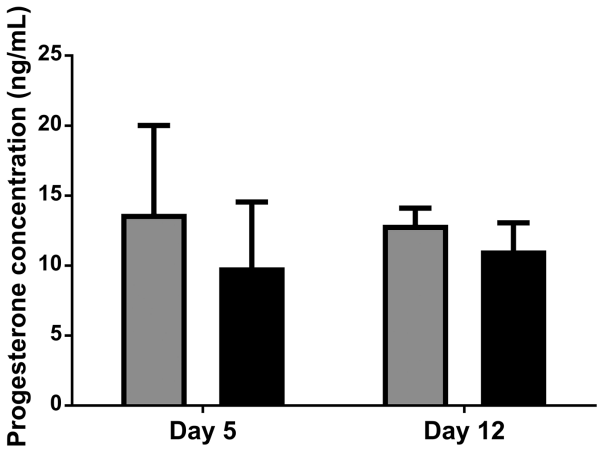

Figure 1—Mean ± SD serum progesterone concentration for 13 mares at 5 and 12 days after ovulation following administration of flunixin meglumine or lactated Ringer solution (placebo treatment) during 33 estrus cycles in which they became pregnant (gray bars) and 10 estrus cycles in which they did not become pregnant (black bars).

Administration of flunixin meglumine did not have a significant effect on the number of days to ovulation, follicular growth rate, or size of the dominant follicle at ovulation **(Table 1)**. Number of days to ovulation and maximum follicular size were not affected by month or replicate within mare. Maximum follicular size before ovulation was significantly related ($r = 0.51$; $P < 0.001$) to the number of days to ovulation but not to month of ovulation. Endometrial edema on the day before ovulation increased as the season progressed, although it was not significantly ($P = 0.39$) affected by replicate within mare.

Pregnancy rates did not differ significantly ($P = 0.26$) between flunixin meglumine treatment (20/24 [83%]) and placebo treatment (13/19 [68%]). Two estrus cycles (both for the placebo treatment) were excluded from this analysis because the mares ovulated before they could be inseminated.

Serum progesterone concentrations on days 5 and 12 after ovulation did not differ between treatments, which indicated that preovulatory administration of flunixin meglumine did not influence postovulatory luteal function (Table 1). Serum progesterone concentrations at day 5 after ovulation did not differ significantly ($P = 0.08$) between pregnant (mean ± SD, 13.70 ± 1.17 ng/mL) and nonpregnant (9.88 ± 1.47 ng/mL) mares **(Figure 1)**. Similarly, mean serum progesterone concentrations at day 12 after ovulation did not differ significantly ($P = 0.44$) between pregnant (12.92 ± 1.19 ng/mL) and nonpregnant (11.08 ± 1.97 ng/mL) mares. Progesterone concentrations in pregnant mares were not related to the number of ovulations or to flunixin meglumine treatment. Independent of pregnancy status or flunixin meglumine treatment, progesterone concentration 5 days after ovulation increased as maximum size of the preovulatory follicle increased

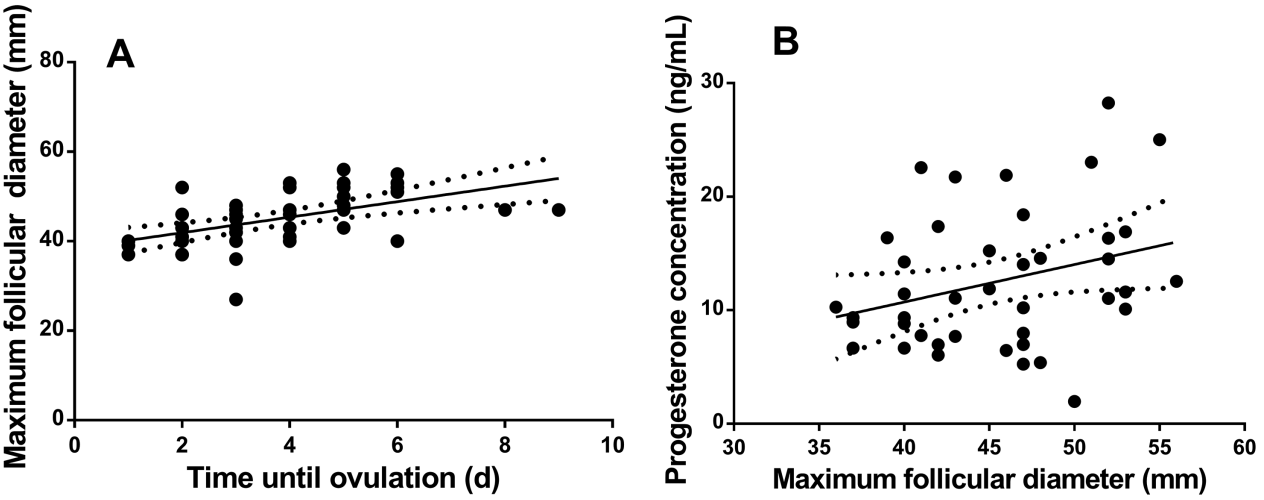

Figure 2—Dot plots of maximum follicular diameter and number of days from treatment until ovulation (A) and serum progesterone concentration 5 days after ovulation and maximum follicular diameter (B) for 45 estrus cycles of 13 mares that received flunixin meglumine or lactated Ringer solution (placebo treatment) at the time of estrus. The mean (solid line) and 95% confidence interval (dashed lines) are indicated.

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(Figure 2). Treatment with flunixin meglumine did not significantly (P = 0.80) affect endometrial edema or behavioral signs of estrus. None of the mares developed a hemorrhagic anovulatory follicle or luteinized unovulated follicle after treatment with flunixin meglumine or the placebo.

Discussion

At the doses used in the study reported here, flunixin meglumine did not delay or prevent ovulation and had no evident effect on postovulatory luteal function. The experiment was designed to have sufficient power to detect a change of 1.3 days in time to ovulation by use of a 2-tailed test. The time to ovulation for the flunixin meglumine treatment was approximately 1 hour less than for the placebo treatment, so it is reasonable to conclude that flunixin meglumine as used in the present study did not postpone ovulation in mares. Experimentally, hemorrhagic anovulatory follicles have been reliably produced in mares after administration of large and repeated doses of flunixin meglumine or phenylbutazone, usually after administration of human chorionic gonadotropin or a gonadotropin-releasing hormone analog.[12,14,16] The doses administered in the present study were comparable to those administered in clinical practice for systemic anti-inflammatory action.[17] Although ovulation was not prevented or postponed by use of flunixin meglumine at this dose and frequency of administration, deleterious effects were not observed; thus, it can be concluded that administration of flunixin meglumine at anti-inflammatory doses during the preovulatory period would not have a detrimental effect on ovulation. Previous reports on NSAID administration during the periovulatory period have described induction of intrafollicular hemorrhage and luteinization of anovulatory follicles.[10,12–14,16] In contrast to previous experiments, mares in the study reported here received lower doses of flunixin meglumine less frequently to reflect clinical application of flunixin meglumine for proposed postponement of ovulation or treatment of other conditions. The preovulatory increase in follicular prostaglandin $F_{2\alpha}$ concentration occurs approximately 10 to 12 hours before ovulation.[14] Therefore, flunixin meglumine may have been administered too early in the present study to impair ovulation. Again, administration in this manner was intended to replicate empirical application of flunixin meglumine in breeding management of mares, wherein it is administered at all stages of the estrus cycle.

No significant effect of treatment on overall pregnancy rate was detected. A limitation of the present study was that evaluation of endometrial inflammation after insemination by use of ultrasonography or endometrial cytologic examination was not included in data collection. However, none of the mares received antimicrobials or ecbolic agents after breeding. In horses, flunixin meglumine is highly protein bound and has a serum half-life of approximately 4 hours.[18] Flunixin meglumine may remain tightly bound to cyclooxygenase-2 for prolonged periods in areas of active inflammation.[17–19] More data need to be collected to evaluate the specific effects of flunixin meglumine on pregnancy rates of horses when the drug is administered at standard doses during the preovulatory period.

Previous reports[6,20] on mares examined during repeated estrus cycles have found a higher peripheral progesterone concentration in pregnant versus nonpregnant mares at 5 days after ovulation. In the study reported here, the low number of estrus cycles for nonpregnant mares may have contributed to the inability to detect a similar difference.

Attempts to postpone ovulation with progestogens have been unsuccessful, which leaves equine practitioners with advancement of ovulation as the only effective means of manipulating ovulation within a given estrus cycle.[6] We concluded that flunixin meglumine was ineffective at postponing ovulation when administered > 24 hours preceding spontaneous (noninduced) ovulation. As such, its use as a management tool for scheduling of inseminations of mares is of little clinical value. Additionally, attempts to administer flunixin meglumine closer to the time of ovulation may increase the risk of inducing a hemorrhagic luteinized unovulated follicle.

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Footnotes

a. SonoSite M Turbo, Fujifilm SonoSite, Bothell, Wash.
b. Banamine (50 mg/mL), Merck, Elkhorn, Neb.
c. Hospira Inc, San Clemente, Calif.
d. EZ Mixin CST, Animal Reproduction Systems, Chino, Calif.
e. Estrumate (250 µg/mL), Merck, Elkhorn, Neb.
f. Stata IC, version 11.0, StataCorp LP, College Station, Tex.

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