

# Effects of flunixin meglumine and transportation on establishment of pregnancy in beef cows<sup>1</sup>

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**ABSTRACT:** Objectives of these studies were to determine the effects of flunixin meglumine (FM) administration on early embryonic mortality and circulating PG and cortisol concentrations in transported and nontransported cows. Cows ( $n = 483$ ) from 3 locations were used to evaluate the effects of transportation and FM approximately 14 d after AI on the establishment of pregnancy and serum concentrations of progesterone, PGF metabolite (PGFM), and cortisol. Treatments were transport ( $n = 129$ ), transport + FM ( $n = 128$ ), no transport ( $n = 130$ ), and no transport + FM ( $n = 96$ ). Multiparous cows ( $n = 224$ ) were used at 2 locations, and nulliparous cows ( $n = 259$ ) were used at 1 location. The no transport + FM treatment was used at only 2 locations. Flunixin meglumine (approximately 1.1 mg/kg of BW; i.m.) was administered before the cows were separated into transportation groups. Transportation included 4 to 6 h of transportation, without calves, via semitractor trailer. Nontransported cows remained penned, with their calves in adjacent pens, during the same period as the transported cows. Blood samples were collected from all cows before and after treatment and, at 2 loca-

tions, approximately 3 h after the onset of treatment. Location affected AI pregnancy rate ( $P < 0.01$ ). Treatment effects, although not significant ( $P = 0.16$ ), were of a magnitude to be considered practically important. Cows that received transportation + FM tended ( $P = 0.07$ ) to have greater AI pregnancy rates (74%) than those that did not receive FM (66%), irrespective of transportation. Cortisol concentration was greater ( $P < 0.05$ ) for transported cows than for nontransported cows. Cows receiving FM had greater ( $P < 0.05$ ) AI pregnancy rates than non-FM cows (71 vs. 61%, respectively). Cows receiving transportation had lower ( $P < 0.01$ ) mean PGFM concentrations than nontransported cows (45.4 vs. 54.6 pg/mL, respectively), and cows receiving FM had lower ( $P < 0.01$ ) mean PGFM concentrations than non-FM cows (39.4 vs. 60.6, respectively). We conclude that transportation of cows approximately 14 d after AI increased serum cortisol concentrations but did not affect AI pregnancy rates. However, treatment of cows with FM increased AI pregnancy rates, irrespective of whether they were transported.

**Key words:** cattle, flunixin meglumine, pregnancy establishment, transportation stress

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## INTRODUCTION

In some regions of the United States, producers using AI often transport cattle after AI to summer pasture. The timing of transport after AI may have negative effects on pregnancy establishment. Harrington

et al. (1995) reported that 74, 62, and 65% of heifers transported on d 1 to 4, 8 to 12, or 29 to 33 after AI were pregnant. When transportation is not feasible early after breeding, producers are faced with the dilemma of transporting females when an AI or subsequent pregnancy may be at increased risk. Producers might choose to transport cattle after the AI pregnancy is at a reduced risk, but we have witnessed a 6% pregnancy loss in transported heifers on d 45 to 60 of pregnancy (T. W. Geary, unpublished data).

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Elevated serum cortisol concentration has been used as an index of stress in cattle (Lefcourt and Elsasser, 1995), and transportation has been demonstrated to increase serum cortisol concentration (Crookshank et al., 1979). In addition, embryonic loss around the time of maternal recognition of pregnancy may occur because some embryos are unable to sufficiently inhibit uterine secretion of PGF<sub>2 $\alpha$</sub>  (Thatcher et al., 2001). Mechanisms by which increased stress, cortisol, or both, might affect PGF<sub>2 $\alpha$</sub>  or initiate pregnancy loss are unknown.

Flunixin meglumine (**FM**) is a potent nonsteroidal, antiinflammatory agent that inhibits cyclooxygenase, thus preventing conversion of arachadonic acid to PGF<sub>2 $\alpha$</sub>  (Anderson et al., 1990; Odensvik, 1995). Treatment with FM has been shown to decrease PGF<sub>2 $\alpha$</sub>  secretion in dairy and beef cows for at least 24 h (Guilbault et al., 1987). Our hypothesis was that transportation stress may reduce fertility by increasing PGF<sub>2 $\alpha$</sub>  and that FM administration to cattle receiving transportation stress 10 to 14 d after AI would decrease PGF<sub>2 $\alpha$</sub>  and embryonic mortality.

The objectives of these studies were to determine the effects of FM administration on early embryonic mortality and serum PG and cortisol concentrations in cows receiving transportation or no transportation stress.

## MATERIALS AND METHODS

### *Animals and Treatments*

Procedures used for all cows were approved by the Montana State University Institutional Animal Care and Use Committee.

Cows from 3 locations were used to evaluate effects of transportation stress and a single administration of FM approximately 14 d after AI on pregnancy establishment and serum concentrations of progesterone, 13, 14-dihydro-15-keto PGF<sub>2 $\alpha$</sub>  metabolite (**PGFM**), and cortisol. Estrous cycles were synchronized, and cows were bred by AI approximately 12 h after the onset of estrus or by timed AI coincident with an injection of GnRH. Cows were assigned within location, AI sire, AI date, and AI technician to treatment, and treatments were applied approximately 14 d after AI. Treatments were transportation, transportation + FM, no transportation, and no transportation + FM and were applied during midmorning to afternoon at each location. Transportation included 4 to 6 h of transportation via semitractor trailer on unpaved and paved roadways. Cows receiving FM (Flunixinamine, Fort Dodge Animal Health, IA) were administered approximately 1.1 mg/kg of BW, i.m., in the neck just after the initial blood collection while sorting the cattle to their respective treatments.

Blood samples were collected from the median caudal vein of all cows before and after application of treatment for measurement of serum cortisol, progesterone, and PGFM concentrations.

Calves were removed from all cows and remained in a separate pen adjacent to the nontransported cows for the duration of the treatments. At each location, cows were exposed to bulls in a single breeding pasture beginning 1 d after treatment (approximately 15 d after AI) for 30 d. The bull-to-cow ratio for locations 1, 2, and 3 was approximately 1:25, 1:25, and 1:20, respectively. Treatments were applied to cows at each location during early to mid June, when forage quality and quantity were at peak levels and were assumed to be sufficient to meet the energy requirements. All cows at each location were managed similarly until diagnosed for pregnancy at 30 to 55 d after AI using transrectal ultrasonography (Aloka 550 with 5-MHz linear array probe; Aloka, Wallingford, CT). Not placing the cows with the bulls until 15 d after AI coupled with early pregnancy diagnosis allowed us to accurately distinguish between pregnancies resulting from AI and natural service.

At the first location, 97 multiparous Angus-cross cows (BW, 636  $\pm$  4.5 kg; mean age, 5.4 yr) were used. Cows received a 33-d melengestrol acetate- (**MGA**) PGF<sub>2 $\alpha$</sub>  treatment [0.5 mg of MGA/d for 14 d followed by PGF<sub>2 $\alpha$</sub>  (25 mg, i.m.; ProstaMate, Phoenix Scientific, Saint Joseph, MO) 19 d after discontinuing the MGA feeding]. All animals were observed for estrus twice daily, from 0600 to 0900 and from 1800 to 2100, for 5 d after PGF<sub>2 $\alpha$</sub>  injection and received AI approximately 12 h after the onset of estrus. Cow body condition was not recorded for individual cows at this location, because they were uniform in condition (range, 5.0 to 5.5). Cows at this location only received 3 treatments (transportation, no transportation, transportation + FM) because of a limited number of animals.

Nontransported cows (n = 33) remained at the ranch and were provided access to water but no feed for 5 h. Transported (n = 32) and transported + FM (n = 32) cows were transported via semitractor trailer for 4 h (mean ambient temperature, 24°C). Rectal temperatures also were recorded for all cows before and after treatment using 1 of 2 M216 Series Hi-Speed digital thermometers (GLA Agricultural Electronics, San Luis Obispo CA). The thermometers registered the same temperature in 5 consecutive cows at the onset of treatment. A pretreatment and posttreatment blood sample was collected from each cow. The mean time interval between blood samplings and rectal temperature recordings was 5 h due to unloading, mixing, and sample collection.

At the second location, 127 multiparous Angus-cross cows (BW, 681  $\pm$  4.5 kg; mean age, 5.4 yr) were utilized. Estrous cycles were synchronized using the 33-d MGA-PGF<sub>2 $\alpha$</sub>  treatment, and cows received AI after detection of estrus as described above. Cow body condition was not recorded for individual cows at this location, because they were very uniform in condition (range, 5.0 to 5.5). Nontransported (n = 32) and nontransported + FM (n = 31) cows remained at the ranch and were

provided access to water but no feed. Transported ( $n = 32$ ) and transported + FM ( $n = 32$ ) cows were transported via semitractor trailer for 4 h (mean ambient temperature, 28.5°C).

Blood samples were collected before application of the treatment, approximately 2 h after the initiation of treatment, and at the completion of treatment. To obtain the intermediate blood sample from the 2 groups of transported cows, they were unloaded from the semitractor trailer 2 h after initiation of treatment at a cattle working facility 76 km from the home ranch and reloaded onto the semitractor trailer to complete their transportation treatment. The intermediate blood sample was collected from the 2 nontransported groups of cows at the home ranch approximately 2 h after initiation of treatment. The final blood sample was collected from all cows as described above at the home ranch approximately 5 h after the first blood sample collection.

At the third location, 259 nulliparous Angus-cross cows ( $5.15 \pm 0.34$  BCS) were utilized. Cows were synchronized using a controlled internal drug release device (CIDR, Pfizer Animal Health, New York, NY) for 7 d, with or without an injection of GnRH (100 µg, i.m.; Fertagyl, Intervet Inc., Millsboro, DE) at CIDR insertion and PGF<sub>2α</sub> (25 mg, i.m.; Lutalyse, Pfizer Animal Health) at CIDR removal. Cows were observed for estrus twice daily from 0600 to 1000 and from 1500 to 2100 for 72 h. Approximately 50% of the cows were fixed-time inseminated coincident with an injection of GnRH (100 µg, i.m.) at 60 h after PGF<sub>2α</sub>, regardless of the expression of estrus. The remainder of the cows that were observed in estrus received AI approximately 12 h after the onset of estrus, until 84 h after PGF<sub>2α</sub> when those not observed in estrus received AI coincident with GnRH (100 µg, i.m.). Cows were blocked by synchronization treatment and AI type (after estrus or timed AI) within AI sire before assignment to no transport ( $n = 65$ ), no transport + FM ( $n = 65$ ), transport ( $n = 65$ ), or transport + FM ( $n = 64$ ). Cows that were not transported remained at the ranch and were provided access to water but no feed. Transported cows were transported via semitractor trailer for 6 h (mean ambient temperature, 27°C).

Blood samples were collected before application of the treatment, approximately 3 h after the initiation of treatment, and at the completion of treatment. To obtain the intermediate blood sample from the transported cows, they were unloaded from the semitractor trailer 3 h after initiation of the treatment at a cattle working facility 90 km from the home ranch and reloaded onto the semitractor trailer to complete their transportation treatment. The intermediate blood sample was collected from nontransported cows approximately 3 h after initiation of treatment at the home ranch. The final blood sample was collected from all cows as described above at the home ranch approximately 7 h after the first blood sample collection. Rectal temperature was recorded at the time of each blood

collection for a subset of cows (30, 29, 24, and 27 transported, transported + FM, nontransported, and nontransported + FM cows, respectively) at this location.

### *Blood Samples and Hormone Assays*

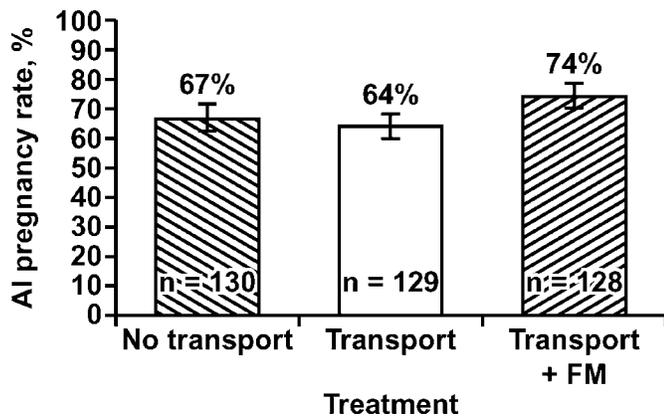
Blood samples were stored at 4°C for approximately 16 h and centrifuged at  $3,000 \times g$  for 20 min to separate serum. Serum was stored at -20°C until analyzed for concentrations of progesterone, cortisol, and PGFM. Serum samples from cows at location 1 only were evaluated in duplicate for progesterone concentration by solid-phase RIA (Coat-a-Count kit, Diagnostic Products Corp., Los Angeles, CA), as described by Bellows et al. (1991). Sensitivity of the assay was 0.08 ng/mL, and the intraassay CV was 1.2%.

Serum samples from all cows were evaluated in duplicate for cortisol concentration using a solid-phase RIA (Coat-a-Count kit; Diagnostic Products Corp.) and standards prepared in our laboratory according to the recommendations of the manufacturer. Standards contained 2-fold dilutions of 1.56 to 400 ng/mL of hydrocortisone (H5885, Sigma-Aldrich Corp., St. Louis, MO) in charcoal-stripped serum from cows that had been ovariectomized and treated with dexamethasone (20 mg, twice daily) for 3 d to inhibit adrenal steroid production. Cross-reactivity of aldosterone, dexamethasone, estriol, estrone, pregnenolone, and progesterone with this antibody was all less than 0.05%. Cross-reactivity of corticosterone, cortisone, deoxycorticosterone, and deoxycortisol was 0.94, 0.98, 0.26, and 11.4%, respectively, for this antibody. Known amounts of cortisol (50, 25, and 12 ng) added to bovine serum were accurately recovered (96.1%). Serial dilutions ( $n = 4$ ) of bovine serum samples collected during the estrous cycle were parallel to the standard curve. Sensitivity of the assay was 2.2 ng/mL, and the intraassay CV was 2.4%.

Serum samples from all cows were evaluated in triplicate for concentrations of PGFM by double-antibody <sup>3</sup>H-PGFM RIA, as described by Homanics and Silvia (1988). The PGFM antiserum (WS4468) was generously provided by W. J. Silvia, University of Kentucky, Lexington. The antiserum was raised in rabbits immunized against a PGFM-BSA conjugate. Cross-reactivity of PGF<sub>2α</sub>, PGE<sub>2</sub>, PGA<sub>2</sub>, and 6-keto-PGF<sub>1</sub> was less than 0.10%. The antiserum was used at a working dilution of 1:6,000. Known amounts of PGFM (250 and 50 pg) added to bovine serum were accurately recovered (94.5%). Serial dilutions ( $n = 4$ ) of bovine serum samples collected during the estrous cycle of 5 cows were parallel to the standard curve. Sensitivity of the assay was 11.5 pg/mL, and the intra- and interassay CV for the low- and high-pool samples were 9.2 and 16.1% and 5.5 and 14.1%, respectively.

### *Statistical Analysis*

Two sets of analyses were conducted. Data from all 3 locations in a randomized complete block design with



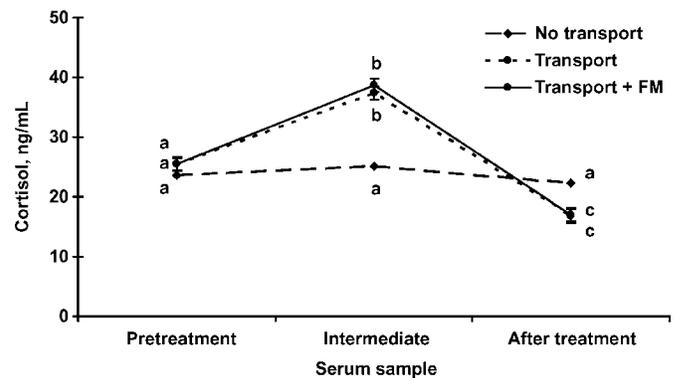
**Figure 1.** Effects of transport (4 to 6 h of transportation via semitractor trailer), transport + flunixin meglumine (FM; approximately 1.1 mg/kg of BW, i.m.), or no transport approximately 14 d after AI on AI pregnancy rates of beef cows from 3 locations. Treatment effect ( $P = 0.16$ ) and a contrast between transportation + FM and the treatments without FM ( $P = 0.07$ ) were evaluated.

no transport, transport, and transport + FM treatments were used in the first set. The second set used data from the second and third locations and all 4 treatments arranged in a 2 (location)  $\times$  2 (transportation)  $\times$  2 (FM) factorial. The latter analyses allowed testing of the interaction of transportation and FM, whereas the former analyses had greater power for testing differences among transportation, transportation + FM, and no transportation. Treatment effects on pregnancy establishment were evaluated using PROC LOGISTIC (SAS Inst. Inc., Cary, NC) with a model that also included location and the interaction of treatment and location. Hormone concentrations were analyzed using PROC MIXED of SAS with a repeated measures model that included the fixed effects of treatment, location, their interaction, time and the treatment  $\times$  time interaction, and a random effect of cow nested within the treatment by location interaction. The default variance component structures (type = VC) were assumed for both random and repeated effects. Significance tests for treatment, location, and treatment  $\times$  location interaction effects used cow nested within the interaction as the error term. Other effects were tested using residual variance as the error term.

## RESULTS

### Locations 1, 2, and 3

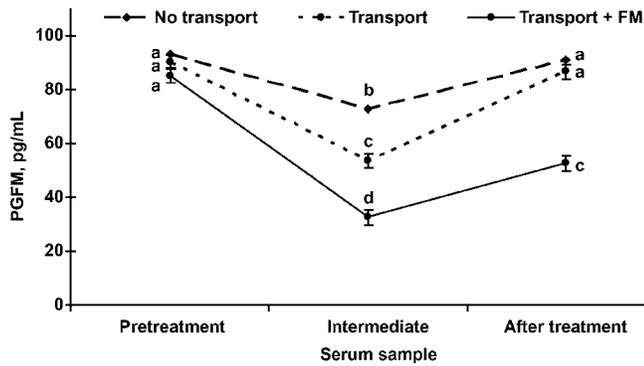
Analyses of the 3 treatments common to each location (no transport, transport, and transport + FM) revealed no treatment  $\times$  location interactions ( $P = 0.96$ ), so data were pooled. Location affected AI pregnancy rate ( $P < 0.01$ ). Treatment effects, although not significant ( $P = 0.16$ ), were of a magnitude to be practically important (Figure 1). Cows that received trans-



**Figure 2.** Serum cortisol concentrations at the initial, intermediate (after 2 to 3 h of treatment), or final blood sampling of cows from 3 locations receiving transport (4 to 6 h of transportation via semitractor trailer), transport + flunixin meglumine (FM; approximately 1.1 mg/kg of BW, i.m.), or no transport approximately 14 d after AI. <sup>a-c</sup>Differences ( $P < 0.05$ ) between treatments and time points are indicated by different letters.

portation + FM tended ( $P = 0.07$ ) to have greater AI pregnancy rates (74%) than those that did not receive FM (66%), irrespective of transportation. Pretreatment serum cortisol and PGFM concentrations did not differ among treatments. Cortisol concentration was greater for transported and transported + FM cows in the intermediate serum sample and lower for transported and transported + FM cows in the posttreatment serum sample than for nontransported cows ( $P < 0.05$ ; Figure 2). Serum PGFM decreased ( $P < 0.05$ ) for all cows but was greatest for nontransported cows, intermediate for transported cows, and lowest for transported + FM cows in the intermediate blood samples and lower for transported + FM cows than nontransported and transported cows in the posttreatment blood sample (Figure 3).

Mean rectal temperatures recorded from multiparous cows were different ( $P < 0.05$ ) among treatments ( $38.8 \pm 0.02$ ,  $38.8 \pm 0.02$ , and  $38.9 \pm 0.02^\circ\text{C}$  for transported, transported + FM, and nontransported cows, respectively), location, treatment  $\times$  location, and treatment  $\times$  time (data not shown). Rectal temperature was similar for all cows at the initial temperature collection ( $39.0 \pm 0.03^\circ\text{C}$ ) and decreased ( $P < 0.05$ ) from the initial to final sample collection for each treatment, with final temperature lowest for transported + FM ( $38.6 \pm 0.03^\circ\text{C}$ ), intermediate for transported ( $38.7 \pm 0.03^\circ\text{C}$ ), and greatest for nontransported ( $38.8 \pm 0.03^\circ\text{C}$ ) cows. Serum progesterone concentration was determined from blood samples collected from cows at location 1 only. Serum progesterone concentration differed ( $P < 0.05$ ) by treatment ( $3.4 \pm 0.2$ ,  $3.3 \pm 0.2$ , and  $2.9 \pm 0.2$  ng/mL for transported, transported + FM, and nontransported, respectively) and sample time ( $2.9 \pm 0.1$  and  $3.6 \pm 0.1$  ng/mL for initial and final blood collec-



**Figure 3.** Serum PGF metabolite (PGFM) concentrations at the initial, intermediate (after 2 to 3 h of treatment), or final blood sampling of cows from 3 locations receiving transport (4 to 6 h of transportation via semitractor trailer), transport + flunixin meglumine (FM; approximately 1.1 mg/kg of BW, i.m.), or no transport approximately 14 d after AI. <sup>a-d</sup>Differences ( $P < 0.05$ ) between treatments and time points are indicated by different letters.

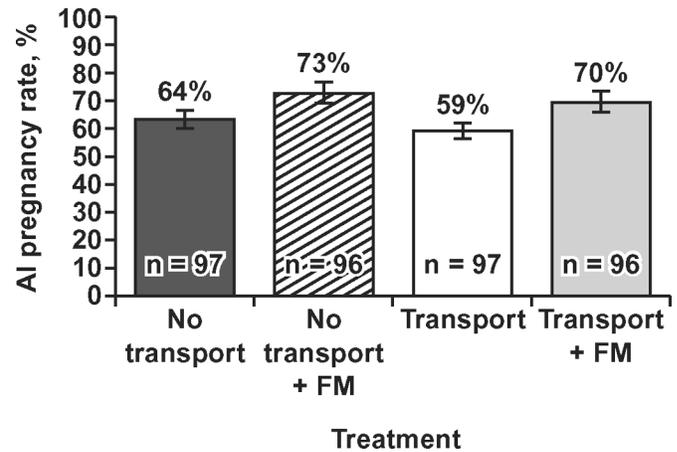
tion, respectively; data not shown). No treatment  $\times$  time interaction was observed ( $P > 0.05$ ).

### Locations 2 and 3

Analyses of the 4 treatments applied to multiparous and nulliparous cows at 2 locations revealed no treatment  $\times$  location interactions ( $P = 0.97$ ), so data were pooled for further analyses. Treatment with FM, but not transportation, affected AI pregnancy rate. Cows receiving FM treatment had greater ( $P < 0.05$ ) AI pregnancy rates than non-FM cows (71 and 61%, respectively). Figure 4 illustrates pregnancy rates of cows receiving each treatment. Pregnancy rates also differed by location, with multiparous cows having greater ( $P < 0.05$ ) AI pregnancy rates than nulliparous cows.

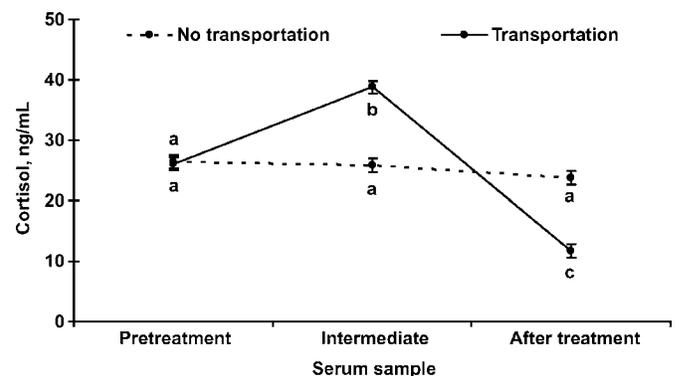
Serum cortisol concentration was affected ( $P < 0.01$ ) by location, sampling time, and a transportation  $\times$  sampling time interaction (Figure 5) but not ( $P > 0.10$ ) by FM or transportation. Serum cortisol concentrations were greater ( $P < 0.01$ ) for nulliparous cows ( $34.7 \pm 0.8$  ng/mL) than for multiparous ( $15.1 \pm 1.0$  ng/mL) cows. Serum cortisol concentrations increased ( $P < 0.01$ ) during the initial transportation period but then decreased ( $P < 0.01$ ) below initial concentrations among transported cows and remained unchanged ( $P > 0.10$ ) among nontransported cows (Figure 5).

Serum concentrations of PGFM were affected ( $P < 0.01$ ) by transportation, FM, sampling time, location, FM  $\times$  sampling time, and transportation  $\times$  FM  $\times$  sampling time. Cows receiving transportation stress had lower ( $P < 0.01$ ) mean PGFM concentrations than nontransported cows ( $45.4 \pm 1.7$  and  $54.6 \pm 1.7$  pg/mL, respectively). Cows receiving FM had lower ( $P < 0.01$ ) mean PGFM concentrations than non-FM cows ( $39.4$

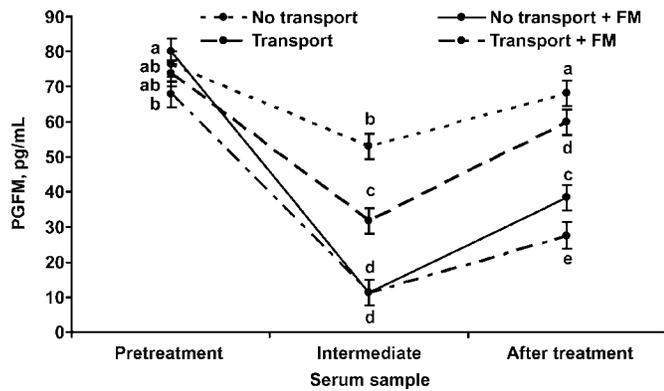


**Figure 4.** Effects of transport (4 to 6 h of transportation via semitractor trailer), transport + flunixin meglumine (FM; approximately 1.1 mg/kg of BW, i.m.), no transport, or no transport + FM approximately 14 d after AI on AI pregnancy rates of beef cows from 2 locations. Cows receiving FM had greater AI pregnancy rates than cows not receiving FM ( $P < 0.05$ ).

$\pm 1.7$  and  $60.6 \pm 1.7$  pg/mL, respectively). Nulliparous cows had greater ( $P < 0.01$ ) mean PGFM concentration than multiparous cows ( $60.0 \pm 1.4$  and  $39.0 \pm 2.0$  pg/mL, respectively). Across all treatments, serum PGFM concentrations decreased during the initial blood sampling period and then increased during the final blood sampling period but did not reach pretreatment levels by the end of sampling except among nontransported cows (Figure 6). This decrease in serum PGFM concentration was magnified among cows receiving FM treatment.



**Figure 5.** Effects of transport on serum cortisol concentrations at the initial, intermediate (after 2 to 3 h of treatment), or final blood sampling of cows receiving transportation (4 to 6 h via semitractor trailer) or no transportation approximately 14 d after AI. <sup>a-c</sup>Differences ( $P < 0.05$ ) between treatments and time points are indicated by different letters.



**Figure 6.** Serum PGF metabolite (PGFM) concentrations at the initial, intermediate (after 2 to 3 h of treatment), or final blood sampling of cows receiving transport (4 to 6 h of transportation via semitractor trailer), transport + flunixin meglumine (FM; approximately 1.1 mg/kg of BW, i.m.), no transport, or no transport + FM approximately 14 d after AI. <sup>a-c</sup>Differences ( $P < 0.05$ ) between treatments and time points are indicated by different letters.

## DISCUSSION

Treatment of cows with FM approximately 14 d after AI resulted in greater AI pregnancy rates, irrespective of whether they were transported. Treatments were administered approximately 14 d after AI, because maximal response to treatment with a PGF<sub>2 $\alpha$</sub>  inhibitor was anticipated at this time. Suppression of PGF<sub>2 $\alpha$</sub>  by interferon- $\tau$  from approximately d 12 to 17 after breeding is imperative to successful establishment of pregnancy in cattle (Bazer et al., 1991; Roberts et al., 1992). Cows receiving FM in the current study had decreased serum concentrations of PGFM consistent with previous reports (Odensvik et al., 1998; Lemaster et al., 1999). If the mechanism by which FM increased pregnancy rates was via suppressed endogenous PGF<sub>2 $\alpha$</sub>  synthesis and secretion, then the window of opportunity for improvements in pregnancy rates with FM may be limited. Thatcher et al. (1992) reported that 17 d after estrus, endometrial tissue from cyclic and pregnant cattle begin to differ in secretion of PGF<sub>2 $\alpha$</sub> .

In the current study, transportation did not decrease pregnancy rate of cows compared with nontransported cows, which contradicts an earlier report by Harrington et al. (1995). However, at each location, transported cows had numerically lower pregnancy rates than cows receiving other treatments. Nulliparous cows had lower pregnancy rates than multiparous cows. Nulliparous cows also had greater serum cortisol concentrations, indicating they perceived these treatments as being stressful. A longer duration of transportation among nulliparous cows is probably not the major factor for increased serum cortisol concentrations, because even peak cortisol concentration (at the

intermediate sample) was increased. A review by Grandin (1997) suggested that multiparous cows in the current study may have been more habituated to animal handling and transportation than nulliparous cows and thus perceived the treatments as being less stressful. Genetic differences in response to stress have also been reported (Le Neindre et al., 1995). The amount of variation between the greatest and lowest mean body temperature was only 0.09°C and probably not a major contributing factor to pregnancy success, especially because nontransported cows had the greatest mean temperature. Because of the antiinflammatory action of FM (Newton et al., 1990; Oka et al., 2001), it is not surprising that FM cows had the lowest mean body temperature, but mean body temperature was above normal body temperature (Sprinkle et al., 2000) for each treatment group, indicating that perhaps all cows were stressed to some degree. The decrease in body temperature for transported cows in the current study seems counterintuitive and disagrees with that of VonBorell (2001), who reported an increase in heart rate and temperature related to stressful conditions in livestock. However, it is also possible that transportation had a cooling effect on body temperature, because the ambient temperature at each location was below normal body temperature at the time of treatment.

The increase in progesterone for cows receiving transportation stress and the increase from the first to final blood sample collection is supported by the work of Collier et al. (1982), who reported increased progesterone among cattle that had undergone stress. Progesterone concentration in serum was not related to pregnancy success, as has been reported previously (Mann et al., 1999; Perry et al., 2005). However, it is possible that transported cows had the greatest pregnancy rate at the time of treatment, but due perhaps to treatment, maintained fewer pregnancies until the time when ultrasound diagnosis of pregnancy was conducted.

Increased cortisol concentrations at the intermediate blood sampling for transported and transported + FM cows indicate they achieved a greater level of stress than nontransported cows and is consistent with response to transport and other stresses (Lefcourt and Elsasser, 1995; Grandin, 1997). The decrease in cortisol concentration from the intermediate to final sample collection for transported cows suggests that either the hypothalamic-pituitary-adrenal axis became refractory to continued stress or the cows themselves adapted to the handling and transportation stress. Crookshank et al. (1979) and Becker et al. (1985) reported a similar increase followed by decrease in cortisol concentration among transported calves or gilts that were tethered to stalls for several hours. Alternatively, the decrease in cortisol concentration as transport time increased may have been related to depletion of releasable stores of ACTH. Cortisol concentrations did not differ between the initial and final blood sam-

pling for nontransported cows, indicating handling cattle to obtain a blood sample does not activate the hypothalamic-pituitary-adrenal axis. Serum cortisol concentrations were not influenced by treatment with FM in the current study, as was reported previously in cows treated with FM and endotoxins (Giri et al., 1991). However, Odensvik and Magnusson (1996) reported that FM treatment prevented endotoxin-induced elevation in serum cortisol concentration in heifers. A mechanism by which FM might affect serum cortisol concentration is not known and was not anticipated in the current study.

The decrease in PGFM due to transportation was unexpected in this study. Because both FM and transportation decreased serum PGFM concentration, it may have been more beneficial to administer FM to cows after transportation to keep PGF<sub>2 $\alpha$</sub>  suppressed longer. Others (Aiumlamai et al., 1990; Buford et al., 1996) have reported that PGFM concentration decreased within 30 min of FM administration but returned to baseline within 6 h. Serum concentration of PGFM was still depressed among cows in the current study 6 to 7 h after FM administration. The elevation of cortisol concentration at the intermediate sampling period for transported cows may have mediated or simply been correlated to the decrease in PGFM through some unknown mechanism. However, administration of ACTH to cows during early pregnancy (approximately d 35) increased serum cortisol concentration without decreasing serum PGFM concentration (T. W. Geary, unpublished data).

In summary, transportation of cows approximately 14 d after AI increased serum cortisol concentrations but did not affect AI pregnancy rates. However, treatment of cows with FM, an inhibitor of PG synthesis, increased AI pregnancy rates irrespective of whether they were transported. Both FM treatment and transportation suppressed serum PGFM concentrations, suggesting that administration of FM after transportation may result in a more prolonged suppression of PG. The amount of stress perceived by nontransported cows in this study is difficult to interpret because serum cortisol concentrations did not increase, but body temperature did increase. We speculate that stress associated with handling cows for sample collection may have been sufficient to elicit a response and that FM mitigated the effect in treated cows. Whether administration of FM, or other PG inhibitors, to cows approximately 14 d after AI would improve pregnancy establishment compared with cows that remain on pasture (without handling) may deserve further study.

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