

# Effects of adrenocorticotrophic hormone and flunixin meglumine on pregnancy retention in beef cows<sup>1</sup>

T. W. Geary<sup>2</sup>

USDA-ARS, Fort Keogh Livestock and Range Research Laboratory, Miles City, MT 59301

**ABSTRACT:** Pregnancy loss in beef cattle after d 28 of gestation is variable, but it has been reported to be as great as 14% and has been related to transportation or handling stress. The primary objective of this study was to determine whether activation of the hypophyseal-adrenal axis with ACTH would mimic a stressful response and cause pregnancy loss in beef cattle. A secondary objective was to determine if a single injection of the PG synthesis inhibitor flunixin meglumine would attenuate the stress response and suppress serum PGF<sub>2α</sub> concentrations to prevent pregnancy loss. Forty nonlactating beef cows that were 34 ± 0.33 d pregnant were used for this study. In a 2 × 3 factorial arrangement, cows were randomly assigned to receive ACTH [0 or 0.5 IU/kg of BW, intramuscularly (i.m.)] at 0 and 2 h of the study and flunixin meglumine (0, 1.1, or 2.2 mg/kg of BW, i.m.) at 0 h. Blood samples were collected from all cows at 0 h and every 30 min for 4 h to measure serum cortisol and PGF<sub>2α</sub> metabolite (PGFM) concentrations. Rectal temperature was collected for each cow at 0, 120, and 240 min. Pregnancy exams were conducted 31 and 58 d after treatment by transrectal ultrasonography, and the presence of a fetal heartbeat was used as an indicator of fetal viability. Se-

rum cortisol concentration was affected ( $P < 0.01$ ) by ACTH, time, and the interaction of ACTH × time, but not by flunixin meglumine ( $P \geq 0.14$ ) or any other interactions. Cortisol concentrations increased ( $P < 0.01$ ) in the serum of ACTH-treated cows immediately after ACTH treatment and remained increased ( $P < 0.01$ ) throughout the 4-h sampling period. Serum PGFM concentration was not affected by ACTH ( $P = 0.97$ ) or by any interactions ( $P > 0.35$ ) with ACTH, but was affected ( $P < 0.01$ ) by flunixin meglumine, time, and the interaction of flunixin meglumine × time. Regardless of dosage (1.1 or 2.2 mg/kg of BW), flunixin meglumine decreased ( $P < 0.01$ ) serum PGFM concentrations in both ACTH-treated and control cows for the duration of the study. Although ACTH treatment induced a prolonged increase in serum cortisol concentration, none of the cows used in this study lost a pregnancy. In conclusion, the activation of the hypophyseal-adrenal axis with ACTH increased serum cortisol concentrations but did not increase serum concentrations of PGFM or cause pregnancy loss during early gestation in cows. Flunixin meglumine treatment suppressed serum PGFM concentrations in control and ACTH-treated cows.

**Key words:** adrenocorticotrophic hormone, cattle, cortisol, flunixin meglumine, pregnancy loss

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## INTRODUCTION

Stressors applied to beef cattle during early pregnancy can affect pregnancy establishment. Transportation of beef heifers on d 8 to 12 or d 29 to 33 after AI

resulted in decreased pregnancy rates compared with transportation from d 1 to 4 after AI (Harrington et al., 1995). Transportation stress applied to beef cows and heifers approximately 14 d after AI reduced pregnancy rates, and administration of 1.1 mg/kg of BW of flunixin meglumine at the time of transportation increased pregnancy rates (Merrill et al., 2007). In addition, pregnant beef heifers transported at 30 to 60 d of gestation experienced approximately 6% pregnancy loss (T. W. Geary, unpublished data). This study evaluated the hypothesis that transportation stress, via activation of the hypophyseal-adrenal axis, causes embryonic mortality during early pregnancy in beef cows. It was further hypothesized that administration of ACTH would mimic a stressful condition, similar to transportation, and would cause pregnancy loss during early gestation.

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<sup>2</sup>Corresponding author: tom.geary@ars.usda.gov

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Flunixin meglumine is a potent nonsteroidal, anti-inflammatory agent that inhibits cyclooxygenase, thereby preventing conversion of arachidonic acid to  $\text{PGF}_{2\alpha}$  (**PGF**; Anderson et al., 1990; Odensvik, 1995). (Guilbault et al., 1987) reported that intramuscular (**i.m.**) treatment with flunixin meglumine decreased serum PGF metabolite (**PGFM**) concentrations in nonlactating cows for at least 12 h. Cows that received flunixin meglumine 14 d after AI had 10% greater AI pregnancy rates and 21% less serum PGFM than controls (Merrill et al., 2007). In the same study, flunixin meglumine had no effect on serum cortisol concentrations (Merrill et al., 2007). Thus, an additional hypothesis of the present study was that flunixin meglumine would decrease PGFM and prevent the pregnancy loss anticipated with an ACTH-induced stress response. The objective of this study was to determine the effects of stress induction, via activation of the hypophyseal-adrenal axis using ACTH, and administration of flunixin meglumine on early pregnancy maintenance and serum concentrations of cortisol and PGFM.

## MATERIALS AND METHODS

This research was conducted in accordance with procedures approved by the Fort Keogh Animal Care and Use Committee.

### *Animals and Treatments*

Forty nonlactating beef cows (predominantly Hereford  $\times$  Angus; BW:  $540 \pm 13$  kg; age:  $6 \pm 0.43$  yr) known to be in early pregnancy [ $34 \pm 0.33$  d (mean  $\pm$  SE); range 32 to 37 d] were used for this study. Cows were randomly assigned to receive saline or 0.5 IU/kg of BW, **i.m.**, of ACTH (Porcine ACTH No. A6303, ACTH fragment 1–39, Sigma-Aldrich Chemical Co., St. Louis, MO) at 0 and 2 h of the study and saline or flunixin meglumine [0 ( $n = 20$ ), 1.1 ( $n = 10$ ), or 2.2 ( $n = 10$ ) mg/kg of BW, **i.m.**; Banamine, Intervet Schering-Plough Animal Health, Millsboro, DE] at 0 h in a  $2 \times 3$  factorial arrangement of treatments. The dosages of ACTH and flunixin meglumine used in this study were based on the reports of Lay et al. (1996), Odensvik and Magnusson (1996), and Merrill et al. (2007). Blood samples (10 mL) were collected from the coccygeal blood vessels of all cows at 0 h (immediately before treatment) and every 30 min for 4 h to measure serum cortisol and PGFM concentrations. Rectal temperature was measured for each cow at 0, 120, and 240 min. To facilitate blood collection, cows were cycled 9 times through the animal handling facility, in which 10 cows could be restrained simultaneously for blood collection. Each blood collection included a separate puncture of the coccygeal blood vessels using a new needle.

Pregnancy was diagnosed at the initiation of treatment, at 31 d, and again 58 d after treatment by using transrectal ultrasonography (Aloka, Wallingford, CT)

with a 5-MHz probe. A fetal heartbeat was confirmed at each pregnancy diagnosis to confirm a viable fetus.

### *Blood Samples and Hormone Assays*

Blood samples were stored at  $4^{\circ}\text{C}$  for approximately 16 h and centrifuged at  $3,000 \times g$  for 20 min at  $4^{\circ}\text{C}$  to separate serum. Serum was stored at  $-20^{\circ}\text{C}$  until analyzed for concentrations of cortisol and PGFM. Serum samples from all cows were evaluated in duplicate for cortisol concentration using solid-phase cortisol RIA (Coat-a-Count, Diagnostic Products Corp., Los Angeles, CA) as described by Merrill et al. (2007). Sensitivity of the assay was 2.2 ng/mL and the intra- and interassay CV were 2.4 and 3.9%, respectively.

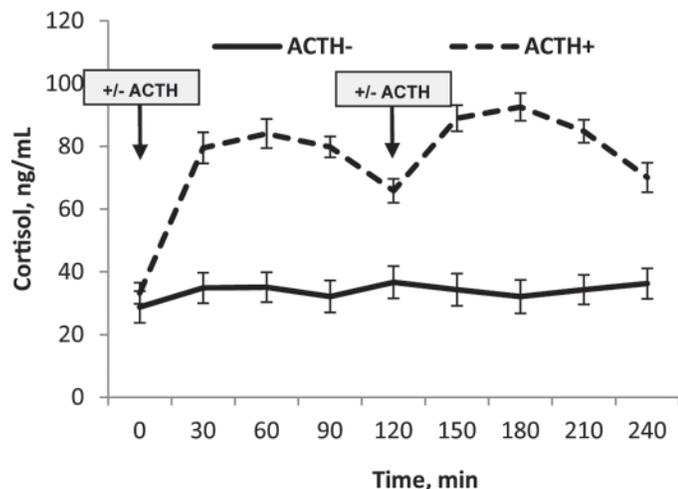
Serum samples from all cows were evaluated in triplicate for concentrations of PGFM by double-antibody  $^3\text{H}$ -PGFM RIA as described by Homanics and Silvia (1988) but modified in our laboratory (Merrill et al., 2007). The PGFM antiserum (WS4468) was generously provided by W. J. Silvia, University of Kentucky, Lexington. Sensitivity of the assay was 11.5 pg/mL, and the intra- and interassay CV were 8.6 and 11.9%, respectively.

### *Statistical Analysis*

Effects of ACTH and flunixin meglumine on pregnancy maintenance, serum cortisol concentration, serum PGFM concentration, and temperature were analyzed using the PROC MIXED procedure (SAS Inst. Inc., Cary, NC) with a repeated measures model that included the fixed effects of ACTH, flunixin meglumine, time, and all interactions. The random effect in this model was cow nested within treatment. Dosage of flunixin meglumine (1.1 or 2.2 mg/kg of BW) did not affect results, so data were pooled for further analyses. The default variance component structures of SAS (TYPE=VC) were assumed for both random and repeated effects. Significance tests for the effects of ACTH, flunixin meglumine, and the ACTH  $\times$  flunixin meglumine interaction used cow nested within the interaction as an error term. Other effects were tested using residual variance as the error term.

## RESULTS AND DISCUSSION

Serum cortisol concentrations of cows did not differ ( $P = 0.19$ ) between treatments at the initiation of treatments (time 0); however, serum cortisol was affected ( $P < 0.01$ ) by ACTH, time, and the interaction of ACTH  $\times$  time (Figure 1). Administration of ACTH (0.5 IU/kg of BW, **i.m.**) increased ( $P < 0.01$ ) serum cortisol concentrations in cows within 30 min of administration, and concentrations remained increased ( $P < 0.01$ ) throughout the blood sampling period (240 min). In fact, serum cortisol concentrations were still increased in ACTH-treated cows at the time of the second ACTH



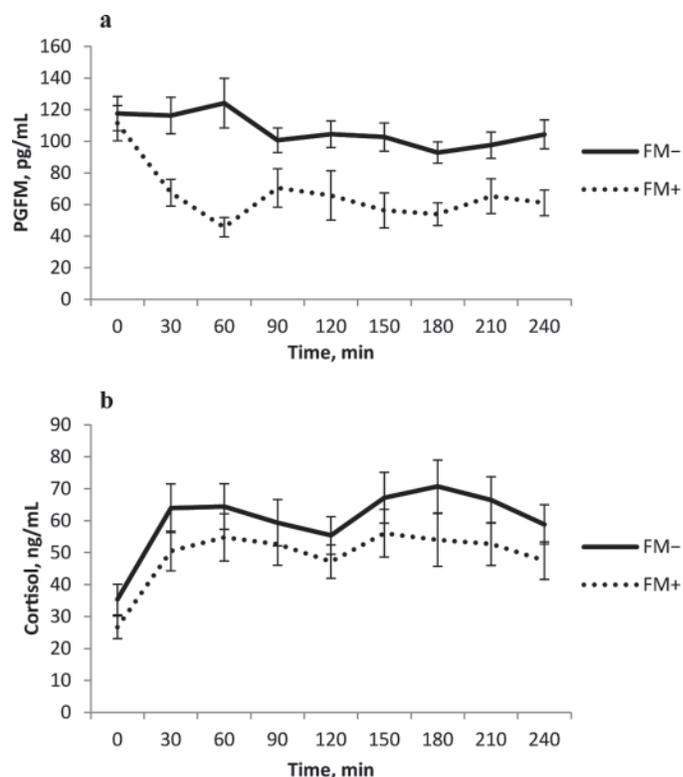
**Figure 1.** Serum cortisol concentrations of cows after administration of ACTH (0.5 IU/kg of BW, intramuscularly; ACTH<sup>+</sup>, dashed line) or saline injection (ACTH<sup>-</sup>, solid line) at 0 and 2 h to mimic a stress response during early pregnancy (d 34 ± 0.33 of gestation; mean ± SEM). Each point represents the mean ± SEM of 20 cows. Injection of ACTH increased ( $P < 0.01$ ) serum cortisol concentrations within 30 min, which remained increased ( $P < 0.01$ ) for the entire 4-h sampling period.

injection but increased even further. Serum cortisol concentrations in cows at the initiation of treatment in the present study were similar to cortisol concentrations of cows before transportation stress was applied, and they increased more than 2-fold above the greatest cortisol concentration in transportation-stressed cows reported by Merrill et al. (2007). Thus, ACTH treatment in this study achieved our goal of mimicking the cortisol concentrations created by transportation stress. However, transportation stress also generally includes restriction from water (similar to the current study during the blood sampling period) and overcrowding, which did not occur in the current study. In addition, the catecholamines epinephrine and norepinephrine were not measured in the current study, but transportation did increase epinephrine concentrations in bulls (Burdick et al., 2010). Nonetheless, the ACTH-induced increase in serum cortisol concentrations did not cause pregnancy loss in the present study. Thus, it is possible that pregnancy loss attributable to transportation stress (Harrington et al., 1995; T. W. Geary, unpublished data) is mediated through overcrowding or increased catecholamine concentrations. Treatment with flunixin meglumine (1.1 or 2.2 mg/kg of BW) did not affect ( $P = 0.14$ ) serum cortisol concentrations (Figure 2b), in agreement with previous reports (Giri et al., 1991; Merrill et al., 2007). However, Odensvik and Magnusson (1996) reported that oral administration of flunixin meglumine prevented an increase in serum cortisol concentrations in endotoxin-treated heifers. To date, the study by Odensvik and Magnusson (1996) is the only one to detect a decrease in cortisol concentrations after administration of flunixin meglumine. It was hypothesized that the increase in cortisol concentrations attributable to transportation stress, as reported by Merrill

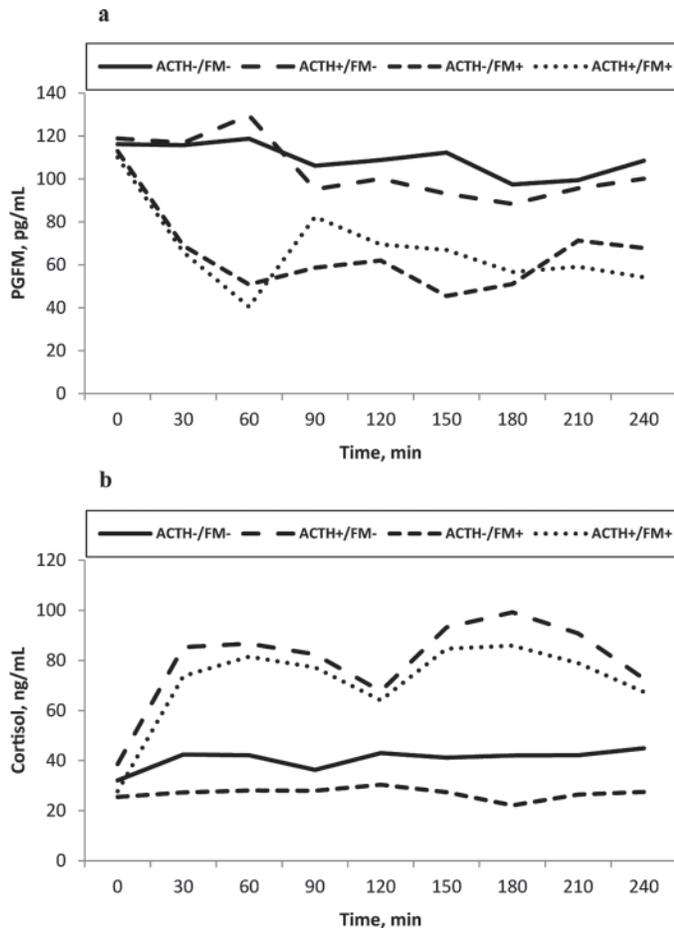
et al. (2007), could be alleviated with a greater dosage of flunixin meglumine [similar to that used by Odensvik and Magnusson (1996)], or a decrease in cortisol as a result of flunixin meglumine could be detected with more frequent blood sampling in the current study. The data in Figures 2 and 3 demonstrate that neither was correct. Given that flunixin meglumine did not affect cortisol concentrations in ACTH-treated cows, it does not appear that the suppressive effects of flunixin meglumine on cortisol are mediated through the adrenocorticotrophic axis.

Rectal temperature increased ( $P < 0.01$ ) with time (101.6, 102.3, and 102.5 ± 0.08°C at 0, 120, and 240 min, respectively) and ACTH, but was not affected ( $P = 0.23$ ) by a time × ACTH interaction. Cows that received ACTH had increased ( $P < 0.01$ ) temperatures at the initiation of the study (101.3 and 101.9 ± 0.12°C for control and ACTH-treated cows, respectively).

Serum PGFM concentrations of cows did not differ ( $P = 0.91$ ) at the initiation of treatments (time 0). Administration of ACTH did not affect ( $P = 0.97$ ) serum PGFM concentrations (data not shown), but



**Figure 2.** Serum PGF<sub>2α</sub> (PGF) metabolite (PGFM) concentrations (a) and cortisol concentrations (b) of cows after administration of flunixin meglumine [either 1.1 (n = 10) or 2.2 (n = 10) mg/kg of BW, intramuscularly; FM<sup>+</sup>, dotted line] or saline injection (FM<sup>-</sup>, solid line) at 0 min to measure the ability of flunixin meglumine to prevent a stress-induced increase in PGFM or cortisol during early pregnancy (d 34 ± 0.33 of gestation; mean ± SEM). Each point represents the mean ± SEM of 20 cows. Both doses of flunixin meglumine decreased ( $P < 0.01$ ) serum PGFM concentrations throughout the 4-h sampling period, but flunixin meglumine had no effect ( $P = 0.14$ ) on serum cortisol concentrations. Injection of ACTH had no effect ( $P = 0.97$ ) on serum PGFM concentration.



**Figure 3.** Serum  $\text{PGF}_{2\alpha}$  (PGF) metabolite (PGFM) concentrations (a) and cortisol concentrations (b) of cows that received saline (ACTH<sup>-</sup>) or ACTH [0.5 IU/kg of BW, intramuscularly (i.m.); ACTH<sup>+</sup>] and saline (FM<sup>-</sup>) or flunixin meglumine (1.1 or 2.2 mg/kg of BW, i.m.; FM<sup>+</sup>) in a 2 × 3 factorial arrangement during early pregnancy (d 34 ± 0.33 of gestation; mean ± SEM). Each point represents the mean of 10 cows. Error bars were removed from this figure so the reader could observe the trends of the interaction of treatments (ACTH × flunixin meglumine). The dose of flunixin meglumine had no effect ( $P = 0.97$ ) on PGFM concentrations, so data were pooled. There were no interactions ( $P > 0.35$ ) between ACTH and flunixin meglumine on serum cortisol or PGFM concentrations.

serum PGFM concentrations were similar to concentrations reported by Merrill et al. (2007). There were effects ( $P < 0.01$ ) of flunixin meglumine, time, and the interaction of flunixin meglumine × time on serum PGFM concentrations (Figure 2a). Flunixin meglumine (1.1 and 2.2 mg/kg of BW, i.m.) decreased ( $P < 0.01$ ) PGFM concentrations within 30 min of administration, but PGFM concentrations did not differ ( $P = 0.97$ ) between flunixin meglumine dosages. This rapid decrease in serum PGFM after the flunixin meglumine injection is consistent with the literature (Aiumlamai et al., 1990; Buford et al., 1996). Serum PGFM was still reduced in flunixin meglumine-treated cows 4 h after the initial treatment, in agreement with the reports of others (Guilbault et al., 1987; Anderson et al., 1990). There was no interaction of ACTH × flunixin meglumine or ACTH × flunixin meglumine × time on serum cortisol or PGFM concentrations ( $P > 0.35$ ; Figure 3).

None of the cows in this study experienced pregnancy loss, which was unexpected because of the results of Merrill et al. (2007) and Geary et al. (2010). In both of those studies, pregnancy rates were decreased by 6% in heifers (but not cows) when animals were handled on approximately d 13 after AI to administer flunixin meglumine compared with heifers remaining on pasture (Geary et al., 2010), and pregnancy rates were decreased by 8% in cows and heifers that did not receive flunixin meglumine with transportation compared with those females that received flunixin meglumine (Merrill et al., 2007). As with any study, it is possible that the number of cattle used in the current study was insufficient to accurately assess involvement of the hypophyseal-adreno-corticotrophic axis in stress-induced pregnancy loss; however, this is unlikely because cortisol concentrations were increased more than 2-fold greater than concentrations measured in the transportation studies mentioned herein.

The incidence of embryonic loss in beef cows is variable but has been reported to be as great as 14% after d 28 of pregnancy (Beal et al., 1992; Humblot, 2001; Perry et al., 2005). Because handling of cattle is necessary to evaluate the earlier pregnancy and because pregnancy rates were decreased in heifers experiencing handling stress during early pregnancy (Geary et al., 2010; T. W. Geary, unpublished data), it was hypothesized that administration of ACTH would mimic a stressful condition and cause pregnancy loss. Although treatment with ACTH increased circulating cortisol concentrations in cows in the present study, the hypothesis that pregnancies would be lost without the suppressive effects of flunixin meglumine on PGF was not supported. Thus, the early embryonic loss in cattle observed previously (Geary et al., 2010) may not be related to increased cortisol as hypothesized. Because ACTH activation of the hypophyseal-adreno-corticotrophic axis did not increase PGFM, it cannot be determined whether this is the correct model to study the effects of transportation stress on pregnancy.

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