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# Flunixin meglumine improves pregnancy rate in embryo recipient beef cows with an excitable temperament

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ABSTRACT

Objectives were to determine effects of: 1) handling temperament and administration of flunixin meglumine, an inhibitor of prostaglandin F2a (PGF2a) synthesis, given at the time of embryo transfer, on pregnancy rates in beef cattle embryo transfer recipients; 2) handling temperament and flunixin meglumine on peripheral concentrations of progesterone, cortisol, substance-P, prostaglandin F metabolites (PGFM, (13,14-dihydro-15-keto-PGF2a) and isoprostane 8-epi PGF2a; and 3) flunixin meglumine treatment on proportion of non-pregnant recipient cows returning to estrus within an expected interval. Angus cross beef cows (n = 710) at 7 locations were assigned a body condition score (BCS: 1, emaciated; 9, obese) and a temperament score [0, calm, slow chute exit; walk (n = 352), 1, excited, fast chute exit; jump, trot or run (n = 358)] and were synchronized with Select-Synch with a controlled internal drug release (CIDR) protocol. Estrus detection aids were applied at CIDR removal and cows were observed thrice daily for estrus until 72 h. Recipient cows that expressed estrus and had a corpus luteum received a frozen-thawed embryo on Day 7 after estrus. At the time of transfer, recipient cows were randomly allocated to receive 10 mL of flunixin meglumine im, immediately after transfer (n = 365) or served as an untreated control (n = 345). In a subset of cows (n = 80), ovarian ultrasonography was performed on the day of embryo transfer to determine corpus luteum volume and blood samples were collected twice, at the time of embryo transfer and 7 d later. All cows received estrus detection aids again on Day 14 (7 d after embryo transfer) and were observed for estrus twice daily until Day 24. Accounting for treatment ($P > 0.1$), embryo transfer difficulty score ($P < 0.1$), temperament by treatment interaction ($P < 0.05$), recipient cows with calm temperament had a higher pregnancy rate compared to those with an excited temperament [59.4 (209/352) vs 51.7% (185/358)]. The pregnancy rate for excitable cows without flunixin meglumine was lower (46.3% 81/175) compared to excitable cows that did received flunixin meglumine [56.8% (104/183)], and calm cows that did [59.3% (108/182)] or did not [59.4% (104/170)] receive flunixin meglumine. Proportions of non-pregnant recipient cows returning to estrus on Days 18–24 were not different between flunixin meglumine and control groups, 87.6% (134/153) and 84.0% (137/163), respectively ($P > 0.1$). At the time of embryo transfer and 7 d later, there were moderate to strong correlations among circulating concentrations of progesterone, cortisol, substance-P, PGFM and isoprostane 8-epi PGF2a. Among excitable cows, progesterone concentrations were lower and cortisol, substance-P, PGFM and isoprostane 8-epi PGF2a concentrations were greater for cows in the control group compared to cows that received flunixin meglumine. In conclusion, administration of flunixin meglumine improved pregnancy rates in excitable recipient cows following embryo transfer without affecting the proportion of non-pregnant cows returning to estrus.



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## 1. Introduction

Assisted reproductive technologies, including embryo transfer, are used to accelerate genetic gain and in some instances to overcome subfertility or infertility in cattle [1]. Variations in pregnancy rates following embryo transfer may due to embryonic, maternal and environmental factors, or combinations of these factors. Early embryonic death can be caused by a poor quality embryo, asynchrony between recipient females and embryos, poor uterine environment, and inadequate uterine-embryo interaction [2,3]. Environmental stress such as heat and handling stress, poor management of recipient nutrition, or inflammatory conditions (mastitis, pneumonia, lameness and uterine diseases in recipients) may also reduce pregnancy rates after embryo transfer in cattle [4–6].

Elevated concentrations of prostaglandin (PG) F2a in the uterine lumen after transfer of an embryo can reduce embryo viability and pregnancy rates [7–11], as PGF2a causes luteolysis and reduces progesterone concentrations, which contributes to early embryonic loss. Interestingly, stress-induced releases of PGF2a had similar pulsatile patterns as those associated with luteolysis [12].

Production of endometrial PGF2a is mainly governed by the rate-limiting enzymes cyclooxygenase (COX) 1 and COX2. These enzymes are responsible for conversion of arachidonic acid into PGH2, the common precursor of various forms of PGs including PGF2a and PGE2. Flunixin meglumine, a non-steroidal anti-inflammatory drug, is a very potent non-specific inhibitor of COX [13,14]. Effects of flunixin meglumine treatment on pregnancy rates in embryo transfer recipients [15,16] have been reported. Scenna et al. (2005) reported that uterine release of PGF2a was increased after embryo transfer and that administration of a PGF2a synthesis inhibitor at embryo transfer improved pregnancy rates in cows [17]. Merrill et al. (2007) demonstrated that transportation of beef cows 14 days after artificial insemination (AI) increased serum cortisol concentrations but did not affect AI pregnancy rates [18]. However, treatment of cows with flunixin meglumine decreased PGFM and increased AI pregnancy rates, irrespective of whether they were transported. Purcell et al. (2005) reported that whether or not flunixin meglumine at embryo transfer increased pregnancy rates were location-dependent [15], perhaps due to cow genetics, nutritional management, and the environment, including cattle handling and temperament. Further, Thatcher et al. (2007) demonstrated that flunixin meglumine failed to improve pregnancy rate and/or late embryo survival in dairy heifers [19].

Temperament is a reaction characteristic of cattle in response to human handling [20]. In general, excitable temperament has detrimental effects on growth, carcass quality, and cattle health [21–24]. Although an excitable temperament decreased pregnancy rate to AI [25,26], effect of excitability on pregnancy rate after embryo transfer has not been reported. The hypothesis of this study was that an excitable temperament decreases pregnancy rate after embryo transfer and that administration of flunixin meglumine improves pregnancy rate in excitable cows. Objectives were to determine effects of: 1) handling temperament and administration of flunixin meglumine, an inhibitor of prostaglandin F2a (PGF2a) synthesis, given at the time of embryo transfer, on pregnancy rates in beef cattle embryo transfer recipients; 2) handling temperament and flunixin meglumine on peripheral concentrations of progesterone, cortisol, substance-P, prostaglandin F metabolites (PGFM, (13,14-dihydro-15-keto-PGF2a) and isoprostane 8-epi PGF2a; and 3) flunixin meglumine treatment on proportion of non-pregnant recipient cows returning to estrus within an expected predetermined interval.

## 2. Materials and methods

The protocol was approved by Institutional Animal Care and Use Committee at Washington State University (ASAF #03922-001).

### 2.1. Animals and treatments

Angus-cross beef cows (n = 710) were processed in standard cattle handling chutes, assigned a body condition score (BCS: 1, emaciated; 9, obese) and a temperament score (0, calm, slow chute exit; walk, 1, excited, fast chute exit; jump, trot or run at the initiation of the protocol. This work was conducted at 7 locations, with 42–178 cows per location. Selected recipient cows had moderate to good body condition. They were at least 60 days post calving. Cows were dewormed and vaccinated (for respiratory and reproductive diseases) as part of total herd health management plan.

#### 2.1.1. Diets

The cows were fed to meet National Research Council (NRC) recommendations [Nutrient requirements of beef cattle: eighth revised edition (2016)]. Beef cows were either allocated to mixed alfalfa or grass hay from 3 months before and 2 weeks after AI and then turned out to pasture or grazed Bermuda grass supplemented with corn silage and a corn soybean meal supplement. Cows received injectable mineral supplement twice a year and provided loose chelated minerals on the pasture throughout the year.

#### 2.1.2. Synchronization treatments

To synchronize ovulation, all cows were treated with a Select-Synch + controlled internal drug release (CIDR) protocol (Fig. 1). Briefly, they were given a 1.3 g progesterone intravaginal insert (CIDR, Eazi-Breed™ CIDR® Cattle Insert; Zoetis Animal Health, New York, NY, USA) and 100 μg of gonadorelin diacetate tetrahydrate (GnRH; 2 mL; Cystorelin®, Merial Inc., Duluth, GA, USA) im, on Day -10. On Day -3, CIDRs were removed and 25 mg of dinoprost (PGF2a; 5 mL; Lutalyse® sterile solution; Zoetis Animal Health) im was administered to all cows. Furthermore, concurrent with CIDR removal, Estrus Alert patches (Western Point Inc., Apple Valley, MN,

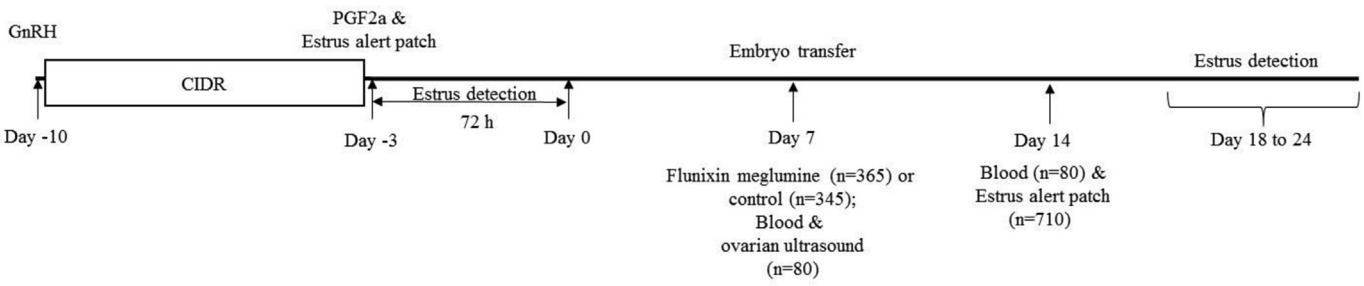


**Fig. 1.** Schematic presentation of estrus synchronization, embryo transfer and treatment.