Effect of flunixin meglumine on early embryonic mortality in stressed beef female
by Melissa Lee Merrill

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Animal and Range Sciences
Montana State University

Abstract:
The objectives of this study were to determine if an injection of flunixin meglumine (1.1 mg/kg BW) would affect early embryonic mortality in stressed beef females. Ninety-seven cows were assigned to one of three treatment groups (Experiment 1; El). Treatments were: 1.) control (CON) 2.) transportation stressed (S) and 3.) transportation stressed with flunixin meglumine (SFM). The following year 259 heifers (Experiment 2; E2) and 127 cows (Experiment 3; E3) were assigned to one of four treatments: the three treatments used in El plus control with flunixin meglumine (CONFM). Approximately 14 d following synchronization of estrus and artificial insemination (Al) treatments were applied in all three experiments. Rectal temperatures were recorded and blood samples were taken for serum concentration of progesterone, PGF metabolite, and cortisol. The CON and CONFM (NTS) remained at the ranch while S and SFM (TS) females were transported for 5-6 h. The SFM and CONFM (FM) treatments received an injection of flunixin meglumine (1.1 kg/mg BW, i.m.) while S and CON did not receive an injection of flunixin meglumine (NFM). Females were not exposed to clean-up bulls until after treatment. Transrectal ultrasonography was used to determine Al pregnancy status 33-35 d for heifers and 55-57 d for cows post-AI. Statistics were determined using a 2x2 factorial design. No differences were detected in progesterone or PGFM serum concentration ($P>0.10$) in El. Serum cortisol concentrations decreased for stressed animals (S and SFM) compared to control animals (CON) between pre- and post-treatment blood samplings ($P<0.05$) in El. In E2 and E3, serum cortisol concentrations were similar ($P>0.10$) at the initial blood sampling, increased for TS compared to NTS ($P<0.01$) at intermediate blood sampling and decreased greater for TS compared to NTS (PO.01) at the final blood sampling. Temperatures were similar for initial temperature collection among treatments of E2 and E3. At the E3 intermediate and E2 final temperature collection, FM was lower ($P<0.05$) than NFM. The Al pregnancy rates were higher ($P=0.03$) for FM (63%) than NFM (53%) when combining E2 and E3. The SFM cows had higher ($P=0.06$; 71%) Al pregnancy rates than S (60%) with CON (63%) being intermediate when combining El, E2, and E3. Final pregnancy rates did not differ. In conclusion, flunixin meglumine decreases the embryonic mortality in beef females that are transportation stressed d 12-14 post-AI but . the stress of handling needs to be explored.

EFFECT OF FLUNIXIN MEGLUMINE ON EARLY EMBRYONIC MORTALITY IN STRESSED BEEF FEMALES

by

Melissa Lee Merrill

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Melissa Lee Merrill

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style and consistency, and is ready for submission to the College of Graduate Studies.

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ABSTRACT

The objectives of this study were to determine if an injection of flunixin meglumine (1.1 mg/kg BW) would affect early embryonic mortality in stressed beef females. Ninety-seven cows were assigned to one of three treatment groups (Experiment 1; E1). Treatments were: 1.) control (CON) 2.) transportation stressed (S) and 3.) transportation stressed with flunixin meglumine (SFM). The following year 259 heifers (Experiment 2; E2) and 127 cows (Experiment 3; E3) were assigned to one of four treatments: the three treatments used in E1 plus control with flunixin meglumine (CONFM). Approximately 14 d following synchronization of estrus and artificial insemination (AI) treatments were applied in all three experiments. Rectal temperatures were recorded and blood samples were taken for serum concentration of progesterone, PGF metabolite, and cortisol. The CON and CONFM (NTS) remained at the ranch while S and SFM (TS) females were transported for 5-6 h. The SFM and CONFM (FM) treatments received an injection of flunixin meglumine (1.1 kg/mg BW, i.m.) while S and CON did not receive an injection of flunixin meglumine (NFM). Females were not exposed to clean-up bulls until after treatment. Transrectal ultrasonography was used to determine AI pregnancy status 33-35 d for heifers and 55-57 d for cows post-AI. Statistics were determined using a 2x2 factorial design. No differences were detected in progesterone or PGFM serum concentration (P>0.10) in E1. Serum cortisol concentrations decreased for stressed animals (S and SFM) compared to control animals (CON) between pre- and post-treatment blood samplings (P<0.05) in E1. In E2 and E3, serum cortisol concentrations were similar (P>0.10) at the initial blood sampling, increased for TS compared to NTS (P<0.01) at intermediate blood sampling and decreased greater for TS compared to NTS (P<0.01) at the final blood sampling. Temperatures were similar for initial temperature collection among treatments of E2 and E3. At the E3 intermediate and E2 final temperature collection, FM was lower (P<0.05) than NFM. The AI pregnancy rates were higher (P=0.03) for FM (63%) than NFM (53%) when combining E2 and E3. The SFM cows had higher (P=0.06; 71%) AI pregnancy rates than S (60%) with CON (63%) being intermediate when combining E1, E2, and E3. Final pregnancy rates did not differ. In conclusion, flunixin meglumine decreases the embryonic mortality in beef females that are transportation stressed d 12-14 post-AI but the stress of handling needs to be explored.

INTRODUCTION

Studies indicate that the rate of conceptus loss in domestic livestock during early pregnancy ranges from 20-40% (Hanly, 1961; Perry and Rowlands, 1962). Embryonic loss was increased when the dam was exposed to one or more of the many stresses that can compromise embryonic survival (Hansen, 2002). The preimplantation embryo was most susceptible to certain types of stress, such as heat, transportation, or handling stress, very early in development (Lefcourt et al., 1995). Fear is a very strong stressor that can result from handling and transportation (Grandin, 1997). Harrington et al. (1995) reported that beef heifers transported between d 8-12 or 29-33 after artificial insemination (AI) had lower synchronized conception rates than those transported 1-4 d after AI.

The adrenal hormones (norepinephrine, epinephrine, and cortisol) are commonly used as indices of stress (Lefcourt et al., 1995) in addition to prostaglandins (Morita, 2002) as they are actively secreted during what is perceived as stressful stimuli.

Maternal recognition of pregnancy in cows occurs about 14 d after estrus and $PGF_{2\alpha}$ is an important hormone involved in luteolysis and maternal recognition. To maximize fertility in the cow, treatments must reduce luteolytic influences such as excess $PGF_{2\alpha}$ or estradiol early after mating and during maternal recognition of pregnancy (Inskeep, 2002).

Guilbault et al., (1987) reported that $PGF_{2\alpha}$ secretion was suppressed for at least 24 h by flunixin meglumine treatment (1 g/ i.m. injection; twice per d) in dairy and beef cows. Flunixin meglumine is a potent, nonsteroidal, anti-inflammatory agent that inhibits cyclooxygenase and prevents the conversion of arachadonic acid to $PGF_{2\alpha}$ (Anderson et al., 1990; Odensvik, 1995). Flunixin meglumine may prevent the loss of embryos resulting from the rise of prostaglandin $F_{2\alpha}$ and luteolysis that may be caused by stress.

The objectives of these three studies were to determine if flunixin meglumine would affect the concentrations of cortisol and/or prostaglandin $F_{2\alpha}$ in stressed or control females approximately 14 d after, AI and its effect on subsequent embryonic mortality.

LITERATURE REVIEW

Introduction

The United States has approximately 99.5 million head of cattle, which was 3.9 % of the GNP and is approximately valued at one billion dollars (National Ag Stat Service, 1997). According to the National Cattleman's Beef Association, in 1992, $760 million of Montana's economy was generated from 2.4 million beef cattle. This accounted for 44.8% of the total economy and 85% of all livestock and poultry receipts. Beef cattle are a large portion of Montana's interests and a substantial amount of the United States' wealth.

Numerous studies have been conducted on factors contributing to reproductive performance in beef cattle; these include nutrition, disease, chromosomal abnormalities, and physiological imbalances. The greatest production loss results from 17.4% of cows not becoming pregnant by the end of the breeding season (Bellows and Short, 1994). According to Thatcher et al. (1994), approximately 30% of repeat breeder cows experience embryonic loss by d 7 of pregnancy largely due to chromosomal abnormalities. Additional losses occur gradually between d 8 to 17 and d 17 to 24 accounting for almost 40% and 24% of total embryonic losses, respectively.

Animals, that experience early embryonic loss, may have problems becoming pregnant within a 45 d breeding season because it may be the third or fourth breeding cycle before they conceive again and maintain a pregnancy. Therefore more cows that experience embryonic loss may repeatedly calve late resulting in younger and lighter calves, or fail to become pregnant.

Using a simulation model, Tess (1999) reported that a post-partum interval beyond 70 d or about 3 estrous cycles, resulted in a decrease in the weight weaned per cow exposed and a decline in ranch gross margin (Figure 1 and 2). These findings further demonstrate the effect reproductive efficiency has on the profitability of a beef herd.

Normal Estrous Cycles and Pregnancy

The normal estrous cycle of a beef cow is an average length of 20 to 21 d and is usually only interrupted by pregnancy. The estrous cycle is broken down into four main stages (Figure 3; Senger, 1999): proestrus, estrus, metestrus, and diestrus. Proestrus is characterized by completion of luteolysis and the presence of a corpus albicans, the formation of the ovulatory follicle, and high estrogen secretion. Estrus, when sexual receptivity occurs, is marked by a peak of estrogen and one ovary having a large, mature follicle that is destined to ovulate 28-32 hours after the onset of estrus. Metestrus involves ovulation, the formation of the corpus luteum, and the rise of progesterone production. Diestrus is characterized by sustained luteal function, follicular waves of growth, and sustained secretion of progesterone.

Luteal Phase

The luteal phase, consisting of metestrus and diestrus, lasts from the time of ovulation until regression of the corpus luteum. After ovulation, the theca interna and granulosal cells undergo luteinization or transformation from ovulatory follicle to luteal tissue. This begins as a corpus hemorrhagicum: during ovulation many small blood vessels rupture and the follicle hemorrhages. The follicle wall collapses and the cells of the theca interna and granulosa interdigitate and a gland is formed. During the early stages of the luteal phase the corpus luteum develops and progesterone concentrations increase.

Mid-way through the luteal phase the corpus luteum is fully functional, and progesterone concentration peaks and then, levels off. Follicular waves occur throughout the luteal phase. The end of the luteal phase is characterized by lysis of the corpus luteum. The two main hormones are oxytocin from the corpus luteum and prostaglandin from the uterine endometrium. Luteal oxytocin receptor formation increases followed by prostaglandin release (Figure 4; Senger, 1999; McCracken et al., 1999). Thus, when peaks become more frequent (approximately 5/d) the threshold concentration of prostaglandin is reached and luteolysis occurs (Thatcher et al., 2001). During the first half of the estrous cycle progesterone blocks oxytocin receptor formation in the uterus; however, after 10-12 days progesterone loses the ability to block oxytocin receptor formation (Lemaster et al., 1999; Bogacki et al., 2002).

Follicular Phase

The follicular phase, consisting of proestrus and estrus, is initiated after luteolysis, which results in a marked reduction in progesterone. As progesterone concentration decreases, follicle stimulating hormone (FSH) and luteinizing hormone (LH) increase together in response to the release of gonadotrophin releasing hormone (GnRH; Crowe et al., 1999). Increased FSH and LH cause the production of estradiol (E_2) by ovarian follicles. The preovulatory surge of GnRH is controlled by the combination of high estrogen and low progesterone (Figure 5; Senger, 1999).

The preovulatory follicles are recruited and selected during proestrus and eventually will dominate during estrus. Elevated concentrations of FSH induce recruitment of follicles from the gonadotropin sensitive pool within the ovary (Adams et al., 1992). Once the follicles are recruited they will produce some estrogen and small amounts of inhibin. As the process merges into selection, the amount of inhibin is increased, thus, creating a negative feedback on the anterior pituitary, creating a reduction in the amount of FSH produced. This causes many FSH-sensitive follicles to become atretic; those that do not regress are LH-dependent. Again, a change is experienced in the hormonal pattern of the follicular wave. Large follicles produce more estrogen and inhibin, thus reducing FSH concentrations. As estrogen concentrations reach a threshold, GnRH is released causing the preovulatory LH surge to occur. Prostaglandin and progesterone concnetrations rise in the ovulatory follicle, resulting in contraction of the ovarian smooth muscle and release of lysosomal enzymes. This creates an increase in follicular pressure and the weakening of follicular walls. This cascade of events leads to ovulation and the end of the follicular phase.

Pregnancy

When fertilization occurs, the host female undergoes many changes. Until the time of pregnancy recognition, uterine physiology is very similar between pregnant and cyclic animals (Binelli et al., 2001). In order for the events of early embryonic development to continue, luteolysis must be prevented and progesterone concentrations must remain elevated (Figure 6 and 7; Senger, 1999). During early pregnancy the pulsatile release of prostaglandin is abolished. However, minute episodic or basal concentration peaks do occur. This does not seem to affect the progesterone secretion from the corpus luteum (Fredriksson et al., 1984). Maternal recognition involves physiological mechanisms that result in protection of the corpora lutea from luteolysis by modification or inhibition of uterine production of luteolytic pulses of $PGF_{2\alpha}$ (Bazer et al., 1991). Maternal recognition of pregnancy occurs through secretion of bovine interferon τ (tau) from the conceptus. This protein provides the signal to prevent luteolysis during pregnancy.

Early Embryonic Mortality

Establishment of pregnancy depends on many different processes. Functional gametes of potentially high fertility must be produced by both sexes. The female must exhibit estrus; estrus must be detected; and mating must occur within the functional lifespan of the gametes (Bellows, 1994). Also included in the establishment of pregnancy, are acquisition of sperm receptor proteins and signaling molecules on the ooplasm to allow for fertilization; accumulation of intracellular stores of calcium for signaling events; execution of the block to polyspermy; nuclear maturation with proper segregation of chromosomes; acquisition of the reducing agents and other molecules required for pronuclear decondensation; development of the cytoskeletal apparatus necessary for syngamy; and synthesis and storage of the mRNA, proteins, and other molecules necessary to support preimplantation development (Hansen, 2002).

Development of the embryo takes place in the reproductive tract and the dam has a major impact on embryonic survival. Components of uterine function responsible for embryonic mortality result from asynchrony, nutrition, or improper endocrine patterns (Jindal et al., 1997; Hansen, 2002; Vallet et al., 2002). The uterus also plays a key role in maternal recognition of pregnancy, embryo elongation, implantation, and support of the developing embryo during early pregnancy (Vallet et al., 2002).

Most early embryonic loss can be associated with chromosomal abnormalities, inadequate rates of embryonic development, and improper uterine environment. In addition, there are the possibilities of insufficient secretion of hormonal signals, a poorly functioning corpus luteum as a result of inadequate preovulatory follicle development and maturation, and perhaps follicular development during early pregnancy that antagonizes corpus luteum maintenance (Thatcher et al., 1994).

The interaction between the conceptus and the uterine endometrium leads to maintenance of the corpus luteum. The ability of embryonic interferon-τ to inhibit uterine secretion of prostaglandin $F_{2\alpha}$ is critical to the establishment of pregnancy in cattle (Thatcher et al., 2001). A high proportion of embryonic losses may be attributed to the period of conceptus inhibition of uterine prostaglandin secretion, suggesting that some loss may be occurring because certain conceptuses are unable to inhibit secretion of $PGF_{2\alpha}$ (Thatcher et al., 2001).

Pregnancy rates in cows will be low if the corpus luteum regresses prematurely, because luteal progesterone is essential throughout pregnancy (McDonald et al., 1953). Excess prostaglandin secretion early in pregnancy causes luteolysis and variability in progesterone synthesis which, may lead to asynchrony between the embryo and the uterus thus causing an embryotoxic effect through a hormonal imbalance (Buford et al., 1996).

A study conducted by Harrington et al. (1995) indicated that the embryonic losses were increased by stress during early embryonic pregnancy (8-33 d). When stress cannot be totally avoided (*i.e.* trailing or hauling to summer pasture) part or all the females within a given herd may be in a critical period such as, maternal recognition or implantation. This is crucial with the use of estrous or ovulation synchronization.

Embryonic production of these interferons begins around d 10-12 of pregnancy and peaks at d 17 in cattle (Bartol et al., 1985). Interferon-τ suppresses the normal pattern of pulsatile release of uterine $PGF_{2\alpha}$ preventing luteolysis at the end of the estrous cycle; leaving the corpus luteum functional (Demmers et al., 2001). Several other factors contribute to the demise or the continuance of pregnancy. Including genetic abnormalities, nutritional deficiencies, maternal incompatibilities, and management decisions as they could affect the amount of embryonic mortality experienced within a herd. Decisions should encompass the knowledge of the entire process including limiting stress because of possible effects on pregnancy and survival of the offspring.

Stress

Stress is a broad term that implies a threat to which the body must adjust or compensate. There are many diverse stimuli that may cause stress, such as pain, hunger, thirst, severe climatic conditions, handling, isolation, or transportation.

Novelty is also a strong stressor especially when an animal is suddenly confronted with unfamiliar conditions. In the wild, novelty and strange sights or sounds are often a sign of danger (Grandin, 1993). Novelty may come in many forms, such as, squeeze chutes, alleyways, pens, or loading and unloading docks. Many of these are present when transporting animals.

Cattle's response to stressors requires a progression of events beginning with sensing the stressor, followed by signaling to various biological mechanisms. These events are followed by activation of neurophysiological mechanisms, which mount a biological effort to resist and prevent major damage (Ewing et al., 1999). The parasympathetic nervous system maintains homeostasis and is mainly responsible for energy conservation and relaxation during stress. Parasympathetic activities are antagonized by sympathetic activities that mobilize energy during stress (VonBorrell, 2001). The secretion of catecholamines from the adrenal medulla is characteristic of the fight or flight syndrome, preparing the body for response to a stressor (VonBorrell, 2001).

The hypothalamic-pituitary-adrenocortical (HPA) axis is a vital neuroendocrine regulatory system for adaptation of animals to environmental changes. The activation of the HPA-axis is mainly dependent on the emotional involvement of the animal; stressors do not necessarily activate the HPA system, the animal does, therefore a response to stimuli may not be detected if the animal does not perceive the situation as stressful (VonBorrell, 2001).

The HPA axis is extremely sensitive to environmental factors and stimuli (Hopster et al., 1999). The adrenal hormones (norepinephrine, epinephrine, and cortisol) are commonly used as indices of stress (Lefcourt et al., 1995). Also, adrenocorticotropic hormone, corticosteroids, prostaglandins and catecholamines are used when studying stress and its effects (VonBorrell, 2001; Negishi and Katoh, 2002).

Prostaglandins are known to be involved in many physiological and pathological processes including inflammation, stress, and ovulation (Morita, 2002). In order for arachidonic acid to be converted to $PGF_{2\alpha}$, a high concentration of the cyclooxygenase-2 (COX-2) enzyme must be induced by some form of stimuli such as inflammation from injections, a head catch, or jostling before d 13 of the estrous cycle, (Arosh et al., 2002; Morita, 2002).

Evaluating the effect of transportation-induced stress is extremely difficult due to the cumulative effect of individual sources of stress associated with the transportation process (Stephens and Perry, 1990). The degree of stress an animal experiences in situations that are nonpainful, such as transportation, is directly determined by the degree of fear evoked during the event (Grandin, 1998). Events that are unfamiliar to animals will often be perceived as dangerous and, even in the absence of pain association, can result in elevated concentrations of cortisol (Table 1; Lay et al, 1997).

Harrington et al. (1995) indicated that the embryonic losses were increased by transportation-induced stress during early embryonic pregnancy (8-33 d). Following a synchronized insemination of 430 heifers, pregnancy rates were evaluated to determine the effects of transportation at three different times. Animals were transported via a tractor-trailer for 7h. These animals were transported 1-4, 8-12, and 29-33 days post-AI; synchronized AI pregnancy rates were 74%, 62%, and 65%, respectively (Table 2). The differences in AI pregnancy rates observed between days transported post-AI were attributed to critical time periods within gestation. The expression of COX-2 enzyme is relatively low on days 1-3 of the estrous cycle, with d 0 being estrus (Arosh et al., 2002) thus, the ability to secrete $PGF_{2\alpha}$ is reduced. This is during metestrus and ovulation, at a period when prostaglandins might serve as a temporary luteotrophic hormone and immunomodulator during establishment of pregnancy (Arosh et al., 2002). On d 10-12, or the beginning of maternal recognition of pregnancy, a prostaglandin rise could have been induced to luteolytic concentrations, thus resulting in mortality (Demmers et al., 2001). This high proportion of losses is coincident with the period of conceptus inhibition of the uterine $PGF_{2\alpha}$ secretion, suggesting that some of the loss may be occurring because certain conceptuses are unable to inhibit secretion of $PGF_{2\alpha}$ (Thatcher et al., 2001). Giri et al. (1991) suggested that inflammation from any source that induces increased prostaglandin production and release may influence reproductive performance in the bovine. Placentation of the embryo begins to occur on d 28 following fertilization the majority being 1.1-2.2 mg/kg BW either administered i.m. or i.v. (Guibault et al., 1987; Aiumlamai et al., 1990; Anderson et al., 1990).

Odensvik et al. (1998) also documented the efficacy of oral granules of flunixin meglumine in comparison to injectable flunixin meglumine were similar in ability to reduce prostaglandin synthesis and luteolysis in cycling dairy heifers and cows. In non-pregnant animals, flunixin meglumine controlled prostaglandin production by delaying normal luteolysis and mimicking a situation of maternal recognition of pregnancy by prolonging a viable estrous cycle in dairy cows (Aiumlamai et al., 1990).

Odensvik et al. (1998) reported that flunixin meglumine, in non-pregnant heifers, lengthened estrous cycles when it was orally administered (2.2 mg/kg BW) three to four times daily. Flunixin meglumine inhibited the peaks of prostaglandin secretion that were normally observed during d 16-19 of the estrous cycle. The estrous cycle was lengthened by approximately 3 d in animals fed flunixin meglumine 3 times per day and luteolysis was not observed in those fed flunixin meglumine four times daily until the termination of the experiment.

Flunixin meglumine reduced luteolysis caused by endotoxins in dairy cows during the first trimester (Giri et al., 1991). Endotoxins are believed to increase prostaglandin synthesis through the lipopolysaccharide component of gram-negative bacterial cell walls. Animals were treated with flunixin meglumine (1.1 mg/kg BW, i.v.) or a volume equivalent of saline prior to an infusion of E. coli endotoxin and 13 h later cows were again, treated with flunixin meglumine or saline. Pregnancy rates were determined via transrectal ultrasonography 5 d later. Cows infused with saline and endotoxins had higher

($P<0.05$) abortion rates compared to those infused with flunixin meglumine and endotoxins (3/5 vs 0/6, respectively).

In summary, flunixin meglumine has several different applications. It inhibits prostaglandin synthesis in both pregnant and non-pregnant cattle. Flunixin meglumine can lengthen the life of the corpus luteum, mimic a situation of maternal recognition of pregnancy, and reduce uterine activity. Flunixin meglumine can also be used to treat endotoxins, equine colic and musculoskeletal disorders and bovine mastitis and uterine involution (Guibault et al., 1987; Anderson et al., 1990).

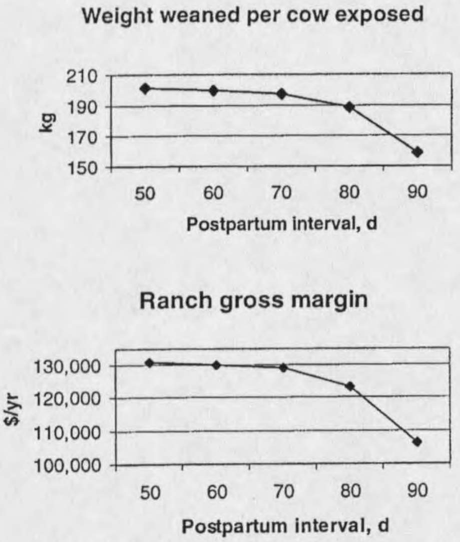


Figure 1 and 2. Effects of post-partum interval on weight weaned per cow exposed and ranch gross margin (copied from Tess, 1999).

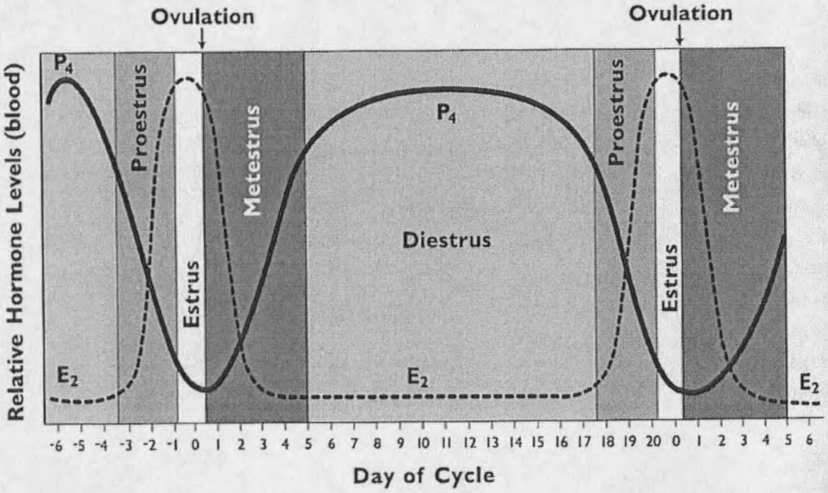


Figure 3. Stages of the estrous cycle (reprinted with permission from Current Conceptions, Inc., Pathways to Pregnancy and Parturition-1st revised edition by P.L. Senger, 1999). Proestrus is characterized by a significant rise in estradiol. When estradiol (E_2) reaches threshold the female enters estrus. Following ovulation, cells of the follicle are transformed into a corpus luteum during metestrus. Diestrus is characterized by a fully functional CL and high progesterone (P_4).

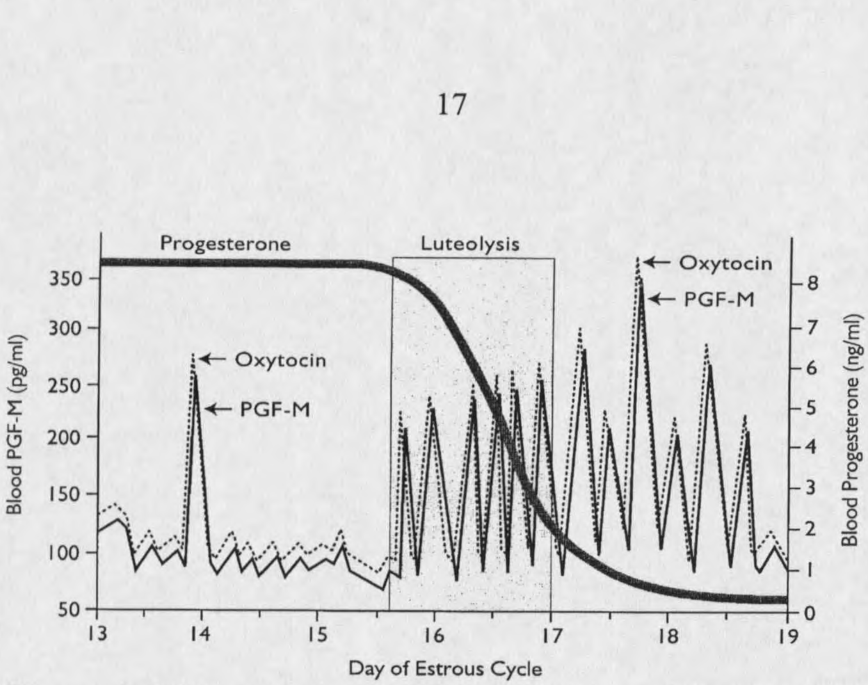


Figure 4. Changes in $PGF_{2\alpha}$ secretion during the last 6 days of the estrous cycle as reflected by prostaglandin $F_{2\alpha}$ metabolites (PGF-M; reprinted with permission from Current Conceptions, Inc., Pathways to Pregnancy and Parturition-1st revised edition by P.L. Senger, 1999). Luteal oxytocin episodes coincide almost perfectly with episodes of PGF-M. When about five pulses of $PGF_{2\alpha}$ occur in a 24-hour period, luteolysis will occur.

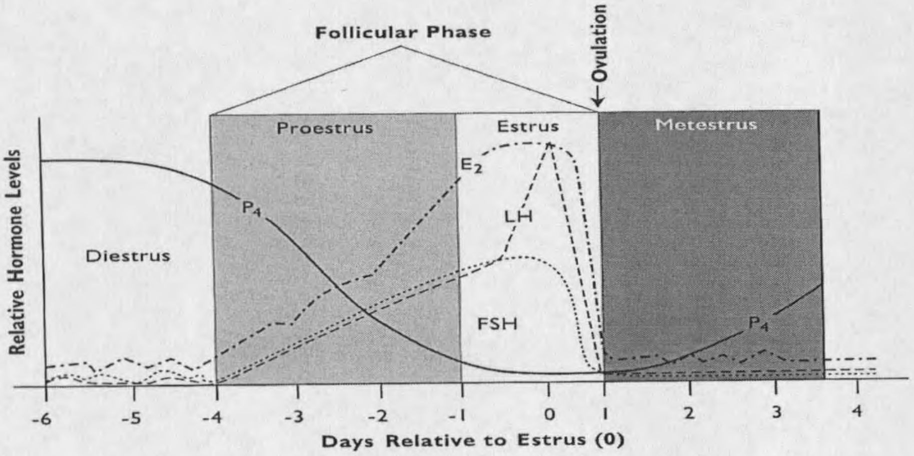


Figure 5. Changes of hormones during the follicular phase (reprinted with permission from Current Conceptions, Inc., Pathways to Pregnancy and Parturition-1st revised edition by P.L. Senger, 1999). As progesterone (P_4) drops, FSH and LH increase together in response to GnRH. FSH and LH cause the production of estradiol (E_2) by ovarian follicles. When the follicle reaches a certain maturational stage, it produces inhibin, which suppresses FSH secretion from the anterior pituitary. Thus, FSH does not surge with the same magnitude as LH. When estrogen reaches a threshold concentration the preovulatory surge of LH occurs, inducing ovulation.

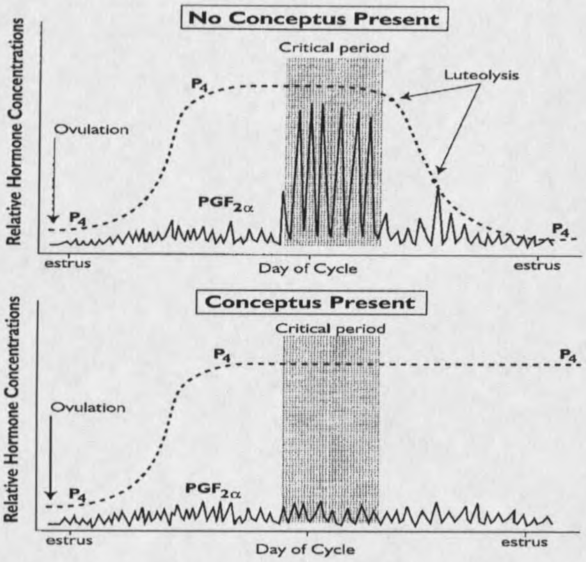


Figure 6 and 7. Comparison between the endocrine condition of a female with conceptus present and a female with no conceptus (reprinted with permission from Current Conceptions, Inc., Pathways to Pregnancy and Parturition-1st revised edition by P.L. Senger, 1999). Maternal recognition of pregnancy must occur prior to luteolysis if the pregnancy is to be maintained.

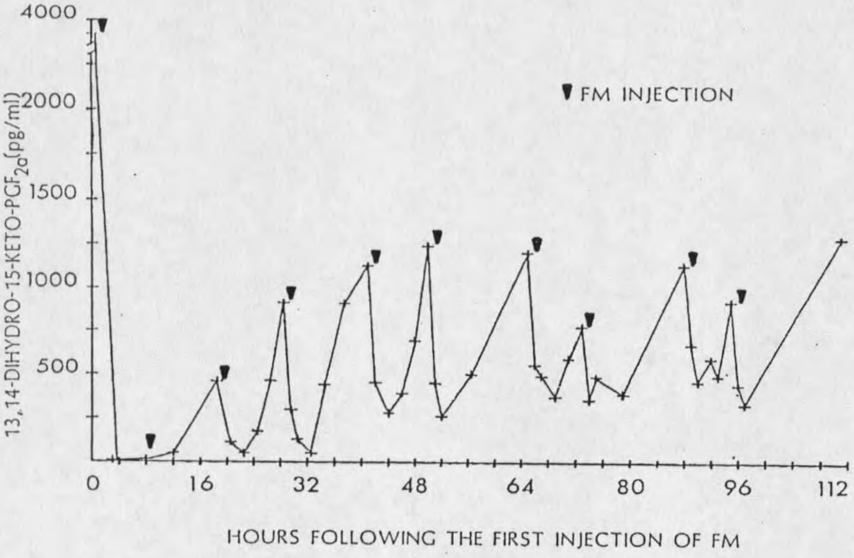


Figure 8. Profile of inhibition of F series prostaglandins, as reflected by concentrations of 15-keto-13, 14-dihydro-$PGF_{2\alpha}$ (PGFM), after repeated i.m. injections of flunixin meglumine (FM) were given twice daily (0800 and 1700 h) to a cow from day 0 to day 5 postpartum (copied from Guilbault et al., 1987).

Table 1. Serum cortisol concentration for calves subjected to stress at 150 d of age[a]. SHAM calves walking through handling facilities, TRANS calves were transported for 24.2 km to holding pens left one hour and returned, and ACTH calves were given an injection of ACTH at 1 IU/kg BW (Lay et al., 1997).

		Time, min[b]								
Trt	n	-15	0	15	30	45	60	90	120	180
		Cortisol, ng/ml								
SHAM	5	19 ± 8	63 ± 9	53 ± 9	46 ± 8^{c}	40 ± 8^{c}	43 ± 7^{c}	36 ± 6^{c}	34 ± 7^{c}	25 ± 7^{c}
TRANS	7	21 ± 7	44 ± 8	60 ± 9	60 ± 7^{cd}	60 ± 7^{d}	52 ± 6^{c}	56 ± 6^{d}	55 ± 7^{d}	50 ± 7^{d}
ACTH	7	20 ± 6	54 ± 8	71 ± 11	76 ± 9^{d}	78 ± 9^{e}	79 ± 8^{d}	75 ± 7^{d}	63 ± 8^{d}	49 ± 9^{d}

[a] Least square means (± SE) are presented for cortisol.

[b] The time is indicated relative to when blood sample was collected

[c,d,e] Numbers within same column with different subscripts differ ($P<0.05$).

Table 2. Effect of time of transport after a synchronized insemination on synchronized pregnancy rate, breeding season pregnancy rate and day of conception in beef heifers (Harrington, et al. 1995).

Variable	1-4 d post-AI	8-12 d post-AI	29-33 d post AI	p-value of model
No. of heifers	143	143	144	
Synchronized pregnancy Rate (%)[a]	73.9^{x}	62.2^{y}	64.7^{y}	0.08
Breeding season pregnancy rate (%)[b]	95.1	93.7	94.4	0.88
Mean day of conception[c]	9.6^{x}	13.4^{y}	13.6^{y}	0.04

[a] Synchronized pregnancy rate is the percentage of heifers pregnant to a synchronized estrus and AI. Means within row without a common superscript differ ($P<0.10$).

[b] Breeding season pregnancy rate is the percentage of heifers pregnant for the entire breeding season of the total in each group.

[c] Mean day of conception is the average day into the breeding season when heifers became pregnant, with d 1 of the breeding season being the date when the first heifer was inseminated. Means within row without a common superscript differ ($P<0.05$).

Materials and Methods

Experiment 1

Ninety-seven, multiparous, Angus-cross cows (BW 636 ± 4.5 kg; mean age 5.4 yr) were utilized to test the effects of flunixin meglumine on early embryonic mortality, PGF$_{2\alpha}$, and cortisol release. These animals were pastured on the Montana State University Red Bluff Research Station in Norris, MT. All cows received a 33 d MGA-PGF$_{2\alpha}$ treatment to synchronize estrus (fed 0.5mg MGA/hd/d for 14 d followed by a 25mg injection of PGF$_{2\alpha}$, 19 d after discontinuance of feeding). All animals were observed for estrus twice daily, from 0600 to 0900 and from 1800 to 2100, for 5 d after PGF$_{2\alpha}$ injection and were artificially inseminated (AI) approximately 12 h after onset of estrus. Approximately 14 d following artificial insemination (AI), cows were assigned to treatments by AI sire, AI date, and AI technician. Treatments were control (CON), induced stress (S), and induced stress with flunixin meglumine (SFM). The cows receiving CON (n=33) remained at the ranch with their calves and were provided access to water but no feed for 5 h. Cows receiving S (n=32) and SFM (n=32) were transported via semi-truck for 4 h (mean ambient temperature 24° C). Before transportation-induced stress, SFM treated cows received flunixin meglumine (1.1 mg/kg, i.m) injection in the neck. At the beginning and completion of treatment, rectal temperatures were recorded and blood samples collected (coccygeal venipuncture) from all cows for measurements of cortisol, progesterone, and PGF metabolite (PGFM) concentrations. Mean time lapse between blood samplings was 5 h due to unloading, mixing, and collection time. Blood was collected using sterile, non-additive, 10 mL, red-topped tubes (Becton Dickinson and Company, Franklin Lakes, NJ) and 18 gauge x 1" blood collection needles (Becton Dickinson and Company, Rutherford, NJ). These samples were placed on ice for approximately 7 h until transport was available to the laboratory in Bozeman, MT (26 km). Blood was allowed to clot at room temperature for one hour. Serum was obtained by centrifugation at 3,000 x g for 20 min, transferred to new tubes, and stored at –20° C until analyzed. Cows were exposed to clean-up bulls beginning the day after treatment for 30 d. Transrectal ultrasonography was used to determine AI pregnancy status 55 to 57 d post-AI. Final pregnancy rates were determined approximately 90 d after AI by rectal palpation.

Serum was analyzed for progesterone concentration using coated tubes (Kit TXPGX; Diagnostic Products Corporation, Los Angeles, CA) as described by Bellows et al., (1991). Sensitivity of the assay was 0.08 ng/ml and intra-assay CV was 1.16 %. Serum cortisol concentration was analyzed using coated tubes (Kit 2100; Diagnostic Systems Laboratories, Webster, TX) using a recently validated assay. Sensitivity of the assay was 2.2 ng/ml and intra-assay CV was 2.41 %. Serum concentrations of 13, 14-dihydro-15-keto prostaglandin $F_{2\alpha}$ metabolite (PGFM) were quantified by RIA as described by Silvia and Niswender (1984). The sensitivity of the assay was 1.25 pg/ml and the intra and interassay CV were 11.7 % and 11.0 %, respectively, across two assays.

Experiment 2

Two hundred and fifty-nine, primiparous, yearling, Angus-cross heifers (5.15 ± 0.34 BCS) were utilized to determine the effects of flunixin meglumine on early embryonic mortality and serum cortisol concentration. These animals were pastured at the Lonestar Ranch, near Springdale, MT. Heifers were blocked by the previous synchronization treatment and randomly assigned to treatments for this study. All heifers were synchronized with EAZI-BREED™ CIDR® a time-released progesterone intravaginal insert. Treatments were GnRH or no GnRH at CIDR insertion and heifers were assigned to AI inseminated either 12 h following estrous detection or by appointment at 72 h following $PFG_{2\alpha}$. Animals were observed for estrus twice daily from 0600 to 1000 and from 1500 to 2100 for 72 h and were artificially inseminated approximately 12 h after onset of estrus or by appointment. Heifers, in the estrous insemination treatments, not detected in estrus within 72 h were timed AI and given GnRH at 72 h. Treatments, in the current study, were control (CON), control with flunixin meglumine (CONFM), induced stress (S), and induced stress with flunixin meglumine (SFM). The heifers receiving CON (n=65) and CONFM (n= 65) remained at the ranch and were provided access to water but no feed for 7 h. Heifers receiving S (n=65) and SFM (n=64) were transported via semi-truck for 7 h (mean ambient temperature 27° C). Before transportation-induced stress, SFM and CONFM treated heifers received flunixin meglumine (1.1 mg/kg, i.m.) injection in the neck. At the beginning of treatment, rectal temperatures were recorded, and blood samples collected (coccygeal venipuncture) from all heifers for measurements of serum cortisol concentration. Blood was collected using sterile, non-additive, 10 mL, red-topped tubes (Becton Dickinson and Company, Franklin Lakes, NJ) and 18 gauge x 1" blood collection needles (Becton Dickinson and Company, Rutherford, NJ). Blood samples and temperatures were collected from heifers receiving S and SFM at Montana State University Teaching and Research Center, Bozeman, MT after 3 h transportation stress and then reloaded and returned to the Lonestar Ranch, near Springdale, MT. Blood samples and rectal temperatures were collected from CON and CONFM heifers that remained at the ranch, during the transporting of the S and SFM heifers. All heifers were again blood sampled a third time at the Lonestar Ranch after S and SFM heifers returned. All blood samples were placed on ice approximately 14 h until transport was available to the laboratory in Bozeman, MT (26 km). Blood was stored overnight (approximately 10 h) on ice then was allowed to clot at room temperature for approximately 1 h. Serum was obtained by centrifugation at 3,000 x g for 20 min, transferred to new tubes, and stored at –20° C until analyzed. Heifers were exposed to clean-up bulls beginning the day after treatment for 30 d. Transrectal ultrasonography was used to determine AI pregnancy status 30-33 d post-AI. Final pregnancy rates were determined approximately 90 d after AI by transrectal ultrasonography.

Serum cortisol concentrations were analyzed using coated tubes (Kit TKC05; Diagnostic Products Corporation, Los Angeles, CA) using a recently validated assay. Sensitivity of the assay was 2.2 ng/ml and inter- and intra-assay CV were 5.8% and 6.3% for high pool and 2.2% and 1.8% for the low pool, respectively.

Experiment 3

One hundred and twenty-seven, multiparous, Angus-cross cows (BW 681± 4.5 kg; mean age 5.4 yr) were utilized to test the effect of flunixin meglumine on early embryonic mortality and serum cortisol concentration. These animals were pastured on the Montana State University Red Bluff Research Station in Norris, MT. All cows received a 33 d MGA-PGF$_{2\alpha}$ treatment to synchronize estrus, as described in experiment 1. All animals were observed for estrus twice daily from 0600 to 0900 and 1800 to 2100 for 5 d after PGF$_{2\alpha}$ injection and were artificially inseminated approximately 12 h after onset of estrus. Approximately 14 d following AI, cows were randomly assigned to treatments. Treatments were control (CON), control with flunixin meglumine (CONFM), induced stress (S), and induced stress with flunixin meglumine (SFM). The cows receiving CON (n=32) and CONFM (n= 31) remained at the ranch and were provided access to water but no feed for 4 h. Cows receiving S (n=32) and SFM (n=32) were transported via semi-truck for 4 h (mean ambient temperature 28.5° C). Before transportation-induced stress, SFM and CONFM treated cows received flunixin meglumine (1.1 mg/kg, i.m.) injection in the neck. At the beginning of treatment, rectal temperatures were recorded, and blood samples collected (coccygeal venipuncture) from all cows for measurements of serum cortisol concentrations. Blood was collected using sterile, non-additive, 10 mL, red-topped tubes (Becton Dickinson and Company, Franklin Lakes, NJ) and 18 gauge x 1" blood collection needles (Becton Dickinson and Company, Rutherford, NJ). Blood samples and temperatures were collected from cows receiving S and SFM at Montana State University Teaching and Research Center, Bozeman, MT after approximately 1.5 h transportation stress and then cows were reloaded and returned to Red Bluff Research Station, in Norris, MT. The CON and CONFM animals remained at the ranch, where blood samples and temperatures were collected again during the transportation of the other cows. Blood samples were collected a third time from all cows at the Red Bluff Research Station after transported cows returned approximately 4 h later. All blood samples were placed on ice (approximately 8 h) until transport was available to facilities. Blood was stored on ice overnight (approximately 10 h) then was allowed to clot at room temperature for approximately 1 h. Serum was obtained by centrifugation at 3,000 x g for 20 min, transferred to new tubes, and stored at –20° C until analyzed. Cows were exposed to clean-up bulls beginning the day after treatment for 30 d. Transrectal ultrasonography was performed on cows approximately 55 to 57 d post-AI. Final pregnancy rates were determined approximately 90 d after AI by rectal palpation and validation of AI pregnancy status was determined.

Serum cortisol concentrations were analyzed using coated tubes (Kit TKC05; Diagnostic Products Corporation, Los Angeles, CA) using a recently validated assay. Sensitivity of the assay was 2.2 ng/ml and CVs were: 5.8% and 6.3% for the inter- and intra-assay, respectively, for high pool and 2.17% and 1.8% for the inter- and intra-assay, respectively, for the low pool.

Statistical Analyses

In experiment 1, differences in cortisol, progesterone, PGFM, and temperature were evaluated between treatments using ANOVA (SAS, 2001). Also in experiment 1, differences in AI pregnancy rate were analyzed with Chi-Square (SAS, 2001).

In experiments 2 and 3, a 2 x 2 factorial design of induced transportation stress (TS) or no transportation stress (NTS) and receiving an injection of flunixin meglumine (FM) or no flunixin meglumine (NFM) was used for analyzing differences in absolute serum cortisol concentration, absolute temperature, change in serum cortisol concentrations, change in temperature and AI and final pregnancy rates. When no transportation stress by flunixin meglumine interaction was present ($P>0.10$), only main effects are discussed.

A 2 x 2 factorial design was also used to determine if there was a year or ranch interaction. When no year by ranch interaction was detected ($P>0.10$) data were combined for re-analysis and reported.

Results and Discussion

Introduction

In year one, there were only three treatments applied to the cows. In year two, a fourth treatment was added: CONFM. Also in year two, another ranch (259 primiparous heifers) was added to the experiment including all four treatments.

Results are discussed by experiment. When no transportation stress by flunixin meglumine interactions were detected ($P>0.10$), only main effects are discussed. When no year by ranch interactions (P>0.10) were detected in pregnancy rates, results are also discussed within combinations evaluated. However, absolute cortisol, absolute temperature, change in cortisol, and change in temperature were not combined due to varying times among sampling periods and variations in cortisol assays.

Progesterone

No differences (P>0.10; Figure 9) in progesterone concentrations were detected among treatments. Buford et al. (1996) reported no differences in progesterone concentrations between control cows (saline treated) and cows treated with flunixin meglumine. There are conflicting results on whether progesterone concentration increases (Collier et al., 1982) or decreases (Abilay et al., 1975) in cattle that have undergone stress.

Prostaglandins

No differences (P>0.10; Figure 10) in PGFM concentration were detected between treatments or sampling periods in experiment 1. These results disagree with Lemaster et al. (1999) who reported a decrease in PGFM in cows treated with flunixin meglumine (1g, i.m.) compared to control cows injected with 25 mL saline on d 5-8 post-AI. Odensvik et al (1998) reported a sharp decrease in the concentration of PGFM shortly after administration of flunixin meglumine in non-pregnant heifers d 14-21 of the estrous cycle. The extended time interval between blood sampling (approximately 5 h) in experiment 1, may have decreased our ability to measure changes in PGFM. As the decrease was seen within 30 min and $PGF_{2\alpha}$ returned to normal concentrations at approximately 6 h (Aiumlamai et al., 1990, Buford et al., 1996).

Cortisol

In experiment 1, cortisol concentrations increased in SFM (18 to 29 ng/ml) and S (23 to 29 ng/ml) cows, but decreased in CON (21 to 18 ng/ml) pre- and post-treatment, respectively($P<0.05$; Figures 11). Changes in cortisol concentrations before and after treatment were not different ($P>0.10$) between S and SFM (Figure 12). Grandin (1997) reported an increase in cortisol concentration in beef cows during transportation or handling stress. Treatment with FM did not affect ($P>0.10$) serum cortisol concentrations among stressed cows in the present study. Hopster et al. (1999) reported that a maximum increase in cortisol concentration from dairy cows sampled five times in one hour was reached within 17 min. Since the samplings in the current experiment were separated by 5 h, this peak would have gone undetected. However, a conflicting study reported no increase in cortisol concentration within 30 min after caudal venipuncture in beef heifers (Veissier and Le Neindre, 1988 as reported by Hopster et al., 1999).

In experiment 2 (Figure 13), no interaction ($P>0.10$) was detected in the initial serum cortisol concentration between treatments, so only main effects will be discussed. Heifers that did not receive an injection of flunixin meglumine (NFM) had lower ($P<0.05$) pre-treatment serum cortisol concentrations (34.51 ng/ml) than FM heifers (39.57 ng/ml). In the intermediate blood sampling, there was an interaction ($P<0.05$) of induced stress and flunixin meglumine on serum cortisol concentrations. Serum cortisol concentration of SFM heifers (48.5 ng/ml; Figure 14) was higher (*P*<0.05) than S, CON, and CONFM, 40.6, 37.0, and 35.6 ng/ml, respectively (Figure 11). Hopster et al. (1999) reported a continued increase in cortisol concentrations after the initial release from venipuncture at 15 min intervals for one hour. The higher serum cortisol concentrations of S and SFM heifers validate an increase in serum cortisol concentrations is associated with induced stressed in cattle. In the final blood sampling, there was no interaction (*P*>0.10) of induced stress by flunixin meglumine on serum cortisol concentrations. However, there was a decrease (*P*<0.01) in serum cortisol concentration for the transportation stressed heifers (TS; 19.39ng/ml; Figure 15) heifers compared to those that had not been stressed (NTS; 33.77 ng/ml). The transportation stressed heifers may have adjusted to the situation and decreased serum cortisol concentrations as a defense mechanism for additional stimuli (Friend, 1991) such that final serum cortisol concentration was below the NTS heifers which had stayed in familiar surroundings and were only worked through the chute. These results are supported by others who have suggested that an increase in serum cortisol concentrations maybe due to loading and the animals recover as transportation continues (Wariss et al., 1995 as reported by Dixit et al., 2001).

When comparing the change in serum cortisol concentration between blood samplings in experiment 2, an interaction between flunixin meglumine and induced stress was detected in the change between the initial and intermediate blood samplings (*P*<0.10). Serum cortisol concentrations decreased (*P*<0.10) in CONFM heifers (4.85 ng/ml) compared to increased serum cortisol concentrations detected in S, SFM, and

CON heifers (7.33, 10.64, 3.48 ng/ml, respectively; Figure 14). No interaction (*P*>0.10) was detected in the change of serum cortisol concentrations from intermediate to final blood sampling. However, serum cortisol concentrations of TS heifers decreased (26.15 ng/ml) more (*P*<0.01) than NTS heifers (3.13 ng/ml). This reduction agrees with Akana and Dallman (1992) who reported that stress-induced facilitation of subsequently stimulated ACTH secretion in mice explains the nonresponsiveness of the mammalian adrenocortical system to repeated stress. There was no interaction (*P*>0.10) detected between the change in serum cortisol concentrations of the initial to the final blood sampling. However, serum cortisol concentrations of TS heifers decreased (17.94 ng/ml) more (*P*<0.01) compared to the NTS heifers (3.60 ng/ml; Figure 15).

In experiment 3 (Figure 16), no interaction or main effect differences (*P*>0.10) were detected at the initial blood sampling for serum cortisol concentrations. In the intermediate blood sampling, no interaction (*P*>0.10) was detected. However, the concentrations of serum cortisol were higher (*P*<0.01) in TS cows (28.02 ng/ml) compared to NTS cows (15.15; Figure 17). These results are similar to what was observed in experiment 2. No interaction (P>0.10) was detected in serum cortisol concentrations of cows following treatment. Concentrations of serum cortisol were lower (*P*<0.01) for TS cows (4.91ng/ml) than for NTS cows (13.44 ng/ml). Becker et al. (1985) reported a similar response in gilts that were tethered to stalls of increased cortisol concentrations followed by decreased serum cortisol concentrations after several hours. Serum cortisol concentration was also lower (*P*<0.05) at the final blood sampling for NFM cows (8.01 ng/ml) than FM cows (10.34 ng/ml; Figure 18). This is similar to Giri et al. (1991) who reported that flunixin meglumine compared to control had no effect on increased serum cortisol concentrations in animals that were injected with endotoxins. When comparing the change in serum cortisol concentration between blood samplings in experiment 3, no interaction was detected in the change of serum cortisol concentration between initial to intermediate blood samplings ($P>0.10$). However, there was an increase ($P<0.01$) in serum cortisol concentrations among TS cows (13.48 ng/ml) in comparison to NTS cows (0.38 ng/ml). As discussed before, the difference in cortisol concentrations can be attributed to the transportation stress (Friend, 1991; Grandin, 1997). No interaction ($P>0.10$) was detected in the change of serum cortisol concentrations between the intermediate and the final blood sampling. However, serum cortisol concentrations decreased more ($P<0.01$) in TS cows (23.95 ng/ml) compared to NTS cows (1.71 ng/ml). This decrease may be due to animals becoming less responsive to the prolonged stressful stimuli as described by Becker et al. (1985) and Akana and Dallman (1992). Also, serum cortisol concentration of the NFM cows decreased more ($P<0.05$; 15.76 ng/ml) than FM cows (9.90 ng/ml). No interaction ($P>0.10$) was detected between the change of serum cortisol concentration between the initial and final blood samplings. However, the serum cortisol concentration decreased more ($P<0.01$) in TS cows (10.04 ng/ml) than NTS cows (0.90 ng/ml).

Temperature

Temperature of cows, in experiment 1, receiving S, SFM, and CON treatments decreased ($P<0.01$) between pre- and post-treatment collections (Figure 19). This disagrees with VonBorrell (2001) who reported an increase in heart rate and temperature related to stressed conditions in livestock.

No interaction (*P*>0.10) or differences in main effects (*P*>0.10) were detected for temperatures of heifers in experiment 2 at the initial or intermediate sampling periods. In the final sampling period, no interaction (*P*>0.10) was detected for temperature, however, temperatures of NFM heifers were higher (*P*<0.05; 39.06 ^{0}C; Figure 20) compared to FM heifers (38.94 ^{0}C). Newton et al. (1990) reported a lower temperature in cattle given flunixin meglumine and bovine interferon-τ compared to those given saline or just bovine interferon- τ . Also, temperatures for NTS heifers (39.07 ^{0}C) were higher (*P*<0.05; Figure 21) at the final sampling period compared to TS heifers (38.94 ^{0}C).

The change in temperature between collection periods for heifers in experiment 2 was also analyzed. No interactions (*P*>0.10) or main effect differences (*P*>0.10) were detected in the change of temperature of heifers from the initial to the intermediate sampling period. No interaction (*P*>0.10) was detected in the change in temperature of heifers from the intermediate to the final sampling period. However, temperature increased (*P*<0.05; Figure 22) in NFM heifers (0.13 ^{0}C) compared to a decrease in FM heifers (0.02 ^{0}C). Oka et al. (2001) reported a decrease in the core body temperature of stressed rats after an injection of indomethacin, a cyclooxygenase inhibitor, in comparison to, control rats that did not receive an injection of indomethacin. The change in temperature of NTS heifers increased (*P*<0.01; 0.15 ^{0}C; Figure 23) compared to a decrease in the TS heifers (0.03 ^{0}C) between the initial and final collection periods. This disagrees with Von Borrell (2001) who stated that an increase in temperature is expected in animals under stressed conditions. No interaction (*P*>0.10) was detected in the change of temperature from the initial to the final sampling periods, however, there was a greater decrease (*P*<0.01) in temperature detected in FM heifers (0.26 ^{0}C) compared to NFM heifers (0.08 ^{0}C). This maybe attributed to the anti-inflammatory effects of the flunixin meglumine (Oka et al., 2001). A greater decrease (*P*<0.05) in temperature between the initial and final collection periods in TS heifers (0.26 ^{0}C) compared to NTS heifers (0.09 ^{0}C) was also detected.

In experiment 3, due to equipment failure, temperatures were collected from only two sampling periods (initial and intermediate). No interactions (*P*>0.10) were detected in the initial temperature sampling period, however, the initial temperature of NTS cows were higher (*P*<0.10; 38.99 ^{0}C) than TS cows (38.93 ^{0}C). No interaction (*P*>0.10) was detected in the intermediate temperature collection period. However, the intermediate temperature of FM cows was lower (*P*<0.05; 38.52 ^{0}C; Figure 24) than NFM cows (38.64 ^{0}C). The lower temperature in FM cows is attributed to the anti-inflammatory action of flunixin meglumine (Newton et al. 1990; Oka et al., 2001).

The change in temperature was analyzed between the initial and intermediate sampling periods for cows in experiment 3. No interaction (*P*>0.10) or main effect differences (*P*>0.10) were detected.

All treatment mean temperatures collected (range 38.4- 39.06 ^{0}C) were numerically above the average temperature for cattle (38.3 ^{0}C) but within biological range (37.7- 39.4 ^{0}C; Sprinkle et al., 2000).

Pregnancy Rates

Pregnancy data for the three trials will be discussed individually, however, because there were no significant interactions between years or ranches detected ($P>0.10$) all data were combined for final analysis.

The AI pregnancy rates of cows in experiment 1 tended to be higher ($P=0.17$) among SFM cows (84%) than S cows (69%), while AI pregnancy rates for CON cows was intermediate (76%; Figure 25). No differences ($P>0.10$) were detected in final (breeding season) pregnancy rates. Buford et al. (1996) reported similar results in pregnant cows. Cows treated with $PGF_{2\alpha}$ had lower pregnancy rates compared to cows treated with flunixin meglumine or saline.

In experiment 2, no interaction ($P>0.10$) was detected in heifer AI or final pregnancy rates (Figure 26). The FM heifers tended ($P=0.15$; 63%; Figure 27) to have higher AI pregnancy rates compared to NFM heifers (54%). No differences ($P>0.10$) existed in final pregnancy rates of heifers among treatments. Giri et al., (1991) reported similar differences between AI pregnancy rates in cows that were infused with endotoxins and had received saline compared to cows that received flunixin meglumine.

In experiment 3 (Figure 28), the Red Bluff cows were utilized again in the 2003 breeding season. No interaction ($P>0.10$) was detected in AI or final pregnancy rates of cows. The FM cows had a higher AI pregnancy rate ($P<0.08$; 80%) compared to NFM (66%; Figure 29). No differences ($P>0.10$) were detected in final pregnancy rates of cows. The differences were similar to those seen in experiment 2 in NFM heifers compared to FM heifers.

There were no ranch or induced stress by flunixin meglumine interactions (*P*>0.10), so heifers and cows were combined from the 2003 breeding season and re-analyzed (Figure 30). The AI pregnancy rates were higher (*P*<0.05) for FM females (68%) compared to NFM females (58%; Figure 31). There was also no year or flunixin meglumine by induced stress interaction (*P*>0.10), so experiments 1 and 3 were combined and re-analyzed without the CONFM treatment of 2003. The AI pregnancy rate was higher (*P*<0.08; Figure 32) for SFM cows (81%) compared to S cows (67%) with CON cows were intermediate (71%). Harrington et al. (1995) reported a 62% AI pregnancy rate in heifers that were stressed 8-12 d post-AI, this is similar to the S cows' 67% AI pregnancy rate, in this study.

Pregnancy data for the three trials were combined, because there was no interaction between years or ranches detected (*P*>0.10), and the CONFM data was omitted, as it was not a treatment in experiment 1. The AI pregnancy rate was higher (*P*=0.06) in SFM females (71%) than S females (60%) while AI pregnancy rate of CON females was intermediate (63%; Figure 33). The higher pregnancy rate of FM females when compared to NFM females has been standard throughout the experiments; however no effect of induced transportation stress has been exposed. These results suggest that flunixin meglumine has an effect on AI pregnancy rates that overcomes transportation stress induced embryonic loss.

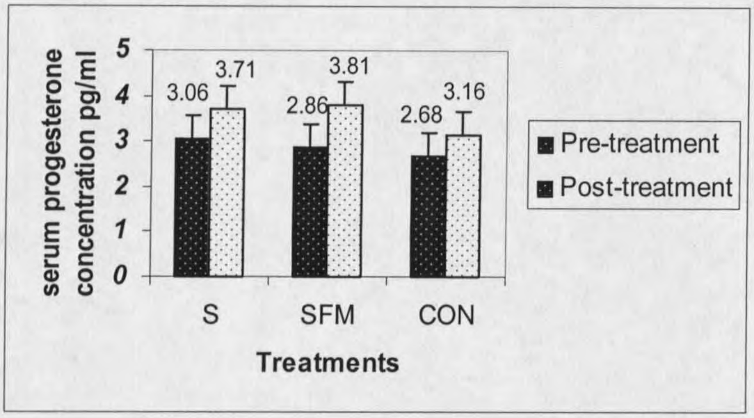


Figure 9. Mean + SE serum progesterone concentration of experiment 1 for control cows (CON) and cows that received 4 h transportation stress (S) or transportation stress with flunixin meglumine (SFM) pre- and post-treatment. No differences were detected (*P*>0.10).

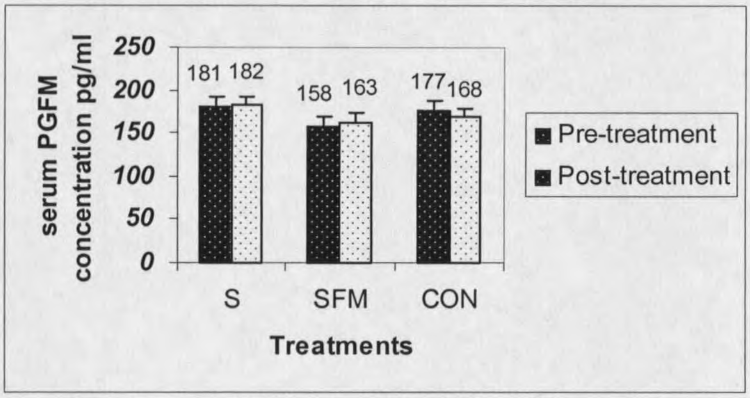


Figure 10. Mean + SE serum PGFM concentrations of experiment 1 for control cows (CON) and cows that received 5 h transportation stress (S) or transportation stress with flunixin meglumine (SFM) pre- and post-treatment. No differences were detected (*P*>0.10).

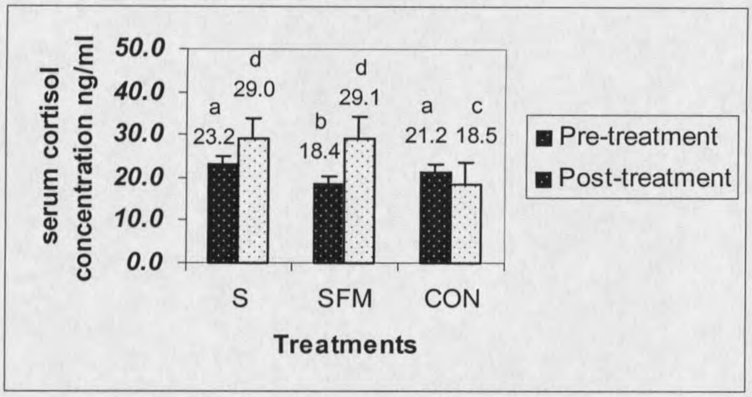


Figure 11. Mean + SE pre- and post-treatment serum cortisol concentrations for control cows (CON) and cows that received 4 h transportation stress (S) or transportation stress with flunixin meglumine (SFM) in experiment 1. [a,b] Pre-treatment cortisol concentration bars that lack common superscripts differ (*P*<0.05). [c,d] Post-treatment cortisol concentration bars that lack common superscripts differ (*P*<0.05).

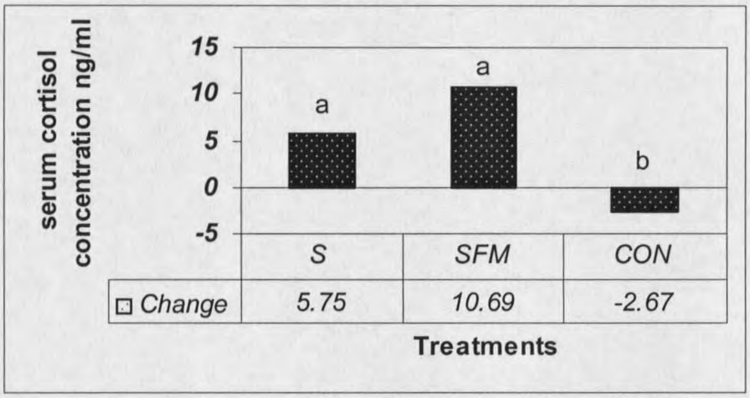


Figure 12. Difference in mean serum cortisol concentrations pre- and post-treatment of control cows (CON) and cows that received 4 h transportation stress (S) or transportation stress with flunixin meglumine (SFM) in experiment 1. Bars that lack a common superscript letter differ (*P*<0.03).

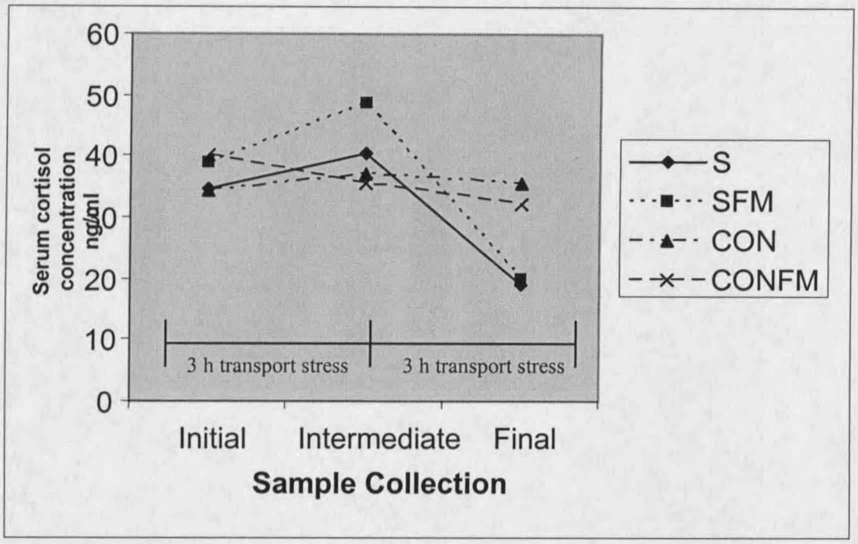


Figure 13. Mean serum cortisol concentrations of heifers for experiment 2 at each of the three blood samples collect from control heifers (CON) and control heifers with flunixin meglumine (CONFM) and heifers that received 6 h transportation stress (S) or transportation stress with flunixin meglumine (SFM).

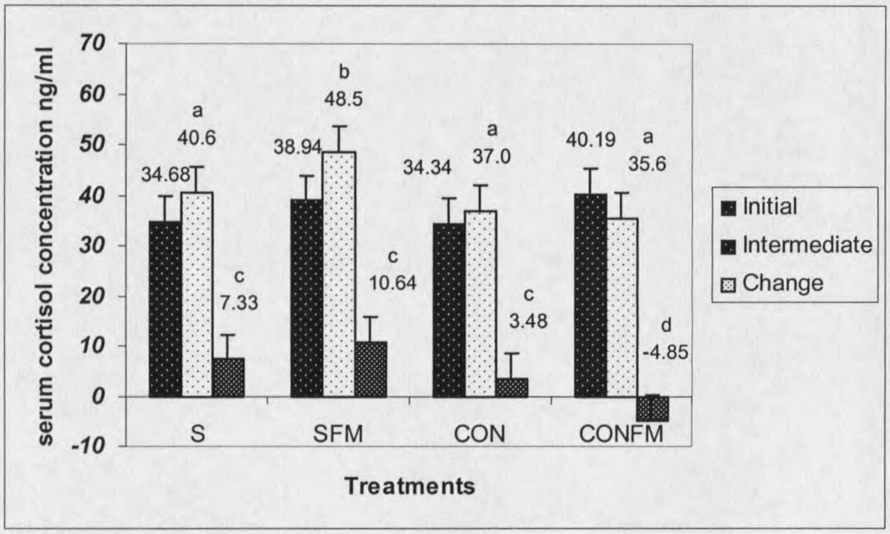


Figure 14. Initial, intermediate, and the change between initial and intermediate blood samplings for mean + SE serum cortisol concentrations of experiment 2 for heifers that received transportation induced stress (S) or transportation induced stress with an injection of flunixin meglumine (SFM) and control heifers (CON) or control heifers that received an injection of flunixin meglumine (CONFM). ^{a,b} Bars that lack common superscripts differ ($P<0.05$) between treatments. ^{c,d} Bars that lack common superscripts differ ($P<0.10$) between treatments.

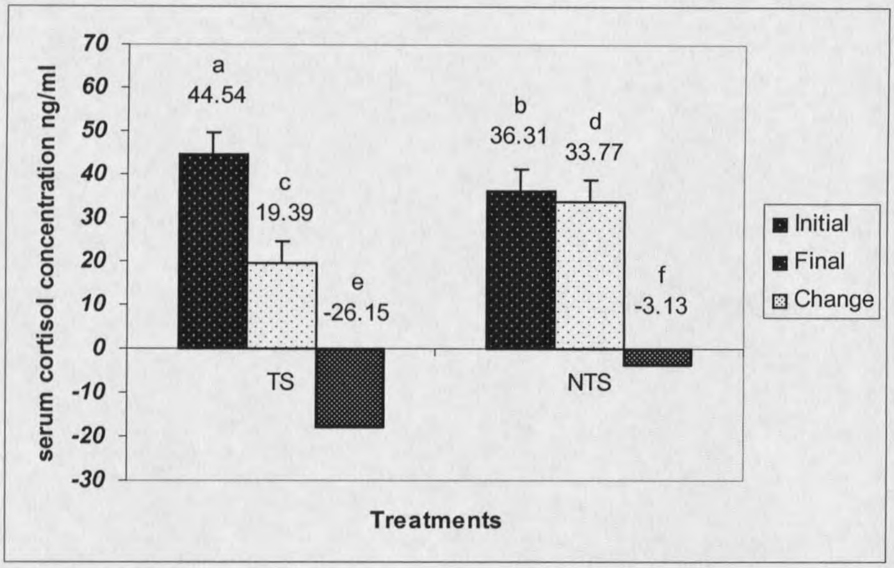


Figure 15. Initial, final, and change between initial and final blood samplings of mean + SE serum cortisol concentrations for experiment 2 of heifers that received transportation stress (TS) and those that did not receive transportation stress (NTS). Bars that lack common superscripts within blood sampling periods differ ($P<0.01$) between treatments.

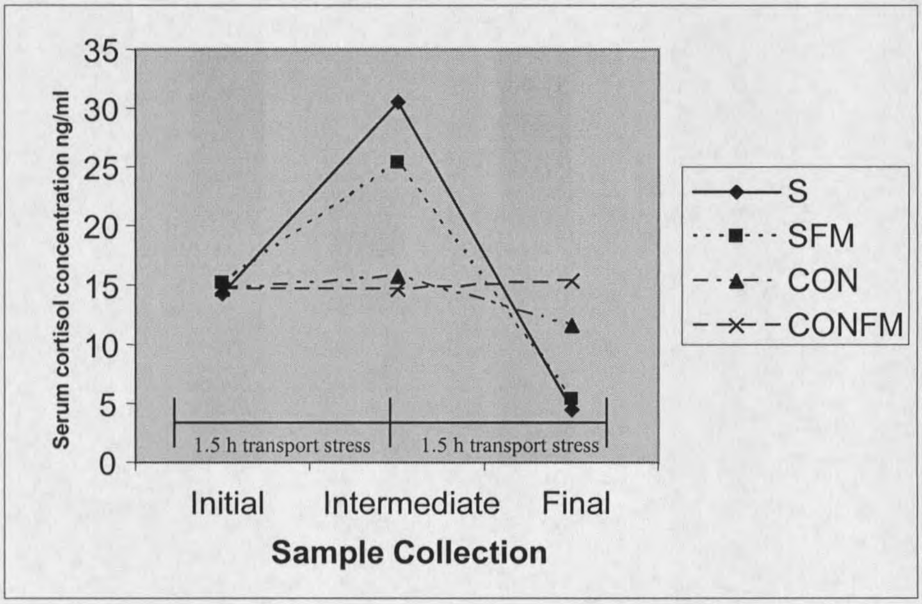


Figure 16. Mean + SE cortisol concentrations of cows for experiment 3 at each of three blood samplings from control cows (CON) and control cows with flunixin meglumine (CONFM) and cows that received 3 h transportation stress (S) or transportation stress with flunixin meglumine (SFM).

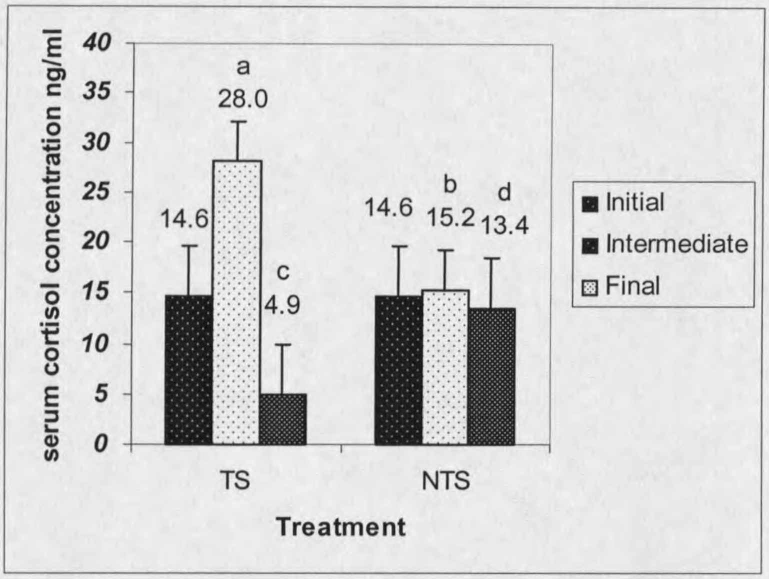


Figure 17. Initial, intermediate, and final blood samplings for mean + SE serum cortisol concentrations of experiment 3 for animals that received transportation stress (TS) and those that did not receive transportation stress (NTS). Bars that lack common superscripts within blood sampling periods differ ($P<0.01$).

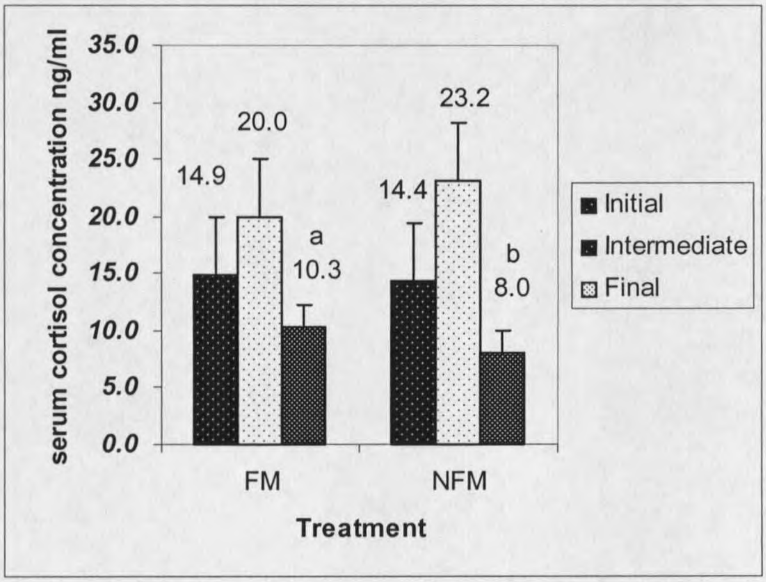


Figure 18. Initial, intermediate, and final mean + SE serum cortisol concentrations for experiment 3 of cows that were injected with flunixin meglumine (FM) and those that were not injected with flunixin meglumine (NFM). Bars that lack common superscripts differ ($P<0.05$) among blood sampling periods.

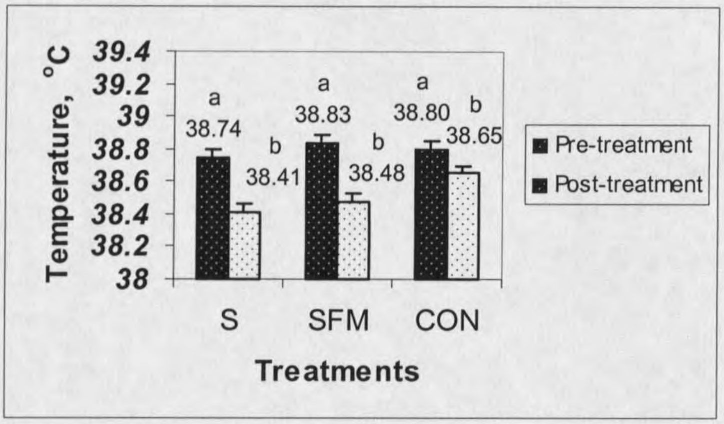


Figure 19. Mean + SE pre- and post treatment temperatures of experiment 1 for control cows (CON) and cows that received transportation stress 4 h (S) or transportation stress with flunixin meglumine (SFM). Bars that lack a common superscript differ ($P<0.01$)

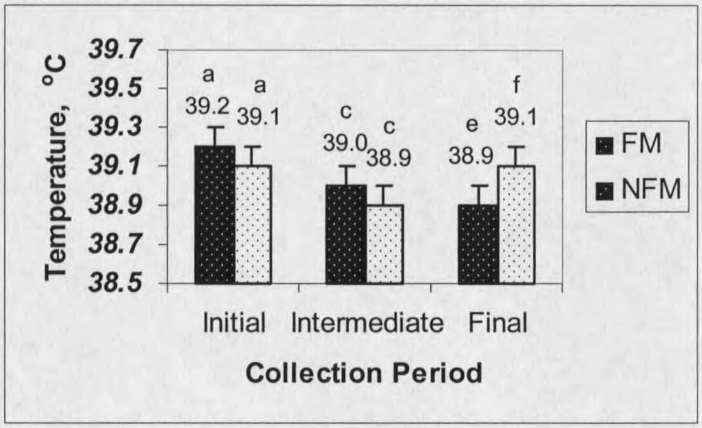


Figure 20. Initial, intermediate, and final collection period of mean + SE temperatures for experiment 2 of heifers that received flunixin meglumine (FM) and those that did not receive flunixin meglumine (NFM). Bars that lack common superscripts differ ($P<0.05$) within sampling period.

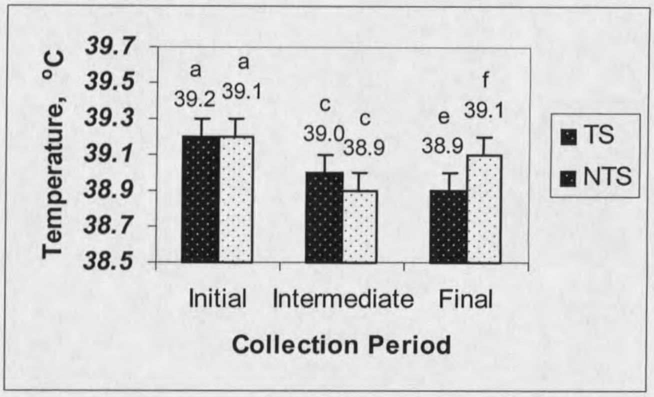
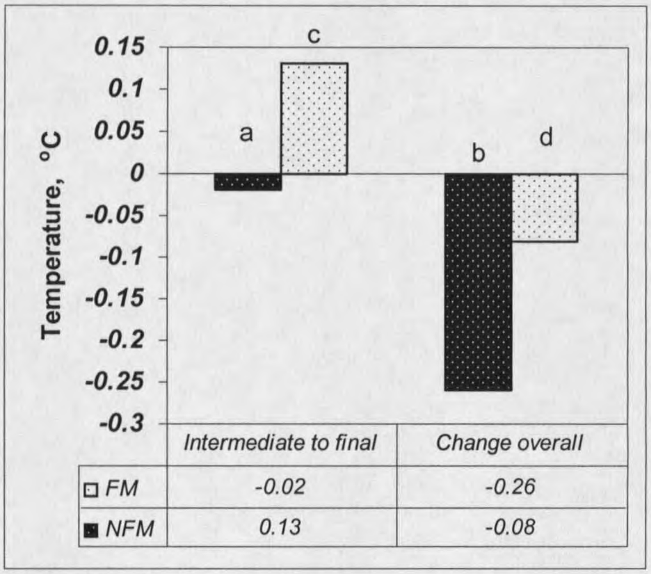


Figure 21. Initial, intermediate, and final collection of mean + SE temperature for heifers in experiment 2 that received transportation stress (TS) and those that did not receive transportation stress (NTS). Bars that lack common superscripts differ ($P<0.05$) with in collection period.



	Intermediate to final	*Change overall*
☐ *FM*	*-0.02*	*-0.26*
■ *NFM*	*0.13*	*-0.08*

Figure 22. Change in mean temperature from intermediate to final and initial to final (overall change) for experiment 2 of heifers that received an injection of flunixin meglumine (FM) and those that did not receive flunixin meglumine (NFM). ^{a,b} Bars that lack common superscripts differ ($P<0.01$) within change period. ^{c,d} Bars that lack common superscripts differ ($P<0.05$) within change period.

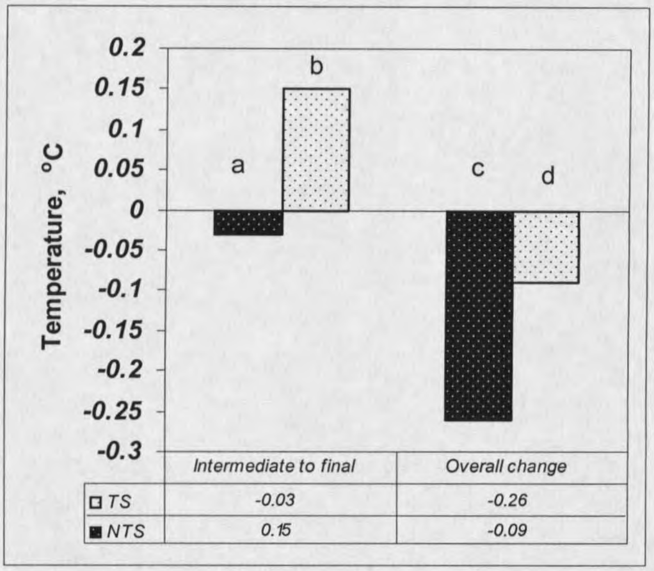


Figure 23. Change in mean temperature from intermediate to final and initial to final (overall change) for experiment 2 of heifers that received transportation stress (TS) and those that did not receive transportation stress (NTS). [a,b] Bars that lack common superscripts differ ($P<0.01$) within change period. [c,d] Bars that lack common superscripts differ ($P<0.05$) within change period.

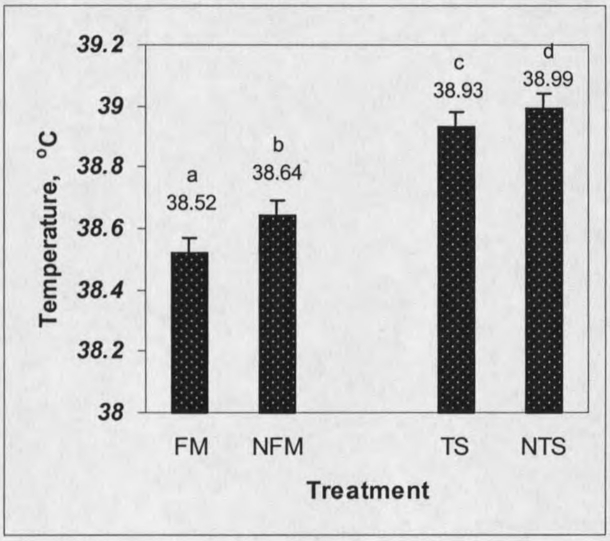


Figure 24. Mean + SE temperature at the intermediate collection period for experiment 3 for main effects of cows that received flunixin meglumine (FM) or cows that did not receive flunixin meglumine (NFM) and cows that received transportation induced stress (TS) or cows that did not receive transportation stress. [a,b] Bars that lack common superscripts differ ($P<0.05$). [c,d] Bars that lack common superscripts differ ($P<0.10$).

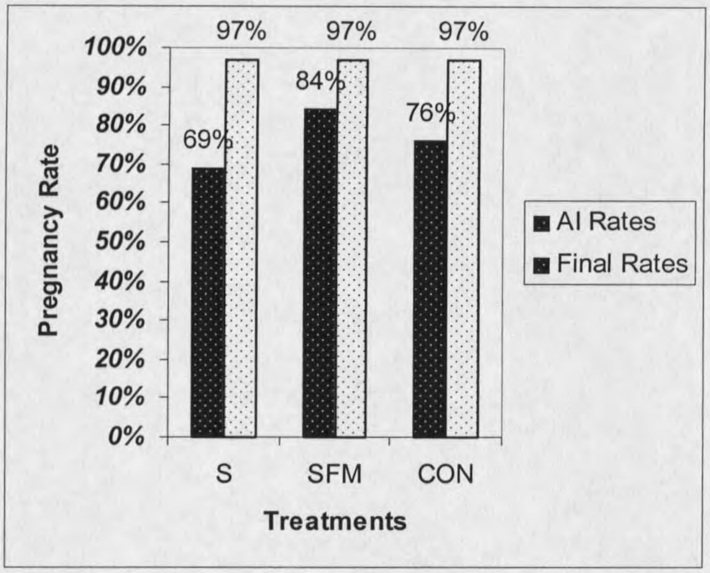


Figure 25. The AI and final pregnancy rates for control cows (CON) and cows that received 4 h transportation stress (S) or transportation stress with flunixin meglumine (SFM; *P*=0.17) in experiment 1.

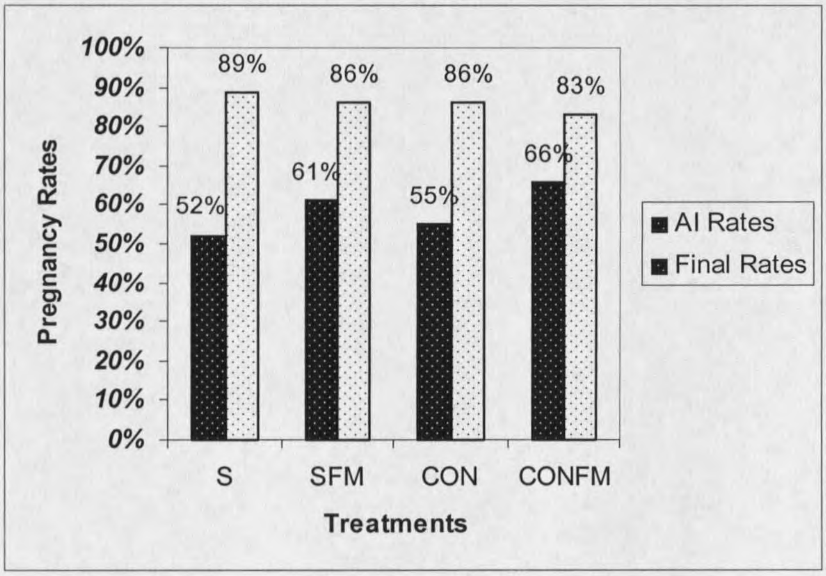


Figure 26. The AI and final pregnancy Rates of experiment 2 for control heifers (CON), control heifers with flunixin meglumine (CONFM) and heifers that received transportation stress (S) or heifers that received transportation stress with flunixin meglumine (SFM; *P*>0.10).

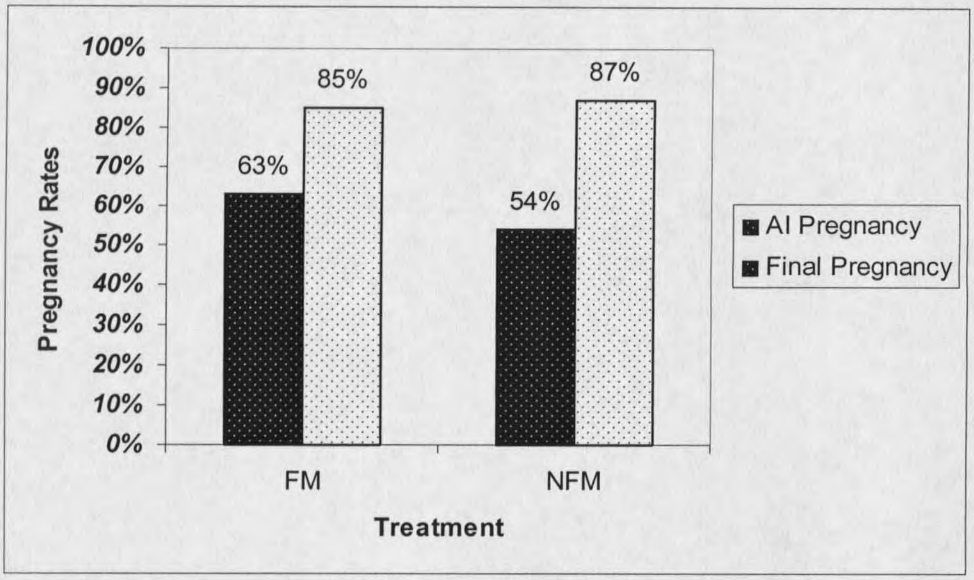


Figure 27. The AI and final pregnancy rates of experiment 2 for heifers that received flunixin meglumine (FM) and heifers that did not receive flunixin meglumine (NFM; *P*=0.15).

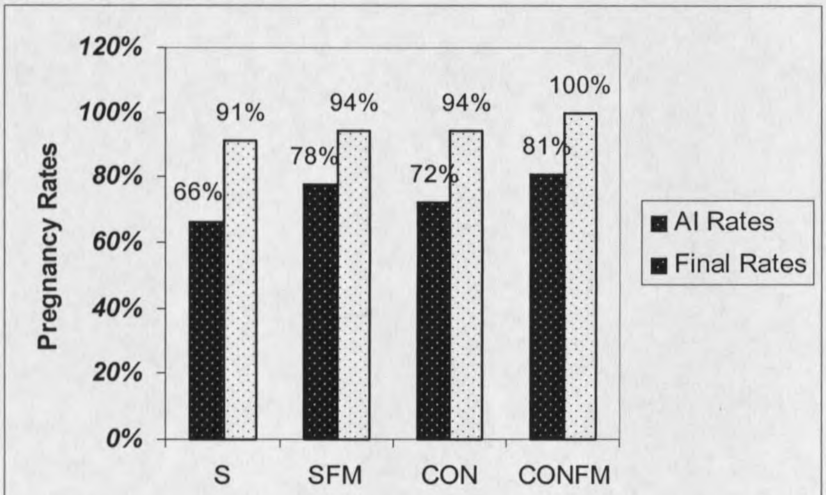


Figure 28. The AI and final pregnancy rates of experiment 3 for control cows (CON) or control cows with flunixin meglumine (CONFM) and cows that received transportation stress (S) or transportation stress with flunixin meglumine (SFM; *P*>0.10).

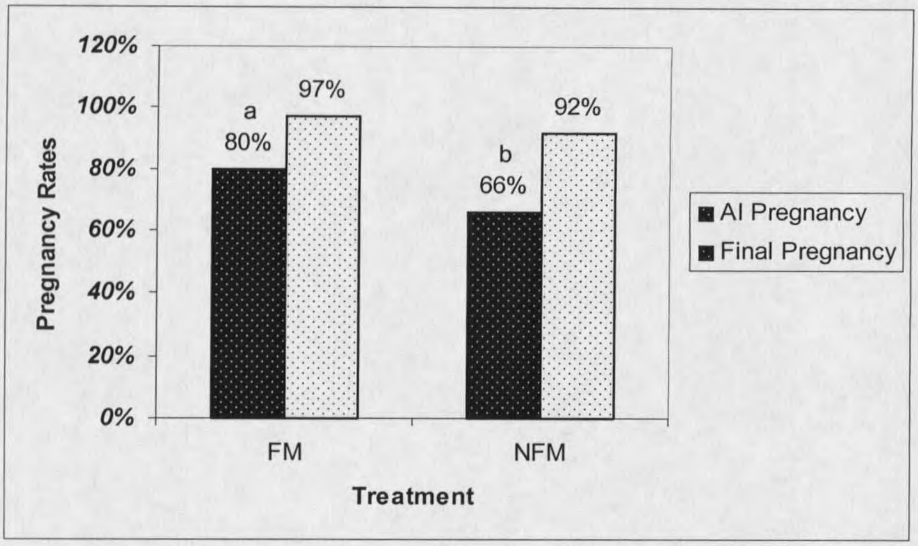


Figure 29. The AI and final pregnancy rates of experiment 3 for cows that received flunixin meglumine (FM) and cows that did not receive flunixin meglumine (NFM). Bars that lack common superscripts differ (*P*=0.08).

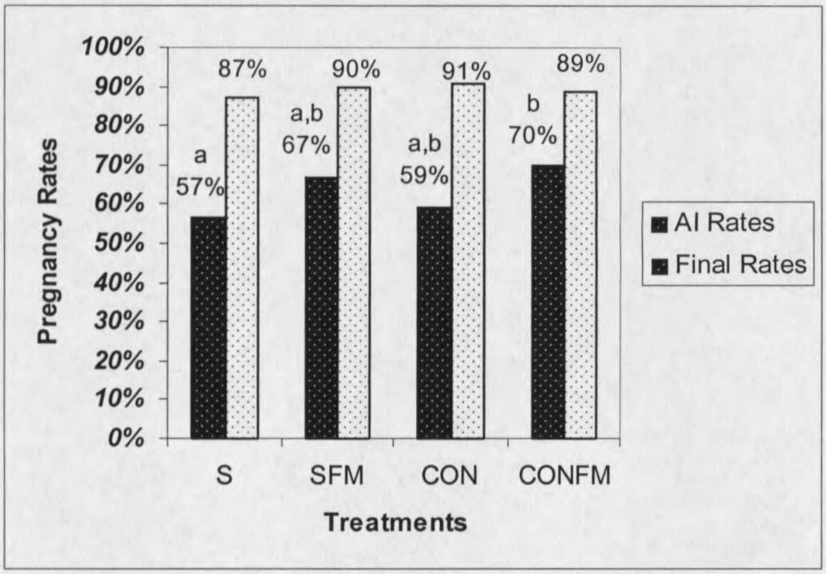


Figure 30. The AI and final pregnancy rates for experiment 2 and 3 combined, of control females (CON), control females with flunixin meglumine (CONFM) and females that received transportation stress (S) or transportation stress with flunixin meglumine (SFM). There was no ranch interaction (*P*<0.10). Bars that lack a common superscript differ (*P*<0.05).

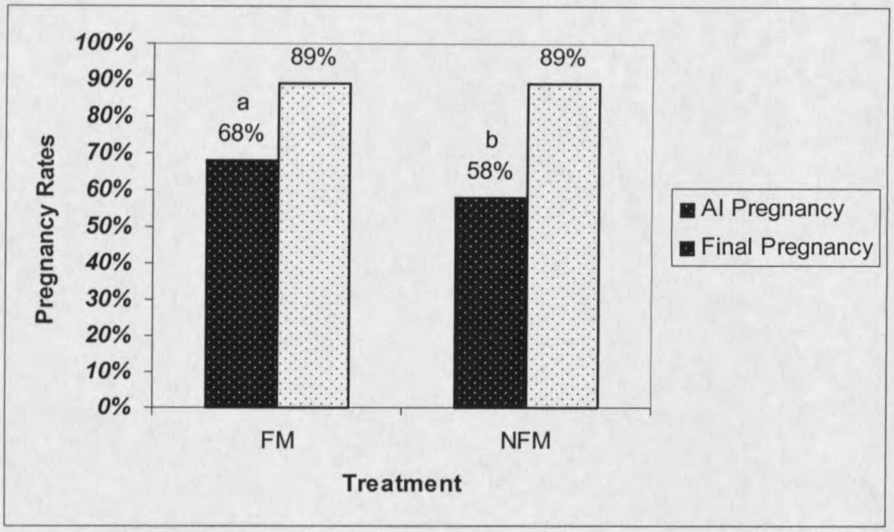


Figure 31. The AI and final pregnancy rates of experiments 2 and 3 for females that received flunixin meglumine (FM) and females that did not receive flunixin meglumine (NFM). Bars that lack common superscripts differ (*P*=0.03).

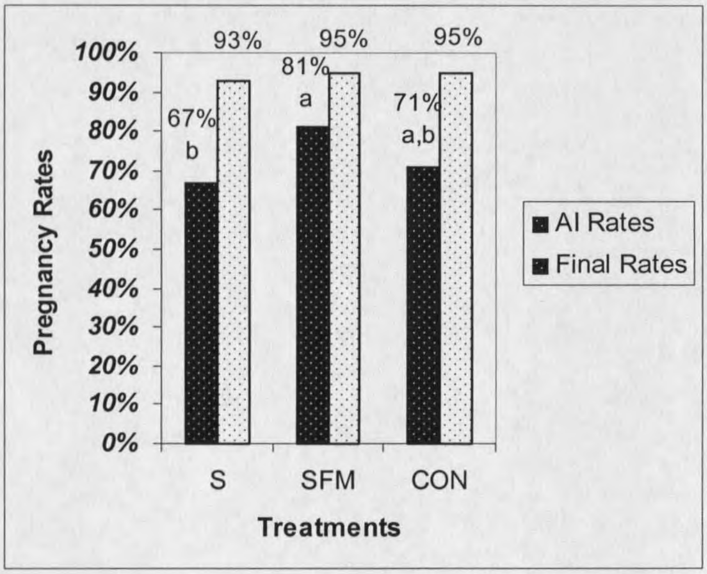


Figure 32. The AI and final pregnancy rates of Red Bluff cows (experiment 1 and 3) for control cows (CON) and cows that received transportation stress (S) or transportation stress with flunixin meglumine (SFM). No interactions were observed between ranch or year (*P*>0.10). Bars that lack common superscripts differ (*P*<0.08).

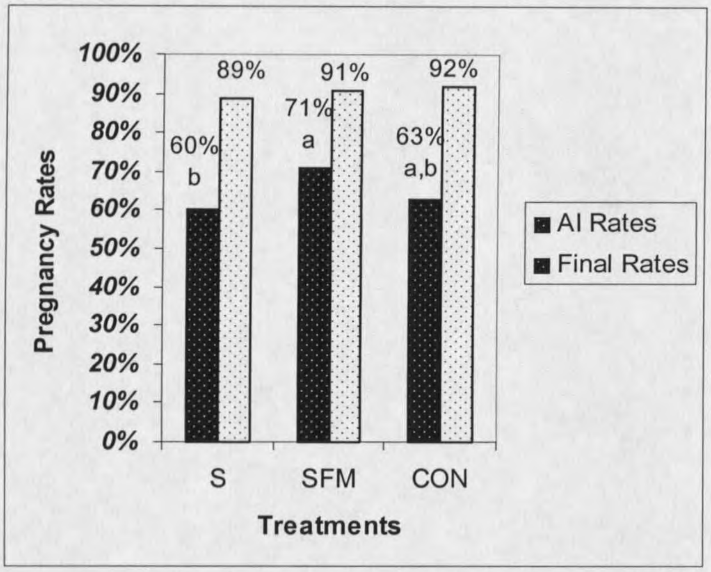


Figure 33. The AI and final pregnancy rates of experiments 1-3 combined, of control females (CON) and females that received transportation stress (S) or transportation stress with flunixin meglumine (SFM). There was no ranch or year interactions (*P*>0.10). Bars that lack common superscripts differ (*P*<0.06).

IMPLICATIONS

These data indicate that flunixin meglumine can be an effective tool in reducing early embryonic mortality due to stress during early pregnancy (12-14 d). However, the mechanisms are still unclear. Changes in serum cortisol concentrations due to flunixin meglumine were not tected in this study. Although, body temperature was reduced in flunixin meglumine treated cattle, the higher temperatures in cattle that did not receive flunixin meglumine were still within the normal biological range. In future trials, an additional control group without handling until pregnancy diagnosis may need to be added to determine if handling and data collection is sufficient stress to impact embryonic mortality.

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