To the Graduate Council:

I am submitting herewith a thesis written by Charles Dexter Young entitled "Reproductive Efficiency following Administration of an Inhibitor of Prostaglandin $F_{2\alpha}$ during Early Embryonic Development in Dairy Cattle". I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

F. Neal Schrick

Major Professor

We have read this thesis and
recommend its acceptance:

J. Lannett Edwards

Matthew Welborn

Accepted for the Council:

Anne Mayhew

Vice Chancellor and
Dean Of Graduate Studies

(Original signatures are on file with official student records.)

Reproductive Efficiency following Administration of an Inhibitor of Prostaglandin $F_{2\alpha}$ during Early Embryonic Development in Dairy Cattle

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Charles Dexter Young
August, 2004

Acknowledgments

I would like to express my appreciation to all the people who have helped and encouraged me during my graduate career. I first want to thank Dr. F. Neal Schrick for his guidance and continued support. He continually challenged my abilities and intellect, while providing a comfortable and friendly atmosphere to work. I would also like to thank Dr. J. Lannett Edwards and Dr. Matthew Welborn for supporting me through my entire graduate career and serving on my graduate committee. I thank Dr. Edwards for her insightful view of Reproductive Physiology, and continuously challenging me to view topics from a different angle. I thank Dr. Welborn for his assistance in completing the research, and always being available with his professional knowledge and opinions.

The graduate students and faculty of The University of Tennessee, Knoxville have always provided support and the opportunity to accomplish my goals. My experience here has always been exciting and challenging. I thank all those that I have met over the years and all those that have assisted me to this point. I would especially like to thank Mitch, Tim, Fernando, Gustavo, Eddie, Jimmy, and David for their friendship and advice over the years. I thank the Knoxville Experiment Station for providing me the opportunity to complete my Master's degree. I especially would like to thank Dr. Mark Campbell for his continued support, patience, and advice while I completed the requirements for this degree.

Finally, I would like to thank my family for their encouragement. Most of all, I thank my wife Miranda for her unwavering support. Thank you for your patience and encouragement at times when I needed it. Thank you for reminding me that you and the boys are why life is important.

Abstract

A study was performed to examine the administration of flunixin meglumine (FM; an inhibitor of prostaglandin $F_{2\alpha}$) following artificial insemination on pregnancy rates of lactating dairy cattle. Jersey and Holstein cows (n=106) were randomly assigned to receive intravenously either FM (2.2 mg/kg BW) or saline (SAL; 1 mL/23 kg BW). Cows were randomly allotted to treatment by lactation number, bovine somatotropin administration, breed, BW, days in milk, milk production, and 305 ME milk production. Study was performed from September through April and all cows were milked twice daily. Following estrous synchronization (cloprostenol, 500 μg im), cows were artificially inseminated 12 ± 6 h after the onset of estrus was detected utilizing electronic monitoring. Administration of FM or SAL was administered 5 d ± 12 h following artificial insemination. Animals underwent verification of pregnancy 28-35 d following artificial insemination using transrectal ultrasonography by an individual blinded to treatment. Pregnancy was determined by the presence or absence of an embryo with a heartbeat. Pregnancy data were analyzed using CRD Mixed ANOVA Analysis. Administration of FM following artificial insemination did not improve pregnancy rates in lactating dairy cows (FM, 37 vs SAL, 31.4%; $P > 0.10$). In conclusion, treatment with flunixin meglumine did not improve pregnancy rates when administered following artificial insemination in lactating dairy cows.

Table of Contents

1. Introduction 1

2. Literature Review 5

2.1 Reproductive Inefficiency in Dairy Cattle 5

2.2 Embryonic Development 9

2.2.1 The sperm and oocyte fusion 9

2.2.2 Maternal recognition of pregnancy 13

2.3 Causes of Embryonic Mortality 16

2.4 $PGF_{2\alpha}$ Association With Early Embryonic Loss 25

2.5 Effects of $PGF_{2\alpha}$ Inhibitors 28

2.6 Summary of the Literature 33

3. Materials and Methods 35

3.1 Statistical Analysis 38

4. Results 39

5. Discussion 51

List of References 59

Vita 69

List of Tables

1. Description of animal numbers and production characteristics. 36

2. Effect of treatment with flunixin meglumine (FM) or saline (SAL) on pregnancy rates for cows administered rBST (rBST +) or not administered rBST (rBST -) and within lactational groups (1, 2, 3 +). 41

3. Effect of lactation (LN) group and rBST administration (rBST +) or no rBST administration (rBST -) on pregnancy rates within lactation group. 50

List of Figures

1. Effect of treatment on pregnancy rates. 40

2. Effect of treatment with flunixin meglumine (FM) or saline (SAL) on pregnancy rates between low, medium, and high milk production groups.....42

3. Effect of treatment with flunixin meglumine (FM) or saline (SAL) on pregnancy rates among different 305 mature equivalent (ME) groups.....43

4. Effect of treatment with flunixin meglumine (FM) or saline (SAL) on pregnancy rates for cows in the low (1-2.5) and moderate (3-5) body condition score groups.....44

5. Effect of treatment with flunixin meglumine (FM) or saline (SAL) on pregnancy rates among different days in milk (DIM) groups.....45

6. Effect of milk production and 305 ME group on pregnancy rates. 46

7. Effect of rBST administration (rBST +) or no rBST administration (rBST -) on pregnancy rates. 48

8. Effects of rBST administration (rBST + or rBST -) on pregnancy rates for cows < 100 days in milk (DIM). 49

1. Introduction

Reproductive failure in dairy cows is a problem that has increased over the last three decades and continues to plague the modern dairy producer (reviewed by Lucy, 2001). When dairy cattle suffer reproductive difficulties, they encounter increased days open, a term that refers to the time during which an animal is not pregnant (reviewed by Lucy, 2001). An increase in days open can result in a loss of income. The average dairy cow costs the producer up to $4.00 per each day open after 85 days postpartum (Britt, 1975). In order to avoid this monetary loss, the producer must reestablish pregnancy approximately 60-90 days postpartum. However, many factors, such as an animal's stress level and nutrition, can lead to the failure of reestablishing and maintaining pregnancy during this time period.

Lactating dairy cows endure continuous stress due to two - and, in some cases, three - milkings every day. Heat stress due to ambient temperature is another factor that tends to hinder pregnancy (reviewed by De Rensis and Scaramuzzi, 2003). Management practices that use stimulating growth hormones (Cole et al., 1991; reviewed by Dohoo et al., 2003) and highly concentrated nutrition can negatively affect days open in dairy cows (O'Callaghan et al., 2000). Since failure to establish and maintain pregnancy in an animal results in monetary loss for the agricultural industry, researchers must determine other means by which reproduction can be made more successful. One course of action is to control the prostaglandins produced during establishment of pregnancy.

Prostaglandins are hormones that are released by most mammalian cells (Bito, 1975). They are involved in inflammation, pain and fever production, control of blood vessel tone, luteolysis, and blood clotting (Nett et al., 1976; Seibert et al., 1994; Blatteis

and Sehic, 1997). The study of luteolysis is central to understanding how to maintain pregnancy. Prostaglandin F_{2alpha} ($PGF_{2\alpha}$) is capable of lysing the corpus luteum (CL) on the mammalian ovary (reviewed by Bazer et al., 1993). The CL is responsible for the production of progesterone, the hormone that maintains pregnancy (Fields, 1991). Prostaglandin $F_{2\alpha}$ not only lyses the corpus luteum, but it also provides a dangerous environment for the embryo. Studies indicate the presence of high concentrations of $PGF_{2\alpha}$ around the embryo at days 5-8 of development have a negative effect on continued development (Schrick et al., 1993; Breuel et al., 1993; Seals et al., 1998; Lemaster et al., 1999; Hockett et al, 2004; Scenna et al., 2004a).

Research has explored effects of $PGF_{2\alpha}$ on the embryo at early stages of development. Studies have been performed in which $PGF_{2\alpha}$ was administered on days 5 through 8 following artificial insemination. These studies involved cows that were supplemented with progestogen, and the results indicated a reduction in pregnancy rates and the development of retarded embryos (Buford et al., 1996; Hockett et al., 2004; Seals et al., 1998; Lemaster et al., 1999). These results indicate a direct and/or indirect effect of $PGF_{2\alpha}$ on embryonic survival in the cow. Schrick and coworkers (1993) reported a negative correlation between embryo quality and high uterine luminal concentrations of $PGF_{2\alpha}$. Breuel and coworkers (1993) reported detrimental effects on 8-cell rat embryos when cultured with $PGF_{2\alpha}$. The culture of these 8-cell embryos with $PGF_{2\alpha}$ caused a decrease in the number of embryos reaching morula, blastocyst, and expanded blastocyst stages (Breuel et al., 1993).

The failure for an embryo to advance to morula and blastocyst stages results in embryonic death. Data presented by Breuel (1993) agree with the indications made by

Schrick (1993) that increased concentrations of $PGF_{2\alpha}$ in the uterine lumen may contribute to embryonic mortality. Scenna and coworkers (2004a) reported that $PGF_{2\alpha}$ had a negative effect on the continued development of both in vitro and in vivo-derived embryos. Reduced blastocyst formation was seen in 16-32 cell stage embryos that were in vitro derived. A reduction in blastocyst formation was not seen in in vivo-derived embryos, but a reduction in hatching blastocysts was observed. Extensive studies have indicated that $PGF_{2\alpha}$ does in fact have an effect on embryo quality and survival; therefore, these studies indicate that $PGF_{2\alpha}$ may be causing early embryonic mortality. Premature release of uterine luminal concentrations of $PGF_{2\alpha}$ can be the result of several factors such as heat stress (Putney et al.,1989; Maylayer et al., 1990), nutrition (reviewed by Butler, 1998), mastitis (Hockett et al., 2000), and manipulation of the reproductive tract during artificial insemination and embryo transfer (Wann and Randel, 1990; Velez et al., 1991; Scenna et al., 2004b).

Artificial insemination is a management tool utilized by the majority of cattle producers in animal agriculture. It is a process that allows an individual to maximize the genetic potential of an animal by choosing from several hundred sires. Studies have shown that manipulation of the reproductive tract during artificial insemination or embryonic transfer causes an increase in $PGF_{2\alpha}$ concentrations (Wann and Randel, 1990; Scenna et al., 2004b). Manipulation of the reproductive tract, and insertion of the artificial insemination gun into the cow's reproductive tract, results in an inflammatory response. Flunixin meglumine is a known inhibitor of certain enzymes involved in inflammatory responses (Odensvik et al., 1989). The inhibition of these enzymes causes a block of 15-ketodihydro-$PGF_{2\alpha}$, the main metabolite of $PGF_{2\alpha}$ (Odensvik et al., 1995;

Konigsson et al., 2003). Administration of flunixin meglumine at the time of embryo transfer has been shown to increase pregnancy rates in cows (Scenna et al., 2004b). The act of transferring an embryo in a cow involves all actions involved in artificial insemination. The administration of flunixin meglumine following artificial insemination, and its effects on pregnancy rates, will be addressed by this thesis.

The specific objective of the continued work in this field is to discover more efficient means of establishing and maintaining pregnancy in dairy and beef cattle; therefore, lowering the cost of production. The specific aim of this study is to increase reproductive performance by decreasing embryonic mortality through the use of prostaglandin inhibitors. It is hypothesized that administration of a prostaglandin inhibitor will improve reproductive efficiency in lactating dairy cattle due to the stress of high milk production and factors associated with high milk production. This task will be attempted by administering flunixin meglumine at day 5 following artificial insemination. A decrease in embryonic mortality will increase pregnancy rates, thus allowing for a better reproductive efficiency. A higher reproductive efficiency will decrease the number of days open of an individual animal, and save the average dairy producer approximately $60,000 per year. The savings of the average producer calculates into millions of dollars for animal agriculture in the nation. A lower production cost allows for a better and more consistent product for the public.

2. Literature Review

2.1 Reproductive Inefficiency in Dairy Cattle

The dairy industry has changed dramatically in the past decades. Milk production per cow has increased as a result of better management, nutrition, and genetic selection. Dairy farms in the United States have grown into larger operations. Nearly 30% of the cows in the United States are on farms with 500 cows or more (reviewed by Lucy, 2001). A shift towards more productive cows has been associated with poorer reproductive performance (Pryce et al., 1999). Cows with the highest milk production tend to have the highest incidence of infertility, but other factors may be affecting reproduction (Butler, 2000).

The need for more milk production per cow can be explained by an increase in demand for dairy products. A growing population has demanded more dairy products and forced the producer towards more production per cow. This growing trend toward more production per cow has led to a smaller number of dairy cows meeting a high demand. Animals are managed so that pregnancy must be established during a highly stressful time for the animal. Animals are usually inseminated around day 60-75 of lactation, the time when peak production is reached and animals are beginning to recover from a loss of body weight (reviewed by Lucy, 2001). Lifetime milk production of an individual cow depends on whether the animal can become pregnant and return for another lactation. Pregnancy drives the lifetime productivity of a dairy animal because parturition results in lactation. Declining fertility in the dairy industry is probably a combination of physiological and management factors that have an additive effect on reproductive efficiency (reviewed by Lucy, 2001).

The decline in reproductive efficiency has been documented in several publications. Butler performed a review (1998) and reported a decline in first-service conception rates from approximately 65% in 1951 to 40% in 1996 on farms studied in New York. Casida et al. (1961) reported that cows artificially inseminated at observed estrus in the 1950s typically reported conception rates of 55%. Dransfield et al. (1998) performed a review of the literature to report conception rates of 45% for inseminations at observed estrus. The fact that conception rates have decreased by such a significant percentage indicates that a problem may exist with establishment of pregnancy in dairy cattle in the United States.

Dairy cattle in the United States are not the only cattle experiencing a decline in reproductive efficiency. Similar decreases have been reported for first-service conception rates in Ireland (Roche et al., 2000), Australia (Macmillan et al., 1996) and the United Kingdom (Royal et al., 2000). A decline in European countries may be due to a move toward selection for high milk yield United States genetics, without changing local management and feeding practices (reviewed by Lucy, 2001). Nevertheless, poor reproductive efficiency is a worldwide problem affecting the dairy industry (reviewed by Lucy, 2001).

The extent of reproductive decline is poorly documented because many dairy producers do not maintain adequate reproductive records. The Dairy Herd Improvement Association (DHIA) is an association that collects herd records for milk production, reproduction, and nutrition. An individual producer must willfully participate and pay for the services that DHIA offers. Records on every herd participating in the program are kept in Raleigh, NC, in the Dairy records Management Systems (DRMS). Silvia et al.

(1998) reviewed the DHIA records for dairy producers in Kentucky. Silvia reported an increase in services per conception of 1.62 to 2.91 from 1972 to 1996. Lucy (2001) observed increases in services per conception, days open, and days to first insemination in 143 dairy herds continuously enrolled in the DHIA record system from 1970 to 1999. Lucy (2001) reported that the greatest decline in reproductive efficiency of these herds occurred after the mid-1980s. The data obtained are not necessarily a good representation of the US dairy herds because producers must voluntarily enroll in the dairy testing program. Nevertheless, data indicate that a reproductive efficiency problem has developed over the past decades.

The decline of reproductive efficiency has been established through DHIA records and personal communication between producers and veterinarians nationwide. The cause of this decline is usually associated with increased milk production and a combination of other management factors. The 2001 USDA National Agricultural Statistics service showed that nearly 30% of all dairy cows in the United States were found on farms of 500 or more cows. The number of herds with fewer than 200 cows has decreased, while the number of farms with greater than 200 cows has increased. These statistics indicate that the current trend is a consolidation of the dairy industry into large farms with more cows (reviewed by Lucy, 2001).

In order for a producer to expand their lactating herd, the producer must purchase or maintain replacement animals. Stahl et al. (1999) reported that pregnant heifers or first-lactation cows are often used to expand a dairy. First-lactation cows have a lower energy balance because they consume less, and they require energy for lactation and growth. Lower energy balance in first lactation cows has been associated with delayed

intervals to first ovulation (Lucy et al., 1992). This delayed ovulation in animals with lower energy balance may explain why the first lactation has been identified as a risk for conception failure (Loeffler et al., 1999). Many herds have a large percentage of first-lactation cows because culling practices usually lead to the removal of older cows. Reduced reproductive efficiency in first-lactation cows may be the reason producers have seen an overall reduction in reproduction during this expansion era.

Inbreeding is another factor that may influence reproductive performance in an expanding dairy industry. The breeding of dairy cows in the past few decades has moved towards maximizing milk production. The use of artificial insemination allows a producer to use a desired bull many times. Artificial insemination can increase the occurrence of inbreeding if a producer is not careful with their matings. Hermas et al. (1987) concluded that a 1% increase of inbreeding led to a 0.17 increase in services per conception, a 2-day increase in days open, and a 3.3% decrease in conception rate. If these estimates are correct, inbreeding could be the sole cause of an increase of 7 days open, 0.6 increase in services per conception, and a 12% decrease in conception rate since 1980 (Hansen, 2000). Present levels of inbreeding are approximately 5% (Hansen, 2000). Hansen (2000) predicts that levels of inbreeding will reach 10% by the year 2020. Inbreeding is an aspect of the growing dairy industry that must be monitored, but it is probably not the only reason for decreased reproductive efficiency.

Numerous factors could have contributed to the decline in reproductive performance in the dairy industry over the past two decades. Unfortunately, few research programs in the United States concentrate on reproduction in the dairy cow. This fact leads to many assumptions about why a decrease in reproductive performance has been

experienced. Further research is needed to identify why reproduction has declined, and what can be done to improve the reproductive efficiency of the dairy cow.

2.2 Embryonic Development

2.2.1 The sperm and oocyte fusion

The development of an embryo occurs over several different stages: fertilization, 1-cell stage, 2-cell stage, 4-cell stage, 8-cell stage, 16-cell stage, morula, blastocyst, expanded blastocyst, hatching blastocyst, maternal recognition of pregnancy, and placental attachment (reviewed by Bazer et al., 1993). One must understand each of these stages in order to identify problems that may cause embryonic mortality.

Fertilization requires three steps to take place: sperm migration through the reproductive tract, sperm attachment and migration through the zona-pellucida, and fusion of sperm and oocyte membranes. Sperm first travels through the vagina, cervix, and uterus, where it begins preparation for fertilization. Sperm preparation, known as sperm capacitation, begins in the uterus and is completed in the isthmus of the oviduct (reviewed by Bazer et al., 1993).

A sperm cell basically consists of the head, mid-piece, and tail. The head is the area in which the chromatin is carried. Chromatin comprises the DNA of a haploid nucleus (reviewed by Bazer et al., 1993). A caplike structure called the acrosome covers the anterior end of the head. This acrosome cap carries enzymes that will be used to penetrate the membranes of the zona pellucida (Tulsiani et al., 1998). Energy for the motility of the sperm cell is generated in the mid-piece. The purpose of the sperm tail is providing a means of travel to the oocyte, and penetrating the oocyte. The oocyte consists of several layers that the sperm must penetrate: the cumulus oophorus, zona

pellucida, perivitelline space, and vitelline membrane. Physical properties of the sperm and oocyte allow each of them to successfully merge and complete the act of fertilization (reviewed by Bazer et al., 1993).

All physical aspects of the sperm and oocyte contribute to the penetration and fusion of genomic properties of each. The presence of a sperm triggers events that change the membrane potential of the oocyte. Binding of the sperm to the zona pellucida is a receptor mediated event (Tulsiani et al., 1998). The specific sperm receptor has been identified as the ZP3 glycoprotein (Bleil and Wasserman, 1980). Only an intact acrosome can bind to this receptor. Once the sperm binds to the zona pellucida, the sperm binding protein stimulates events that lead to the fusion of the plasma membrane and the acrosomal membrane. The acrosomal membrane of a sperm cell contains a lysosome that forms around the anterior portion of the nucleus. This lysosome contains enzymes that are responsible for digesting the zona pellucida so entry of the sperm may occur. The tail movement of the sperm is also used for entry into the zona pellucida. Once the sperm has penetrated the zona pellucida, it moves into the vitelline space and contacts the vitelline membrane. Following fusion with the oocyte, the sperm nuclear envelope breaks down. Chromatin from the sperm and oocyte migrate to the center of the ovum, and the chromosomes of each intertwine. These two haploid cells now produce a diploid cell which contains the genomic make up of the offspring. These steps conclude the act of fertilization and lead to the development of a 1-cell embryo (reviewed by Bazer et al., 1993).

The development of the embryo continues at the one-cell stage and is taken through several stages until a fetus is formed. The joining of the two haploid cells, or

chromatin, will come together to form a diploid cell. It is at this stage that the embryo is considered to be one-cell. The one-cell embryo will rapidly divide into 2, 4, 8, and 16 cells through a process called mitosis. The dividing of the cells is referred to as cleavage division. Mitotic division results in daughter cells, in which each cell receives the full assortment of chromosomes. Cells that are undergoing cleavage are known as blastomeres. Following the formation of a 16-cell embryo, the blastomeres begin to form tight junctions with one another. These blastomeres flatten on each other to form a rounded embryo and an internal cell mass. Surface microvilli are also formed and become asymmetrically positioned in a process called polarization (reviewed by Bazer et al., 1993).

The combined processes of flattening and polarization are referred to as compaction (reviewed by Bazer et al., 1993). The formation of tight junctions and compaction result in the separation of cells. One population of cells produces the inner cell mass and gives rise to the embryo and fetus. The other cells produce the trophectoderm. The trophectoderm acquires the ability to transport nutrients and water from the uterine environment to the blastocoelic cavity. The continued accumulation of fluid due to compaction causes the blastocyst to expand, forming an expanded blastocyst. Eventually a crack develops in the zona pellucida, and the blastocyst is released, forming a hatching blastocyst. The steps by which the embryo forms all occur inside the zona pellucida, but the embryo continuously moves down the reproductive tract while developing inside (reviewed by Bazer et al., 1993).

The portion of the reproductive tract in which the embryo travels to the uterus is called the oviduct. The oviduct consists of four distinct regions: infundibulum, ampulla,

ampullary-isthmic junction, and isthmus (reviewed by Ellington, 1991). An oocyte is released by the ovulatory follicle into the infundibulum and transported to the ampulla. The ampulla is the area in which the oocyte is matured and prepared for sperm binding. Sperm are stored in the isthmus where they undergo capacitation and are prepared to fertilize the oocyte. The sperm and oocyte meet at the ampullary-isthmic junction and are bound together. A fertilized embryo will remain in the ampullary-isthmic junction for 2.5-3 days. Early embryos are unable to cleave and develop in the uterus; therefore, they must remain in the oviduct until approximately 72 hours post ovulation. Once the embryo has reached the 16-cell stage, it travels into the uterus, where it remains for the duration of gestation (reviewed by Ellington, 1991). The uterus consists of the uterine body and two horn-like structures on either side. The horns each have an ovary at the tip, which will provide adequate hormones for maintaining pregnancy (reviewed by Bazer et al., 1993).

Hatching of the blastocyst occurs around day 9-11 of development. The shedding of the zona pellucida is coincided with an accelerated growth of the blastocyst. This rapid growth involves the enclosure of the blastocoele (inner cell mass) to form a bilaminar blastocyst. The bilaminar blastocyst elongates to allow for attachment in the uterus later. The extraembryonic endoderm cells involved in the closure develop the equivalent to the yolk sac. This membrane will be involved in supplying nutrients to the early embryo. Day 13 in the bovine species exhibits a logarithmic elongation of the blastocyst. The corpus luteum (CL) that is responsible for pregnancy maintenance is located on the ovary ipsilateral to the uterine horn containing the embryo. Generally, the elongated blastocyst will develop in the horn that has an ovary with a CL. The embryo

will develop in the uterine horn until later elongation into the uterine body (reviewed by Bazer et al., 1993).

2.2.2 Maternal recognition of pregnancy

Once the embryo is elongated and residing in the uterine horn, other changes must take place. Certain processes must take place in order for the mother's body to recognize that she is pregnant. This process is known as maternal recognition of pregnancy. The conceptus must release certain proteins in order for pregnancy recognition to take place. Bovine interferon-tau (IFN-τ) is the protein responsible for the maintenance of pregnancy (Bowen and Burghardt, 2000). Production of $PGF_{2\alpha}$ begins towards the end of a natural estrous cycle by the endometrium. This release of $PGF_{2\alpha}$ stimulates the release of OT by luteal cells, which enhances the release of $PGF_{2\alpha}$ by the endometrium (Fuchs, 1987). Endometrial $PGF_{2\alpha}$ excretion and continued OT release by the corpus luteum (CL) ensure a pulsatile release of $PGF_{2\alpha}$. This pulsatile $PGF_{2\alpha}$ lyses the CL; therefore, restarting the estrous cycle or causing embryonic mortality (Bowen and Burghardt, 2000). Interferon-tau must be released by the conceptus in order to prevent OT from causing luteal death (Bowen and Burghardt, 2000).

Bovine conceptus secretory hormones will enter the uterus about day 15 and inhibit uterine production of $PGF_{2\alpha}$. The endometrium of pregnant cows does not respond to estradiol- and OT-induced uterine production of $PGF_{2\alpha}$, and has very low estrogen and oxytocin receptors on days 14-21, as opposed to cycling nonpregnant cows (reviewed by Bazer et al., 1993). Prostaglandin $F_{2\alpha}$ is the luteolytic agent in cattle, and is primarily secreted from the surface of the epithelium of the uterus. The epithelium of the uterus is a glandular surface responsible for the release of regulatory proteins. Oxytocin

is responsible for the pulsatile release of $PGF_{2\alpha}$, resulting in the lysing of the CL. Oxytocin stimulates the release of $PGF_{2\alpha}$ from the endometrium, and uterine oxytocin receptors (OTR) to determine the sensitivity of the endometrium to OT stimulation. In order for pregnancy to be maintained, the pulsatile action of the $PGF_{2\alpha}$ must be eliminated. Interferon-tau is produced by the trophoblast of the conceptus (Thatcher et al., 1997). The secretion of this protein decreases the OTRs and therefore decreases the sensitivity of the endometrium to OT stimulation (Okuda et al., 2002). The exact mechanism of how IFN-τ operates is not completely understood. One mechanism theorized is that IFN-τ operates by reducing estradiol receptor number, therefore reducing estrogen-induced increase in OTR numbers. Studies have indicated that IFN-τ also decreases the amount of cyclooxygenase-2 (COX-2), which is a precursor to the production of $PGF_{2\alpha}$. This mechanism of decreasing COX-2 is believed to be independent of changes in OTR (Xiao et al., 1999).

Although IFN-τ reduces OTRs in the endometrium, this is probably not the sole effect the embryo has on $PGF_{2\alpha}$ suppression. Linoleic acid has been identified as inhibiting $PGF_{2\alpha}$ synthesis in cows (Thatcher et al., 1994). This inhibitor is released by the endometrium of pregnant cows. Linolenic acid competes with arachidonic acid for the active site on the cyclooxygenase enzyme. The cyclooxygenase enzyme is the key rate-limiting enzyme responsible for the conversion of arachidonic acid to precursors of $PGF_{2\alpha}$ production (Xiao et al., 1999). Interferon-tau can suppress the detrimental effects of $PGF_{2\alpha}$ by acting on the oxytocin receptor gene. The oxytocin receptor gene expression is induced by estrogen. Studies suggest that IFN-τ affects oxytocin receptors through inhibitory actions on the estrogen receptors. Type 1 interferons, such as IFN-τ, activate

protein kinase C (Thatcher et al., 1997). Protein kinase C reduces concentrations of estrogen receptors by increasing the turnover of estrogen receptor mRNA. Bazer et al. (1997) suggests that binding of type 1 interferon to its receptor activates the latent kinases, JAK1, and tyk2, which phosphorylate tyrosine residues of signal transducers and activators of transcription 1 (STAT1), STAT1A, and STAT2. The activation of kinases JAK1 and tyk2 leads to a cascade of mechanisms that activate the negative transcription factor IRF-2. Bazer's studies suggest IFN-τ suppresses the estrogen receptor directly by binding to an IFN-τ responsive element in the estrogen receptor gene. The exact mechanism of how IFN-τ down regulates the negative effects of OT and its receptors is not fully understood (Demmers et al., 2001).

The uterus must undergo critical changes in order for the embryo to survive. The exact changes that are necessary are not fully understood. The presence of progesterone, produced by the CL, stimulates the glandular epithelium of the uterus. This epithelium is responsible for the secretion of proteins that become essential for conceptus development. Exact proteins secreted at the time of conceptus entrance into the uterus have not been identified in the bovine. Human, pigs, and rats have been studied extensively, but the regulatory proteins are mainly species specific. It is known that the growth factor, insulin-like growth factor II (IGF-II), produced by the uterine tissue and the embryo are essential for embryonic survival (reviewed by Bazer et al., 1993). Though not much is known about the precise secretions needed for conceptus survival in the bovine uterus, certain known proteins are essential.

Changes in protein concentrations and physiological changes from the embryo and the uterus are needed for embryonic attachment. The bovine trophoblast will first

develop finger-like villi (papillae) that will project into the lumen of the uterine glands. The papillae will provide a temporary attachment. A loss of trophoblastic microvilli allows for close contact with the uterine epithelial microvilli. The uterine epithelium presses into the trophoblastic surface, interlocking with the cytoplasmic projections on the trophoblastic surface. This close surface contact between the epithelium of the uterus and the trophoblast of the conceptus allows a redevelopment of trophoblastic microvilli, and a more complex attachment (reviewed by Bazer et al., 1993).

2.3 Causes of Embryonic Mortality

Reproductive wastage is a major cause of production loss in the beef and dairy industry. Fertilization failure and embryonic mortality are suggested to be the main causes of reproductive wastage (reviewed by Ayalon, 1978). Several factors are known to contribute to early embryonic mortality. Nutrition, genetic make-up, heat stress, and management techniques are the main factors that have been examined through research. Early embryonic mortality is referred to as the losses experienced between fertilization and day 42 of gestation (Dunne et al., 2000). Nutrition is thought to be a major factor contributing to reproductive failure in dairy animals.

Poor nutrition has always been directly associated with poor reproductive performance in dairy cows (Butler, 2000). All dairy animals will experience a negative energy balance following parturition (O'Callaghan and Boland, 1999). A negative energy balance occurs when the animal is unable to consume enough nutrients to account for the milk produced. In order to meet the nutrient demands of high milk production following parturition, the animal will mobilize energy from its body reserves. This process causes a loss of weight for the individual early in lactation. The association

between negative energy balance and weight loss leads to a reduction in body condition score. Body condition score (BCS) is a tool utilized by producers to evaluate the amount of reserves possessed by an animal. The dairy industry uses a scale of 1-5. A one score indicates a severe loss of reserve fat, usually associated with starvation. A two score is more commonly seen in the early lactation animals. A score of five is excessive fat reserves over the entire body; usually no bones are distinctly visible. A negative energy balance has been shown to have a direct association with BCS (Snijders et al., 2001). Cows that experience weight loss early postpartum will exhibit a low BCS, and experience a negative energy balance comparable to the BCS (Snijders et al., 2001). A low BCS has been shown to have a direct association with poor reproductive performance of dairy animals (Snijders et al., 2001).

Nutritional factors such as BCS and overall intake have been shown to have an effect on reproductive performance. Severe body condition losses have been associated with a lower conception rate at first service (Butler and Smith, 1989). This information indicates a link between the amount of body reserves and the chance of establishing pregnancy. High nutritional intake has also shown negative effects on the developmental capacity of embryos (O'Callaghan et al., 2000). Animals grouped in high and low intake groups were compared for reproductive performance. The study indicated that animals experiencing high intake had lower conception rates on the first service (O'Callaghan et al., 2000). High intake is needed for animals to compensate for high milk production, and the loss of body reserves in early lactation. The mechanism of how high intake affects reproduction has been studied, but is not fully understood.

The relationship between high intake and poor reproduction may be more associated with the oocyte than the embryo. Studies revealed that high dietary intake and high metabolic load are detrimental to oocyte development and the establishment of pregnancy (O'Callaghan et al., 2000). Supplementation of dietary fats through ration balancing has shown to slightly improve the developmental competence of the oocyte. Dietary fat supplementation is known to increase the size of preovulatory follicles (Lucy et al., 1993), increase the number of follicles (Wehrman et al., 1991), and increase the growth rate of dominant follicles (Oldick et al., 1997). Studies have also shown that dietary fat supplementation increases the longevity of the corpus luteum (Williams, 1989) and increase production of progesterone (Hightshoe et al., 1991).

The increase of dietary fat in an animal's ration provides a direct increase in conception rate (Staples et al., 1998). The use of dietary fat to increase reproductive performance is not without negative attributes. Increasing fat content of the diet can cause a dramatic weight gain, and result in metabolic problems later in lactation. The reproductive parameters are increased, but only minimally enough to see significant differences statically. The slight differences seen reproductively usually do not outweigh the metabolic problems experienced in later lactation. The method of increasing dietary fat is used, but used very carefully. The need for dietary changes to increase reproductive performance is mainly due to high milk production in early lactation. The genetic make-up of an animal is a contributing factor to high milk production.

High milk production is a genetic aspect that is examined by every dairy breeder in the industry. Increased production costs have forced producers to obtain as much milk from each animal as possible. Ideally this allows a more efficient operation and a lower

cost overall. High milk yield has long been associated with poor reproductive performance. Milk yield genetics are determined by examining the performance of the animal's mother, and her father's daughters. Performance evaluation calculates into a number known as genetic merit. Genetic merit predicts the productive performance of an animal based on her mother and siblings' milk performance. Studies have examined the effects of milk genetic merit on reproductive performance (Snijders et al., 1999; O'Callaghan et al., 2000; Snijders et al., 2001).

Reproductive performance comparisons of high and medium genetic merit animals have shown that high genetic merit animals have poorer reproductive performance (Snijders et al., 1999; Snijders et al., 2001). High genetic merit (HGM) cows are animals that are bred to produce the maximum amount of milk. Medium genetic merit (MGM) cows are bred to produce an average amount of milk compared over the entire breed. Studies examining nutritional values of HGM and MGM cows were coupled with examining reproductive parameters (O'Callaghan et al., 2000). Several differences have been seen between HGM and MGM cows that indicate genetics play a major role in reproductive performance by influencing milk production and body condition (Snijders et al., 1999; Snijders et al., 2001). Snijders et al. (1999) collected oocytes form high and medium genetic merit cows. The number of oocytes collected did not differ between the two groups, but percent cleavage rate and number of blastocysts formed were significantly lower for high genetic merit cows (Snijders et al., 1999). High genetic merit cows also had a lower body condition score at the time of calving (Snijders et al., 1999). This data indicates that BCS may have an impact on oocyte quality. In a later study Snijders and coworkers (2001) looked closer at the relationship of BCS and

reproductive performance. HGM cows exhibited a lower body condition score during early lactation than MGM cows (Snijders et al., 2001). A lower body condition score indicates a greater loss of body fat and decreased nutrients for oocyte development. Glucose and IGF-1 binding proteins were significantly lower for HGM cows at 2-10 weeks postpartum (Snijders et al., 2001). Glucose is needed for proper ovary function, and a lack of excessive glucose will result in poor ovulatory function (Snijders et al., 2001). HGM cows also exhibited a higher dietary intake than MGM cows (Snijders et al., 2001). Studies performed by O'Callaghan and coworkers (2000) indicated that animals with a higher intake experienced a lower reproductive performance.

The comparison of HGM and MGM cow's nutrition and reproductive performance indicated that genetic merit is a major contributing factor in reproductive failure. Snijders et al. (2001) indicated that the glucose and IGF-1 were lower for HGM cows at 2-10 weeks postpartum. HGM and MGM cows were bred during this same study and compared. The plasma concentrations of glucose did not differ between HGM and MGM cows that established pregnancy on the first service (Snijders et al., 2001). This information indicates that genetic merit is a greater negative factor than nutrition for reproductive problems (Snijders et al., 2001). Nutrition would have been a greater problem, according to this study, if the plasma concentrations of glucose had been lower. Snijders and coworkers' (2001) study also indicated that HGM cows had a lower conception rate on first and second services when compared to MGM cows. There were also more HGM cows not pregnant at the end of the breeding season than MGM cows (Snijders et al., 2001). Snijders and coworkers (2001) found no differences in the number of services per conception for HGM cows, but more services were needed for

HGM cows as a group. These studies performed by Snijders et al. (1999, 2001) indicate that genetic merit plays a greater role in reproductive failure than once thought.

Heat stress is an environmental factor that is known to negatively influence reproductive performance (reviewed by De Rensis and Scaramuzzi, 2003). De Rensis and Scaramuzzi (2003) conducted a review of the research that indicates heat stress can have a negative effect on the oocyte, ovary, and early embryonic development. All of these negative factors lead to a decrease in reproductive performance, pregnancy rates, and an increase in days open. Decreases in pregnancy rates due to heat stress have always plagued the animal industry and cost producers money.

Early studies in reproductive performance indicated that heat stress was causing a decrease in pregnancy of dairy cows. Stott and Williams (1962) found that pregnancy rates were decreased by the summer heat of Arizona. A pregnancy rate of 61.6% was reported in May, and the percentage fell throughout June and July. The lowest conception rate (17.1%) occurred in August. September recorded a 58.2% conception rate, which indicates that the heat had a dramatic effect on pregnancy rates. However, heat was not the sole factor on the decreased conception rate during August. Humidity also seems to affect pregnancy, as the percentage of humidity was greatest during the month of August.

Investigators hypothesized that another reason conception rates were low during the summer months was lack of estrous behavior. Cows reportedly experienced long estrual intervals. Long estrual intervals were identified as cows that were found not pregnant at the 35 day pregnancy check, but did not exhibit estrus before 35 days post insemination. The long estrual intervals indicate that the heat affects the estrous cycle in

some manner (Stott and Williams, 1962). The mechanism by which heat stress affects the estrous cycle of the cow is not fully understood by investigators in this field.

Heat stress is known to negatively affect the ovary and embryo. Intensive study of the ovary during times of heat stress has provided some insight of the mechanism. Howell et al. (1994) examined the growth and function of the corpus luteum during spring and summer. Summer months resulted in heat stressed dairy cows due to high ambient temperatures and relative humidity. Spring months allowed animals to remain at a thermoneutral temperature. Howell and coworkers reported that season did not affect the size of the corpus luteum, or the length of the luteal phase (Howell et al., 1994). It was also reported that the peak progesterone concentration was significantly lower for the animals experiencing heat stress during the summer (Howell et al., 1994). The authors concluded that hyperthermia may alter the functional characteristics of the corpus luteum cells (Howell et al., 1994).

Wilson et al. (1998) found that ovaries under the influence of heat stress had fewer follicles. Wilson's finding indicates that there is a disturbance in the follicular growth and function process. Small ovulatory follicles and large subordinate follicles were found on ovaries of heat stressed animals. Wilson et al. (1998) also found that the cows undergoing heat stress had lower concentrations of estradiol in blood serum. The low levels of estradiol may contribute to the small ovulatory follicle size. Estradiol also plays an important role in the luteolysis process in the cow. Estradiol is released from the preovulatory follicle and increases blood concentrations. Increased concentrations of estradiol will up-regulate the synthesis of the endometrial oxytocin receptor and activate enzymes associated with $PGF_{2\alpha}$ production. Oxytocin and estradiol bind to their

respective uterine receptors to initiate the pulsatile release of $PGF_{2\alpha}$. The pulsatile release of $PGF_{2\alpha}$ leads to the lysing of the corpus luteum and another estrous cycle. The lack of estradiol delays the luteolysis process and allows the corpus luteum to remain intact (Wilson et al., 1998).

The failure of luteolysis is most likely why Stott and Williams (1962) observed a longer estrual interval during hot summer months. Wilson et al. (1998) concluded that heat stress inhibited follicular growth during the preovulatory period. Inhibition, as seen by a decrease in estradiol level, may cause small dominant follicles and excessive follicular waves each estrous cycle. The conclusions were interpreted as an abnormal ovarian function due to heat stress (Wilson et al., 1998). The effects of heat stress on ovarian function have been linked to a negative effect on embryonic development.

Poor follicular function leads to an incompetent oocyte. Al-Katanani et al. (2002) collected oocytes from warm and cool season ovaries. These oocytes were cultured and fertilized in vitro. Oocytes and cleaved embryos from warm season collections had lower blastocyst development than cool season collections (Al-Katanani et al., 2002). There was no reduction of cleavage rate of oocytes from warm season oocytes. This suggests that heat stress does not affect oocyte maturation, sperm binding, and fertility. Zygotes from warm season oocytes had a reduced competence to develop to blastocyst. These findings suggest that the effects of heat stressed oocytes greatly affect early embryonic development (Al-Katanani et al., 2002). Edwards and Hansen (1997) reported a reduced 2 and 4 cell embryo development to blastocyst due to heat stress. The development of compacted morulae was not affected by heat stress. It was concluded that heat stress has the greatest effect on early embryonic development. The embryo seems to develop a

resistance to heat stress following day 3 of development, and becomes less susceptible as it develops (Edwards and Hansen, 1997). The fact that heat stress has a negative effect on early embryonic development leads to questions of how to prevent these effects.

Early studies indicated that embryonic loss due to heat stress occurred before day 35 of gestation (Stott and Williams, 1962). It was believed that cooling animals under heat stress prior to insemination would help establish pregnancy. Al-Katanani et al. (2002) cooled animals to a temperature of 39.2 ± .1 °C for 42 days using fans and foggers before predicted ovulation. Reports stated that the oocyte quality had not improved following this cooling (Al-Katanani et al., 2002). Conclusions stated that cooling was not effective for several possible reasons: 1) the degree of cooling was not sufficient to prevent adverse effects of heat stress; 2) heat stress damaged the oocyte earlier in development than cooling was initiated, or 3) seasonal effects represent factors other than heat stress (Al-Katanani et al., 2002). Conclusions from various studies indicate that heat stress affects the oocyte early in development. It has been reported that primary follicles begin growth about 84 to 85 days before ovulation (Picton et al., 1998; McNatty et al., 1999). Adverse effects of heat stress on the oocyte potentially begin during the first steps of antral follicle development. These general conclusions correspond with poor fertility in early cool seasons. This may be due to oocytes experiencing heat stress during summer heat and being released as damaged oocytes in early cool seasons.

Studies are currently being conducted to further understand the effects of heat stress on early oocyte development. Heat stress is known to decrease reproductive performance of lactating dairy cows. The mechanism by which heat stress affects the oocyte, ovary, and early embryo is not fully understood. Heat stress is one of several

factors that cause early embryonic mortality. Nutrition, genetic merit, milk production, and heat stress all can have negative effects on reproductive performance, and all must be monitored in order to gain better reproductive performance.

2.4 $PGF_{2\alpha}$ Association With Early Embryonic Loss

Prostaglandins are biochemical compounds derived from arachidonic acid, a polysaturated fatty acid. Prostaglandins are involved in several physiological processes such as inflammation, production of pain and fever, control of blood vessel tone and arterial blood pressure, luteolysis, and blood clotting (Nett, 1976; Seibert, 1994; Blatteis and Sehic, 1997). The possible effects of prostaglandin on the early embryo are the focus of the following review.

Studies using "short cycle" cows indicate that a high level of $PGF_{2\alpha}$ in the uterus may also cause early embryonic loss. Short cycle animals are those that experience an estrous cycle less than or equal to 14 days (Schrick et al., 1993). A normal cycling animal will have an estrous cycle of approximately 21 days. Short cycle animals experience a premature release of $PGF_{2\alpha}$ by a postpartum uterus after first exposure to progesterone (Cooper et al., 1991). This premature release of $PGF_{2\alpha}$ causes an early lysing of the corpus luteum. A short cycle is often associated with the first estrus following parturition (Ramirez-Godinez et al., 1982). Conception rates of 28% were reported on cows experiencing a short cycle (Butcher et al., 1992). A 56% conception rate was reported on cows with a normal estrous cycle (Butcher et al., 1992). These significantly different conception rates indicate that short cycle cows have an improper uterine environment for the oocyte or embryo, on or before day 7 of embryonic development (Butcher et al., 1992).

Schrick et al. (1993) studied the quality of embryos from short cycle cows and normal cycling cows. Schrick reported that the quality of embryos at day 6 of development did not differ in short cycle cows when compared to normal cycling cows. Pregnancy rates did not differ between short and normal cycling embryos when placed in a normal cycling uterine environment. This information indicates that the lower conception rates seen in short cycling cows are taking place around day 7 or shortly after. Concentrations of $PGF_{2\alpha}$ from uterine flushings were also recorded by Schrick. Schrick reported that $PGF_{2\alpha}$ concentrations were much higher in short cycling cows when compared to normal cycling cows. High uterine concentrations of $PGF_{2\alpha}$ indicate that the uterine environment in short cycle cows may be too poor for embryonic development. The findings by Schrick et al. (1993) indicate that the embryo is not affected prior to day 6 of embryonic development in a high $PGF_{2\alpha}$ concentration environment.

The decreased pregnancy rates in short cycling and normal cycling cows have been suggested to be the result of a regressing corpus luteum. A regressing corpus luteum will produce and direct oxytocin towards the uterus for continued $PGF_{2\alpha}$ production. In vitro treatment of 8-cell rat embryos with $PGF_{2\alpha}$ prevented development to the morula stage (Breuel et al., 1993). The treatment of rabbit embryos with $PGF_{2\alpha}$ prevented the formation of expanded or hatched blastocysts (Maurer and Beier, 1976). These studies suggest that embryonic development may be compromised by a direct action of $PGF_{2\alpha}$ or an embryo toxic factor from the regressing corpus luteum. Seals et al. (1998) studied in vivo embryonic development in progestogen supplemented cattle with a removed corpus luteum. Animals involved in the study were bred and pregnancy checked at 30 days post artificial insemination (AI). A control group was simply bred AI

and pregnancy checked. Another group was administered $PGF_{2\alpha}$ following breeding. The final group was administered $PGF_{2\alpha}$ following breeding and underwent a lutectomy. Lutectomy is a procedure in which the corpus luteum is ruptured, or removed, in vivo by applying digital pressure. Animals that underwent a lutectomy and $PGF_{2\alpha}$ administration had increased pregnancy rates compared to those that were only administered $PGF_{2\alpha}$. Seal's study suggested that a regressing corpus luteum may be needed to elevate concentrations of $PGF_{2\alpha}$ high enough to lower embryonic survival rates.

Hockett et al. (2004) studied the effects of $PGF_{2\alpha}$ administration on embryonic development in cattle. Animals were assigned a control treatment of saline or administered $PGF_{2\alpha}$. Hockett reported elevated concentrations of 13-14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ (PGFM), the metabolized form of $PGF_{2\alpha}$, in the blood of $PGF_{2\alpha}$ treated animals compared to animals that received saline. A reduction in the quality score of the day 8-recovered embryos was observed in animals treated with $PGF_{2\alpha}$. The development of the embryos between the two treatment groups was also studied. Hockett et al. reported that many of the $PGF_{2\alpha}$-treated embryos did not develop beyond the morula stage, while all of the saline treated embryos had developed to maturity corresponding to the day of development. Hockett concluded that embryos under the influence of $PGF_{2\alpha}$ during days 5-8 of development suffer detrimental effects with respect to quality and development (Hockett et al., 2004).

Scenna et al. (2004a) studied the effects of $PGF_{2\alpha}$ on pre-compacted in vitro-derived embryos and compacted in vivo-derived embryos. The study found that addition of $PGF_{2\alpha}$ to culture medium of pre-compacted embryos had a direct negative effect on the embryo's ability to develop to the blastocyst stage. The addition of $PGF_{2\alpha}$ to culture

medium of compacted in vivo-derived embryos did not affect the embryo's ability to develop to blastocyst stage, but did significantly reduce hatching rates. These results indicate that a negative effect of $PGF_{2\alpha}$ may be occurring as early as or before day 5 of development. The authors concluded that $PGF_{2\alpha}$ may be disrupting tight junctions to result in decreased blastocyst development and hatching rates (Scenna et al., 2004a).

2.5 Effects of $PGF_{2\alpha}$ Inhibitors

Nonsteroidal anti-inflammatory drugs (NSAIDs) are drugs that inhibit production of prostaglandins associated with an inflammatory response (Vane, 1971). Inflammatory responses are those associated with pain, fever, swelling, redness of tissue, and other injury related experiences (Vane and Botting, 1998). The act of manipulating the reproductive tract during rectal palpation, an act involved in artificial insemination and embryo transfer, can cause an inflammatory response and the release of $PGF_{2\alpha}$ (Wann and Randel, 1990; Velez et al., 1991). Prostaglandin $F_{2\alpha}$ exposure to an early embryo can compromise embryonic survival (Schrick et al., 1993; Breuel et al., 1993; Seals et al., 1998; Lemaster et al., 1999; Hockett et al., 2004; Scenna et al., 2004a). Prostaglandins released by an inflammatory response during artificial insemination and embryo transfer may be inhibited by NSAIDs.

Therapeutic activities of NSAIDs occur through inhibition of cyclooxygenase (COX). The enzyme COX is involved in the production cascade required for the production of prostaglandins. Research has indicated that there are at least two COX enzymes, COX-1 and COX-2. Prostaglandins that protect the stomach and kidneys from damage are produced by COX-1. Prostaglandins that contribute to the pain and swelling of inflammation are produced by COX-2. Selecting NSAIDs to inhibit COX-2 should

prevent an anti-inflammatory response without damaging the stomach or kidneys (Vane and Botting, 1998).

Several NSAIDs have been used in research in an attempt to inhibit COX-2, which should block prostaglandins. Prostaglandins are derived from arachidonic acid, which is oxygenated by COX-2. Ibuprofen lysinate, aspirin, and flunixin meglumine are all NSAIDs that have been used in research to attempt to inhibit the production of prostaglandins. The administration of flunixin meglumine has been shown to result in lower levels of $PGF_{2\alpha}$ in the blood when administered 4 times daily (Aiumlamai et al., 1990). The $PGF_{2\alpha}$ concentrations were reduced so to prevent luteolysis of the corpus luteum and prolong the estrous cycle (Aiumlamai et al., 1990). Treatment with flunixin meglumine did not alter the $PGF_{2\alpha}$ production cascade permanently. Animals that underwent flunixin meglumine treatment experienced a significant decrease of $PGF_{2\alpha}$ metabolite for 32, 20, and 16 hours following the final treatment. All animals resumed normal hormonal activity following the conclusion of the treatment end (Aiumlamai et al., 1990). The intensive treatment of animals with flunixin meglumine indicates that this NSAID has the ability to inhibit the production of $PGF_{2\alpha}$ and extend the life of the corpus luteum, which is essential for the maintenance of pregnancy. The fact that flunixin meglumine has the ability to inhibit $PGF_{2\alpha}$ production leads to the question of whether $PGF_{2\alpha}$ is released during manipulation of the reproductive tract.

Velez et al. (1991) and co-workers performed a study in which PGFM concentrations were measured following uterine manipulation. Peripheral concentrations of PGFM reflect the endogenous uterine production of $PGF_{2\alpha}$. Animals received gentle uterine manipulation for 2 minutes at a time. One group received manipulation weekly

from days 9-51 postpartum. Another group received manipulation one time between 29-35 days postpartum. Blood collections were taken from each group and PGFM concentrations were measured. Results indicated that PGFM concentrations elevated during the weekly manipulation, and elevated for the one time manipulation at day 30 postpartum on average. These results indicated that uterine manipulation does increase the uterine production of $PGF_{2\alpha}$ (Velez et al., 1991). Uterine manipulation for this study occurred over a two minute time period, which may be longer than most artificial insemination and embryo transfer manipulations.

Odensvik and coworkers (1993) performed a study in which the levels of $PGF_{2\alpha}$ were measured following non-surgical embryo transfer in cattle. Prostaglandin metabolite levels in the blood were measured to indicate $PGF_{2\alpha}$ levels. Blood was collected every 10 minutes, beginning 50 minutes before embryo transfer and continuing for about 5 hours following embryo transfer. Each transfer was classified as easy, medium or difficult based on the amount of effort required to insert the catheter in the correct location of the uterine horn. Standard embryo transfer procedure requires a rubber catheter to be inserted two-thirds of the way into the uterine horn. Most embryos are at 7 days of development when transfer is performed; therefore, they must be placed where development is occurring for a 7 day embryo. The investigators found no significant change in prostaglandin $F_{2\alpha}$ metabolite level pattern during embryo transfer. Furthermore, concentrations of PGFM did not differ between the transfer difficulty groups. The results of this study indicate that the manipulation of the reproductive tract during embryo transfer did not increase concentrations of PGFM (Odensvik et al., 1993).

Studies have been performed in humans and cattle to determine whether NSAIDs may increase reproductive performance following embryo transfer. Rubinstein and coworkers (1999) treated human patients with low-dose aspirin during in vitro fertilization procedures. The study found that the number of follicles, number of oocytes retrieved, implantation rate, and pregnancy rates were significantly higher for patients receiving low-dose aspirin treatment. These results indicate that the NSAID aspirin can increase reproductive performance and pregnancy rates by potentially inhibiting prostaglandin (Rubinstein et al., 1999).

Lemaster et al. (1999) performed a study indicating that the NSAID flunixin meglumine was effective in blocking the negative effects of $PGF_{2\alpha}$ and therefore increasing pregnancy rates. Animals were treated with oxytocin + flunixin meglumine (OT + FM), OT, or OT + lutectomy (LUT) following breeding. Lemaster et al. reported that animals administered OT + FM resulted in pregnancy rates similar to that of the controls. Pregnancy rates of OT and OT + LUT treated animals were significantly reduced compared to controls and OT + FM. This information indicates that flunixin meglumine may inhibit the detrimental effects of $PGF_{2\alpha}$ on early embryonic development (Lemaster et al., 1999).

Elli and coworkers (2001) performed study using ibuprofen lysinate, an NSAID, in bovine embryo transfer. Embryos were transferred into heifers that were classified as being acceptable to receive an embryo. Half of the animals received an injection of ibuprofen lysinate before embryo transfer; the other half received a placebo treatment. Pregnancy rates for animals receiving ibuprofen lysinate and controls were 82% and 56% respectively. A significant improvement was reported for those animals that received ibuprofen lysinate (Elli et al., 2001).

The use of flunixin meglumine has been studied in cattle in an attempt to improve reproductive performance during embryo transfer. McNaughtan et al. (2001) conducted a study in which heifers were administered FM prior to embryo transfer. Pregnancy rates were not statistically improved with administration of FM (McNaughtan et al., 2001). However the authors reported that a logistics analysis of the odds ratio indicated that heifers were 1.22 times more likely to become pregnant when administered FM prior to transfer (McNaughtan et al., 2001). Scenna et al. (2004b) performed a study in which animals were administered flunixin meglumine or saline at the time of embryo transfer. The results of the study indicated that a significant improvement in pregnancy rates occurred for those animals administered flunixin meglumine. Animals administered flunixin meglumine resulted in pregnancy rates of 65%, while animals administered saline resulted in 60%. Flunixin meglumine also significantly increased pregnancy rates for quality score 2 embryos (64.5 vs 53.5%; respectively), and morula and blastocyst stage embryos (64.5 vs 58.9%: 66.5 vs 59.5%; respectively). This administration was a single dose and resulted in improvement of pregnancy rates by potentially inhibiting the effects of $PGF_{2\alpha}$ (Scenna et. al., 2004b). These results contradict those of Aiumlamai et al. (1990) with respect to dosage. Aiumlamai et al. (1990) reported that intensive dosing (4 doses per day) was required to have positive effects with flunixin meglumine. Flunixin meglumine is preferred in cattle because it has other therapeutic uses and is readily available to the animal producer.

Embryo transfer is a procedure that involves many of the uterine manipulations involved in artificial insemination. Reports indicate that NSAIDs have a positive effect on pregnancy rates during embryo transfer in humans (Rubinstein et al., 1999) and in cattle (Elli et al., 2001; Scenna et al., 2004b). The fact that flunixin meglumine has been

reported to have positive effects is promising for the animal producer. Flunixin meglumine could be used during artificial insemination to increase pregnancy rates since uterine manipulations are similar. The key to flunixin meglumine administration is discovering the best time to administer the NSAIDS to maximize the possible positive effect. Embryos are transferred at 7 days of development, but the embryo does not exist when artificial insemination is performed. Researchers involved in this field predict that days 4-6 following artificial insemination may be the best time to administer flunixin meglumine. Studies have not been performed to test this hypothesis.

2.6 Summary of the Literature

Early embryonic death is a problem that plagues dairy producers worldwide. High milk demands have forced producers to feed highly concentrated rations, breed for high milk yield, and concentrate more animals in a centralized location. These factors have contributed to a decline in reproductive efficiency of dairy animals over the past decades. Research has been conducted in attempts to improve reproductive performance without compromising milk yield. Unfortunately, more studies are needed before acceptable reproductive levels are reached.

The presence of $PGF_{2\alpha}$ has been shown to have a negative effect on early embryonic development. Controlling $PGF_{2\alpha}$ during early embryonic development may decrease early loss. Flunixin meglumine has successfully been used to block $PGF_{2\alpha}$ synthesis in cattle. Flunixin meglumine has also been shown to increase pregnancy rates when administered immediately following embryo transfer. The use of flunixin meglumine on day five following artificial insemination may improve pregnancy rates for the dairy industry.

The objective of this study is to determine if administration of flunixin meglumine on day 5 following artificial insemination can increase reproductive efficiency in lactating dairy cattle.

3. Materials and Methods

All experimental materials were provided by Schering-Plough Animal Health Corporation (SPAHC; Union, NJ) unless otherwise noted. The experiment was conducted from September, 2002 through April, 2003 at the University of Tennessee Knoxville Experiment Station (KES) Dairy Unit. Animal/and nutritional management were performed as regular facility practices at the KES Dairy Unit. A total of 121 Holstein and Jersey cows (Table 1) were randomly assigned to receive a treatment of flunixin meglumine or saline following estrous synchronization and artificial insemination. Ultimately, 106 animals were used for final data analysis as discussed in the results section.

All animals involved in estrous synchronization and treatment assignment were at least 60 days in milk, ≥ 364 kg body weight (BW), and identified as being reproductively sound by the attending herd veterinarian. Animals underwent estrous synchronization using a two-injection protocol of Estrumate (cloprostenol, 500 μg). One injection of Estrumate (2 mL) was administered intramuscularly to initiate estrous synchronization. Animals were fitted with a radiotelemetric estrous detection system (HeatWatch Estrus Detection System, DDX, Inc., Denver, CO). Standing estrus was defined as ≥ 3 mounts of ≥ 2 sec within a 4-hour period as detected by HeatWatch, or visual confirmation of an animal standing to be mounted by a herd mate. Animals exhibiting standing estrus were artificially inseminated 12 ± 6 h following first detection of standing estrus utilizing one 0.5 mL straw of semen. Semen was used from various Holstein and Jersey bulls of proven fertility (semen obtained by University of Tennessee KES). Animals not exhibiting standing estrus following the first injection of Estrumate

Table 1. Description of animal numbers and production characteristics.

Variable	Number Animals	SAL	FM
Cows	106	51	55
1st Lactation	50	24	26
2nd Lactation	30	15	15
3+ Lactation	26	12	14
Holstein	71	34	37
Jersey	35	17	18
rBST	71	34	37
MP (kg/d)[a]		33.5	33.9
305 ME (kg)[b]		10,959	10,636
BW (kg)[c]		528	548
BCS[d]		2.8	2.9
DIM (d)[e]		131	149

[a]MP = Milk Production

[b]305 ME = 305 Mature Equivalent

[c]BW = Body weight

[d]BCS = Body Condition Score (1, thin to 5, fat)

[e]DIM = Days in Milk

received a second injection 11 days following the initial injection. Animals experiencing standing estrus following the second injection of Estrumate were inseminated as defined previously. Animals not exhibiting standing estrus following the two-injection protocol were recycled into the estrous synchronization program for no more than two additional attempts.

Treatment with flunixin meglumine or saline was randomly assigned between primiparous and multiparous cows. Treatment was administered intravenously in the vena cava (FM, 2.2 mg/kg BW; SAL, 1 mL/23 kg of BW) 5 days ± 12 h following artificial insemination. Body weight was determined using certified scales (Tru-Test SR2000, Tru-Test Limited, Auckland, New Zealand) immediately prior to treatment administration. Milk from FM treated cows was discarded for 36 hours (3 milkings) following treatment as directed by the FDA.

Pregnancy was determined at 28-35 days post artificial insemination by the presence of an embryo with a heartbeat by transrectal ultrasonography (SIUI, CTS-200V; Shantou, Guangdong, China) equipped with a 5.0 MHz linear transducer. The ultrasound technician was blinded with respect to treatment. Any animal not pregnant following pregnancy determination was not re-enrolled in the experiment.

Additional variables of interest (Table 1) included breed, lactation number (LN), days in milk (DIM), actual milk production (MP), 305 mature equivalent (ME), BW, body condition score (BCS), and if the animal was under the influence of recombinant bovine somatotropin (rBST; POSILAC®, Monsanto Company, St. Louis, MO). Days in milk was determined on the day of treatment with FM or SAL. Data for 305 ME were calculated by PC-DART (Dairy Records Processing Center, Raleigh, NC). Milk

production was calculated by averaging the daily milk weights (obtained by HerdMaster® Management System, Alfa-Laval Agri Inc., Kansas City, MO) of 3 days prior, the day of, and 3 days following treatment. Lactation number was divided into three groups of 1, 2, and 3+. Days in milk were grouped as high (> 150 d), medium (75-150 d), and low (< 75 d). Daily milk production was grouped as high (> 36 kg), medium (36-27 kg), and low (< 27 kg). Groups of 305 ME were established as high (> 11,818kg), medium (10,000-11,818 kg), and low (< 10,000 kg). Body weight was grouped as high (> 591 kg), medium (454-591 kg), and low (< 454 kg). Recombinant BST administration was determined as whether or not a cow was under the influence of rBST at the time of artificial insemination. Body condition score was assigned to an individual at the time of pregnancy determination by one of two qualified technicians.

3.1 Statistical Analysis

Differences in pregnancy rates were analyzed by using Mixed Models of SAS (SAS, 2000) in a completely randomized design. All cows were equally assigned to treatment with regards to breed, lactation number, rBST, BCS, BW, MP, DIM, and 305 ME and all interactions as fixed effects were used to compare differences among treatments. Animal (treatment x lactation x breed) was included as a random effect. Differences in individual least squares means were evaluated using LSD. Also, pregnancy rates were validated using Fisher's Exact Test (SAS, 2000). This test requires the fewest assumptions when dealing with percentage data. A value of $P < 0.05$ was considered statistically different.

4. Results

A total of 24 estrous synchronization repetitions were performed during the course of the study. The 24 replicates averaged 8.3 animals for a total of 199 animals entering the estrus synchronization protocol. Some of the 199 animals synchronized were synchronized in more than one replicate due to no response during the previous synchronization attempt. Seventy-nine animals (65.3%) responded with estrus following the first injection of Estrumate, while 42 animals (34.7%) responded following the second injection. A total of 121 animals responded to synchronization and were artificially inseminated.

Of 121 cows utilized for the study, two animals were removed from the study prior to pregnancy confirmation due to ambulatory health problems. Six animals were artificially inseminated outside of the 12 ± 6 h window following standing estrus. Four animals were mistakenly artificially inseminated on spontaneous estrus prior to confirmation of pregnancy. Two animals were treated outside of the 5 d ± 12 h window following artificial insemination. One animal mistakenly received an injection of $PGF_{2\alpha}$ by farm personnel prior to pregnancy confirmation. All fifteen of these animals mentioned were not included in the data analysis since none of these procedures met the protocol of the study. A total of 106 animals were ultimately utilized for data analysis (Table 1).

Administration of FM 5 days following artificial insemination did not improve pregnancy rates compared to controls (FM= 37.0, SAL = 31.4%; $P > 0.10$; Figure 1). No significant differences were detected between FM and SAL treatment within rBST administration group or lactation group (Table 2). Animals in the high, medium, and low

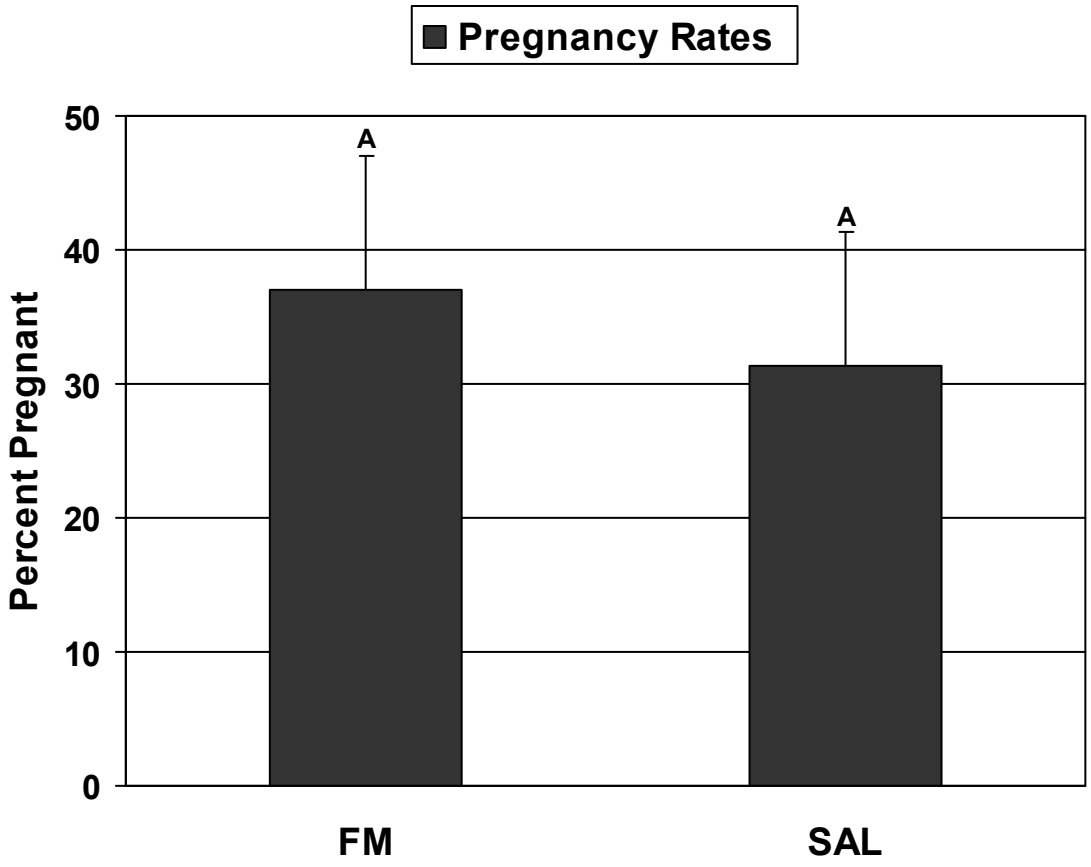

Figure 1. Effect of treatment on pregnancy rates. Treatment with flunixin (FM) or saline (SAL) did not alter pregnancy rates. (^{A} $P > 0.10$)

Table 2. Effect of treatment with flunixin meglumine (FM) or saline (SAL) on pregnancy rates for cows administered rBST (rBST +) or not administered rBST (rBST -) and within lactational groups (1, 2, 3 +). Treatment with FM or SAL did alter pregnancy rates regardless of rBST administration or lactational group (^{ABCDE} P > 0.10).

	FM	SAL
rBST -	**39.1^{A}**	**28.0^{A}**
rBST +	**40.8^{B}**	**54.2^{B}**
LN 1	**42.3^{C}**	**45.8^{C}**
LN 2	**40.0^{D}**	**40.0^{D}**
LN 3 +	**28.6^{E}**	**8.3^{E}**

milk production groups did not differ in pregnancy rates between the two treatments (Figure 2). Cows in the high, medium, and low 305 ME groups did not differ in pregnancy rates between the two treatments (Figure 3). Treatment with FM or SAL did not alter pregnancy rates within low and moderate BCS groups (Figure 4). However, treatment with FM did increase pregnancy rates for moderate BCS cows compared to low BCS cows treated with FM (Figure 4). Cows treated with FM or SAL within different DIM groups did not differ in pregnancy rates (Figure 5).

Factors associated with high milk production were also investigated to determine if these factors had an effect on pregnancy rates regardless of treatment. Cows in the high, medium, and low milk production and 305 ME groups did not differ in pregnancy rates (Figure 6). Breed did not effect pregnancy rates to a significant level. Holstein

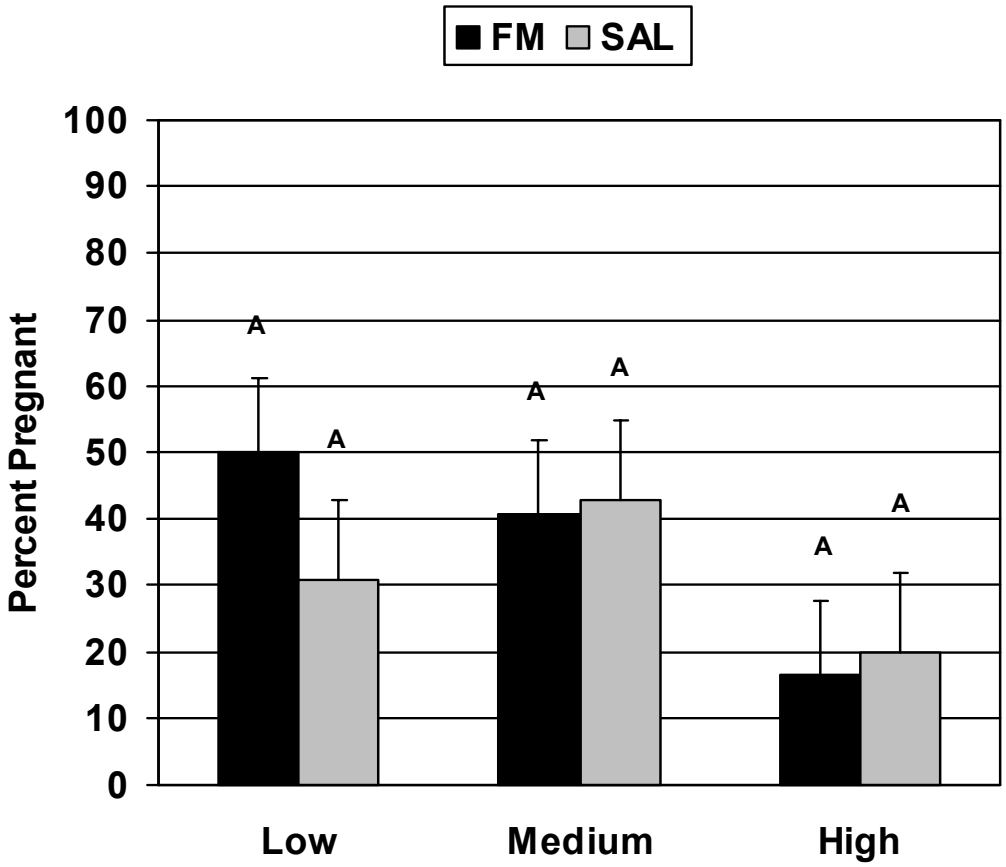

Figure 2. Effect of treatment with flunixin meglumine (FM) or saline (SAL) on pregnancy rates between low, medium, and high milk production groups. Neither treatment with FM nor SAL affected pregnancy rates among the different milk production groups (^{A} $P > 0.10$).

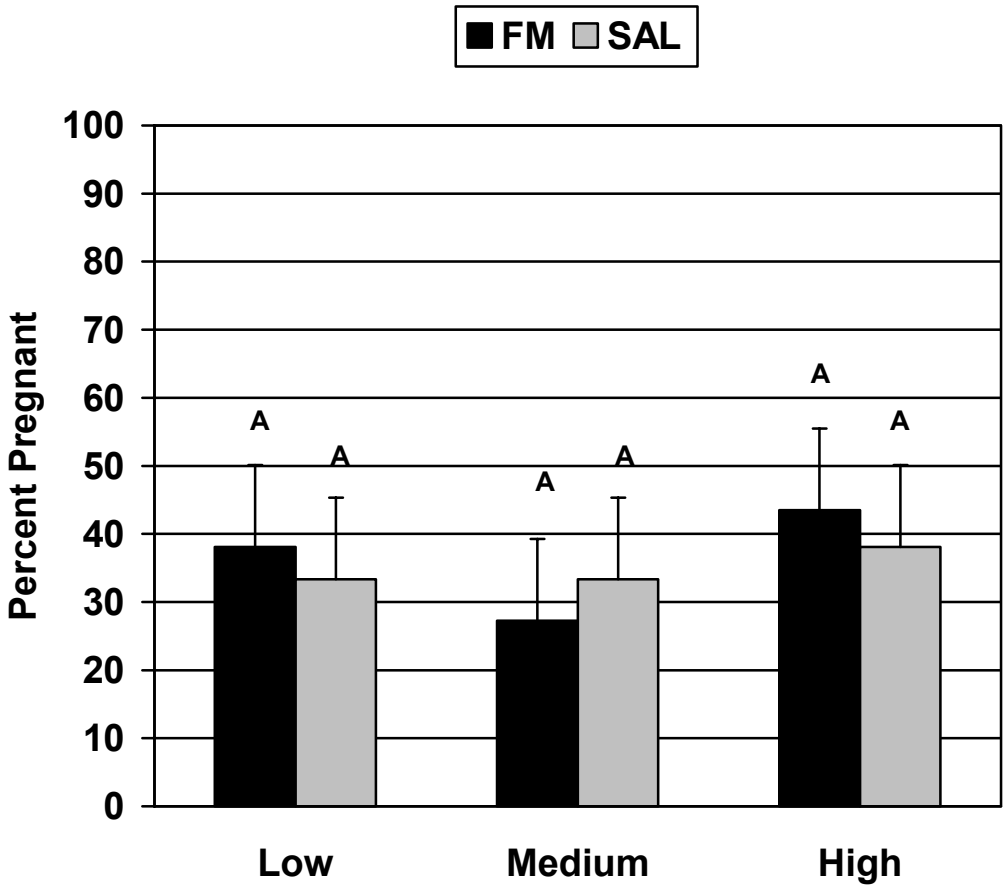

Figure 3. Effect of treatment with flunixin meglumine (FM) or saline (SAL) on pregnancy rates among different 305 mature equivalent (ME) groups. Treatment with FM or SAL did not result in any significant differences in pregnancy rates between the low, medium and high 305 ME groups (^{A} $P > 0.10$).

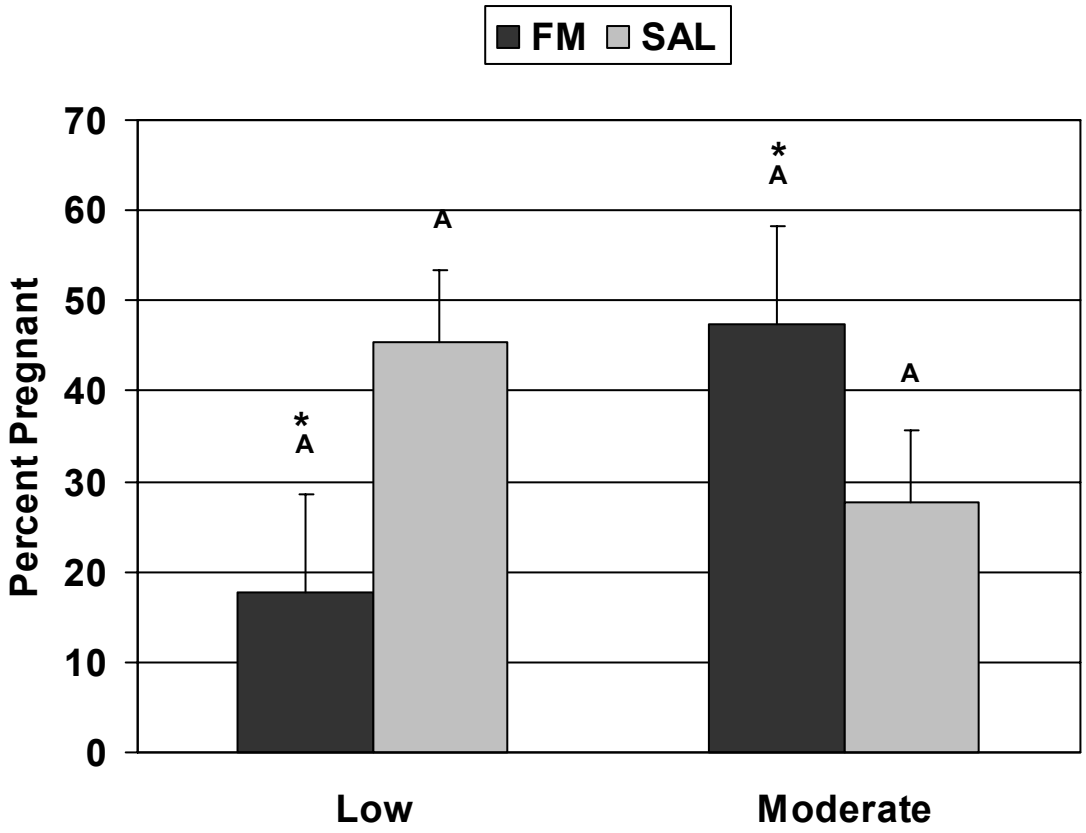

Figure 4. Effect of treatment with flunixin meglumine (FM) or saline (SAL) on pregnancy rates for cows in the low (1-2.5) and moderate (3-5) body condition score groups. Cows within each body condition score group did not differ in pregnancy rates regardless of treatment. Cows treated with FM in the moderate body condition score group did have higher pregnancy rates compared to cows in the low body condition score group treated with FM (* $P < 0.05$, ^{A} $P > 0.10$).

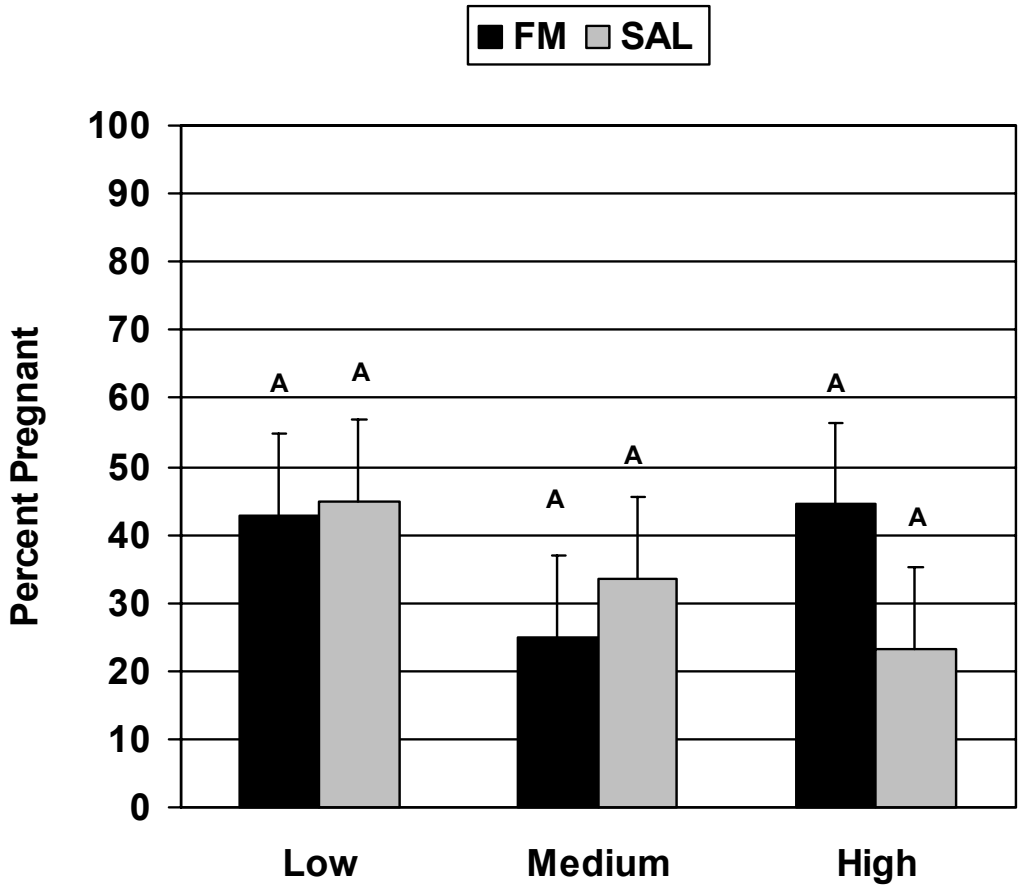

Figure 5. Effect of treatment with flunixin meglumine (FM) or saline (SAL) on pregnancy rates among different days in milk (DIM) groups. Treatment did not result in a significant difference in pregnancy rates for cows in the low, medium, and high DIM groups (^{A} $P > 0.10$).

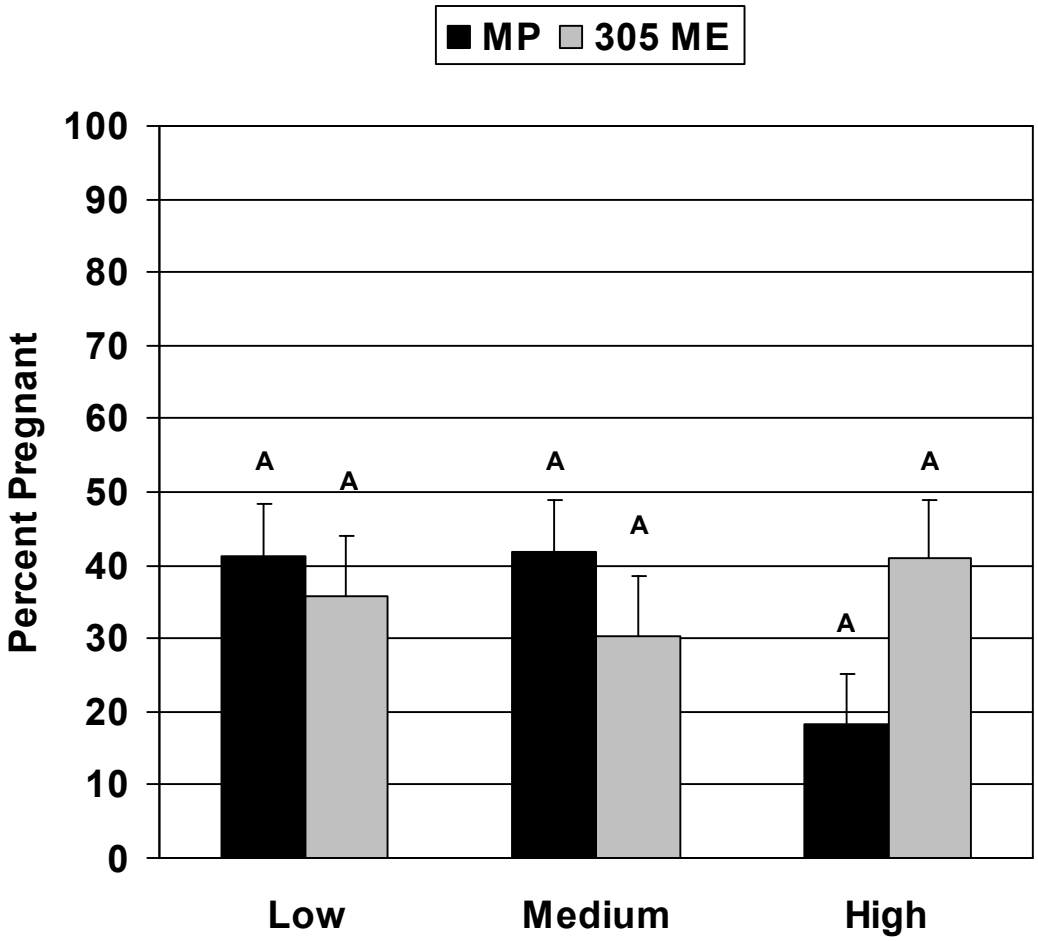

Figure 6. Effect of milk production and 305 ME group on pregnancy rates. Milk production (MP) and 305 mature equivalent (305 ME) did not have any significant effect on pregnancy rates (^{A} P > .10).

cows had a pregnancy rate of 33.8%, while Jersey cows had a pregnancy rate of 42.9% ($P > 0.10$). Administration of rBST did have an effect on pregnancy rates by lowering pregnancy rates for all cows and Jersey cows; however, no differences were detected within the Holstein breed (Figure 7). Administration of rBST to all cows and Jersey cows < 100 DIM also lowered pregnancy rates, but no differences were detected within the Holsteins (Figure 8). Lactation (LN) group tended to decrease ($P = 0.09$) pregnancy rates with increasing lactations, but rBST administration did not alter pregnancy rates within LN group (Table 3). Jersey cows in LN 1 had higher pregnancy rates (72.7%), while Jersey cows in LN 2 and 3+ were lower (38.5 and 18.2%, respectively; $P < 0.05$). However, Holstein cows did not have a significant difference in pregnancy rates with respect to LN number (1=35.9, 2=41.2, and 3+ =20%; $P > 0.10$).

Cows with a BCS of ≤ 2.5 and ≥ 3 resulted in pregnancy rates of 31.5 and 37.5%, respectively ($P > 0.10$). However, cows with a BCS of ≥ 3 tended to have lower pregnancy rates when administered rBST (33.4%) compared to those not administered rBST (60.4%; $P = 0.07$).

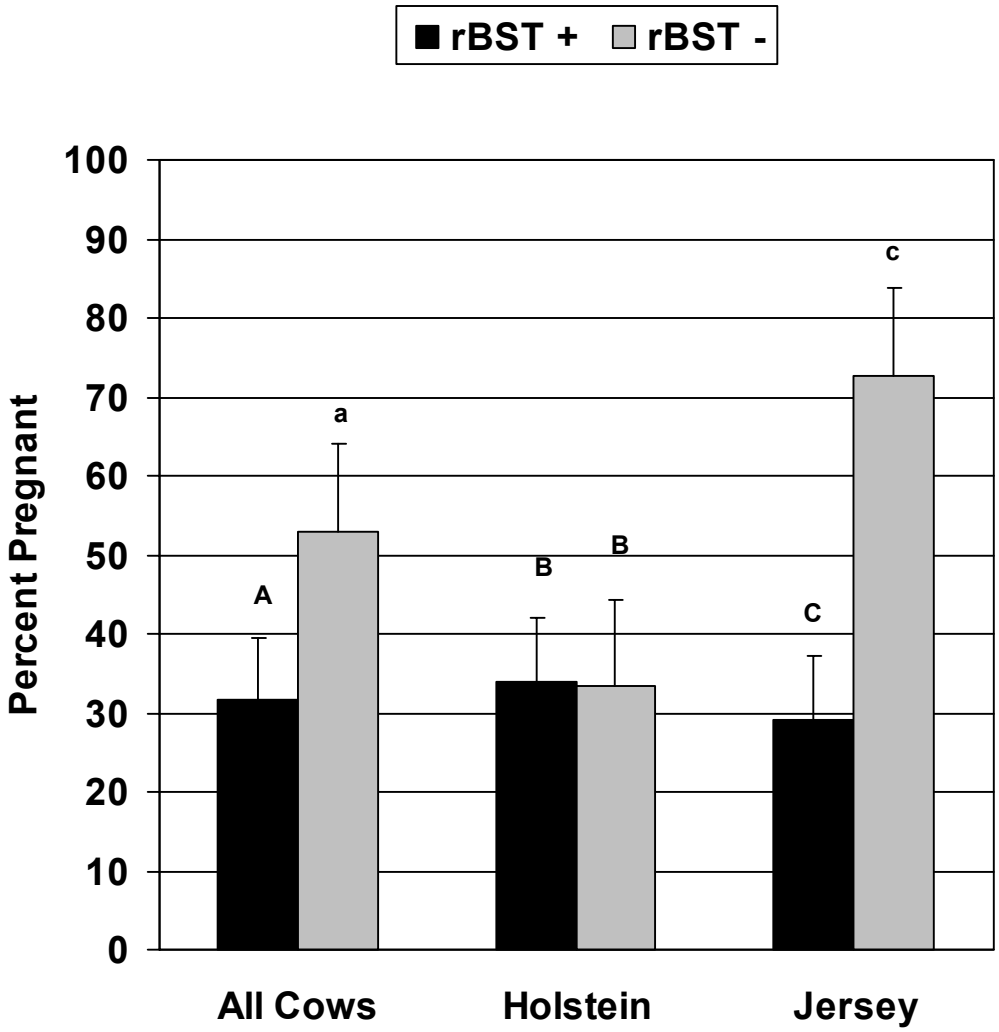

Figure 7. Effect of rBST administration (rBST +) or no rBST administration (rBST -) on pregnancy rates. Administration of rBST significantly decreased pregnancy rates in all cows ($^{A, a}$ $P < 0.05$). However, administration of rBST did not reduce pregnancy rates in Holstein cows (^{B} $P > 0.10$). Jersey cows administered rBST did exhibit a significant decrease in pregnancy rates ($^{C, c}$ $P < 0.05$).

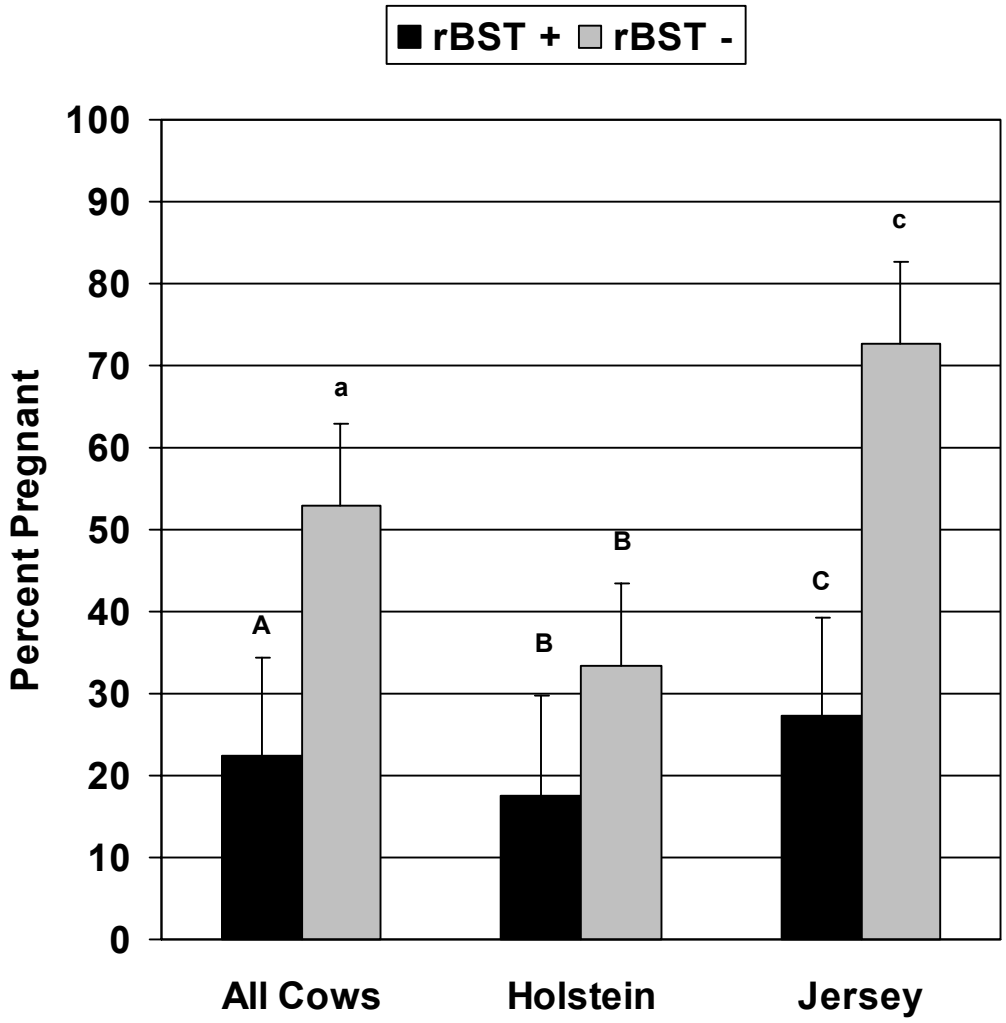

Figure 8. Effects of rBST administration (rBST + or rBST -) on pregnancy rates for cows < 100 days in milk (DIM). Administration of rBST significantly decreased pregnancy rates compared to cows not receiving rBST ($^{A, a}$ $P < 0.05$). However, only a breed by rBST administration interaction was noted in Jersey ($^{C, c}$ $P < 0.05$) but not Holstein cows (^{B} $P > 0.10$).

Table 3. Effect of lactation (LN) group and rBST administration (rBST +) or no rBST administration (rBST -) on pregnancy rates within lactation group. Pregnancy rates tended to decrease between LN 1 and LN 3 + (^{Aa} P = 0.08). Administration of rBST did not alter pregnancy rates within lactation group (^{BCD} P > 0.10).

	1	2	3 +
n	**50**	**30**	**26**
	44.1^{A}	**40^{Aa}**	**18.5^{a}**
rBST +	**42.3^{B}**	**37.4^{C}**	**13.6^{D}**
rBST -	**46.5^{B}**	**50.0^{C}**	**41.7^{D}**

5. Discussion

The present experiment was conducted to examine the effects of flunixin meglumine administration on the establishment of pregnancy following artificial insemination. Interest was also focused towards other factors (rBST, BCS, DIM, 305ME, lactation number, and breed) that may have negative effects on reproductive performance in lactating dairy cattle. It was hypothesized that the stressor of high milk production, and factors associated with high milk production, would result in an increase of $PGF_{2\alpha}$ levels 5 days following artificial insemination such that a positive effect of FM on pregnancy rates would be observed.

No significant differences were detected between FM and SAL treatment for overall pregnancy rates and within rBST administration group, lactation group, milk production group, or 305 ME group. A significant affect of treatment on pregnancy rates was detected with respect to BCS. Animals with a BCS of ≥ 3 experienced increased pregnancy rates compared to a BCS of ≤ 2.5 when treated with FM. However, all results should be interpreted with caution due to the limited number of animals available and utilized in this study.

It is established that FM can be utilized as an adequate inhibitor of $PGF_{2\alpha}$ (Odensvik, 1995; Konigsson et al., 2003). Positive effects of FM (a NSAID) on pregnancy rates seemed logical following the demonstrations of Elli et al. (2001) and Scenna et al. (2004b) that ibuprofen lysinate and FM, respectively, increased pregnancy rates following embryo transfer. Aiumlamai et al. (1990) and Odensvik et al. (1998) also demonstrated that the use of FM could be used to prevent luteolysis and prolong the estrous cycle. The most detrimental effects of $PGF_{2\alpha}$ on embryonic development tend to

occur during morula, compacted morula, and blastocyst development (Seals et al., 1998; Hockett et al., 2004; Scenna et al., 2004a). This stage of development corresponds to day 5-7 of development; thus, treatment with FM on day 5 following artificial insemination seemed logical. However, treatment on day 5 may have been too early considering successful uses of FM with embryo transfer (Scenna et al., 2004b) involved embryos at day 7 of development.

Another factor that may have contributed to similar pregnancy rates between FM and SAL treatments is the absence of an adequate stressor. It was hypothesized that high milk production and factors associated with high milk production would serve as an adequate stressor to increase uterine $PGF_{2\alpha}$ levels. According to the data analysis, milk production and these factors did not have an influence on pregnancy rates between the two treatment groups. Previous studies involving embryo transfer and the use of FM involved the stressor of embryo transfer on animals that did not encounter intense human contact. The intense daily contact involved with lactating dairy animals may have alleviated a stressor commonly involved with beef cattle and dairy heifers. Further studies with FM in dairy cattle in this research area may need to take place during a time of additional stress, such as elevated ambient temperature.

Route of administration of FM is another issue that may have resulted in few differences in pregnancy rates between treatment groups. Three routes of administration of FM have been studied in the past: oral (granules) (Odensvik, 1995; Odensvik et al., 1998; Konigsson et al., 2003), intramuscular (Konigsson et al., 2003; Scenna et al., 2004b;) and intravenous (Odensvik et al., 1993; Odensvik, 1995; Aiumlamai et al., 1990; Konigsson et al., 2003). Odensvik et al. (1995) and Konigsson et al. (2003) found no

differences in route of administration and the ability of FM to inhibit the synthesis of $PGF_{2\alpha}$. Intensive oral administration of FM granules was found to significantly increase the length of the estrous cycle (Odensvik et al., 1998). Intensive intravenous administration of FM was found to also delay normal luteolysis and prolong the estrous cycle (Aiumlamai et al., 1990). However, we are unaware of any publications indicating successful increases in pregnancy rates utilizing an intravenous administration of FM. Successful increases in pregnancy rates were obtained by Elli et al. (2001) and Scenna et al. (2004b) utilizing an intramuscular administration of ibuprofen lysinate and FM, respectively.

First-lactation cows involved in this study experienced a higher pregnancy rate than cows in lactation 2 and 3+. These results are contradictory to work concerning first-lactation animals and negative energy balance. First-lactation cows have a lower energy balance because they consume less and have higher energy requirements for growth and lactation (reviewed by Lucy, 2001). Lucy et al. (1992) associated a lower energy balance in first-lactation cows with delayed intervals to first ovulation. Negative energy balance, which may be more severe in first parity has been associated with poor reproductive performance (Butler and Smith, 1989; Butler, 2000; Snijders et al., 2001; Butler, 2003). Loeffler et al. (1999) identified first parity as a risk for conception failure at first service. Several studies indicate that reproductive performance should be better with multiparous cows. Milk production is the only logical explanation as to why reproductive performance was better in first-lactation cows. First lactation cows generally give less milk than multiparous cows; therefore, the stress of high milk production is not hindering reproduction. Factors associated with high milk production and actual production has

been associated with lowered reproductive performance (Snijders et al., 1999; O'Callaghan et al., 2000; Lucy, 2001; Snijders et al., 2001; Lopez et al., 2004).

The level of milk production of the cattle involved in this study did not result in significant differences in pregnancy rates for all cows. This data disagrees with the work of several investigators, which indicate that milk production and genetic selecting for high milk production can decrease fertility in dairy cattle. O'Callaghan et al. (2000) and Snijders et al. (2001) both indicated that selecting for high producing cows can decrease fertility. The work of O'Callaghan et al. (2000) and Snijders et al. (2001) focused on the nutritional needs of high producing animals. These investigators concluded that selecting for high producing cows forces a high dietary intake, which leads to a decrease in reproductive performance. Lopez and coworkers (2004) indicated that high producing cows have a lowered intensity of estrus. Pregnancy rates for animals in the low, medium, and high MP groups in this study were 41.4, 41.8, and 18.2%, ($P = 0.10$) respectively. Although not statistically different, the high MP group had lower pregnancy rates numerically. Perhaps a greater number of cows would have resulted in a statistically significant decrease of pregnancy rates related to milk production.

The stage of lactation for animals involved in this study significantly affected pregnancy rates. A significant difference in pregnancy rates was detected between Holstein and Jersey cows less than 100 DIM. These results are most likely the result of increased milk production by Holstein cows. High milk production and selection for high milk production has been associated with poor reproductive performance (Snijders et al., 1999; O'Callaghan et al., 2000; Snijders et al., 2001; Lopez et al., 2004). Animals less than 100 DIM and administered rBST resulted in a significant difference in pregnancy

rates for all cows, and Jersey cows. Cole at al. (1991) reported that treatment with rBST in early lactation resulted in a downward shift of energy balance that did not return to control level for 12 weeks. This information may explain why cows less than 100 DIM and administered rBST had significantly lower pregnancy rates. These cows in early lactation were most likely already experiencing a negative energy balance, and administration of rBST may have maintained or increased the severity of the negative energy balance.

Recombinant BST did have a negative effect on reproductive performance for the cows involved in this study. A decrease in pregnancy rates was greatest for cows less than 100 DIM. Other reports have indicated that rBST has a negative effect on pregnancy in lactating dairy cattle (Cole et al., 1991; Cole et al., 1992; reviewed by Dohoo et al., 2003). Dohoo and coworkers (2003) concluded in a review of published data that rBST use in non-pregnant cows increased the risk of a cow not becoming pregnant by 40%. Cole and coworkers' (1991) observations for reduced pregnancy rates of rBST-treated cows also were detected in early lactation cows. In contrast, Santos et al. (2004) reported that rBST use tended to reduce embryonic mortality. The mechanism in which rBST may or may not hinder reproductive performance is not fully understood, but several theoretical explanations have been published.

The most consistent explanation for decreased reproductive performance due to rBST administration in the reviewed literature has been a decrease in expression of estrus (Cole et al., 1991; Morbeck et al., 1991; Waterman et al., 1993; Santos et al., 2004). Along with decreased estrus expression, it has been reported that rBST is related to increased days to first service, services/conception, and days open (Cole et al., 1991;

Morbeck et al., 1991). The best possible explanation for reduced reproductive performance related to rBST found in a review of the literature is an alteration in follicular development (De La Sota et al., 1993; Lucy et al., 1993; Lucy et al., 1994; Lucy et al., 1995; Kirby et al., 1997a; Kirby et al., 1997b; Santos et al., 2004). De La Sota (1993) reported that the second largest follicle involved in the second follicular wave was larger in cows treated with rBST. De La Sota concluded that rBST might change the relationship between the dominant and subordinate follicles to which the dominant follicle loses some ability to suppress the subordinate follicle. Lucy et al. (1994) and Kirby et al. (1997a) reported that rBST treatment resulted in reduced period of dominance for the first follicular wave and an earlier recruitment of the second wave follicle. This earlier recruitment allows for an extended period of dominance for the second wave follicle. Austin et al. (1999) reported that an extended period of dominance is associated with reduced oocyte viability and subsequent fertility. Kirby et al. (1997b) reported that cows treated with rBST have reduced FSH; therefore, resulting in a faster turnover of the dominant follicle and a change in the timing of the follicular waves. Santos et al. (2004) concluded that all factors related to alteration follicular development might be the cause of poor reproductive performance associated with rBST. If rBST is causing a change in the viability of the second follicular wave and beyond, then anything but a first follicular wave insemination may be in jeopardy. Nevertheless, much research is still needed to fully understand the effect rBST on reproduction.

Body condition score did not have a significant impact on the pregnancy rates of the cattle involved in this study. Body condition score has been shown to have a direct association with energy balance (Snijders et al. 2001). Butler and Smith (1989) reported

that severe body condition loss was associated with lower conception rate for first service. Butler (2000) reported that poor fertility in high producing cattle was partially the result of a negative energy balance. Studies have shown that BCS can be directly associated with reproductive performance. Gillund et al. (2001) reported that cows with BCS loss of ≥ 1.25 units following calving were half as likely to conceive at first service as cows with more modest loss. Stevenson et al. (1999) reported that conception rates increased by 10% for every unit increased in BCS. A large study found that cows with a BCS of 3 at insemination are most likely to become pregnant (Loeffler et al., 1999). The information indicating that negative energy balance and BCS at the time of insemination are related to reproductive performance agrees on some levels with the data presented in this study. Overall there was no statistical difference detected in pregnancy rates with respect to BCS, but cows with a BCS of 3.5 were numerically higher at 62.5% compared to 44.44, 30.0, and 36.84% for BCS of 2, 2.5, and 3. These results agree with the indication that cows must be in a sound state of energy balance and BCS in order to perform well reproductively. However, these results disagree with Loeffler et al. (1999) that a BCS of 3 result in the highest pregnancy rates.

Results of this study also indicated that rBST along with BCS had a tendency to affect pregnancy rates. The fact that rBST had a tendency to lower pregnancy rates for cows with a BCS of 3 is puzzling considering no effect was detected for rBST administered cows at a BCS of 2. The information from previous investigators indicates that rBST may decrease reproductive performance (Cole et al., 1991; Cole et al., 1992; reviewed by Dohoo et al., 2003). A low body condition score of 2 along with administration of rBST should decrease reproductive performance. However, the animals

with the ideal BCS of 3 were experiencing the greatest loss of reproductive function when administered rBST. Perhaps an animal must be in a state of adequate body condition before a difference can be noticed.

In conclusion administration of FM on day 5 following artificial insemination did not result in increased pregnancy rates. This finding may be due to timing of administration, route of administration, absence of an adequate stressor, or the limited number of animals utilized. More research is needed to address the timing and route of administration of FM following artificial insemination. Also, no significant effect on pregnancy rates were detected with respect to breed, DIM, BCS, MP or 305ME. However, pregnancy rates had a tendency to decrease for cows in LN 3+ compared to LN 1. Recombinant bST was found to decrease pregnancy rates of lactating dairy cattle regardless of treatment. The greatest decrease in pregnancy rates was detected for animals < 100 DIM and administered rBST. More research is needed to maximize efficiency of FM as a $PGF_{2\alpha}$ inhibitor during artificial insemination, and development of management practices to minimize the detrimental effects of rBST on reproductive performance during early lactation.

List of References

Aiumlamai, S., K. Odensvik, G. Stabenfeldt, H. Kindahl. 1990. Regulation or prostaglandin biosynthesis with flunixin meglumine in the bovine species. J Vet Med 37:16-22.

Al-Katanani, F., F. Paula-Lopes, P. J. Hansen. 2002. Effect of season and exposure to heat stress on oocyte competence in Holstein cows. J Dairy Sci 85:390-396.

Austin, E. J., M. Mihm, M. P. Ryan, D. H. Williams, J. F. Roche. 1999. Effect of duration of dominance of the ovulatory follicle on onset of estrus and fertility in heifers. J Anim Sci 77:2219-2226.

Ayalon, N. 1978. A review of embryonic mortality in cattle. J Reprod Fertil 54:483-493.

Bazer, F. W., R. D. Geisert, M. T. Zavy. 1993. Fertilization, cleavage and implantation. In: Reproduction in Farm Animals. E.S.E. Hafez (Ed.), Lea and Fefiger, Philadelphia pp. 188-212.

Bazer, F. W., T. E. Spencer, T. L. Ott. 1997. Interferon tau: A novel pregnancy recognition signal. Am J Reprod Immunol 37:412-420.

Bito, L. Z. 1975. Are prostaglandins intracellular, transcellular or extracellular autocosis? Prostaglandins 45:229-235.

Blatteis, C. M. and E. Sehic. 1997. Fever: How many circulating pyrogens signal the brain? News Physiol Sci 12:1-9.

Bleil, J. D. and P. M. Wassarman. 1980. Mammalian sperm-egg interaction: Identification of a glycoprotein in mouse egg zonae pellucidae possessing receptor activity for sperm. Cell 20:873-882.

Bowen, J. A. and R. C. Burghardt. 2000. Cellular mechanisms of implantation in domestic farm animals. Semin Cell Dev Biol 11:93-104.

Breuel, K. F., A. Fukuda, F. N. Schrick. 1993. Effect of prostaglandin $F_{2\alpha}$ on development of 8-cell rat embryos in vitro. Biol Reprod 48 (Suppl. 1):173.

Britt, J. H. 1975. Early postpartum breeding in dairy cows. A review. J Dairy Sci 58:266-271.

Buford, W. I., N. Ahmad, F. N. Schrick, R. L. Butcher, P. E. Lewis, E. K. Inskeep. 1996. Embryotoxicity of a regressing corpus luteum in beef cows supplemented with progestogen. Biol Reprod 54:531-537.

Butcher, R. L., J. E. Reber, A. W. Lishman, K. F. Breuel, F. N. Schrick, J. C. Spitzer, E. K. Inskeep. 1992. Maintenance of pregnancy in postpartum beef cows having short-lived corpora lutea. J Anim Sci 70:3831-3837.

Butler, W. R. 2003. Energy balance relationship with follicular development, ovulation and fertility in postpartum dairy cows. Livest Prod Sci 83:211-218.

Butler, W. R. 2000. Nutritional interactions with reproductive performance in dairy cattle. Anim Reprod Sci 60:449-457.

Butler, W. R. 1998. Review: effect of protein nutrition on ovarian and uterine physiology in dairy cattle. J Dairy Sci 81:2533-2539.

Butler, W. R. and R. D. Smith. 1989. Interrelationships between energy balance and postpartum reproductive function in dairy cattle. J Dairy Sci 72:767-783.

Casida, L. E. 1961. Present status of the repeat-breeder cow problem. J Dairy Sci 44:2323-2329.

Cole, W. J., P. J. Eppard, B. G. Boysen, K. S. Madsen, R. H. Sorbet, M. A. Miller, R. L. Hintz, T. C. White, W. E. Ribelin, B. G. Hammond. 1992. Response of dairy cows to high doses of a sustained-release bovine somatotropin administered during two lactations. 2. Health and reproduction. J Dairy Sci 75:111-123.

Cole, W. J., K. S. Madsen, R. L. Hintz, R. J. Collier. 1991. Effect of recombinantly-derived bovine somatotropin on reproductive performance of dairy cattle. Theriogenology 36:573-595.

Cooper, D. A., D. A. Carver, P. Villeneuve, W. J. Silvia, E. K. Inskeep. 1991. Effects of progestagen treatment on concentrations of prostaglandins and oxytocin in plasma from the posterior vena cava of post-partum beef cows. J Reprod Fertil 91:411-421.

De La Sota, R. L., M. C. Lucy, C. R. Staples, W. W. Thatcher. 1993. Effects of recombinant bovine somatotropin on ovarian function in lactating and nonlactating dairy cattle. J Dairy Sci 76:1002-1013.

Demmers, K. J., K. Derecka, A. Flint. 2001. Trophoblast interferon and pregnancy. Reproduction 121:41-49.

De Rensis, F. and R. J. Scaramuzzi. 2003. Heat stress and seasonal effects on reproduction in the dairy cow-a review. Theriogenology 60:1139-1151.

Dohoo, I. R., L. DesCoteaux, K. Leslie, A. Fredeen, W. Shewfelt, A. Preston, P. Dowling. 2003. A meta-analysis review of the effects of recombinant bovine somatotropin 2. Effects on animal health, reproductive performance, and culling. Can J Vet Res 67:252-264.

Dransfield, M. B., R. L. Nebel, R. E. Pearson, L. D. Warnick. 1998. Timing of insemination for dairy cows identified in estrus by a radiotelemetric estrous detection system. J Dairy Sci 81:1874-1882.

Dunne, L. D., M. G. Diskin, J. M. Sreenan. 2000. Embryo and foetal loss in beef heifers between day 14 of gestation and full term. Anim Reprod Sci 58:39-44.

Edwards, J. L. and P. J. Hansen. 1997. Differential responses of bovine oocytes and preimplantation embryos to heat shock. Mol Reprod Dev 46:138-145.

Elli, M., B. Gaffuri, A. Figerio, M. Zanardelli, D. Covini, M. Candiani, M. Vignali. 2001. Effect of single dose of ibuprofen lysinate before embryo transfer on pregnancy rates in cows. Reproduction 121:151-154.

Ellington, J. E. 1991. The bovine oviduct and its role in reproduction: A review of the literature. Cornell Vet 81:313-328.

Fields, P. A. 1991. Relaxin and other luteal secretory peptides: cell localization and function in the ovary. In: Familiari, G., Makabe, S., Motta, P.M. (Eds.), Ultrastructure of the Ovary. Kluwer Academic Press, Boston pp. 177-198.

Fuchs, A. 1987. Prostaglandin $F_{2\alpha}$ and oxytocin interactions in ovarian and uterine function. J Steroid Biochem 27:1073-1080.

Gillund, P., O. Reksen, Y. T. Grohn, K. Karlberg. 2001. Body condition related to ketosis and reproductive performance in Norwegian dairy cows. J Dairy Sci 84:1390-1396.

Hansen, L. B. 2000. Consequences of selection for milk yield from a geneticist's viewpoint. J Dairy Sci 83:1145-1150.

Hermas, S. A., C. W. Young, J. W. Rust. 1987. Effects of mild inbreeding on productive and reproductive performance of Guernsey cattle. J Dairy Sci 70:712-715.

Hightshoe, R. B., P. C. Cochran, L. R. Corah, G. H. Kiracofe, D. L. Harmon, R. C. Perry. 1991. Effects of calcium soaps of fatty acids on postpartum reproductive function in beef cows. J Anim Sci 69:4097-4103.

Hockett, M. E., F. M. Hopkins, M. J. Lewis, A. M. Saxton, H. H. Dowlen, S. P. Oliver, F. N. Schrick. 2000. Endocrine profiles of dairy cows following experimentally induced clinical mastitis during early lactation. Anim Reprod Sci 58:241-251.

Hockett, M. E., N. R. Rohrbach, F. N. Schrick. 2004. Effect of administration of $PGF_{2\alpha}$ on embryonic development and quality in cows supplemented with exogenous progestogen. Prostaglandins 73:227-236.

Howell, J. L., J. W. Fuquay, A. E. Smith. 1994. Corpus luteum growth and function in lactating Holstein cows during spring and summer. J Dairy Sci 77:735-739.

Kindahl, H., L. E. Edqvist, A. Bane, E. Granstrome. 1976. Blood levels of progesterone and 15-keto-13,14-dyhydro-prostaglandin $F_{2\alpha}$ during the normal estrous cycle and early pregnancy in heifers. Acta Endocrinol. (Copenh) 82:134-149.

Kirby, C. J., M. F. Smith, D. H. Keisler, M. C. Lucy. 1997a. Follicular function in lactating dairy cows treated with sustained release bovine somatotropin. J Dairy Sci 80:273-285.

Kirby, C. J., S. J. Wilson, M. C. Lucy. 1997b. Response of dairy cows treated with bovine somatotropin to a luteolytic dose of prostaglandin $F_{2\alpha}$. J Dairy Sci 80:286-294.

Konigsson, K., K Torneke, I. V. Engeland, K. Odensvik, H. Kindahl. 2003. Pharmacokinetics and pharmacodynamic effects of flunixin after intravenous, intramuscular and oral administration to dairy goats. Acta Vet Scand. 44:153-159.

Lemaster, J. W., R. C. Seals, F. M. Hopkins, F. N. Schrick. 1999. Effects of administration of oxytocin on embryonic survival in progestogen supplemented cattle. Prostaglandins 57:259-268.

Loeffler, S. H., M. J. de Vries, Y. H. Schukken, A. C. de Zeeuw, A. A. Dijkhuizen, F. M. de Graaf, A. Brand. 1999. Use of AI technician scores for body condition, uterine tone and uterine discharge in a model with disease and milk production parameters to predict pregnancy risk at first AI in Holstein dairy cows. Theriogenology 51:1267-1284.

Lopez, H., L. D. Satter, M. C. Wiltbank. 2004. Relationship between level of milk production and estrous behavior of lactating dairy cows. Anim Reprod Sci 81:209-233.

Lucy, M. C. 2001. *ADSA Foundation Scholar Award* Reproductive loss in high-producing dairy cattle: Where will in end? J. Dairy Sci 84:1277-1293.

Lucy, M. C., T. L. Curran, R. J. Collier, W. J. Cole. 1994. Extended function of the corpus luteum and earlier development of the 2nd follicular waves in heifers treated with bovine somatotropin. Theriogenology 41:561-572.

Lucy, M. C., R. L. De La Sota, C. R. Staples, W. W. Thatcher. 1993. Ovarian follicular populations in lactating dairy cows treated with recombinant bovine somatotropin (Sometribove) or saline and fed diets differing in fat content and energy. J Dairy Sci 76:1014-1027.

Lucy, M. C., C. R. Staples, W. W. Thatcher, P. S. Erickson, R. M. Cleale, J. L. Firkins, M. R. Murphy, J. H. Clark, B. O. Brodie. 1992. Influence of diet composition, dry matter intake, milk production, and energy balance on time of postpartum ovulation and fertility in dairy cows. Anim Prod 54:323-331.

Lucy, M. C., W. W. Thatcher, R. J. Collier, F. A. Simmen, Y. Ko, J. D. Savio, L. Badinga. 1995. Effects of somatotropin on the conceptus, uterus, and ovary during maternal recognition of pregnancy. Domest Anim Endocrinol 12:73-82.

Macmillan, K. L., I. J. Lean, C. T. Westwood. 1996. The effects of lactation on the fertility of dairy cows. Aust Vet J 73:141-147.

Maurer, R. R. and J. M. Beier. 1976. Uterine proteins and development in vitro of rabbit preimplantation embryos. J Reprod Fert 48:33-41.

Maurer, R. R. and J. R. Chenault. 1983. Fertilization failure and embryonic mortality in parous and nonparous beef cattle. J Anim Sci 56:1186-1189.

Malayer, J. R., P. J. Hansen, T. S. Gross, W. W. Thatcher. 1990. Regulation of heat shock-induced alterations in the release of prostaglandins by the uterine endometrium of cows. Theriogenology 34:219-230.

McNatty, K. P., D. A. Heath, T. Lundy, A. E. Fidler, L. Quirke, A. O'Connel, P. Smith, N. Groome, D. J. Tisdall. 1999. Control of early ovarian follicular development. J Reprod Fertil (Suppl) 54:3-16.

McNaughtan, J. W., R. J. Yelland, S. Lingard, A. Abel, R. W. Silcox. 2001. The effect of prostaglandin inhibitor on pregnancy rates of heifer embryo transfer recipients. Theriogenology 55:551. (Abstr)

Morbeck, D. E., J. H. Britt, B. T. McDaniel. 1991. Relationships among milk yield, metabolism, and reproductive performance of primiparous Holstein cows treated with somatotropin. J Dairy Sci 74:2153-2164.

Nett, T. M., M. C. McClellan, G. D. Niswender. 1976. Effects of prostaglandins on the ovine corpus luteum: blood flow, secretion of progesterone and morphology. Biol Reprod 15:66-78.

O'Callaghan, D. and M. P. Boland. 1999. Nutritional effects on ovulation, embryo development and the establishment of pregnancy in ruminants. Anim Sci 68:299-314.

O'Callaghan, D., J. M. Lozanot, J. Fahey, V. Gath, S. Snijders, M. P. Boland. 2000. Recent developments in the effect of nutrition on fertility in dairy cows. Ir Vet J 53:417-425.

Odensvik, K. 1995. Pharmacokinetics of flunixin and its effect on prostaglandin $F_{2\alpha}$ metabolite concentrations after oral and intravenous administration in heifers. J Vet Pharmacol Therap 18:254-259.

Odensvik, K., N. Cort, S. Basu, H. Kindahl. 1989. Effect of flunixin meglumine on prostaglandin $F_{2\alpha}$ synthesis and metabolism. J Vet Pharmacol Therap 12:307-311.

Odensvik, K., M. Duchens, H. Gustafsson. 1993. Does mechanical manipulation of the reproductive organs cause a prostaglandin release in the heifer during embryo transfer. Acta Vet Scand 34:219-221.

Odensvik, K., H. Gustafsson, H. Kindahl. 1998. The effect of luteolysis by intensive oral administration of flunixin granules in heifers. Anim Reprod Sci 50:35-44.

Okuda, K., Y. Miyamoto, D. J. Skarzynski. 2002. Regulation of endometrial prostaglandin $F_{2\alpha}$ synthesis during luteolysis and early pregnancy in cattle. Domest Anim Endocrinol 23:255-264.

Oldick, B. S., C. R. Staples, W. W. Thatcher, P. Gyawu. 1997. Abomasal infusion of glucose and fat effect on digestion, production, and ovarian and uterine functions of cows. J Dairy Sci 80:1315-1328.

Picton, H., D. Briggs, R. Gosden. 1998. The molecular basis of oocyte growth and development. Mol Cell Endocrinol 145:27-37.

Pryce, J. E., B. L. Nielsen, R. F. Veerkamp, G. Simm. 1999. Genotype and feeding system effects and interactions for health and fertility traits in dairy cattle. Livest Prod Sci 57:193-201.

Putney, D. J., S. Mullins, W. W. Thatcher, M. Drost, T. S. Gross. 1989. Embryonic development in superovulated dairy cattle exposed to elevated ambient temperatures between the onset of estrus and insemination. Anim Reprod Sci 19:37-51.

Ramirez-Godinez, J. A. G. H. Kiracofe, R. R. Schalles, G. D. Niswender. 1982. Endocrine patterns in the postpartum beef cow associated with weaning: a comparison of the short and subsequent normal cycles. J Anim Sci 55:153-158.

Roche, J. F., D. Mackey, M. D. Diskin. 2000. Reproductive management of postpartum cows. Anim Reprod Sci 60-61:703-712.

Royal, M. D., A. O. Darwash, A. P. F. Flint, R. Webb, J. A. Woolliams, G. E. Lamming. 2000. Declining fertility in dairy cattle: changes in traditional and endocrine parameters of fertility. Anim Sci 70:487-502.

Rubinstein, M., A. Marazzi, E. P. de Fried. 1999. Low-dose aspirin treatment improves ovarian responsiveness, uterine and ovarian blood flow velocity, implantation, and pregnancy rates in patients undergoing in vitro fertilization: a prospective, randomized, double-blind placebo-controlled assay. Fertil Steril 71:825-829

Santos, J. E. P., S. O. Juchem, R. L. A. Cerri, K. N. Galvao, R. C. Chebel, W. W. Thatcher, C. S. Dei, C. R. Bilby. 2004. Effect of bST and reproductive management on reproductive performance of Holstein dairy cows. J Dairy Sci 87:868-881.

SAS(2000) SAS/STAT User's Guide. Cary, NC.

Scenna, F. N., J. L. Edwards, N. R. Rohrbach, M. E. Hockett, A. M. Saxton, F. N. Schrick. 2004a. Detrimental effects on prostaglandin $F_{2\alpha}$ on preimplantation bovine embryos. Prostaglandins 73:215-226.

Scenna, F. N., M. E. Hockett, T. M. Towns, A. M. Saxton, N. R. Rohrbach, N. E. Wert, M. Mimms, D. Ezcurra, M. E. Wehrman, F. N. Schrick. 2004b. Administration of a prostaglandin inhibitor at the time of embryo transfer improves pregnancy rates in cattle. Prostaglandins (Submitted).

Schrick, F. N., E. K. Inskeep, R. L. Butcher. 1993. Pregnancy rates for embryos transferred from early postpartum beef cows into recipients with normal estrous cycles. Biol Reprod 49:617-621.

Seals, R. C., J. W. Lemaster, F. M. Hopkins, F. N. Schrick. 1998. Effects of elevated concentration of prostaglandin $F_{2\alpha}$ on early embryonic survival in progestogen supplemented cattle. Prostaglandins 56:377-389.

Seibert, K., Y. Zhang, K. Leahy. 1994. Pharmacological and biochemical demonstration of the role of cyclooxygenase 2 in inflammation and pain. Proc Natl Acad Sci 91:12013-12017.

Silvia, W. J. 1998. Changes in reproductive performance of Holstein dairy cows in Kentucky from 1972 to 1996. J Dairy Sci 81(Suppl.1):244. (Abstr.)

Snijders, S. E. M., P. G. Dillon, K. J. O'Farrell, M. Diskin, A. R. G. Wylie, D. O'Callaghan, M. Rath, M. P. Boland. 2001. Genetic merit for milk production and reproductive success in dairy cows. Anim Reprod Sci 65:17-31.

Snijders, S. E. M., P. G. Dillon, K. J. O'Farrell, M. Diskin, D. O'Callaghan, M. Rath, M. P. Boland. 1999. Relationship between genetic merit, milk production, body condition, blood parameters and reproductive performance of dairy cows. *Proceedings of the British Society of Animal Science Occasional Meeting.* Abstr 94.

Stahl, T. J., B. J. Conlin, A. J. Seykora, G. R. Steuernagel. 1999. Characteristics of Minnesota dairy farms that significantly increased milk production from 1989-1993. J Dairy Sci 82:45-51.

Staples, C. R., J. M. Burke, W. W. Thatcher. 1998. Influence of supplemental fat on reproductive tissues and performance of lactating cows. J Dairy Sci 81:856-871.

Stevenson, J. S., Y. Kobayashi, F. N. Thompson. 1999. Reproductive performance of dairy cows in various programmed breeding systems including ovsynch and combinations of gonadotropin-releasing hormone and prostaglandin $F_{2\alpha}$. J Dairy Sci 82:506-515.

Stott, G. H. and R. J. Williams. 1962. Causes of low breeding efficiency in dairy cattle associated with seasonal high temperatures. J Dairy Sci 45:1369-1375.

Thatcher, W. W., M. Binelli, J. Burke, C. R. Staples, J. D. Ambrose, S. Coelho. 1997. Antiluteolytic signals between the conceptus and endometrium. Theriogenology 47:131-140.

Thatcher, W. W., C. R. Staples, G. Danet-Desnoyers, B. Oldick, E. P. Schmitt. 1994. Embryo health and mortality in sheep and cattle. J Anim Sci 72 (Suppl 3):16-30.

Tulsiani, R. P., A. Abou-Haila, C. R. Loeser, B. M. J. Pereira. 1998. The biological and functional significance of the sperm acrosome and acrosomal enzymes in mammalian fertilization. Exp Cell Res. 240:151-164.

Vane, J. R. 1971. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nature New Biol 231:232-235.

Vane, J. R. and R. M. Botting. 1998. Anti-inflammatory drugs and their mechanism of action. Inflamm Res 47, (Suppl. 2):S78-S87.

Velez, J. S., R. D. Randel, D. A. Neuendorff. 1991. Effect of uterine manipulation on postpartum fertility and plasma 13,14-dihydro-15keto-prostaglandin $F_{2\alpha}$ in Brahman cows and first-calf heifers. Theriogenology 36:987-998.

Wann, R. A. and R. D. Randel. 1990. Effect of uterine manipulation 35 days after parturition on plasma concentration of 13, 14-dihydro-15-keto prostaglandin $F_{2\alpha}$ in multiparous and primiparous Brahman cows. J Anim Sci 68:1389-1394.

Waterman, D. F., W. J. Silvia, R. W. Hemken, G. Heersche, Jr., T. S. Swenson, R. G. Eggert. 1993. Effect of bovine somatotropin on reproductive function in lactating dairy cows. Theriogenology 40:1015-1028.

Wehrman, M. E., T. H. Jr Welsh, G. L. Williams. 1991. Diet-induced hyperlipidemia in cattle modifies the intrafollicular cholesterol environment, modulates ovarian follicular dynamics and hastens the onset of luteal activity. Biol Reprod 45:514-522.

Williams, G. L. 1989. Modulation of luteal activity in postpartum beef cows through changes in dietary lipid. J Anim Sci 67:785-793.

Wilson, S. J., R. S. Marion, J. N. Spain, D. E. Spiers, D. H. Keisler, M. C. Lucy. 1998. Effect of controlled heat stress on ovarian function in dairy cattle: I. Lactating cows. J Dairy Sci 1:2124-2131.

Xiao, C. W., B. D. Murphy, J. Sirois, A. K. Goff. 1999. Down-regulation of oxytocin-induced cyclooxygenase-2 and prostaglandin F synthase expression by interferon-τ in bovine endometrial cells. Biol Reprod 60:656-663.

VITA

Charles Dexter Young was born August 6th, 1979 in Athens, Tennessee. He attended elementary school in Etowah, Tennessee. Charles graduated from McMinn Central High School in 1997. In the spring of 2001 he graduated with a B. S. degree in Animal Science from the University of Tennessee, Knoxville. In the summer of 2004, he graduated with a M. S. degree in Animal Science from the University of Tennessee, Knoxville.