Comparison of Luteolysis and Timed Artificial Insemination Pregnancy Rates after Administration of $PGF_{2\alpha}$ in the Muscle or the Ischiorectal Fossa in Cattle

Sarah Christine Holland

Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

Master of Science
In
Biomedical and Veterinary Sciences

William S. Swecker, Jr., Chair
William D. Whittier
Sherrie C. Clark

May 26, 2015
Blacksburg, VA

Keywords: Prostaglandin, luteolysis, estrus synchronization, beef quality assurance

Comparison of Luteolysis and Timed Artificial Insemination Pregnancy Rates after Administration of $PGF_{2\alpha}$ in the Muscle or the Ischiorectal Fossa in Cattle

Sarah C. Holland

Abstract: Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is commonly given to female cattle intramuscularly (IM) for the synchronization of estrus. A novel site for administration of PGF2α that improves beef quality assurance is the ischiorectal fossa (IRF). The objective of this study was to determine whether administration of $PGF_{2\alpha}$ in the IRF results in a similar physiologic response to administration of $PGF_{2\alpha}$ given IM.

Yearling angus-cross heifers (n=112) were blocked by weight and randomly assigned within blocks to be injected with 5 mL $PGF_{2\alpha}$ either IM in the neck or in the IRF. Blood samples were taken at 0, 8, 16, 24, 36, and 48 h post-injection. Serum samples were analyzed for progesterone concentration using a radioimmunoassay. Progesterone concentration curves for each heifer were plotted to determine luteolysis. The median times to luteolysis for neck and IRF injections were 18.1 hrs and 20.0 hrs, respectively (p=0.06).

Angus cross commercial beef cows (n=1471) at least 30 days post-partum were blocked by age and randomly assigned to be injected with 5 mL $PGF_{2\alpha}$ either IM in the neck muscle or in IRF as part of a 7-Day CO-Synch + CIDR ovulation protocol. Pregnancy diagnosis was performed via ultrasound at 60 days post insemination. Results were analyzed with Proc Glimmix (SAS). Pregnancy rates for neck and IRF injections were 52.6% and 57.2%, respectively (p=0.06).

In summary, injection of $PGF_{2\alpha}$ in the IRF for estrus synchronization and lysis of the corpus luteum did not differ from injection in the neck muscle. Utilizing the ischiorectal fossa as an injection site for $PGF_{2\alpha}$ may be considered as an alternative that more closely aligns with beef quality assurance objectives.

Dedication

I dedicate this work to my husband, Jimmy Holland. Over the course of 12 years and 3 degrees he has been more gracious, loyal, encouraging, and patient than anyone should ever have to be and I am forever grateful for him and to him.

Acknowledgements

I am very grateful to the many people who assisted me with the research that went into this project. I have learned so much from so many and could never have completed this work alone. Special thanks are due to:

Dr. Terry Swecker, Jr.
Dr. Dee Whittier
Dr. Sherrie Clark
Dr. Keith Inskeep
Dr. Jim Lauderdale
Dr. John Chenault
Dr. Mike McGilliard
David Fiske
Shenandoah Valley Agricultural Research and Extension Center staff
Chad Joines
Dr. Hillary Feldman
Lee Johnson
Virginia Department of Corrections Agribusiness

Funding for this project was supplied by a VMCVM IRC grant
Cattle provided by the Virginia Tech College of Agriculture and Life Sciences and Virginia Department of Corrections

Comparison of Luteolysis and Timed Artificial Insemination Pregnancy Rates after Administration of $PGF_{2\alpha}$ in the Muscle or the Ischiorectal Fossa in Cattle

Table of contents

List of Tables

List of Figures

Chapter I

Introduction

Cows are administered injectable products (vaccines, antibiotics, analgesics, and reproductive hormones) to improve health and productivity. The recommended site for intramuscular and subcutaneous injections is in the neck. However, this site is not easily accessed in some restraint systems. Therefore, many cows are given injections in the muscles of the rear legs, which affects the quality of the carcass at slaughter. A novel alternative site for injection in the back half of the cow that minimizes damage to the carcass is the ischiorectal fossa (IRF), located adjacent to the tailhead. This thesis reviews the current literature pertaining to the administration of the hormone prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in the ischiorectal fossa and validates this injection location for administration of dinoprost, a synthetic analogue of $PGF_{2\alpha}$.

Prostaglandin $F_{2\alpha}$ causes the corpus luteum on the ovary to stop producing progesterone through a process known as luteolysis and is used to shorten the cycle in female cattle and, subsequently, induce estrus. Prostaglandin $F_{2\alpha}$ is difficult to detect and measure in serum due to its very short half-life, but researchers can monitor the physiologic response to $PGF_{2\alpha}$ by observing a decrease in serum progesterone after treatment with $PGF_{2\alpha}$. In the first study, beef heifers were randomly assigned to be administered an injection of $PGF_{2\alpha}$ in the IRF or intramuscularly (IM) in the neck. Multiple blood samples were taken over 48 hours in order to monitor blood progesterone levels. Serum progesterone levels for each heifer were plotted over time and analyzed for differences in time to luteolysis.

Prostaglandin $F_{2\alpha}$ is also used for estrus synchronization when producers want to artificially inseminate a group of cows. In the second study, 1471 beef cows were synchronized with a common protocol that includes $PGF_{2\alpha}$. Cows were randomly assigned to receive the dose of $PGF_{2\alpha}$

either in the IRF or IM in the neck. The cows were then artificially inseminated at the end of the protocol and diagnosis of pregnancy was performed approximately two months later. Estrus expression and pregnancy rates in treatment and control cows were compared to analyze for an effect of injection location on fertility.

Chapter II

Review of Literature

Introduction

The veterinary profession has improved the health and well-being of its patients in part through the use of injectable pharmaceuticals and biologics. Equipped with parenteral vaccines and antibiotics, veterinarians have successfully prevented and treated many diseases in the past century. In particular, injectable products are especially useful in ruminants because this avenue of administration avoids the fore-stomachs; oral administration of many compounds would result in those compounds being rendered ineffective in the rumen where fermentation occurs (Rebhun, 1995). The ability to give biologics in the vein, in the muscle, and under the skin of cattle has greatly augmented the bovine veterinarian's potency in regards to disease. For example, *Clostridium chauvoei*, commonly known as blackleg, is an infectious disease of cattle with a very high mortality rate. From 1897 to 1922 the Department of Agriculture distributed 47 million doses of an injectable blackleg vaccine, first developed in France in 1883; death losses decreased from 10 percent to 0.5 percent over those years (Stein, 1956). The previously available oral powders and pills subsequently fell out of favor due to ineffectiveness as compared with the new injectable vaccine. While it is sometimes necessary to give cattle injections for health reasons, these injections are not without negative consequences. Clostridial vaccines given in the muscle in 2-3 month old calves cause lesions in the muscle at slaughter 67%-94% of the time (George, Heinrich, et al., 1995). The beef industry has been challenged in trying to preserve the efficacy of injectable products while limiting or eliminating carcass damage from the same products. This effort coalesced into a program called Beef Quality Assurance.

The success of the beef industry in the US means that it has become an important part of our agricultural economy. In 2013, farm gate receipts for cattle on feed totaled $44 billion (USDA NASS). Maintaining the stability of this industry both internally and in export markets is of interest to many stakeholders. In the December, 2014 issue of Beef magazine, John Brook, United States Meat Export Federation regional director for Europe, Russia, and the Middle East, is quoted as saying, "It's the consistency of quality that has made the market so successful" (Ishmael, 2014). The consistency of the beef product in this country can, in large part, be attributed to the work accomplished by the Beef Quality Assurance program. Finding ways to augment this program and further its objectives while still allowing for effective treatment of cattle will be paramount in protecting the beef industry in the years to come.

An opportunity to improve beef quality could come in the form of reducing injection site lesions in the muscles that will eventually become edible beef. One obvious option is to give products subcutaneously rather than in the muscle. Another option is to identify all possible locations on the live animal where injections can be given without damaging cuts of beef. The beef industry has had great success using these two strategies. The dairy industry, however, continues to see losses from injection site lesions, partly due to the use of injectable reproductive hormones. These hormones are often administered in the muscles of the back legs, primarily due to the restraint systems used on dairy farms. One of the pharmaceuticals commonly administered to cattle is prostaglandin F2α ($PGF_{2\alpha}$). While commonly administered in the muscles of the rear legs, this compound could be given in the ischiorectal fossa in order to preserve beef quality assurance. This review covers a brief history of the significance of beef quality assurance, the administration of $PGF_{2\alpha}$ to both dairy and beef cows and the subsequent ramifications, physiologic

luteolysis in response to endogenous $PGF_{2\alpha}$, iatrogenic luteolysis in response to exogenous $PGF_{2\alpha}$, and the feasibility and efficacy of an alternative injection location: the ischiorectal fossa.

Beef Quality Assurance

In the 1960's NASA sought a method to ensure food safety for astronauts in space. The Pillsbury Company and the US Army Natik Laboratory collaborated to develop the Hazard Analysis Critical Control Point (HACCP) system ("Beef Quality Assurance Manual," 2015). The USDA subsequently accepted the system for use in food production across many different industries. With the new emphasis on food safety, the beef industry started a program in the late 1970's called Beef Safety Assurance as a means of guaranteeing that beef was safe and wholesome, with a primary focus on eliminating violative residues and other adulterants ("Beef Quality Assurance Manual," 2015). Producers took notice of the increase in regulation and in 1985 three cattle companies volunteered to collaborate with FSIS to become certified as "Verified Production Control" feedyards as part of the Pre-harvest Beef Safety Production Program. The criteria implemented at that time have evolved to become what is known today as the Beef Quality Assurance Program ("Beef Quality Assurance Manual," 2015).

In 1991, Gary Smith of Colorado State University completed the first Beef Quality Audit to provide a benchmark for the beef industry. Smith reported that injection site lesions were among the top three concerns of purveyors, restauranteurs, retailers, and packers (Smith et al., 1991). Most injection site lesions cannot be located from external examination of the carcass at the packer but are exposed at the meat market or steak cutter during processing of subprimals. Therefore, Dexter et al. conducted 6 audits of steak cutting plants in 8 states over 3 years in order to determine the incidence of blemishes in top sirloin (Dexter et al., 1994). The prevalence of blemishes in July 1991 was reported to be 21% +/- 8%. The authors noted that the variability in prevalence was due

to the variability in sources of cattle. Over the next few years the prevalence fell and by the last audit in March 1993 the prevalence was 11% +/- 3%. The authors calculated that the decrease in blemishes saved the US beef industry $26.9 million dollars during that time. The authors also reported that 80-90% of the lesions were classified as chronic, meaning that the injections had most likely occurred during the cow-calf, stocker, or early finishing phase, rather than the late finishing phase.

Although the scientific community already knew that parenteral products could cause damage (Rasmussen, 1978), the results of the audit led to a long investigation of the effects of specific injectable pharmaceuticals used in beef cattle at different times during production. Apley et al. analyzed carcass lesions in steers that were given either a 2 ml or 5 ml dose of clostridial vaccine 95 days prior to slaughter (Apley, Wray, & Armstrong, 1994). For the 2 mL and 5 mL vaccines, 12.5% and 50%, respectively, of steers were found to have surface level lesions at the injection location. Trimming revealed deeper lesions in 3.5% and 30% of the steers for the 2 mL and 5 mL products. This study was one of the first prospective trials comparing lesions from specific injectable products and demonstrated the benefit of lowering the volume to be injected. Up to this point, the focus on improvement of injection techniques was happening at the feedlot stage of production. However, George et al, in 1995, measured the effects of several vaccines, a vitamin supplement, and an antibiotic administered prior to the feedlot stage, at branding and at weaning, on carcasses at slaughter (George, Heinrich, et al., 1995). Clostridial vaccines of 2 mL and 5 mL volumes caused lesions an average of 45% and 80% of the time, respectively. A vitamin AD product caused lesions an average of 10% of the time, and oxytetracycline (antibiotic) administered at the labeled dose caused lesions an average of 92% of the time. The average amount of trim for the 2 mL and 5 mL vaccines, vitamin AD, and oxytetracycline products were, on

average, 39 g, 78 g, 65 g, and 96 g, respectively. One unexpected finding was that the lesions from injections given earlier in the calf's life (branding) were actually bigger than the lesions from injections given later (weaning). One theory the authors offered was that the lesion is created at the time of injection but then grows with the calf. Regardless of how it happens, however, products injected into calves create lesions that are present in the carcass. Thus, both the volume of a given product or the timing of product administration can influence the size and presence of a lesion in the carcass.

Injection lesions may or may not be visible at carcass inspection and may differ in severity. Some lesions are not visible on the surface of the muscle and are not discovered until primals and subprimals are processed by purveyors. These injection lesions are costly not only because damaged product must be discarded, but because when an abscess is encountered during fabrication, the work station is shut down until the area can be cleaned and disinfected. Not all lesions, however, result in abscesses. Some lesions are no more than discoloration/streaking and remain unnoticed in cuts of meat that end up packaged and on grocery store shelves. George et al. took their research one step further and quantified the changes in tenderness in lesions from bottom-round subprimals obtained at 3 different purveying facilities in 1994. Tenderness can be approximated using the Warner-Bratzler shear test in which the force (in kilograms) required to cut a 1 cubic centimeter muscle sample is measured; this method objectively simulates biting into meat. Lesions were found in 8% of round cuts (George, Morgan, et al., 1995). Warner-Bratzler shear values were measured in steaks with lesions at the center of the lesion and 1, 2, and 3 inches away and at corresponding locations in control steaks. Values for steaks with lesions were 14, 10, 8, and 6 kg and values for control steaks were 4, 4, 4, and 4 kg (according to the US National Beef Tenderness Survey, tenderloin tends to measure around 2 kg and top round steak tends to measure

around 5 kg). The tenderness of the steak was affected not only at the lesion but in a radius of 3 inches, as well. This data demonstrates the negative effect that injection site lesions can have on a consumer's eating experience.

Once the prevalence and negative ramifications of injection site lesions were established, BQA programmers began delivering guidelines and training to producers in an effort to curtail the damage. Their methods involved a certification program that required producers and their employees to be trained and to then pass a test about drug residue avoidance and injection techniques. The Beef Quality Assurance guidelines include specific instructions and recommendations concerning the production of quality beef. The term "quality beef" can be considered to encompass many aspects of beef production. One of the priorities of the program is to ensure the delivery of a safe and wholesome product to the consumer. This pertains to violative residues as well as other adulterants such as broken needles. At its inception the program was called "Beef Safety Assurance" but has since broadened its scope to include eating characteristics such as tenderness and palatability. Current recommendations, in regards to the administration of animal health products, are to inject products subcutaneously, if possible, and to administer products in front of the shoulder in order to avoid causing lesions in the more valuable cuts of meat ("Beef Quality Assurance Manual," 2015).

The success of this strategy can be evaluated based on the trends in injection site prevalence over the years. Dexter et al reported a decrease in prevalence of injection site lesions in top sirloin butts between July 1991 (21.3%) and March 1993 (10.9%). Similarly, George et al reported that the prevalence of injection-site lesions in beef top sirloin butts was 10.9% in July 1993, but the prevalence of lesions did not decrease between then and July 1995 (10.2%) (George, Cowman, Tatum, & Smith, 1996). The National Cattlemen's Beef Association continued to fund audits over

the next several years. In 2001, Roeber et al reported the incidence of injection site lesions in beef top sirloin butts over 5 years (Roeber, Cannell, et al., 2001). In March 1995 the incidence was 11.4%, which correlates with the data from George, et al. In contrast to the 1996 George paper and similarly to the 1994 Dexter paper, the prevalence of lesions over those 5 years decreased to 2.1% in July, 2000. The overall decrease in the incidence of injection site lesions in beef top sirloin butts from July 1991 to July 2000 was approximately 90%. The industry has attributed much of this success to the efficacy of the Beef Quality Assurance program and its widespread adoption not only in the feedlot sector but in the cow-calf industry, as well.

The Canadian beef industry has undergone a similar process. The first audit conducted in Canada was published by Van Donkersgoed et al in 1997 and described the results of surveying 5 packing plants in Ontario and Alberta. The prevalence of injection site lesions in top butts from November 1996 to January 1997 was 18.8% (Van Donkersgoed, Dixon, Brand, & VanderKop, 1997). Interestingly, when the authors separated Canadian fed cattle from American fed cattle, they saw a difference in the incidence of lesions. The Canadian fed cattle had an incidence of 22.3% while the American fed cattle had an incidence of 9.0%. Canadian and American beef production practices are similar, but one reason for the difference might be the lag in time between when BQA started in America vs Canada. Canada's Verified Beef Production program started in 1994, whereas the grass roots origin of America's BQA program started nearly a decade prior to that. By spring of 1997, just a few months later, the prevalence of lesions in the top butt of fed cattle in Canada had decreased to 13.3% (Van Donkersgoed, Dixon, & VanderKop, 1998a). The decrease in prevalence may be an artifact of the study rather than the result of change in the industry.

The economic ramifications of this trend are impressive. In 1994, Dexter et al estimated losses to the beef industry in 1991 from injection site lesions in the top butt to be $55 million based on an average lesion size of 141.7 g (Dexter et al., 1994). In 1995 George et al estimated that losses to the beef industry from injection sites in the rounds were around $28 million on an annual basis (George, Morgan, et al., 1995). Using the reduction in incidence of lesions in the top butt over the next few years, the authors calculated that by 1993 the improvement in injection site location and technique saved the industry $26.9 million. If one considers the decrease in prevalence in lesions in the top butt from 1991 until 2000, the savings is estimated at $76 million (Roeber, Cannell, et al., 2001). The Beef Quality Assurance program can certainly be credited with saving the industry money by teaching producers to protect the high value primal cuts.

Beef Quality Assurance has established the importance of earning and keeping the consumers' trust and has spent a lot of time and money researching the demand for consistency among beef consumers ("Beef Quality Assurance Manual," 2015). All stakeholders in the beef industry share a common interest in producing quality beef, from the time a calf is conceived until the product lands on the consumer's plate. Even though Beef Quality Assurance guidelines have traditionally been focused on feedlot steers and heifers, beef consumed in this country comes from all varieties of bovines: dairy cattle and beef cattle, steers and heifers, cows and bulls. Most consumers and even some farmers and veterinarians do not consider dairy and beef cows to be an important part of the beef supply in the country. However, on an annual basis, beef and dairy cows make up 20% of the cattle slaughtered in this country ("National Agricultural Statistics Service," 2015). In 2013, 6.2 million cows were slaughtered for beef ("National Agricultural Statistics Service," 2015). The average dressed weight for cow carcasses in 2013 was 620 pounds. The total pounds of dressed weight from beef and dairy cows slaughtered in the US in 2013 therefore

totaled 3.8 billion pounds. Dairy and beef cows are an important contributing factor to our beef supply, and producers need to structure their management of cows with the final product in mind. Although BQA procedures should also be applied to beef and dairy cows, compliance by producers is in question. Many producers give shots in the back half of the animal despite BQA recommendations (Adams, Olea-Popelka, Grandin, Woerner, & Roman-Muniz, 2014; Aly et al., 2014; Glaze & Chahine, 2009). Part of this lack of compliance may be associated with frequent use of injections to support reproduction. This relationship is explained below.

Reproductive technology

Artificial insemination (AI) has become a common tool in bovine reproductive management. According to the National Animal Health Monitoring System (NAHMS) survey completed in 2007, 72.5% of all dairy operations implement AI. Nearly half of dairy operations in the US do not keep any bulls on the premises (System, 2007b). Estrus detection becomes paramount in order to achieve reproductive success in an AI program. Historically, estrus detection efficiency is nowhere near 100%. The average dairyman only finds 51% of eligible cows in estrus, based on records from 6,800 dairy herds in the US (Stevenson, 2015). Estrus detection can be improved with the implementation of technologies such as pedometers and accelerometers, but farms that use these tools are only able to achieve between 60 and 70% estrus detection (Valenza et al., 2012). The fault does not lie entirely with the dairyman's ability to be vigilant. The modern dairy cow is only in estrus for an average of 6-8 hours (Sveberg et al., 2015) and stands to be mounted an average of 4 times (Palmer, Olmos, Boyle, & Mee, 2010). All of these factors have led to the popularity of synchronization technology. The administration of hormones and/or their analogs such as prostaglandin F2alpha ($PGF_{2\alpha}$) and gonadotropin releasing hormone (GnRH) to synchronize estrus has alleviated the need for estrus detection. Caraviello reported that

87% of 103 large US dairies utilize estrus synchronization and timed AI (TAI) (Caraviello et al., 2006). The NAHMS 2007 dairy survey, which included farms of all sizes, reported that 58% of farms utilized TAI. Larger farms are more likely to utilize estrus synchronization and TAI, which means more animals are being exposed to this technology. Artificial insemination is utilized at a lower frequency in the US beef industry. As of 2007, 7.6% of all beef cow-calf operations use AI, which translates to 12% of heifers and 4% of cows (System, Beef 2007-08 Part II: Reference of Beef Cow-calf Management Practices in the United States, 2007). Commonly, estrus synchronization accompanies AI in the cow-calf setting due to the inherent difficulty of detecting heat in pastured animals. Of interest, 7.9% of operations use synchronization, which is slightly higher than the percentage that use AI (System, 2007a). Occasionally owners will implement synchronization even when a herd bull is being used in order to achieve a higher number of calves born earlier in the calving season (Lamb et al., 2008; Whittier, Caldwell, Anthony, Smith, & Morrow, 1991).

In the cow-calf setting synchronization typically occurs once per year and the average beef cow lives to be 8 years old (Ag 101: Beef Production, 2012). Based on the most popular synchronization protocols (repro task force), a beef cow in a herd where synchronization and TAI are utilized will be given 21 IM injections of $PGF_{2\alpha}$ and GnRH. Dairy cows do not have as long a life span; the average dairy cow lives to be 5 years old but in the dairy setting fertility is lower, thus a cow may be synchronized multiple times per lactation (Ag 101: Dairy Phases, 2012). Given current reproductive management practices, the average dairy cow therefore will be given 23 injections in the muscle over her lifetime from synchronization alone. These hormones should be injected in front of the shoulder to comply with BQA guidelines, thus injection site lesions should not exist in the back half of the cow. Unfortunately, this is not reflected in cow carcass data.

Injection site lesions in beef and dairy cows

Data regarding injection site lesions were first collected in fed steers, but it wasn't long until researchers began investigating the incidence of lesions in cows, as well. In 1994 the first National Non-fed Beef Quality Audit was conducted to assess the quality and consistency of mature cows and bulls sold as culls at market; this data would then serve as a benchmark for similar studies in the future (Roeber, Mies, et al., 2001). The authors looked specifically at the prevalence and cause of quality defects in live animals, carcasses, and offal. Surveillance of injection site lesions was not performed, since this data is not accessible until the carcasses have been processed, but the authors noted a high incidence of bruising, poor body condition, and poor quality grading, indicating that these animals were not managed with the goal of preserving the end beef product. In a subsequent audit, carcass data was collected from multiple packing plants in 1998, 1999, and 2000 (Roeber et al., 2002). Two hundred outside rounds from each plant were selected and sectioned to look for lesions. The overall incidence of injection site lesions was 49% and 26% in dairy and beef cow carcasses, respectively. The incidence decreased over the three year period from 60% to 35% in dairy cow carcasses and from 31% to 20% in beef cow carcasses. These numbers correlate with data from Canada during a similar time period; in outside rounds from cull cows and bulls, the injection site lesion prevalence was 35% (dairy and beef animals were not differentiated) (Van Donkersgoed, Dixon, & Vanderkop, 1998b). As discussed previously, the prevalence of lesions in fed cattle has decreased over time; a similar trend has been noted in non-fed beef. In 2007 the most recent National Market Cow and Bull Beef Quality Audit was conducted and assessed beef quality both in packing plants and in purveying plants. Compared to a similar audit conducted in 1999, retailers reported that injection site blemishes were decreased in both dairy and beef cows (National Market Cow and Bull Beef Quality Audit, 2007). However,

the prevalence of lesions is still alarmingly high. Defects were detected in 33% of bottom rounds which is less than the 60% incidence noted in 1998 but no different from the 35% prevalence noted in 2000. Further surveying would allow better characterization of the lesions in terms of temporal and regional trends.

Surveys regarding management practices pertaining to BQA in cows have shed some light on the source of these lesions. According to a national USDA survey of cow-calf operations in 1997, owners were giving 35% of IM injections in the neck and 78% of SQ injections in the neck (National Animal Health Monitoring and Surveillance Beef Part IV: Changes in the US Beef Cow-Calf Industry, 1993-1997, 1997). Veterinarians gave 50% of IM injections in the neck and 82% of SQ injections in the neck. The survey was repeated in 2007 and the trend showed an increase in BQA compliance: owners gave 65% of IM injections in the neck and 84% of SQ injections in the neck (System, Beef 2007-08 Part II: Reference of Beef Cow-calf Management Practices in the United States, 2007). Veterinarians had also improved in BQA compliance as they gave 77% of IM injections in the neck and 87% of SQ injections in the neck. This trend can likely be attributed to the popularity of the national BQA program in the beef industry. The dairy industry has lagged behind the beef industry in changing injection practices. A survey of California dairy farms in 1999 identified that although almost all dairymen agreed that safety of meat and milk leaving their farm was important, only about 25% of dairymen had a written quality assurance plan (Payne, Bruhn, Reed, Scearce, & O'Donnell, 1999). Sixty percent of dairymen said that they would be willing to adopt a program initiated by producers and most indicated that they would prefer the program to be managed by the milk processor, or creamery. At the time of that study, the industry had not yet developed a comprehensive nationwide dairy beef quality assurance program tailored to dairy farms, but in 2009, the Dairy Herd Improvement Association and the National BQA

Program collaborated to start the Dairy Animal Care and Quality Assurance program. In a 2005 survey, Pennsylvania dairy farmers reported that most injections were not, in fact, given according to BQA recommendations (Tozer, Varga, Henning, & Holden, 2005). Only 13% of estrus synchronization products were given in the neck; the rest were given in the back half of the cow. Dairy producers administered a vaccine for mastitis caused by coliform bacteria in the neck 28% of the time but other vaccines were administered in the neck only 17% of the time. Of the farms surveyed, 38% reported that no injections were given in the neck, and only 1.5% of farms reported that all injections were given in the neck. BQA compliance may have been low because there was no national program to promote BQA on dairy farms at the time the study was completed. However, even with a national program to raise awareness, dairymen are still reluctant to adopt the practice of giving all injections in the neck. Owners and herdsmen from Colorado dairies were questioned in 2013 about their preferred and actual injection locations (Adams et al., 2014). Although 11/15 owners stated that the neck was their preferred site for IM injections, 12/15 stated that they actually gave the injection either in the neck or hindquarters; only 3/15 owners claimed to always give IM injections in the neck. Owners also ranked traits of a new handling facility in order of importance. Efficiency, animal safety, human safety, ease of animal handling, and ease of operation were all ranked as more important than BQA compliance. The authors concluded that one reason dairymen might be so unconcerned with dairy BQA is a lack of economic incentive since income from cull cows makes up less than 5% of the revenue on a dairy.

Another possible explanation for producers deviating from BQA recommendations has to do with the facilities in which the animals are housed and treated. As mentioned above, ease of BQA compliance is not high on the priority list when new dairy facilities are being designed. It is not difficult for producers in either the beef or dairy industry to follow injection guidelines on

individual cattle when appropriate restraint is used, namely a head catch. However, many times vaccines and reproductive hormones are not administered on an individual basis, but rather to cohorts of animals. On dairy farms, headlocks are commonly used as a management tool for restraining entire pens of cattle simultaneously. Headlocks restrain the head and allow access to the rear of the animal for reproductive purposes but significantly limit access to the neck. California dairy producers surveyed in 2011 associated restraining a cow using a stanchion (headlock) or behind a gate and approaching a cow from the front (as when they are in headlocks) with increased difficulty of giving injections in the neck region (Aly et al., 2014). On beef farms, large numbers of animals are often worked through a chute, which allows for administration of products to animals one at a time in a setting where the head is restrained. This setup goes a long way in facilitating BQA compliance. A survey completed in 2004 and published in 2008 includes data regarding injections given to calves as well as cows on Idaho dairies (Glaze & Chahine, 2009). The authors report that for cows 64-87% of IM injections and 75-87% of SQ injections are given in the neck. The numbers are similar for calves: 59-61% of IM injections and 78-81% of SQ injections are given in the neck. Fifty-one percent of California dairy producers surveyed in 2011 stated that they actually preferred IM to SQ as an injection route (Aly et al., 2014); dairymen are far from making a concerted effort to comply with BQA standards. The most recent NAHMS survey on dairy heifer management included information regarding the route of administration of various injectable products and the facilities which were used to accomplish those treatments but did not include any information regarding the location of the injections and whether or not BQA guidelines were observed (National Animal Health Monitoring and Surveillance Dairy: Heifer Calf Health and Management Practices, 2007). This in and of itself speaks for the gap between the beef industry and the dairy industry when it comes to adopting BQA practices.

In conclusion, the beef industry has improved carcass quality and consistency, namely through the efforts of the Beef Quality Assurance program. However, beef quality of non-fed cattle, namely dairy cows and beef cows, still lags behind that of fed steers and heifers. According to a BQA audit conducted in 2007, 6% of hanging beef carcasses and 11% of hanging dairy carcasses had injection site lesions that were prominent enough to notice in the packing plant(Association, 2007). Two percent of dairy cows had lesions so large that an entire primal was lost. Despite the fact that injection lesions in cows contribute $15 in losses per head or $93 million per year for the beef industry in the US, dairymen and cow-calf producers may not be motivated to change their injection practices in cows due to the limitations of current facilities or handling systems. Producers find it convenient to administer reproductive hormones, vaccines, and even antibiotics in the rear legs of both dairy and beef cows and will continue to do so unless a solution can be found.

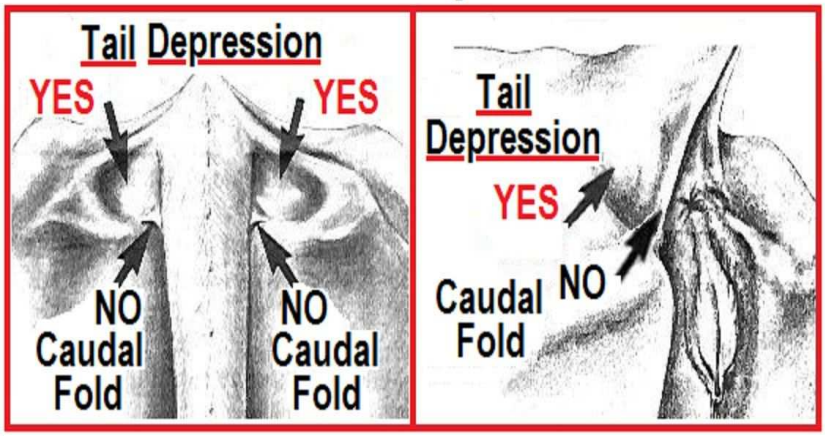
Provision of an alternate injection site that can be accessed from behind the cow may improve BQA compliance. A novel site for SQ administration is the ischiorectal fossa (IRF) located next to the tailhead, craniodorsal and medial to the tuber ischii. Given that many cows are given injections from someone standing at their rear, this site provides a convenient alternative to the muscles of the back leg. Currently, Posilac® (recombinant bovine somatotropin) is the only commercial product in the United States labeled for administration in the IRF, but the potential exists for other products to be given in this location without damaging the carcass.

Anatomy of the Ischiorectal Fossa

The ischiorectal fossa is located medial to the tuber ischii and lateral to the tailhead. The craniodorsal border is formed by the ligament of the tailhead (Figure 2.1). Tissue composition in

this location varies with overall condition of the animal; in more heavily conditioned cows, there is more fat found in this location (Isensee et al., 2014), which may affect drug absorption.

Figure 2.1 **Potential SQ Injection Site**



Griffin, D. Personal correspondence, 2014. Used with permission.

Both dairy and beef producers use $PGF_{2\alpha}$ to manipulate the estrous cycle of cows. These producers commonly give this compound in the muscles of the hind leg even though intramuscular injections of $PGF_{2\alpha}$ are known to cause significant muscle damage (Fajt, Wagner, Pederson, & Norby, 2011). Producers may be able to give this drug in the ischiorectal fossa rather than the muscle, thus sparing the rounds of injection site lesions. The following sections explain the role of $PGF_{2\alpha}$ in the beef and dairy industry.

Physiologic Luteolysis

In general, prostaglandins act on smooth muscle to affect blood flow (Inskeep, 1973). Scientists discovered the specific role that $PGF_{2\alpha}$ plays in the estrous cycle almost 100 years ago. In 1923, Loeb discovered that the corpora lutea of guinea pigs failed to regress after hysterectomy, demonstrating that the luteolytic agent was coming from the uterus (Loeb, 1923). In the late

1960's, $PGF_{2\alpha}$ was identified as the luteolytic agent in rats (Pharriss & Wyngarden, 1969). In the early 1970's $PGF_{2\alpha}$ was shown to be luteolytic in cattle (J.W. Lauderdale, 1972) (Liehr, G.B., & Olson, 1972) (T.M. Louis, Hafs, & Morrow, 1972) (Rowson, Tervit, & Brand, 1972) and sheep (McCracken et al., 1972). Since then, researchers have studied the natural release of this prostaglandin, a 20-carbon fatty acid with two double bonds and three hydroxyl groups, from the endometrium of the uterus. Spontaneous physiologic luteolysis involves multiple signaling pathways. After ovulation, the corpus luteum forms and releases progesterone. Although progesterone generally suppresses the release of $PGF_{2\alpha}$ by the endometrium, 10-14 days of exposure to progesterone sensitizes the endometrium to release $PGF_{2\alpha}$ in response to oxytocin (Silvia, Lewis, McCracken, Thatcher, & Wilson, 1991). The release of $PGF_{2\alpha}$ from the endometrium starts a cascade of events that eventually results in the regression of the corpus luteum on the ovary and the end of diestrus. This release happens in a pulsatile fashion in ruminants (Barcikowski, Carlson, Wilson, & McCracken, 1974; Kindahl, Edqvist, Granstrom, & Bane, 1976; Nancarrow et al., 1973; Peterson, Fairclough, Payne, & Smith, 1975). The length of each pulse and the number of pulses vary with species but in the bovine these pulses occur every 6-8 hours and can last for 24 hours. (Ginther, Rodrigues, Ferreira, Araujo, & Beg, 2008; Silvia et al., 1991). The initiation of the cascade begins when estradiol from the preovulatory follicle causes pulses of oxytocin to be released from the posterior pituitary gland (McCracken, Custer, Eldering, & Robinson, 1996). The endometrium releases a pulse of $PGF_{2\alpha}$ in response, which enters the utero-ovarian vein by way of a countercurrent exchange mechanism and then travels to the ovary (Ginther, 1974). This allows $PGF_{2\alpha}$ to avoid passing through the lungs where it is metabolized (Piper, Vane, & Wyllie, 1970). Most of the receptors for $PGF_{2\alpha}$ in the corpus luteum are concentrated in large steroidogenic luteal cells (Braden, Gamboni, & Niswender, 1988). The

binding of $PGF_{2\alpha}$ to these receptors stimulates the corpus luteum itself to release oxytocin. The result is a positive feedback loop in which $PGF_{2\alpha}$ and oxytocin build on each other. The mechanism by which the pulses occur is hypothesized to be a period of refractoriness during which the uterus ceases to respond to oxytocin by releasing $PGF_{2\alpha}$ (McCracken et al., 1996). Also in response to the presence of $PGF_{2\alpha}$, luteal blood flow decreases, causing a decline in the steroidogenic activity of the large luteal cells; in the first 36 hours after the release of $PGF_{2\alpha}$, serum progesterone concentrations drop precipitously and these cells decrease in size but not in number (Braden et al., 1988). Apoptosis of the large luteal cells occurs after the decline in serum progesterone concentration has occurred. Once progesterone concentrations have subsided, the release of estradiol is no longer inhibited and estrus occurs in 2-5 days.

Iatrogenic Luteolysis

Since the discovery of $PGF_{2\alpha}$, analogs such as dinoprost, cloprostenol, and fenprostalene have been used to induce luteolysis and manipulate the bovine estrous cycle (Cooper, 1974; Deletang, 1975; Inskeep, 1973; J. W. Lauderdale et al., 1974; Roche, 1976; Tervit, Rowson, & Brand, 1973). The development of artificial insemination techniques in the early 1920's precipitated the need for ways to control the timing of ovulation in the bovine. Progesterone was discovered around the same time as $PGF_{2\alpha}$ and prolonged administration and subsequent removal of this hormone sometimes led to ovulation; however, the variation in time to the event was so variable that the usefulness of this progesterone by itself was quite limited (J.W. Lauderdale, 2007). The discovery of $PGF_{2\alpha}$ proved to be the answer to the problem of variability in the time to estrus. Lauderdale determined that shortening the length of the cycle by administering exogenous $PGF_{2\alpha}$ did not affect fertility (J. W. Lauderdale et al., 1974). The dose, route, and duration of administration of these analogues used by researchers varied initially. In the first study

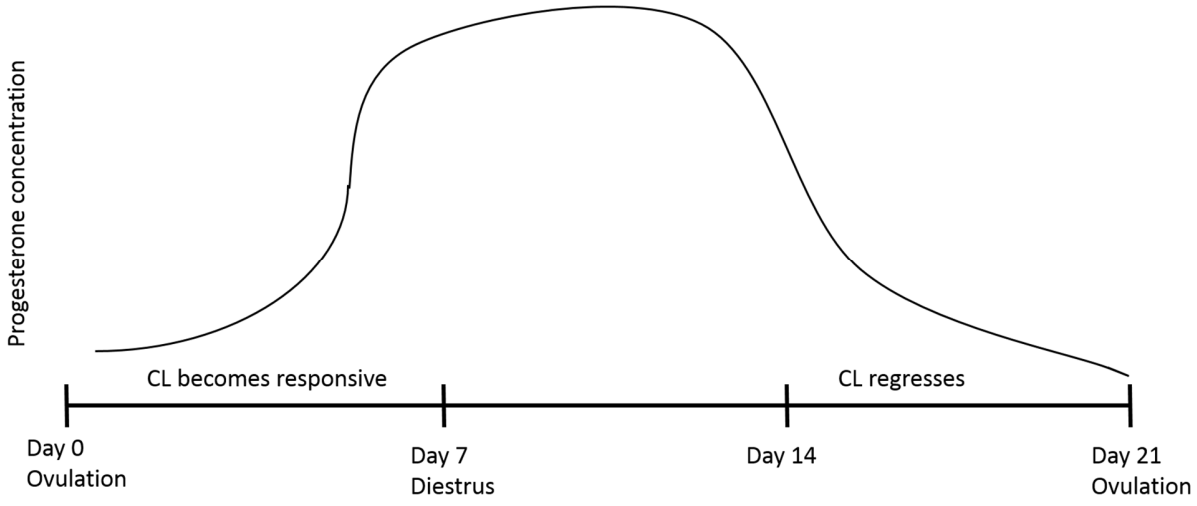
published on the administration of exogenous $PGF_{2\alpha}$, the compound was placed directly in the uterine horn via the cervix (Rowson et al., 1972). In this study, 0.5 mg of $PGF_{2\alpha}$ (rather than an analogue) was administered at various times during the cycle and was either given once or on two consecutive days. Administration of 2 doses during days 5-16 of the estrous cycle was effective in "almost every case" while the same treatment on days 1-4 was ineffective. The authors report that a single dose gave intermediate results. As discussed above, the uterus is unable to participate in the positive feedback loop with oxytocin until prolonged exposure to progesterone has occurred. On days 1-4 of the cycle one would expect the administration of $PGF_{2\alpha}$ to be insufficient for complete luteolysis because prolonged exposure to progesterone has not occurred yet and the corpus hemorrhagicum is still transitioning to a corpus luteum. Further, two consecutive doses compared to a single dose would more closely mimic the release of endogenous $PGF_{2\alpha}$ and would be more effective in jumpstarting the oxytocin- $PGF_{2\alpha}$ cascade of events between the ovary and uterus. According to the authors, synchronization resulting from 2 consecutive doses later in the cycle was "very exact, most animals showing [estrus] on the morning of the 3rd day after treatment."

Synchronization with $PGF_{2\alpha}$

Several other authors reported the same pattern as Rowson: $PGF_{2\alpha}$ given in the first 4 or 5 days after estrus may result in a temporary decline in progesterone but it does not result in complete luteolysis (Inskeep, 1973; J.W. Lauderdale, 1972; Liehr, Marion, & Olson, 1972; T. M. Louis, Hafs, & Seguin, 1973). Lauderdale reported that fertility was not different between cows who were inseminated based on heat detection after $PGF_{2\alpha}$ administration and cows who were inseminated twice at 72 and 90 hrs after $PGF_{2\alpha}$ administration (J. W. Lauderdale et al., 1974). However, cows were only enrolled in the study if they had a palpable corpus luteum. Therefore,

cows who were still transitioning from estrus to diestrus and who would not have responded to the $PGF_{2\alpha}$ may have been excluded. This refractory period of the cycle where a bovine female is unable to respond to a dose of prostaglandin was seen as a significant impediment to using this compound to synchronize large groups of animals; Inskeep proposed a two dose regimen where the first dose might be given to all animals and that a second dose could be given to any animal that did not respond to the first dose (Inskeep, 1973). King and Robertson developed a two injection protocol with 10 days between injections (King & Robertson, 1974). They hypothesized that any animals that did not respond to the first dose because they were in days 0-5 of their cycle would be able to respond 10 days later and that any animals that responded to the first dose would also respond to the second dose as by that time they would be in diestrus again, as shown in Figure 2.2.

Figure 2.2: Progesterone concentration and diestrus during the bovine estrous cycle



Thirty Holstein heifers were injected on day 0 and 13/30 came into estrus after the first injection. All thirty animals were injected again on day 10 and 25/30 came into estrus. Graves also used 10 days between doses and found similar results: 27/29 cows were found to be in estrus

after the second dose of $PGF_{2\alpha}$ (Graves et al., 1974). This protocol became widely accepted and is still used today as a means for pre-synchronization prior to more advanced protocols for timed AI (Moreira et al., 2001).

Dose and Route of Administration of $PGF_{2\alpha}$

To review, Rowson et al reported that consecutive doses were more effective than one single dose at affecting luteolysis (Rowson et al., 1972). Other studies have confirmed this finding (J.W. Lauderdale, 1972; Nancarrow, Hearnshaw, Mattner, Connell, & Restall, 1974; Tervit et al., 1973). Initially, $PGF_{2\alpha}$ was administered solely into the uterus in microgram doses; researchers investigated different doses over different time periods in the horn both ipsilateral and contralateral to the CL (Inskeep, 1973; Liehr, G.B., et al., 1972; T.M. Louis et al., 1972). Almost immediately, however, scientists began experimenting with intramuscular and subcutaneous routes of administration and found those methods to be satisfactory (J.W. Lauderdale, 1972). Several authors noted that intrauterine administration was often very difficult and that subcutaneous administration led to more consistent results (Hearnshaw, Mattner, Nancarrow, & Restall, 1974). Certain analogs were found to be more potent than others and choice of analogue as well as route of administration (intrauterine vs intramuscular or subcutaneous) and day of the cycle affect the dose needed to achieve successful luteolysis (Tervit et al., 1973). As long as the dose was modulated, intramuscular or subcutaneous administration was just as effective as intrauterine administration and was more convenient (Nancarrow et al., 1974). Several researchers injected $PGF_{2\alpha}$ in the mucosa of the vulva or vagina and found the route to be effective but time-consuming (Chauhan, Mgongo, Kessy, & Gombe, 1986; Holy, 1984).

$PGF_{2\alpha}$ is metabolized in the lungs and has a very short half-life (on the order of minutes) (Shrestha, Beg, Burnette, & Ginther, 2012). Given that physiologic luteolysis occurs in a pulsatile

fashion, Peters attempted to lengthen the exposure time by delivering the compound using a mini osmotic pump which was implanted subcutaneously (Peters, 1984). The pump was compared to a single injection and the combination of a single injection and the pump. Injection by pump by itself failed to induce luteolysis so the technology was not widely adopted. For the most part, a single injection has been found to be sufficient for inducing luteolysis during diestrus and multiple injections at narrow intervals are not considered practical relative to production management. However, researchers have developed a novel synchronization protocol, the 5-Day CO-Synch + CIDR, and have reported that 50 mg of dinoprost (2x the labeled dose) increased pregnancy rate to TAI compared to 25 mg, although there was no difference between giving 25 mg twice, 8 hours apart, or 50 mg once (Bridges et al., 2012).

In 1975, Stellflug et al. took on the task of determining the IM dose of $PGF_{2\alpha}$-tham salt, later named dinoprost (Stellflug, Louis, Hafs, & Seguin, 1975). Based on previous success of using a 30 mg dose (J.W. Lauderdale, 1972; J.W. Lauderdale, Chenault, Sequin, & Thatcher, 1973), dinoprost was administered as two 15 mg doses with a 6 hour interval, a single 30 mg dose, and a single 60 mg dose IM to heifers 6-11 days post estrus. No differences were found in decline in progesterone, LH surge, onset of estrus, or interval to ovulation. Kimball et al., investigated 14 $PGF_{2\alpha}$ analogues and reported that the binding affinities were similar, less than, or greater than that of naturally occurring $PGF_{2\alpha}$ with dose ranges between 5 mg and 25 mg (Kimball, Lauderdale, Nelson, & Jackson, 1976). The accepted dose for $PGF_{2\alpha}$–tham salt was determined to be 25 mg by Lauderdale at the Upjohn Company using the Walker-Carmer technique of evaluation (J.W. Lauderdale, 1979). Around the same time, other analogues such as cloprostenol and fenprostalene were approved for use in cattle. Researchers compared these products head to head and found no difference in the luteolytic effects of any of the analogues at their labeled doses (Halbert, Leslie,

Walton, & Betteridge, 1989; Schams & Karg, 1982). Desaulniers reported that intervals to estrus were similar among dinoprost, cloprostenol, or fenprostalene given during the normal estrous cycle or superovulation (Desaulniers, Guay, & Vaillancourt, 1990). However, the authors noted a tendency for delayed luteolysis (>11d) when fenprostalene was given after embryo collection, compared to the other analogues. One possible explanation for the difference is the route of administration; fenprostalene is labelled for SQ administration while dinoprost and cloprostenol are labeled for IM administration.

Both IM and SQ administration of $PGF_{2\alpha}$ have been investigated separately but rarely in the same study. Edqvist et al gave $PGF_{2\alpha}$–tham salt either IM or SQ to 16 heifers in diestrus, followed the decline in progesterone, palpated ovaries, and observed for signs of estrus (Edqvist, Settergren, & Astrom, 1975). Although this study had a limited sample size, all heifers responded by ovulating and displaying signs of estrus. For one analogue, fenprostalene, the half-life for SQ administration was 3-6 hours longer than for IM administration (Tomlinson, Spires, & Kent, 1984). This study was primarily focused on tissue residues so luteolytic efficacy was not measured. To this author's knowledge, no robust studies comparing SQ and IM administration of the same $PGF_{2\alpha}$ analogue exist.

In conclusion, $PGF_{2\alpha}$ analogues approved for use in this country are labeled for intramuscular (IM) injection and are almost exclusively given in this manner in order to synchronize estrus. Several researchers have attempted to lower the dose of prostaglandin by giving it in the vaginal mucosa or directly into the uterine horn, but these techniques were judged to be too time-consuming and impractical and have, for the most part, been abandoned in favor of IM administration. Thus, administration of $PGF_{2\alpha}$ continues to be almost exclusively IM.

The Ischiorectal Fossa (IRF) as a potential injection site

Several research groups have investigated the IRF as an alternate site to give Lutalyse® and Estrumate®, analogs of $PGF_{2\alpha}$ that are labeled for IM administration. This product proves to be a suitable drug to use for proof of concept given that the physiological outcome variable (luteolysis) is reliably and accurately detectable. Colazo et al. conducted several pilot studies regarding the feasibility of this injection location. In the first of three experiments, 21 dry, non-pregnant Holstein cows were given 100 ug of GnRH followed 7 days later by 25 mg dinoprost in the IRF (Colazo, Martinez, Kastelic, Mapletoft, & Carruthers, 2002). Blood samples were collected at 0, 24, 48, and 72 hours post injection. Behavioral responses to the injections of dinoprost were coded from 0 to 3 and at each blood sample collection the injection site in the IRF was assessed both visually and by palpation. Sixteen of the 21 cows were determined to have a functional corpus luteum at the time of dinoprost injection based on a serum progesterone concentration of greater than 1 ng/mL. The average serum progesterone concentration of those cows at 24 hours post-injection was 0.4 ng/mL (range 0.1-0.6) and therefore luteolysis was achieved in all of the cows with a corpus luteum. The authors did not detect any injection site lesions and minimal behavioral responses to injections (average score 0.4). In a second experiment, 74 yearling Holstein heifers were injected with 25 mg of dinoprost in the IRF. Serum progesterone was measured at time of injection and 24-30 hours post injection to determine corpus luteum status and response. If a heifer was detected in estrus within 5 days post-injection, she was either artificially inseminated or exposed to a bull. Pregnancy was diagnosed via rectal palpation at 45-60 days post breeding. Fifty-one heifers had a functional corpus luteum at time of injection. Eighty-four percent (43/51) had a serum progesterone concentration of less than 1 ng/mL at 24-30

hours post injection; the other eight heifers had a concentration of greater than 1 ng/mL at 24-30 hours. Of the 43 heifers with low progesterone at 24 hours, 29 were found to be in estrus. Of the 8 heifers with high progesterone at 24 hours, 5 were found to be estrus. A total of thirty four heifers were therefore bred. The palpation pregnancy rate at 45-60 days was 61.8% (21/34). This injection location is therefore feasible to use for dinoprost; not only do females treated in this manner lyse their corpora lutea, but they subsequently ovulate fertile oocytes and become pregnant when inseminated. An important point to consider is the definition of luteolysis. Historically, literature pertaining to ovarian physiology has used a cut point of either 0.5 or 1 ng/mL for serum progesterone to define the presence of a functional corpus luteum. This number is largely arbitrary (personal communication, John Chenault). The authors also looked at dose and route effects on the efficacy of dinoprost. Forty-eight two-year old heifers were synchronized to ovulate the same day using a CIDR, progesterone injection, and estradiol injection. On day 7 after ovulation, heifers were allocated to 5 different treatment groups and given one of the following treatments of dinoprost: 25 mg IM (gluteal), 25 mg IRF, 10 mg IRF, 10 mg SQ (cervical), and 10 mg intravulvar-submucosal (IVSM). Ovulation was monitored via behavior estrus and ultrasound exams at 12 hour intervals for 6 days. Blood was collected daily for serum progesterone. All heifers given 10 mg of dinoprost either IM (n=9) or in the IRF (n=10) ovulated. Five out of 10 heifers given 25 mg dinoprost in the IRF ovulated, 8 out of 10 heifers given 25 mg of dinoprost IM ovulated, and 1 out of 9 heifers given 25 mg dinoprost in the IVSM ovulated. Ovulation rates for all treatment groups were found to be different from each other with the exception of 10 mg IM and 10 mg IRF, however the biological significance of these differences is unclear. A lower dose of dinoprost should not result in a higher percentage of successful luteolysis. The difference may be an artifact of the small sample size in this study.

Colazo et al evaluated response to closprostenol, a synthetic analog of $PGF_{2\alpha}$, administered either subcutaneously (n=9) or intramuscularly (n=9) in beef heifers determined to have a CL via ultrasonography, (Colazo, Martinez, Kastelic, & Mapletoft, 2002). Heifers were monitored for behavioral estrus and for ovulation (via ultrasonography) every 12 hours. Blood samples were also collected for serum progesterone concentrations prior to treatment and at each exam. A difference in initial CL diameter, serum progesterone concentration, or follicle size at ovulation between treatment groups was not detected. However, heifers given cloprostenol SQ came into estrus later (75 hours) than heifers given cloprostenol IM (59 hours). Despite this finding, there was no difference between treatment groups in time to actual ovulation (106.5 hours), or in the proportion of heifers that ovulated or were found to be in estrus. The results of this study should be interpreted relative to the small sample size and thus limited power to detect a biologically relevant difference in ovulation time. The authors commented that time to ovulation is affected by follicular status at the time of cloprostenol treatment but did not address the fact that time to behavioral estrus was different between treatment groups and time to ovulation was not. The potential difference becomes concerning in the setting of heat detection. If heifers given cloprostenol SQ displayed estrus later but ovulated at the same time as heifers given cloprostenol IM, then the interval from estrus to ovulation was shorter for SQ heifers, which could impact fertility when breeding based on estrus detection. A breeder might need to modify the interval between observed estrus and breeding. Solid conclusions cannot be drawn from this data set alone. The study ought to be repeated to determine if the outcome is repeatable. If so, the mechanism for increased interval to behavioral estrus needs to be elucidated.

The second part of this study used similar treatment groups to the previous experiment. This time, heifers were synchronized with a CIDR, injectable progesterone, and estradiol. Day 0

was determined to be the day of ovulation and the end of synchronization. On day 7, heifers were given cloprostenol either IM (n=9) or SQ (n=9). Heifers were observed for estrus and blood samples were taken for serum progesterone concentration every 12 hours. Blood samples were taken for serum LH concentration every 4 hours between 48 to 96 hours. There were no differences in time to estrus, LH surge, or ovulation between treatment groups, but small sample sizes mean that the differences would have to be very large in order to detect them.

Finally, Chebel et al. compared the efficacy of different routes for dinoprost in lactating Holstein cows (Chebel, Santos, Rutigliano, & Cerri, 2007). In the first part of the study, cows at different stages of lactation were given $PGF_{2\alpha}$ IM 14 days before treatment. On the day of treatment, cows were assigned to be given dinoprost IM in the rear leg (n=39), SQ in the neck (n=40), or in the IRF (n=39). Blood samples for serum progesterone concentration were taken at 0, 12, 24, 36, and 48 hr post treatment. Only cows with serum progesterone greater than 1 ng/mL at time 0 were included in the analysis. No differences were detected among treatment groups in the proportion of cows experiencing luteolysis (serum progesterone < 1 ng/mL or less than 40% of initial concentration) by 12, 24, 36, and 48 hrs post treatment. This data set is slightly more robust than the previous studies described and is the first instance of the IRF being validated in lactating dairy cows. The second part of the study is even more robust but includes less data points. Cows 39-53 days in milk were given $PGF_{2\alpha}$ IM 14 days before treatment. On the day of treatment, cows were assigned to receive dinoprost IM in the rear leg (n=186) or in the IRF (n=193). Blood samples for serum progesterone concentration were taken at 0 and 48 hr post treatment. There were 153/186 cows in the control group and 157/193 in the IRF group with a progesterone concentration > 1 ng/mL at 0 hours and therefore included in the analysis. Days in milk, milk production, and body condition score did not vary between groups. No difference was found

between the proportion of control cows and the proportion of IRF cows experiencing luteolysis at 48 hours (86.3% and 86.6%, respectively) (Chebel et al., 2007). Thus, researchers have established the viability of the IRF as a possible injection location for $PGF_{2\alpha}$.

Conclusion

To date, researchers have documented enough evidence to warrant further investigation of the IRF as a novel site of administration in the caudal part of the bovine for hormones, vaccines, and antibiotics as an alternative to the muscles of the rear legs. However, many parameters need to be determined. The tissue types in this region and how they vary with differing body condition scores needs to be established. The timing of shots given in synchronization protocols is optimized to achieve the maximum response and conception rate. If the majority of the drug is deposited in adipose tissues, absorption may be slower and luteolysis may be delayed. Understanding the dynamics of progesterone concentrations after administration of $PGF_{2\alpha}$ in the IRF will be essential to implementing this technique in existing synchronization protocols. If the bioequivalence of this route of injection with IM administration can be established, this technique can be implemented confidently on farms where BQA compliance is low without affecting production. The Dairy Animal Care and Quality Assurance program already has standards in place, and utilization of the IRF in place of IM injections in the rear legs could easily be added to the list of recommendations.

Half of California dairymen surveyed in 2014 stated that the personnel on their farms received no education regarding Dairy Animal Care and Quality Assurance (Aly et al., 2014). For those who had received information regarding BQA recommendation, veterinarians were the most common source. The dairy industry and veterinary profession need to work together to instill the standards set by the DACQA program, standards necessary to improve dairy beef quality assurance.

Literature Cited

Adams, A. E., Olea-Popelka, F. J., Grandin, T., Woerner, D. R., & Roman-Muniz, I. N. (2014). Dairy cow handling facilities and the perception of Beef Quality Assurance on Colorado dairies. *J Dairy Sci, 97*(2), 798-804. doi: 10.3168/jds.2013-7146

Aly, S. S., Rossow, H. A., Acetoze, G., Lehenbauer, T. W., Payne, M., Meyer, D., . . . Hoar, B. (2014). Survey of beef quality assurance on California dairies. *J Dairy Sci, 97*(3), 1348-1357. doi: 10.3168/jds.2013-6856

Apley, M., Wray, M., & Armstrong, D. (1994). Subcutaneous Injection Site Comparison of Two Multiple Valent Clostridial Bacterin/Toxoids in Feedlot Cattle. *Agri-Practice*, 9-12.

Association, N. C. s. B. (2007). National Market Cow and Bull Beef Quality Audit. Centennial, CO.

Barcikowski, B., Carlson, J. C., Wilson, L., & McCracken, J. A. (1974). The effect of endogenous and exogenous estradiol-17beta on the release of prostaglandin F2alpha from the ovine uterus. *Endocrinology, 95*(5), 1340-1349. doi: 10.1210/endo-95-5-1340

Beef Quality Assurance Manual. (2015). *Beef Quality Assurance*. http://www.bqa.org/CMDocs/bqa/NationalManual.pdf

Braden, T. D., Gamboni, F., & Niswender, G. D. (1988). Effects of prostaglandin F2 alpha-induced luteolysis on the populations of cells in the ovine corpus luteum. *Biol Reprod, 39*(2), 245-253.

Bridges, G. A., Ahola, J. K., Brauner, C., Cruppe, L. H., Currin, J. C., Day, M. L., . . . Whittier, W. D. (2012). Determination of the appropriate delivery of prostaglandin F2alpha in the five-day CO-Synch + controlled intravaginal drug release protocol in suckled beef cows. *J Anim Sci, 90*(13), 4814-4822. doi: 10.2527/jas.2011-4880

Caraviello, D. Z., Weigel, K. A., Fricke, P. M., Wiltbank, M. C., Florent, M. J., Cook, N. B., . . . Rawson, C. L. (2006). Survey of management practices on reproductive performance of dairy cattle on large US commercial farms. *J Dairy Sci, 89*(12), 4723-4735. doi: 10.3168/jds.S0022-0302(06)72522-X

Chauhan, F. S., Mgongo, F. O., Kessy, B. M., & Gombe, S. (1986). Effects of intravulvo-submucosal cloprostenol injections on hormonal profiles and fertility in subestrous cattle. *Theriogenology, 26*(1), 69-75.

Chebel, R. C., Santos, J. E., Rutigliano, H. M., & Cerri, R. L. (2007). Efficacy of an injection of dinoprost tromethamine when given subcutaneously on luteal regression in lactating Holstein cows. *Theriogenology, 67*(3), 590-597. doi: 10.1016/j.theriogenology.2006.09.007

Colazo, M. G., Martinez, M. F., Kastelic, J. P., & Mapletoft, R. J. (2002). Effects of dose and route of administration of cloprostenol on luteolysis, estrus and ovulation in beef heifers. *Anim Reprod Sci, 72*(1-2), 47-62.

Colazo, M. G., Martinez, M. F., Kastelic, J. P., Mapletoft, R. J., & Carruthers, T. D. (2002). The ischiorectal fossa: an alternative route for the administration of prostaglandin in cattle. *Can Vet J, 43*(7), 535-541.

Cooper, M. J. (1974). Control of oestrous cycles of heifers with a synthetic prostaglandin analogue. *Vet Rec, 95*(10), 200-203.

Deletang, F. (1975). Synchronisation of oestrus in cattle using a progestagen (SC 21,009) and a synthetic analogue of prostaglandin F2alpha (cloprostenol). *Vet Rec, 97*(23), 453-454.

Desaulniers, D. M., Guay, P., & Vaillancourt, D. (1990). Estrus induction with prostaglandin F2alpha, cloprostenol or fenprostalene during the normal estrous cycle, superovulation and after embryo collection. *Theriogenology, 34*(4), 667-682.

Dexter, D. R., Cowman, G. L., Morgan, J. B., Clayton, R. P., Tatum, J. D., Sofos, J. N., . . . Smith, G. C. (1994). Incidence of injection-site blemishes in beef top sirloin butts. *J Anim Sci, 72*(4), 824-827.

Edqvist, L. E., Settergren, I., & Astrom, G. (1975). Peripheral plasma levels of progesterone and fertility after prostaglandin-2alpha induced oestrus in heifers. *Cornell Vet, 65*(1), 120-131.

Fajt, V. R., Wagner, S. A., Pederson, L. L., & Norby, B. (2011). The effect of intramuscular injection of dinoprost or gonadotropin-releasing hormone in dairy cows on beef quality. *J Anim Sci, 89*(6), 1939-1943. doi: 10.2527/jas.2010-2923

George, M. H., Cowman, G. L., Tatum, J. D., & Smith, G. C. (1996). Incidence and sensory evaluation of injection-site lesions in beef top sirloin butts. *J Anim Sci, 74*(9), 2095-2103.

George, M. H., Heinrich, P. E., Dexter, D. R., Morgan, J. B., Odde, K. G., Glock, R. D., . . . Smith, G. C. (1995). Injection-site lesions in carcasses of cattle receiving injections at branding and at weaning. *J Anim Sci, 73*(11), 3235-3240.

George, M. H., Morgan, J. B., Glock, R. D., Tatum, J. D., Schmidt, G. R., Sofos, J. N., . . . Smith, G. C. (1995). Injection-site lesions: incidence, tissue histology, collagen concentration, and muscle tenderness in beef rounds. *J Anim Sci, 73*(12), 3510-3518.

Ginther, O. J. (1974). Internal regulation of physiological processes through local venoarterial pathways: a review. *J Anim Sci, 39*(3), 550-564.

Ginther, O. J., Rodrigues, B. L., Ferreira, J. C., Araujo, R. R., & Beg, M. A. (2008). Characterisation of pulses of 13,14-dihydro-15-keto-PGF2alpha (PGFM) and relationships between PGFM pulses and luteal blood flow before, during, and after luteolysis in mares. *Reprod Fertil Dev, 20*(6), 684-693.

Glaze, J. B., Jr., & Chahine, M. (2009). Assessment of management and basic beef quality assurance practices on Idaho dairies. *J Dairy Sci, 92*(3), 1265-1271. doi: 10.3168/jds.2008-1194

Graves, N. W., Short, R. E., Randel, R. D., Bellows, R. A., Kaltenbach, C. C., & Dunn, T. G. (1974). *Estrus and pregnancy following MAP, PGF2a, and GnRH.* Paper presented at the Proceedings: the American Society of Animal Science.

Halbert, G. W., Leslie, K. E., Walton, J. S., & Betteridge, K. J. (1989). *Evaluation of return to estrus in superovulated dairy heifers following prostaglandin treatment.* Paper presented at the Proceedings: Theriogenology.

Hearnshaw, H., Mattner, P. E., Nancarrow, C. D., & Restall, B. J. (1974). Proceedings: The effect of the mode of administration of prostaglandin F 2alpha on the synchronization of oestrus in cattle. *J Reprod Fertil, 36*(2), 486-487.

Holy, L. (1984). [Submucosal vestibulo-vaginal administration of cloprostenol (Oestrophan-Spofa) in cows in relation to estrus activity, conception, and the level of progesterone in milk]. *Vet Med (Praha), 29*(9), 513-519.

Inskeep, E. K. (1973). Potential uses of prostaglandins in control of reproductive cycles of domestic animals. *J Anim Sci, 36*(6), 1149-1157.

Isensee, A., Leiber, F., Bieber, A., Spengler, A., Ivemeyer, S., Maurer, V., & Klocke, P. (2014). Comparison of a classical with a highly formularized body condition scoring system for dairy cattle. *Animal, 8*(12), 1971-1977. doi: 10.1017/S1751731114001888

Ishmael, W. (2014, December). Beef Demand Shines. *Beef Magazine,* 10.

Kimball, F. A., Lauderdale, J. W., Nelson, N. A., & Jackson, R. W. (1976). Comparison of luteolytic effectiveness of several prostaglandin analogs in heifers and relative binding affinity for bovine luteal prostaglandin binding sites. *Prostaglandins, 12*(6), 985-995.

Kindahl, H., Edqvist, L. E., Granstrom, E., & Bane, A. (1976). The release of prostaglandin F2alpha as reflected by 15-keto-13,14-dihydroprostaglandin F2alpha in the peripheral circulation during normal luteolysis in heifers. *Prostaglandins, 11*(5), 871-878.

King, G. J., & Robertson, H. A. (1974). A two injection schedule with prostaglandin F2alpha for the regulation of the ovulatory cycle of cattle. *Theriogenology, 1*(3), 123-128.

Lamb, G. C., Dahlen, C. R., Vonnahme, K. A., Hansen, G. R., Arseneau, J. D., Perry, G. A., . . . Arthington, J. D. (2008). Influence of a CIDR prior to bull breeding on pregnancy rates and subsequent calving distribution. *Anim Reprod Sci, 108*(3-4), 269-278. doi: 10.1016/j.anireprosci.2007.08.012

Lauderdale, J. W. (1972). *Effects of PFG2a on pregnancy and estrous cycle of cattle.* Paper presented at the Proceedings: American Society of Animal Science, Blacksburg, VA.
Lauderdale, J. W. (1979). *Efficacy of Lutalyse Sterile Solution.* Paper presented at the Proceedings of the Lutalyse Symposium, Augusta, MI.
Lauderdale, J. W. (2007). *Product development for estrous synchronization: beef cattle.* Paper presented at the Proceedings: Applied Reproductive Strategies in Beef Cattle, Billings, Montana.
Lauderdale, J. W., Chenault, J. R., Sequin, B. E., & Thatcher, W. W. (1973). *Fertility of cattle after PGF2alpha treatment.* Paper presented at the Proceedings: American Society of Animal Science.
Lauderdale, J. W., Seguin, B. E., Stellflug, J. N., Chenault, J. R., Thatcher, W. W., Vincent, C. K., & Loyancano, A. F. (1974). Fertility of cattle following PGF2 alpha injection. *J Anim Sci, 38*(5), 964-967.
Liehr, R. A., G.B., M., & Olson, H. H. (1972). *Effects of prostaglandin on cattle estrus cycles.* Paper presented at the Proceedings: American Society of Animal Science, Blacksburg, VA.
Liehr, R. A., Marion, G. B., & Olson, H. H. (1972). *Effects of prostaglandin on cattle estrus cycles.* Paper presented at the Proceedings: the American Society for Animal Science, Blacksburg, VA.
Loeb, L. (1923). Mechanism of the sexual cycle with special reference to the corpus luteum. *Proceedings for the Society of Experimental Biology*, 441-443.
Louis, T. M., Hafs, H. D., & Morrow, D. A. (1972). *Estrus and ovulation after uterine PGF2a in cows.* Paper presented at the Proceedings: American Society of Animal Science, Blacksburg, VA.
Louis, T. M., Hafs, H. D., & Seguin, B. E. (1973). Progesterone, LH, estrus and ovulation after prostaglandin F 2 in heifers. *Proc Soc Exp Biol Med, 143*(1), 152-155.
McCracken, J. A., Carlson, J. C., Glew, M. E., Goding, J. R., Baird, D. T., Green, K., & Samuelsson, B. (1972). Prostaglandin F 2 identified as a luteolytic hormone in sheep. *Nat New Biol, 238*(83), 129-134.
McCracken, J. A., Custer, E. E., Eldering, J. A., & Robinson, A. G. (1996). The central oxytocin pulse generator: a pacemaker for the ovarian cycle. *Acta Neurobiol Exp (Wars), 56*(3), 819-832.
Moreira, F., Orlandi, C., Risco, C. A., Mattos, R., Lopes, F., & Thatcher, W. W. (2001). Effects of presynchronization and bovine somatotropin on pregnancy rates to a timed artificial insemination protocol in lactating dairy cows. *J Dairy Sci, 84*(7), 1646-1659. doi: 10.3168/jds.S0022-0302(01)74600-0
Nancarrow, C. D., Buckmaster, J., Chamley, W., Cox, R. I., Cumming, I. A., Cummins, L., . . . Thorburn, G. D. (1973). Hormonal changes around oestrus in the cow. *J Reprod Fertil, 32*(2), 320-321.
Nancarrow, C. D., Hearnshaw, H., Mattner, P. E., Connell, P. J., & Restall, B. J. (1974). Proceedings: Hormonal changes occurring in cattle following the administration of prostaglandin F 2alpha. *J Reprod Fertil, 36*(2), 484-485.
National Agricultural Statistics Service. (2015, April 27). *United States Department of Agriculture*. Retrieved from http://www.nass.usda.gov/Charts_and_Maps/
Palmer, M. A., Olmos, G., Boyle, L. A., & Mee, J. F. (2010). Estrus detection and estrus characteristics in housed and pastured Holstein-Friesian cows. *Theriogenology, 74*(2), 255-264. doi: 10.1016/j.theriogenology.2010.02.009
Payne, M., Bruhn, C. M., Reed, B., Scearce, A., & O'Donnell, J. (1999). On-farm quality assurance programs: a survey of producer and industry leader opinions. *J Dairy Sci, 82*(10), 2224-2230. doi: 10.3168/jds.S0022-0302(99)75469-X
Peters, A. R. (1984). Luteolysis in cows using the prostaglandin F2 alpha analogue, tiaprost, and the effect of mode of administration. *Vet Rec, 114*(17), 418-421.
Peterson, A. J., Fairclough, R. J., Payne, E., & Smith, J. F. (1975). Hormonal changes around bovine luteolysis. *Prostaglandins, 10*(4), 675-684.
Pharriss, B. B., & Wyngarden, L. J. (1969). The effect of prostaglandin F 2alpha on the progestogen content of ovaries from pseudopregnant rats. *Proc Soc Exp Biol Med, 130*(1), 92-94.

Piper, P. J., Vane, J. R., & Wyllie, J. H. (1970). Inactivation of prostaglandins by the lungs. *Nature, 225*(5233), 600-604.

Rasmussen, F. (1978). Tissue Damage at the Injection Site after Intramuscular Injection of Drugs. *Veterinary Science Communications*, 173-182.

Rebhun, W. C. (1995). Abdominal Diseases *Diseases of Dairy Cattle* (pp. 134). Media, PA: Williams and Wilkins.

Roche, J. F. (1976). Fertility in cows after treatment with a prostaglandin analogue with or without progesterone. *J Reprod Fertil, 46*(2), 341-345.

Roeber, D. L., Cannell, R. C., Belk, K. E., Scanga, J. A., Cowman, G. L., & Smith, G. C. (2001). Incidence of injection-site lesions in beef top sirloin butts. *J Anim Sci, 79*(10), 2615-2618.

Roeber, D. L., Cannell, R. C., Wailes, W. R., Belk, K. E., Scanga, J. A., Sofos, J. N., . . . Smith, G. C. (2002). Frequencies of injection-site lesions in muscles from rounds of dairy and beef cow carcasses. *J Dairy Sci, 85*(3), 532-536. doi: 10.3168/jds.S0022-0302(02)74105-2

Roeber, D. L., Mies, P. D., Smith, C. D., Belk, K. E., Field, T. G., Tatum, J. D., . . . Smith, G. C. (2001). National market cow and bull beef quality audit-1999: a survey of producer-related defects in market cows and bulls. *J Anim Sci, 79*(3), 658-665.

Rowson, L. E., Tervit, R., & Brand, A. (1972). The use of prostaglandins for synchronization of oestrus in cattle. *J Reprod Fertil, 29*(1), 145.

Schams, D., & Karg, H. (1982). Hormonal responses following treatment with different prostaglandin analogues for estrous cycle regulation in cattle. *Theriogenology, 17*(5), 499-513.

Shrestha, H. K., Beg, M. A., Burnette, R. R., & Ginther, O. J. (2012). Plasma clearance and half-life of prostaglandin F2alpha: a comparison between mares and heifers. *Biol Reprod, 87*(1), 18, 11-16. doi: 10.1095/biolreprod.112.100776

Silvia, W. J., Lewis, G. S., McCracken, J. A., Thatcher, W. W., & Wilson, L., Jr. (1991). Hormonal regulation of uterine secretion of prostaglandin F2 alpha during luteolysis in ruminants. *Biol Reprod, 45*(5), 655-663.

Smith, G. C., Savell, J. W., Clayton, R. P., Field, T. G., Griffin, D. B., Hale, D. S., . . . Lambert, C. D. (1991). Improving the consistency and competitiveness of beef. *The Final Report of the National Beef Quality Audit*, 1-237.

Stein, C. D. (1956). Blackleg *Animal Diseases* (pp. 263-264). Washington, D.C.: The United States Government Printing Office.

Stellflug, J. N., Louis, T. M., Hafs, H. D., & Seguin, B. E. (1975). Luteolysis, estrus and ovulation, and blood prostaglandin F after intramuscular administration of 15, 30 or 60 mg prostaglanding F2alpha. *Prostaglandins, 9*(4), 609-615.

Stevenson, J. (Producer). (2015, January 30). Hoard's Dairyman. *Reproduction: New Technology Improves Heat Detection Rates*. Retrieved from http://www.hoards.com/E_reproduction/REP31

Sveberg, G., Rogers, G. W., Cooper, J., Refsdal, A. O., Erhard, H. W., Kommisrud, E., . . . Ropstad, E. (2015). Comparison of Holstein-Friesian and Norwegian Red dairy cattle for estrus length and estrous signs. *J Dairy Sci*. doi: 10.3168/jds.2014-8905

System, N. A. H. M. (2007a). Beef 2007-08 Part II: Reference of Beef Cow-calf Management Practices in the United States. *USDA-APHIS*. http://www.aphis.usda.gov/animal_health/nahms/beefcowcalf/downloads/beef0708/Beef0708_dr_PartII.pdf

System, N. A. H. M. (2007b). Dairy Part II: Changes in the US Dairy Cattle Industry, 1991-2007. Washington, DC: United States Department of Agriculture, Animal and Plant Health Inspection Service.

Tervit, H. R., Rowson, L. E., & Brand, A. (1973). Synchronization of oestrus in cattle using a prostaglandin F2alpha analogue (ICI 79939). *J Reprod Fertil, 34*(1), 179-181.

Tomlinson, R. V., Spires, H. R., & Kent, J. S. (1984). Absorption, excretion and tissue residue in feedlot heifers injected with the synthetic prostaglandin, fenprostalene. *J Anim Sci, 59*(1), 164-169.

Tozer, P. R., Varga, G. A., Henning, W. R., & Holden, L. A. (2005). Do Dairy Producers Use Effective Management Practices to Improve the Value of Market Cows? *The Professional Animal Scientist*, 272-277.

Valenza, A., Giordano, J. O., Lopes, G., Jr., Vincenti, L., Amundson, M. C., & Fricke, P. M. (2012). Assessment of an accelerometer system for detection of estrus and treatment with gonadotropin-releasing hormone at the time of insemination in lactating dairy cows. *J Dairy Sci, 95*(12), 7115-7127. doi: 10.3168/jds.2012-5639

Van Donkersgoed, J., Dixon, S., Brand, G., & VanderKop, M. (1997). A survey of injection site lesions in fed cattle in Canada. *Can Vet J, 38*(12), 767-772.

Van Donkersgoed, J., Dixon, S., & VanderKop, M. (1998a). Injection site survey in Canadian-fed cattle: spring 1997. *Can Vet J, 39*(2), 97-99.

Van Donkersgoed, J., Dixon, S., & Vanderkop, M. (1998b). Injection site surveys in Canadian yearling cattle and cull cows and bulls: fall 1997. *Can Vet J, 39*(8), 497-499.

Whittier, J. C., Caldwell, R. W., Anthony, R. V., Smith, M. F., & Morrow, R. E. (1991). Effect of a prostaglandin F2 alpha injection 96 hours after introduction of intact bulls on estrus and calving distribution of beef cows. *J Anim Sci, 69*(12), 4670-4677.

Chapter III

Comparison of serum progesterone concentration curves in beef heifers during luteolysis after administration of dinoprost in the muscle or the ischiorectal fossa

Abstract: A novel site for injections in the rear of the cow that may preserve beef quality assurance is the ischiorectal fossa (IRF), located next to the tailhead. The objective of this study was to determine whether $PGF_{2\alpha}$ given in the IRF results in a similar physiologic response as $PGF_{2\alpha}$ given IM in the neck. The null hypothesis was that location of injection would not alter the timing of luteolysis. Yearling angus-cross heifers (n=112) in 3 cohorts were randomly assigned to one of two treatments: 25 mg $PGF_{2\alpha}$ in the neck, or 25 mg $PGF_{2\alpha}$ in the IRF. Blood samples were taken at 0, 8, 16, 24, 36, and 48 h post-treatment. Serum samples were analyzed for progesterone concentration using a radioimmunoassay. Heifers with lysis of a functional corpus luteum were included in the analysis. Time to luteolysis was compared between treatment groups in 3 ways. Since the literature does not provide a clear endpoint for luteolysis, the authors used three concentrations as the lower threshold for serum progesterone concentration: 1.5 ng/mL, 1.25 ng/mL, and 1 ng/mL. The time it took each animal's progesterone concentration to reach each threshold was calculated by fitting the progesterone curves with a sigmoidal logistic regression model. A Kaplan-Meier survival analysis was run on the time to luteolysis for each group at the different thresholds. The median times to luteolysis for neck and IRF injections using 1.5, 1.25. and 1 ng/mL were 18.1 hrs and 20.0 hrs (p=0.06), 22.8 hrs and 24.5 hrs (p=0.29), and 30.1 hrs and 29.0 hrs (p=0.74), respectively. Given that a biologically significant difference in time to luteolysis was not detected, the IRF may be considered an alternative injection site in the back half of the cow for $PGF_{2\alpha}$ that preserves beef quality assurance.

Key words: prostaglandin, injection lesion, luteolysis, progesterone, ischiorectal fossa

Introduction

Artificial insemination has become a prevalent tool in bovine reproductive management and has necessitated the administration of hormones and/or their analogs such as prostaglandin F2alpha ($PGF_{2\alpha}$) and gonadotropin releasing hormone (GnRH) via injection in order to synchronize estrus. Given current reproductive management practices, the average dairy cow may therefore receive as many as 20+ injections over her lifetime (Tozer, Varga, Henning, & Holden, 2005).

Injections should be given to cattle subcutaneously (SQ), if possible, and should be administered in front of the shoulder in order to minimize lesions in the more valuable cuts of meat. Beef Quality Assurance (BQA) guidelines apply to beef and dairy cows as well as fed cattle, since the former make up 15-20% of the cattle slaughtered in this country ("National Agricultural Statistics Service," 2015). Despite BQA guidelines, many drugs are administered in the semimembranosis, semitendinosis, and gluteal muscles of cows; in more than 32% of Iowa dairies surveyed, producers admitted to giving injections in the rear legs of cows (Glaze & Chahine, 2009). In Pennsylvania dairies, 70% of estrous synchronization shots were given in the hip/rump/flank (Tozer et al., 2005), which may be due to the prevalent use of headlocks as a management tool on many dairies. Provision of an acceptable injection site that can be accessed from the rear of the cow may improve BQA compliance.

A novel site for SQ administration is the ischiorectal fossa (IRF) located next to the tailhead, craniodorsal and medial to the tuber ischii. Currently, Posilac® (recombinant bovine somatotropin) is the only commercial product in the United States labeled for administration in the

IRF, but the potential exists for other products to be given in this location. Several research groups have investigated the IRF as an appropriate site to give analogues of $PGF_{2\alpha}$ labeled for IM administration. Colazo et al. (2002) reported that the corpus luteum regressed in 100% of dairy cows (n=16) after $PGF_{2\alpha}$ injection in the IRF (Colazo, Martinez, Kastelic, Mapletoft, & Carruthers, 2002). Additionally, 50 of 51 Holstein heifers determined to have a CL experienced luteal regression in response to $PGF_{2\alpha}$ given in the IRF. Finally, Chebel et al. (2006) compared progesterone concentrations in Holstein cows given $PGF_{2\alpha}$ either IM (n=165) in the neck or in the IRF (n=166) and found no difference in the decline of progesterone concentration at 12, 24, 36, and 48 hr post treatment between the two groups (Chebel, Santos, Rutigliano, & Cerri, 2007).

Although rate of response to $PGF_{2\alpha}$ in the IRF is similar to IM in the neck, there is some evidence that using the IRF for injection of $PGF_{2\alpha}$ may have an effect on the time to response. Colazo et al. conducted an additional study in which 18 beef heifers were given $PGF_{2\alpha}$ either IM in the rear legs or SQ behind the shoulder (Colazo, Martinez, Kastelic, & Mapletoft, 2002). While the percent in estrus did not differ, the time to estrus was 6.5 hours longer for heifers given the SQ injection as compared to heifers given the IM injection. The components of estrus synchronization protocols are carefully timed so that subsequent injections are given at the appropriate interval. This objective of this study was to compare the time to luteolysis for $PGF_{2\alpha}$ given IM in the neck versus in the IRF. This information will be useful in determining whether adjustments need to be made if $PGF_{2\alpha}$ is given in the IRF as part of a synchronization protocol.

Experimental Procedures

Yearling angus-cross beef heifers (n=112) were managed in 3 cohorts in 2 locations on fescue and orchard grass pasture, supplemented with hay and grain as needed. The first cohort consisted of heifers born in February and March of 2012 (n=49). In August of 2013 the heifers

were randomly assigned to either control (CN) or treatment (IRF) groups and palpated for the presence of a corpus luteum. Heifers with a corpus luteum on ultrasound (n=36) were given 25 mg of dinoprost, an analogue of $PGF_{2\alpha}$ (Lutalyse®; Pharmacia & Upjohn Animal Health, Orangeville, Ontario) either intramuscularly in the neck (CN) with an 18 g x 1.5" needle or in the IRF with an 18 g x 1" needle. Blood was collected at 0, 8, 16, 24, 36, and 48 hours post-injection in evacuated red top tubes from the jugular veins or tail vein. Samples were allowed to clot on ice for 1 hour. Tubes were then centrifuged at 1500 x *g* for 10 minutes. Serum was removed from the clot immediately and frozen in duplicate aliquots at -18° C until further processing. Serum progesterone concentration was determined using a commercial radioimmunoassay (Coat-A-Count Progesterone; Diagnostic Products, Los Angeles, California). The intra-assay and inter-assay coefficients of variation were 7.3% and 8.9%, respectively.

The second cohort of heifers was born in the fall of 2012 (n=22). In November of 2013 the heifers were given 25 mg of $PGF_{2\alpha}$ 12 days prior to the day of treatment. This initial dose of $PGF_{2\alpha}$ functioned as a set-up treatment so that all cyclic heifers would be in diestrus on the day of treatment; therefore, the day of treatment they were not palpated for the presence of a corpus luteum. Heifers were randomly allocated to CN and IRF groups and treated as described for the first cohort. Blood samples were also collected as described for the first cohort.

Data from the first two cohorts of animals was used to complete a power calculation to determine the size of the third and final cohort. In order to detect a difference of 4 hours, 36 animals were needed per treatment group (β=0.8).

The third cohort of heifers was born in the spring of 2013 (n=54). In August of 2014 the heifers were given a set-up dose of 25 mg of $PGF_{2\alpha}$ 11 days prior to the day of treatment and were not palpated the day of treatment for a corpus luteum. Heifers were randomly allocated to CN and

IRF groups and treated as described for the first cohort. Blood samples were also collected as described for the first cohort.

Only heifers with a competent corpus luteum at time zero that responded to $PGF_{2\alpha}$ were included in the analysis (n=78/112). Heifers were considered to have a competent corpus luteum if initial serum progesterone concentration was greater than 2 ng/mL and were considered to have responded if serum progesterone decreased to less than 1.5 ng/mL or to less than 40% of initial concentration. Reasoning for these cut-offs is explained in the discussion. Examples of serum progesterone curves for heifers that did not have a competent corpus luteum, heifers that did have a competent corpus luteum but did undergo luteolysis, and heifers that had a competent corpus luteum that did undergo luteolysis can be found in Appendix 1. For each heifer included in the analysis, a sigmoidal logistic model was used to predict when serum progesterone decreased to less than 1.5, 1.25, and 1 ng/mL (Turino et al, 2010). Reasoning for the use of multiple thesholds is explained in the discussion. These times were used as the "time of death" for the corpus luteum from each heifer. A Kaplan-Meier survival analysis was performed to determine median time for serum progesterone to fall below each threshold using JMP. The Log-Rank test was used to compare survival curves. Significance was set at $p<0.05$.

Results and Discussion

Heifers weighed an average of 334 ± 21 kg at enrollment. In the first cohort of heifers, 20 out of 36 heifers had a functional corpus luteum at the time of treatment based on a serum progesterone concentration greater than 2 ng/mL. Eighteen of the 20 heifers with a functional corpus luteum were considered to have responded based on serum progesterone concentration falling below 1.5 ng/mL. In the second cohort of heifers, 20 out of 22 heifers had a functional corpus luteum at the time of treatment, and 18 heifers were considered to have responded. In the

third cohort of heifers, 42 of 54 heifers were considered to have a competent corpus luteum at the time of treatment, and all 42 heifers were considered to have responded. This data is summarized in Table 3.1.

Table 3.1 Heifers in each treatment group enrolled in the study, found to have a corpus luteum at enrollment, and considered to be responders.

N	*CN*	*IRF*
Enrolled	56	56
Heifers with serum P4 > 2 ng/mL[1]	42	40
Responders[2]	40	38

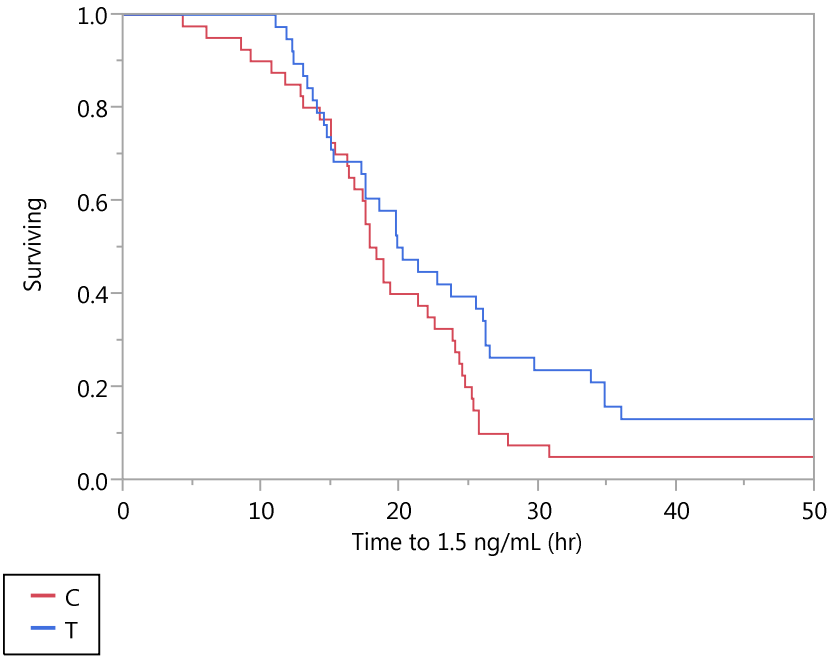
[1]Heifers were considered to have a functional corpus luteum at time zero if initial serum P4 concentration was greater than 2 ng/mL.

[2]Heifers were considered to have responded if serum P4 concentration was less than 1.5 ng/mL or less than 40% of initial concentration 48 hours after treatment.

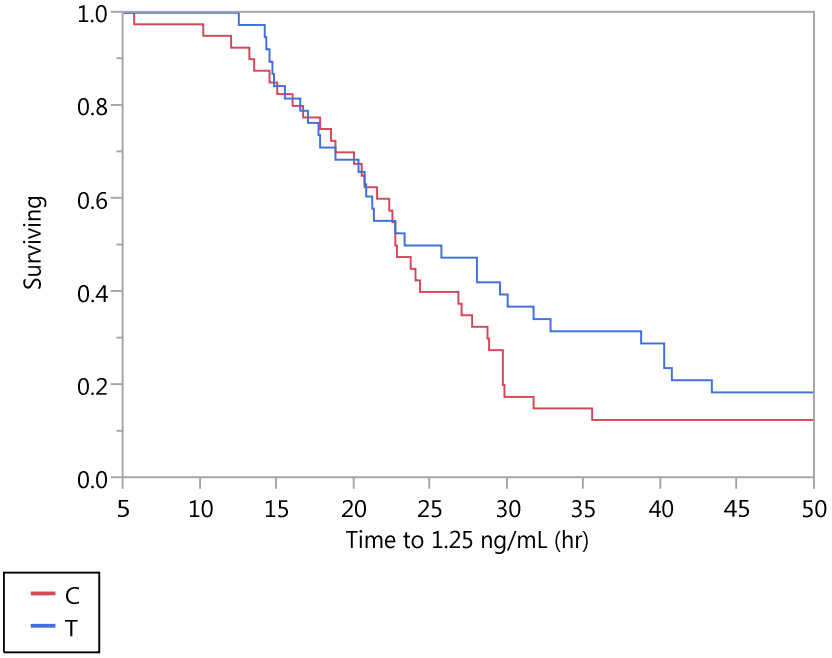
Censored values in this data set were from heifers whose progesterone never decreased below the threshold(s). Given that all censored data was singly Type I right-censored, a Kaplan-Meier survival analysis was performed. The survival curves for time to each threshold for serum progesterone concentration are depicted in Figure 3.1. The median time to 1.5 ng/mL for treatment and control groups was 20.0 and 18.1 hours, respectively, ($p=0.06$) as depicted in Table 3.2. The median time to 1.25 ng/mL for treatment and control groups was 24.5 and 22.8 hours, respectively ($p=0.29$). The median time to 1 ng/mL for treatment and control groups was 29.0 and 30.1 hours, respectively ($p=0.74$). A difference in these times was not detected.

Figure 3.1 Kaplan-Meier Survival curves depicting percent of heifers with serum P4 concentration greater than A) 1.5 ng/mL, B) 1.25 ng/mL, and C) 1 ng/mL over 48 hours after Lutalyse administration in either the neck (C) or the ischiorectal fossa (T).

A)



B)



C)

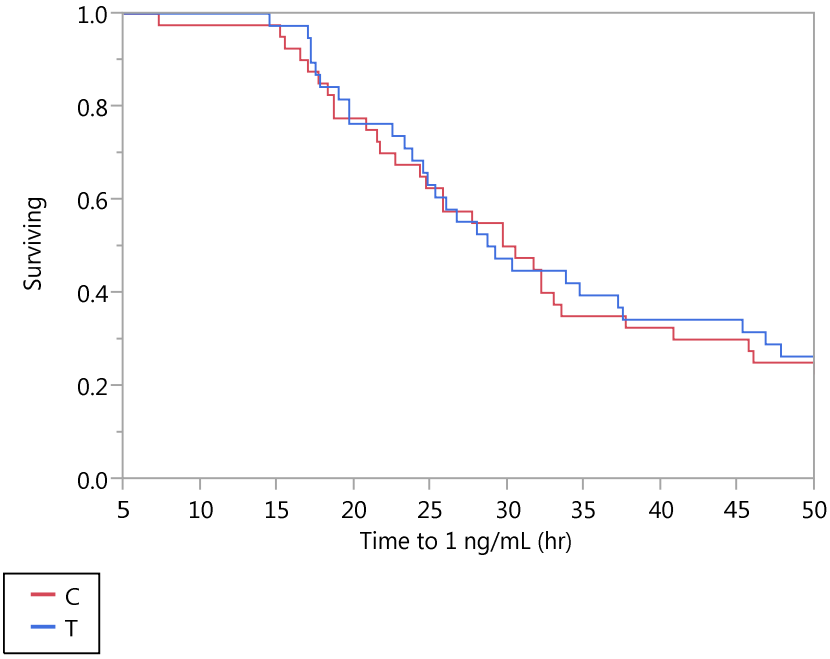

1.0
0.8
0.6
0.4
0.2
0.0
Surviving
5
10
15
20
25
30
35
40
45
50
Time to 1 ng/mL (hr)
C
T

Table 3.2 Median survival times (95% Confidence Interval) for serum progesterone concentrations declining below threshold

Threshold	*CN*	*IRF*	*P value*
1.5 ng/mL	18.1 (16.3-22.0)	20.0 (17.2-26.0)	0.06
1.25 ng/mL	22.8 (20.5-27.0)	24.5 (20.3-31.7)	0.29
1 ng/mL	30.1 (24.3-33.5)	29.0 (24.5-37.5)	0.74

In the first cohort, only 36/49 heifers were found to have a corpus luteum on rectal palpation. Some heifers may not have been cycling yet, and others may have been at days 0-2 or 17-21 in their cycle and would therefore not have a functional corpus luteum at that time. In order to avoid this problem in subsequent cohorts, heifers were given an injection of $PGF_{2\alpha}$ IM in the neck 11-12 days prior to the day of treatment. This amendment did not change the percent of animals that were cycling, but it increased the number of cycling animals that would have a functional and responsive corpus luteum on the day of treatment. In the first cohort, only 20/36 of the heifers with a palpable corpus luteum had a serum progesterone treatment of > 2 ng/mL prior to treatment. The palpator may not have been able to differentiate a corpus hemorrhagicum or a corpus albicans from a corpus luteum; either of the former would result in low serum progesterone concentration.

Typically, in a pharmacokinetic study to establish bioequivalence, the compound administered or a metabolite of that compound would be measured directly. However, the half-life of $PGF_{2\alpha}$ is only about 20 minutes, making this very difficult; hence, the physiologic response to that compound, in this case luteolysis as demonstrated by a decrease in serum progesterone concentration, is measured instead. Defining ovarian events via serum progesterone concentration can be challenging. Serum progesterone concentrations have been utilized to 1) establish the presence of a CL to categorize a female as cycling and 2) to determine if and/or when a CL has lysed.

Many studies utilize serum progesterone concentration to categorize females as cycling or anestrous. In this instance, the age of the CL does not matter, and often a cut-off value of 1 ng/mL is used (Bader et al., 2005; Geary, Whittier, Hallford, & MacNeil, 2001; Giles et al., 2013; Lamb et al., 2006; Lamb et al., 2001; Larson et al., 2006; Mialot et al., 2003; Stevenson et al., 2003) or

even 0.5 ng/mL (Busch et al., 2008; Nash et al., 2012; Schafer et al., 2007; Wilson et al., 2010). Corpora lutea producing between 0.5 and 2 ng/mL are likely either just forming (day 1-4 of the estrus cycle) or in the process of regressing (day 18-20) (Stabenfeldt, Ewing, & McDonald, 1969). In the present study, the authors were interested in CLs that were established enough to respond to prostaglandin and that were not already regressing on their own (day 6-16 of the cycle). This is why a more conservative cut-off value of 2 ng/mL was used to define a functional/responsive CL. Of 112 heifers treated, 82 had a functional corpus luteum (CL) at the time of treatment (serum progesterone concentration > 2 ng/mL).

Serum progesterone concentrations have also been used to determine the endpoint of luteolysis. The threshold for progesterone concentration used to define complete luteolysis has differed between investigators. Several groups have utilized 1.0 ng/mL (Ginther, Araujo, Palhao, Rodrigues, & Beg, 2009; Peters, 1984; Shrestha, Beg, Siddiqui, & Ginther, 2010; Stellflug, Louis, Hafs, & Seguin, 1975). Other authors have used 1.6 ng/mL as a cutoff for the transition from diestrus to estrus (Chauhan, Mgongo, Kessy, & Gombe, 1986). Alternatively, some authors have classified cows as having experienced luteolysis when progesterone concentration fell below 40% of the initial value (Chebel et al., 2007; Rivera, Sterry, & Fricke, 2006). Colazo et al. measured serum progesterone in 74 heifers at the time of $PGF_{2\alpha}$ administration, measured serum progesterone again 24-30 hrs later, and monitored for behavioral signs of estrus for 5 days (Colazo, Martinez, Kastelic, Mapletoft, et al., 2002). Fifty-one heifers were found to have a CL at the time of treatment and were analyzed for luteolysis. Forty-three heifers had a subsequent serum progesterone concentration of < 1 ng/mL at 24-30 hrs after treatment with $PGF_{2\alpha}$ and were considered to have undergone successful luteolysis. However, of the heifers who did not meet this criteria (n=8), 5 heifers with an average serum progesterone concentration of 2 ng/mL at 24-30 hrs

after treatment were found to be in estrus several days later. This data highlights the difficulty of using one arbitrary threshold across many animals to define luteolysis. In the present study, the authors used three thresholds to look for a difference between treatment groups: 1.5 ng/mL, 1.25 ng/mL, and 1 ng/mL.

Another factor to consider when choosing a threshold for luteolysis is the dynamic curve of the radioimmunoassay being used. The standard curve for the assay used in this experiment was 0.1 ng/mL, 0.5 ng/mL, 2 ng/mL, 10 ng/mL, 20 ng/mL, and 40 ng/mL. It can be assumed that the assay is most accurate between 2 and 10 ng/mL and less accurate as the concentration of progesterone decreases from 2 and increases from 10 ng/mL. Therefore, the biological meaning of 1.1 vs 0.9 ng/mL when using this RIA could be somewhat suspect. In the present study, three different thresholds for luteolysis were used in order to account for this. The highest of those thresholds was used a cutoff to determine which animals would be included in the analysis (1.5 ng/mL). Seventy-eight of 82 heifers were found to have undergone complete luteolysis according to this definition.

Researchers have differing opinions on how much time it should take for luteolysis to occur. Schams and Karg reported that the progesterone will fall below 1 ng/mL within 24 hours when luteolysis has taken place (Schams & Karg, 1982). Desaulniers et al. describe a "delayed luteolysis" where the serum progesterone concentration of one heifer failed to fall below 1 ng/mL by 48 hours but estrus was still observed by 72 hours (Desaulniers, Guay, & Vaillancourt, 1990). Serum progesterone was measured until 48 hours to capture all instances of luteolysis. Alternatively, in the case of a refractory corpus luteum (day 1-5 of the cycle), an initial decline in serum progesterone concentration may occur followed by a rebound. The rebound should happen by 48 hours, however, it is possible for the subsequent rise in progesterone to be delayed to 72

hours (Chauhan et al., 1986). Since serum progesterone was only measured until 48 hours in the present study, it is possible that some heifers with a refractory corpus luteum were mistakenly identified as having undergone luteolysis.

Handling of the samples post collection may influence serurm progesterone concentration. In a study of the stability of progesterone in different collection tubes at different temperatures, the concentration of progesterone in a red top tube at 4° C declined numerically by 24 hours and significantly (41%) by 72 hours (Reimers, McCann, & Cowan, 1983). Nash et al. in 2012 used 0.5 ng/mL as the cutoff for cyclicity, however, samples in this study were allowed to clot in red top tubes at 4° C for 24 hours prior to centrifugation and further processing (Nash et al., 2012). The resulting progesterone concentrations in that study could have been artificially decreased, which would bias the data. The handling of samples, therefore, needs to be taken into account. In this study, samples were allowed to clot for one hour to minimize losses in serum progesterone concentration.

Chebel et al reported that the route of administration (IRF, SQ, or IM) of the same dose of $PGF_{2\alpha}$ did not affect the percentage of cows experiencing luteolysis by 12, 24, 36, or 48 hrs post treatment (Chebel et al., 2007). The sensitivity of this study was limited to 12 hour intervals, however. Synchronization protocols are designed to optimize the physiologic response at each step in the protocol in order to maximize pregnancy rates. Previously, adjustments of as little as 8 hours have been made in order to accomplish the highest possible pregnancy rates (Bridges et al., 2012; Dobbins et al., 2009). Therefore, the sample size of this study and analysis of the data was designed to detect a difference of 4 hours in time to luteolysis. While progesterone was not physically measured every 4 hours, fitting each progesterone curve with a sigmoidal logistic model (Turino et al., 2010) allowed for a more precise time to luteolysis to be estimated. No difference

in time to luteolysis between treatment groups was found for each threshold examined. This is in contrast, however, with the findings of Colazo, et al, who found that heifers given 500 µg of an analogue of $PGF_{2\alpha}$ subcutaneously came into heat an average of 17 hours later than heifers given the same dose IM. Time to ovulation, however, did not differ between treatment groups. Each treatment group in this study consisted of 9 heifers. The small sample size and individual variation among heifers may have biased the data in this study. It is also possible that the ischiorectal fossa cannot be considered subcutaneous and therefore the comparison is inappropriate.

Implications

The ischiorectal fossa can be used as the injection site for $PGF_{2\alpha}$ and may be implemented in synchronization protocols without amending subsequent injections. This conclusion should be validated using a clinical trial. In addition, the efficacy of the ishiorectal fossa as an injection site for $PGF_{2\alpha}$ has not been validated in beef cattle. The composition of the tissue found in the IRF has not been well described, but fat is readily deposited in the IRF with increasing body condition score thus the anatomy of the region may as body condition changes. Dairy and beef animals differ in body condition and rate of metabolism and therefore the injection site should be validated in both types of cattle and in animals of varying body conditions.

Literature Cited

Bader, J. F., Kojima, F. N., Schafer, D. J., Stegner, J. E., Ellersieck, M. R., Smith, M. F., & Patterson, D. J. (2005). A comparison of progestin-based protocols to synchronize ovulation and facilitate fixed-time artificial insemination in postpartum beef cows. *J Anim Sci, 83*(1), 136-143.

Bridges, G. A., Ahola, J. K., Brauner, C., Cruppe, L. H., Currin, J. C., Day, M. L., . . . Whittier, W. D. (2012). Determination of the appropriate delivery of prostaglandin F2alpha in the five-day CO-Synch + controlled intravaginal drug release protocol in suckled beef cows. *J Anim Sci, 90*(13), 4814-4822. doi: 10.2527/jas.2011-4880

Busch, D. C., Schafer, D. J., Wilson, D. J., Mallory, D. A., Leitman, N. R., Haden, J. K., . . . Patterson, D. J. (2008). Timing of artificial insemination in postpartum beef cows following administration of the CO-Synch + controlled internal drug-release protocol. *J Anim Sci, 86*(7), 1519-1525. doi: 10.2527/jas.2008-0925

Chauhan, F. S., Mgongo, F. O., Kessy, B. M., & Gombe, S. (1986). Effects of intravulvo-submucosal cloprostenol injections on hormonal profiles and fertility in subestrous cattle. *Theriogenology, 26*(1), 69-75.

Chebel, R. C., Santos, J. E., Rutigliano, H. M., & Cerri, R. L. (2007). Efficacy of an injection of dinoprost tromethamine when given subcutaneously on luteal regression in lactating Holstein cows. *Theriogenology, 67*(3), 590-597. doi: 10.1016/j.theriogenology.2006.09.007

Colazo, M. G., Martinez, M. F., Kastelic, J. P., & Mapletoft, R. J. (2002). Effects of dose and route of administration of cloprostenol on luteolysis, estrus and ovulation in beef heifers. *Anim Reprod Sci, 72*(1-2), 47-62.

Colazo, M. G., Martinez, M. F., Kastelic, J. P., Mapletoft, R. J., & Carruthers, T. D. (2002). The ischiorectal fossa: an alternative route for the administration of prostaglandin in cattle. *Can Vet J, 43*(7), 535-541.

Desaulniers, D. M., Guay, P., & Vaillancourt, D. (1990). Estrus induction with prostaglandin F2alpha, cloprostenol or fenprostalene during the normal estrous cycle, superovulation and after embryo collection. *Theriogenology, 34*(4), 667-682.

Dobbins, C. A., Eborn, D. R., Tenhouse, D. E., Breiner, R. M., Johnson, S. K., Marston, T. T., & Stevenson, J. S. (2009). Insemination timing affects pregnancy rates in beef cows treated with CO-Synch protocol including an intravaginal progesterone insert. *Theriogenology, 72*(7), 1009-1016. doi: 10.1016/j.theriogenology.2009.06.025

Geary, T. W., Whittier, J. C., Hallford, D. M., & MacNeil, M. D. (2001). Calf removal improves conception rates to the Ovsynch and CO-Synch protocols. *J Anim Sci, 79*(1), 1-4.

Giles, R. L., Ahola, J. K., Whittier, J. C., French, J. T., Repenning, P. E., Kruse, S. G., . . . Peel, R. K. (2013). Administration of a GnRH analog on day 9 of a 14-day controlled internal drug release insert with timed artificial insemination in lactating beef cows. *J Anim Sci, 91*(4), 1866-1873. doi: 10.2527/jas.2012-5497

Ginther, O. J., Araujo, R. R., Palhao, M. P., Rodrigues, B. L., & Beg, M. A. (2009). Necessity of sequential pulses of prostaglandin F2alpha for complete physiologic luteolysis in cattle. *Biol Reprod, 80*(4), 641-648. doi: 10.1095/biolreprod.108.072769

Glaze, J. B., Jr., & Chahine, M. (2009). Assessment of management and basic beef quality assurance practices on Idaho dairies. *J Dairy Sci, 92*(3), 1265-1271. doi: 10.3168/jds.2008-1194

Lamb, G. C., Larson, J. E., Geary, T. W., Stevenson, J. S., Johnson, S. K., Day, M. L., . . . Landblom, D. G. (2006). Synchronization of estrus and artificial insemination in replacement beef heifers using gonadotropin-releasing hormone, prostaglandin F2alpha, and progesterone. *J Anim Sci, 84*(11), 3000-3009. doi: 10.2527/jas.2006-220

Lamb, G. C., Stevenson, J. S., Kesler, D. J., Garverick, H. A., Brown, D. R., & Salfen, B. E. (2001). Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin F2alpha for ovulation control in postpartum suckled beef cows. *J Anim Sci, 79*(9), 2253-2259.

Larson, J. E., Lamb, G. C., Stevenson, J. S., Johnson, S. K., Day, M. L., Geary, T. W., . . . Arseneau, J. D. (2006). Synchronization of estrus in suckled beef cows for detected estrus and artificial insemination and timed artificial insemination using gonadotropin-releasing hormone, prostaglandin F2alpha, and progesterone. *J Anim Sci, 84*(2), 332-342.

Mialot, J. P., Constant, F., Dezaux, P., Grimard, B., Deletang, F., & Ponter, A. A. (2003). Estrus synchronization in beef cows: comparison between GnRH+PGF2alpha+GnRH and PRID+PGF2alpha+eCG. *Theriogenology, 60*(2), 319-330.

Nash, J. M., Mallory, D. A., Ellersieck, M. R., Poock, S. E., Smith, M. F., & Patterson, D. J. (2012). Comparison of long- versus short-term CIDR-based protocols to synchronize estrus prior to fixed-time AI in postpartum beef cows. *Anim Reprod Sci, 132*(1-2), 11-16. doi: 10.1016/j.anireprosci.2012.03.013

National Agricultural Statistics Service. (2015, April 27). *United States Department of Agriculture*. Retrieved from http://www.nass.usda.gov/Charts_and_Maps/

Peters, A. R. (1984). Luteolysis in cows using the prostaglandin F2 alpha analogue, tiaprost, and the effect of mode of administration. *Vet Rec, 114*(17), 418-421.

Reimers, T. J., McCann, J. P., & Cowan, R. G. (1983). Effects of storage times and temperatures on T3, T4, LH, prolactin, insulin, cortisol and progesterone concentrations in blood samples from cows. *J Anim Sci, 57*(3), 683-691.

Rivera, H., Sterry, R. A., & Fricke, P. M. (2006). Presynchronization with gonadotropin-releasing hormone does not improve fertility in Holstein heifers. *J Dairy Sci, 89*(10), 3810-3816. doi: 10.3168/jds.S0022-0302(06)72422-5

Schafer, D. J., Bader, J. F., Meyer, J. P., Haden, J. K., Ellersieck, M. R., Lucy, M. C., . . . Patterson, D. J. (2007). Comparison of progestin-based protocols to synchronize estrus and ovulation before fixed-time artificial insemination in postpartum beef cows. *J Anim Sci, 85*(8), 1940-1945. doi: 10.2527/jas.2006-836

Schams, D., & Karg, H. (1982). Hormonal responses following treatment with different prostaglandin analogues for estrous cycle regulation in cattle. *Theriogenology, 17*(5), 499-513.

Shrestha, H. K., Beg, M. A., Siddiqui, M. A., & Ginther, O. J. (2010). Dynamic progesterone responses to simulation of a natural pulse of a metabolite of prostaglandin F(2alpha) in heifers. *Anim Reprod Sci, 118*(2-4), 118-123. doi: 10.1016/j.anireprosci.2009.06.021

Stabenfeldt, G. H., Ewing, L. L., & McDonald, L. E. (1969). Peripheral plasma progesterone levels during the bovine oestrous cycle. *J Reprod Fertil, 19*(3), 433-442.

Stellflug, J. N., Louis, T. M., Hafs, H. D., & Seguin, B. E. (1975). Luteolysis, estrus and ovulation, and blood prostaglandin F after intramuscular administration of 15, 30 or 60 mg prostaglanding F2alpha. *Prostaglandins, 9*(4), 609-615.

Stevenson, J. S., Lamb, G. C., Johnson, S. K., Medina-Britos, M. A., Grieger, D. M., Harmoney, K. R., . . . Marple, T. J. (2003). Supplemental norgestomet, progesterone, or melengestrol acetate increases pregnancy rates in suckled beef cows after timed inseminations. *J Anim Sci, 81*(3), 571-586.

Tozer, T. R., Varga, V. A., Henning, W. R., & Holden, L. A. (2005). Do Dairy Producers Use Effective Management Practices to Improve the Value of Market Cows? *The Professional Animal Scientist*, 272-277.

Turino, L. N., Mariano, R. N., Cabrera, M. I., Scandolo, D. E., Maciel, M. G., & Grau, R. J. (2010). Pharmacokinetics of progesterone in lactating dairy cows: gaining some insights into the metabolism from kinetic modeling. *J Dairy Sci, 93*(3), 988-999. doi: 10.3168/jds.2009-2519

Wilson, D. J., Mallory, D. A., Busch, D. C., Leitman, N. R., Haden, J. K., Schafer, D. J., . . . Patterson, D. J. (2010). Comparison of short-term progestin-based protocols to synchronize estrus and ovulation in postpartum beef cows. *J Anim Sci, 88*(6), 2045-2054. doi: 10.2527/jas.2009-2627

Chapter IV

Comparison of timed artificial insemination pregnancy rates in beef cows after administration of dinoprost in the muscle or the ischiorectal fossa

Abstract: Producers synchronizing beef cows for timed artificial insemination often administer hormones in the muscles of the hips and back legs. As reported in the previous chapter, $PGF_{2\alpha}$ given in the ischiorectal fossa (IRF) results in luteolysis in a similar amount of time as $PGF_{2\alpha}$ given IM. The objective of this study was to determine whether implementing the ischiorectal fossa for $PGF_{2\alpha}$ administration in a timed artificial insemination (AI) protocol would result in a similar AI pregnancy rate as IM in the neck. Angus cross commercial beef cows (n=1471) 3-10 years of age in 12 herds were enrolled in the study. In each herd, cows at least 30 days post-partum were blocked by age group (n=4) and randomly assigned within blocks to be injected with 25 mg $PGF_{2\alpha}$ in either the neck (IM) or IRF as part of a 7-Day CO-Synch + CIDR timed AI protocol. Estrus expression was recorded at insemination and pregnancy diagnosis was performed via ultrasound at approximately 60 days post insemination. Linear mixed models were used to analyze results (Proc GLIMMIX). Estrus expression for IM and IRF cows did not differ (47.6% versus 47.8%, p=0.56). Artificial insemination (AI) pregnancy rate for IM and IRF cows also did not differ (52.6% versus 57.2%, p=0.06). Given that a biologically significant difference in timed AI pregnancy rate was not detected, the IRF may be considered an alternative injection site in the back half of the cow for $PGF_{2\alpha}$ that preserves beef quality assurance.

Key words: prostaglandin, estrus synchronization, injection lesions, timed artificial insemination

Introduction

Dairy and cow-calf producers frequently administer prostaglandin F2alpha ($PGF_{2\alpha}$) via injection in order to synchronize estrus and breed groups of cows by appointment. Intramuscular

$PGF_{2\alpha}$ causes muscle damage as demonstrated by a rise in serum creatinine kinase (CK) levels after injection (Fajt, Wagner, Pederson, & Norby, 2011). Muscle damage from injections, even injections of small volumes, can result in lesions that persist until slaughter (Apley, Wray, & Armstrong, 1994; George, Heinrich, et al., 1995).

The prevalence of injection site lesions in valuable subprimals in the carcasses of fed cattle has decreased over the past several decades (Dexter et al., 1994; George, Morgan, et al., 1995; Roeber, Cannell, et al., 2001). The change can in part be attributed to the Beef Quality Assurance program, which specifies that injections are to be given to cattle subcutaneously, if possible, and should be administered in front of the shoulder in order to avoid causing lesions in the more valuable cuts of meat in the back legs ("Beef Quality Assurance Manual," 2015). The prevalence of injection site lesions in subprimals in the carcasses of market cows is considerably higher than fed cattle (Roeber et al., 2002; Roeber, Mies, et al., 2001). The high prevalence of lesions found at slaughter correlates with the results of a survey conducted by Glaze and Chahine. The authors report that in more than 32% of Iowa dairies drugs are administered in the semimembranosis, semitendinosis, and gluteal muscles of cows (Glaze & Chahine, 2009). In addition, 70% of estrous synchronization shots on Pennsylvania dairies were given in the hip/rump/flank (Tozer, Varga, Henning, & Holden, 2005).

Headlocks are a common method of restraint on dairy farms and limit access to the neck, which may explain the trend of injections given in the back half of dairy cows. Provision of an acceptable injection site that can be accessed from the rear of the cow may alleviate injection site lesions in valuable subprimals on dairy cow carcasses.

Posilac® (recombinant bovine somatotropin) is labeled for administration in the ischiorectal fossa (IRF), located next to the tailhead, craniodorsal and medial to the tuber ischii.

Several authors have reported that giving $PGF_{2\alpha}$ in the IRF is equivalent to giving $PGF_{2\alpha}$ in the muscle (Chebel, Santos, Rutigliano, & Cerri, 2007; Colazo, Martinez, Kastelic, Mapletoft, & Carruthers, 2002). To the author's knowledge, there are no reports on using the IRF as the site of injection for $PGF_{2\alpha}$ as part of a synchronization protocol in conjunction with timed artificial insemination (AI). The components of estrous synchronization protocols are carefully timed so that subsequent injections are given at the appropriate interval to optimize physiologic response. Based on data from a previous study (Chapter III), the authors believe that the IRF could be implemented in a synchronization and timed AI protocol without affecting the efficacy of the protocol. The objective of this study was to compare timed AI pregnancy rates in beef cows when $PGF_{2\alpha}$ was given IM in the neck or in the IRF. This information will be useful in validating previous data that time to luteolysis does not differ when site of injection of $PGF_{2\alpha}$ is moved from the muscle to the IRF and that this adjustment would not alter the efficacy of a synchronization protocol.

Experimental Procedures

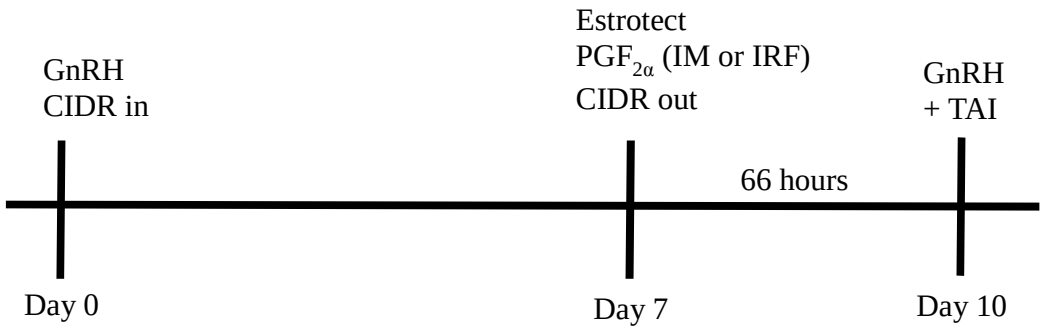
Angus-Simmental cross commercial beef cows (n=1471) in 12 different herds across the Commonwealth of Virginia were used in this study. Cows were pastured on native and improved cool and warm season grasses and were supplemented with hay and grain as needed. The experiment was conducted during the spring (5 herds) and fall (7 herds) of 2014.

Calving records were utilized to block cows by age (4 categories: 2 years old, 3 years old, 4-6 years old, 7 years and older) at each farm and cows within blocks were randomly assigned within block to either treatment (IRF) or control (CN) groups. Cows had to be 30 days post-partum (DPP) on the day of artificial insemination in order to be enrolled in the study. Body condition score (BCS, 1 = thin; 9 = very fat) was recorded on day 0 of the study.

All cows were synchronized using a 7-Day CO-Synch + CIDR protocol, that is they were injected with gonadotropin releasing hormone (GnRH; 100 µg, IM; Cystorelin®, Merial, Athens, GA) and an EAZI-BREED™ CIDR® CATTLE INSERT

(1.38 g progesterone; Zoetis Animal Health, New York, NY) was placed in the vagina on day 0. Cows were given $PGF_{2\alpha}$ (dinoprost; 25 mg; Lutalyse Sterile Solution; Pfizer Animal Health) according to their treatment allocation (IRF or CN) and the CIDR was removed on day 7. Cows in the IRF group were given 25 mg of Lutalyse via a 1 inch 18g needle in the ishiorectal fossa and cows in the CN groups were given 25 mg of Lutalyse via a 1.5 inch 18g needle IM in the neck. Figure 4.1 illustrates the timeline of the synchronization procedure. An Estrotect™ patch was placed on the tailhead at the time of CIDR removal. Sixty-six hours later, cows were given GnRH IM, bred via artificial insemination, and Estrotect™ patch color (gray, red, partial, missing) as well as time and technician were recorded. Angus and Simmental AI sires were matched to cows on the basis of breed and location. In general, two to three sires were used in a herd, with a total of 14 sires used across all farms. Herd bulls were introduced 5 days after artificial insemination and remained with the cows for approximately 65 days.

Figure 4.1 Seven-Day CO-Synch + CIDR protocol



Five experienced veterinarians, 4 of whom used an ultrasound, palpated cows for pregnancy at 47-85 days post insemination. AI pregnancy rate was defined as the number of pregnant cows whose days of pregnancy coincided with days after AI breeding divided by the number of cows who were bred artificially. Cows that were not inseminated were excluded from the analysis. Cows were also excluded from the analysis if a CIDR was missing on day 7 or if a CIDR was mistakenly left in.

Differences between treatment groups in age, days postpartum (DPP), and body condition score (BCS), and time between prostaglandin and insemination were analyzed using Proc TTEST (SAS Inst. Inc., Cary, NC). The authors were interested in the effect of treatment on the expression of estrus because if the prostaglandin injection was ineffective, the cow would not show estrus. Missing and partial patches were not included in the analysis. Differences between treatment groups in estrus expression and AI pregnancy rate were analyzed using Proc GLIMMIX. The models for estrus expression and AI pregnancy rate included the effects of treatment and season and considered age, BCS, and DPP to be covariates. Farm was considered to be a random variable. The models were run with all effects initially; any non-significant effects ($p > 0.1$) were removed.

Results and Discussion

Descriptive statistics for cows enrolled in the study are presented in Table 4.1. No differences were detected in these variables between treatment groups. A mixed linear model was run using Proc Glimmix for estrotect patch color. In the model for estrus expression, the effect of season was removed because it was not significant. Age and DPP were left in the model ($p=0.08$ and $p=0.07$, respectively). BCS had a significant effect on estrus expression ($p<0.0001$). The effects and their significance in the final model are listed in Table 4.2. There was no difference in

the percentage of cows showing estrus at the time of breeding between the CN and IRF groups (Table 4.3; 47.6% versus 47.8%, p=0.56).

Table 4.1 Characteristics of angus-cross commercial cows in 12 herds across Virginia, randomly assigned to be injected with Lutalyse either in the neck (CN) or the ischiorectal fossa (IRF).

Item	*CN*	*IRF*	*SE*	*P value*
N	721	750		
Mean Days Post-Partum[1]	79.4	78.7	0.6	0.39
Mean Age (years)	5.2	5.2	0.1	0.91
Mean Body Condition Score[2]	5.3	5.2	0.04	0.33
Mean Hours PG – Breed[3]	66.4	66.3	0.1	0.54

[1]Days Post-Partum was calculated as the number of days from previous calving until the day of insemination.
[2]Body Condition Score (very thin=1, obese=9) was assessed by trained technicians on day 0 of the study.
[3]Hours PG-Breed was calculated as the time that elapsed from when a cow was given Lutalyse to when she was inseminated.

Table 4.2 Fixed effects in a mixed linear model predicting estrus expression in beef cows given a 7-Day CO-Synch + CIDR protocol

Effect	*F Value*	*Pr > F*
Treatment[1]	0.33	0.56
Days Post-Partum	3.21	0.07
Age	3.15	0.08
Body Condition Score	32.40	<0.0001

[1]Dinoprost was administered either in the muscle (CN) or the ischiorectal fossa (IRF)

Table 4.3 Treatment effects on estrus expression and AI pregnancy rate

Item	*Control*	*IRF*	*F Value*	*Pr > F*
Estrus Expression[1]	47.6%	47.8%	.35	0.56
AI pregnancy rate	52.6%	57.2%	3.44	0.06

[1]Positive estrus expression was defined as a red Estrotect patch. Cows with missing and partially activated patches were not included in the analysis of estrus expression.

In the model for AI pregnancy rate, the effects of season and age were not significant and were dropped from the model. BCS and DPP were both left in the model ($p<0.0001$ and $p=0.04$, respectively). Table 4.4 lists the effects in the final model and their significance. The AI pregnancy rate for the IRF group was numerically higher than the AI pregnancy rate for the CN group (Table 4.3; 57.2% and 52.6%, respectively), although a difference between the two was not detected ($p=0.06$). This numerical difference may have been due to greater attention paid by technicians performing injections in the IRF, since it was a new location to give an injection. This author's opinion is that the difference is not biologically meaningful. Using the standard error from the model, a sample size of 735 animals per treatment group, and assuming $1-\beta = 0.80$, the smallest detectable difference was 6.7%. The power ($1-\beta$) for the difference detected (4.9%) was 0.53.

Table 4.4 Fixed effects in a mixed linear model predicting AI pregnancy rate in beef cows given a 7-day CO-Synch + CIDR protocol

Effect	*F Value*	*Pr > F*
Treatment	3.44	0.06
Days Post-Partum	4.09	0.04
Body Condition Score	20.35	<0.0001

For the purposes of this study, injections given in the IRF were assumed to be given subcutaneously, although this has never been validated in the literature. There are very few studies directly comparing IM and SQ administration of $PGF_{2\alpha}$; both routes have been investigated separately but rarely in head to head comparisons. Intravenous administration has been compared to SQ administration (Lauderdale, 1972) and IM administration (Stellflug, Louis, Hafs, & Seguin, 1975) and researchers generally conclude that a higher dose is needed when administration is IM or SQ in order to achieve plasma levels comparable to that of a lower dose given IV. Edqvist et al. gave sixteen Holstein heifers the same dose of dinoprost either SQ or IM and reported no differences in decline in progesterone, ovarian structures, or signs of estrus between the two groups (Edqvist, Settergren, & Astrom, 1975).

More recently, Colozo et al. investigated giving 500 µg of cloprostenol, a synthetic analogue of $PGF_{2\alpha}$, IM or SQ to beef heifers (n=18) (Colazo, Martinez, Kastelic, & Mapletoft, 2002). The authors report that while there was no difference in the number of animals detected in estrus, the number of animals ovulating, and time to ovulation, heifers given cloprostenol SQ came into heat an average of 6.5 hours later than heifers given cloprostenol IM. Time to ovulation was not different, so it is possible that a difference of 6.5 hours was an artifact of the small sample size or a limitation of the interval of estrus detection (12 hours). In a similar but separate experiment from the same study by Colazo et al., the number of heifers detected in estrus and ovulating was higher in the SQ treatment group than in the IM treatment group. There were 9 heifers in each treatment group and this difference may have been an artifact of the small sample size. In contrast, Chebel et al. gave dinoprost either IM (n=33), SQ (n=36), or in the IRF (n=32) to lactating dairy cows and reported that treatment group had no effect on the decline in serum progesterone concentration or the proportion of cows experiencing luteolysis at 12, 36, and 48 hrs after treatment

(Chebel et al., 2007). In a similar but larger study, Chebel et al. gave lactating dairy cows dinoprost IM (n=153) and in the IRF (n=157) and reported that treatment group had no effect on decline in serum progesterone concentration or the proportion of cows experiencing luteolysis at 48 hours after treatment (Chebel et al., 2007).

Implications

Based on the data from this clinical trial, the ischiorectal fossa can be used as the injection site for $PGF_{2\alpha}$ without affecting efficacy and can be implemented in synchronization protocols without amending the components of those protocols. This management change will alleviate damage done to the muscles of the rear legs: the gluteals, semimembranosis, and semitendinosis. Preserving these muscles will increase the value of dairy and beef cow carcasses and will save the beef industry money at the level of the packer and purveyor.

The efficacy of using the IRF as the injection location for other pharmaceuticals such as vaccines and antibiotics has yet to be validated in controlled studies. The anatomy of the anatomical region has not been fully described; most authors assume that injections given in the IRF go into subcutaneous tissues but this has never been validated. In addition, the ramifications of giving multiple injections over the lifetime of a cow in such a small area should be assessed. It is possible that chronic damage to nerves and ligaments in that area could occur over time with many injection given in the IRF, which could negatively impact welfare.

Literature Cited

Apley, M., Wray, M., & Armstrong, D. (1994). Subcutaneous Injection Site Comparison of Two Multiple Valent Clostridial Bacterin/Toxoids in Feedlot Cattle. *Agri-Practice*, 9-12.

Beef Quality Assurance Manual. (2015). *Beef Quality Assurance*. http://www.bqa.org/CMDocs/bqa/NationalManual.pdf

Chebel, R. C., Santos, J. E., Rutigliano, H. M., & Cerri, R. L. (2007). Efficacy of an injection of dinoprost tromethamine when given subcutaneously on luteal regression in lactating Holstein cows. *Theriogenology, 67*(3), 590-597. doi: 10.1016/j.theriogenology.2006.09.007

Colazo, M. G., Martinez, M. F., Kastelic, J. P., & Mapletoft, R. J. (2002). Effects of dose and route of administration of cloprostenol on luteolysis, estrus and ovulation in beef heifers. *Anim Reprod Sci, 72*(1-2), 47-62.

Colazo, M. G., Martinez, M. F., Kastelic, J. P., Mapletoft, R. J., & Carruthers, T. D. (2002). The ischiorectal fossa: an alternative route for the administration of prostaglandin in cattle. *Can Vet J, 43*(7), 535-541.

Dexter, D. R., Cowman, G. L., Morgan, J. B., Clayton, R. P., Tatum, J. D., Sofos, J. N., . . . Smith, G. C. (1994). Incidence of injection-site blemishes in beef top sirloin butts. *J Anim Sci, 72*(4), 824-827.

Edqvist, L. E., Settergren, I., & Astrom, G. (1975). Peripheral plasma levels of progesterone and fertility after prostaglandin-2alpha induced oestrus in heifers. *Cornell Vet, 65*(1), 120-131.

Fajt, V. R., Wagner, S. A., Pederson, L. L., & Norby, B. (2011). The effect of intramuscular injection of dinoprost or gonadotropin-releasing hormone in dairy cows on beef quality. *J Anim Sci, 89*(6), 1939-1943. doi: 10.2527/jas.2010-2923

George, M. H., Heinrich, P. E., Dexter, D. R., Morgan, J. B., Odde, K. G., Glock, R. D., . . . Smith, G. C. (1995). Injection-site lesions in carcasses of cattle receiving injections at branding and at weaning. *J Anim Sci, 73*(11), 3235-3240.

George, M. H., Morgan, J. B., Glock, R. D., Tatum, J. D., Schmidt, G. R., Sofos, J. N., . . . Smith, G. C. (1995). Injection-site lesions: incidence, tissue histology, collagen concentration, and muscle tenderness in beef rounds. *J Anim Sci, 73*(12), 3510-3518.

Glaze, J. B., Jr., & Chahine, M. (2009). Assessment of management and basic beef quality assurance practices on Idaho dairies. *J Dairy Sci, 92*(3), 1265-1271. doi: 10.3168/jds.2008-1194

Lauderdale, J. W. (1972). *Effects of PFG2a on pregnancy and estrous cycle of cattle.* Paper presented at the Proceedings: American Society of Animal Science, Blacksburg, VA.

Roeber, D. L., Cannell, R. C., Belk, K. E., Scanga, J. A., Cowman, G. L., & Smith, G. C. (2001). Incidence of injection-site lesions in beef top sirloin butts. *J Anim Sci, 79*(10), 2615-2618.

Roeber, D. L., Cannell, R. C., Wailes, W. R., Belk, K. E., Scanga, J. A., Sofos, J. N., . . . Smith, G. C. (2002). Frequencies of injection-site lesions in muscles from rounds of dairy and beef cow carcasses. *J Dairy Sci, 85*(3), 532-536. doi: 10.3168/jds.S0022-0302(02)74105-2

Roeber, D. L., Mies, P. D., Smith, C. D., Belk, K. E., Field, T. G., Tatum, J. D., . . . Smith, G. C. (2001). National market cow and bull beef quality audit-1999: a survey of producer-related defects in market cows and bulls. *J Anim Sci, 79*(3), 658-665.

Stellflug, J. N., Louis, T. M., Hafs, H. D., & Seguin, B. E. (1975). Luteolysis, estrus and ovulation, and blood prostaglandin F after intramuscular administration of 15, 30 or 60 mg prostaglanding F2alpha. *Prostaglandins, 9*(4), 609-615.

Tozer, T. R., Varga, V. A., Henning, W. R., & Holden, L. A. (2005). Do Dairy Producers Use Effective Management Practices to Improve the Value of Market Cows? *The Professional Animal Scientist*, 272-277.

Chapter V

Summary and Conclusion

Beef and dairy producers have been giving cattle injections in order to increase health and productivity for years and the benefits of these injections are well-documented. More recently, the detriments of these injections have also been documented as injection site lesions that decrease the carcass value of fed cattle and market cows alike. The beef industry has exerted a significant effort towards ameliorating this problem by changing injection techniques for cattle on feed; improvements in injection techniques in beef and dairy cows, however, have lagged behind and market cow carcasses continue to have a high prevalence of injection site lesions in valuable primal cuts such as the rounds. The lesions must be trimmed out of the carcass and discarded, which results in a loss of revenue for the beef industry.

Contributing factors to this problem include a lack of producer education and the design of current restraint systems. Headlocks are a common management tool used on dairies to restrain groups of cows for the administration of various injectable products and restrict access to the neck while allowing excellent exposure to the muscles of the back legs. Provision of an alternative site of injection in the rear of the cow that avoids the muscles should provide a compromise that improves carcass quality within the existing restraint facilities. The ischiorectal fossa has been investigated for the administration of $PGF_{2\alpha}$ but had not been fully validated prior to this thesis.

Timing of reproductive hormone administration is important; often estrus synchronization protocols involve a series of injections and the interval between these injections has been carefully determined to optimize physiologic response. We report that not only is the ischiorectal fossa a feasible injection location for $PGF_{2\alpha}$ but that the time to physiologic response was similar, within 4 hours, to an intramuscular injection.

Several researchers had demonstrated that an isolated injection of $PGF_{2\alpha}$ given in the ischiorectal fossa would result in luteolysis. This study was unique in that we examined implementing the ischiorectal fossa as the injection location for $PGF_{2\alpha}$ as part of an estrus synchronization protocol and found that route of injection did not affect either estrus expression at breeding or timed AI pregnancy rate.

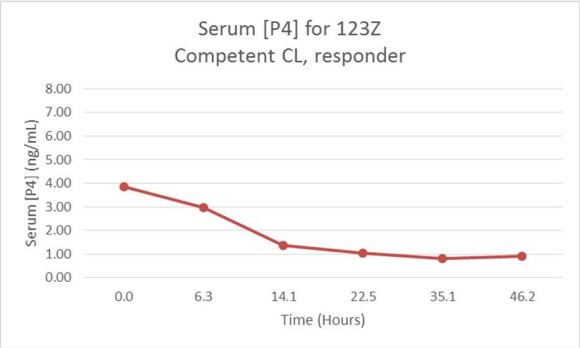
In conclusion, the ischiorectal fossa can be implemented by producers as an alternative injection location for $PGF_{2\alpha}$ in estrus synchronization protocols that will not affect fertility. Using the ischiorectal fossa rather than the muscles of the rear legs as an injection location for $PGF_{2\alpha}$ should improve carcass quality and augment revenue for the beef industry.

Appendix 1

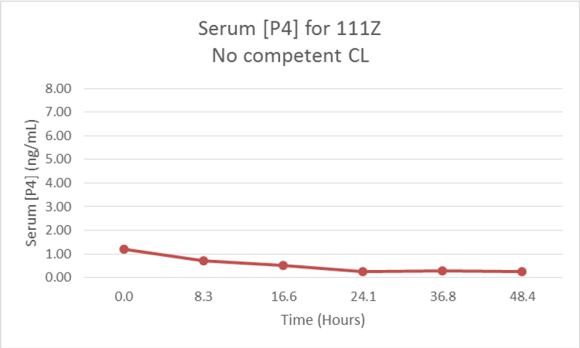
Serum Progesterone Curves

Figure A.1: Examples of serum progesterone curves for A) a heifer that had a competent corpus luteum that did undergo luteolysis, B) a heifer that did not have a competent corpus luteum, and C) a heifer that did have a competent corpus luteum but did not undergo luteolysis.

A)



B)



C)