# REPRODUCTIVE AND ECONOMIC OUTCOMES FOLLOWING PRE-SYNCHRONIZATION OF DAIRY HEIFERS

A Thesis

# Presented in Partial Fulfillment of the Requirements for the

Degree of Master of Science

with a

Major in Animal Science

in the

College of Graduate Studies

at the University of Idaho

by

Courtney K. Claypool

Major Professor: Joseph C. Dalton, Ph.D.

Committee Members: Amin Ahmadzadeh, Ph.D. and Neil Rimbey, Ph.D.

Department Administrator: Mark McGuire, Ph.D.

May 2015

# AUTHORIZATION TO SUBMIT THESIS

This thesis of Courtney Claypool, submitted for the degree of Master of Science with a Major in Animal Science and titled "Reproductive and economic outcomes following presynchronization of dairy heifers," has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

Major Professor: _		Date:
	Joseph Dalton, Ph.D.	
Committee Members:		
_		Date:
	Amin Ahmadzadeh, Ph.D.	
_		Date:
	Neil Rimbey, Ph.D.	
Department		
Administrator:		Date:
	Mark McGuire, Ph.D.	

# ABSTRACT

Hypotheses were that pre-synchronization of estrus in dairy heifers, in comparison with no pre-synchronization, would 1) reduce days to first AI, and 2) result in an increased proportion of heifers pregnant within the first week of the breeding program. The 14 d CIDR group (n=119) received a controlled internal drug releasing insert (CIDR) on d -30, which was removed on d -16, followed by an injection of prostaglandin  $F_{2a}$  (PG) on d 0. The 2X PG group (n=118) received an initial injection of PG on d -11, and a second injection of PG on d 0. The control group (1X PG; n=121) received an injection of PG on d 0. Heifers received AI upon detected estrus. Pre-synchronization affected (P<0.05) days to first AI and the proportion of heifers pregnant within the first week of breeding eligibility. The 14 d CIDR treated heifers had reduced days on feed (P<0.05) (breeding pen entry to projected calving date) compared to control heifers.

Keywords: dairy heifers, pre-synchronization, CIDR

# ACKNOWLEDGMENTS

I would like to thank the following people and groups for their support and encouragement throughout my graduate program at the University of Idaho:

My major professor Dr. Joseph Dalton for his outstanding mentorship and passion for education.

My committee members, Dr. Amin Ahmadzadeh and Dr. Neil Rimbey for their expertise and guidance.

Dr. Bahman Shafii and Dr. Bill Price for their assistance in statistical analysis.

Jennifer Spencer, Darryl Kerby, Saulo Zoca, and Darin Mann for helping make my research trial possible.

# DEDICATION

To my friends and family members who have told me to never settle for less than the best.

AUTHORIZATION TO SUMBIT THESIS	ii
ABSTRACT	iii
ACKNOWLEDGMENTS	iv
DEDICATION	v
TABLE OF CONTENTS	vi
LIST OF FIGURES	ix
LIST OF TABLES	x
ABBREVIATION KEY	xi
INTRODUCTION	1
CHAPTER ONE: A REVIEW OF LITERATURE	
Reproductive Management	3
The Bovine Estrous Cycle	
Follicular Phase	4
Luteal Phase	5
Estrous Behavior and Detection in Cattle	7
Estrous Synchronization	8
Prostaglandin	9

# TABLE OF CONTENTS

Progestins	9
Artificial Insemination	13
Semen Handling	14
Economics of Heifer Rearing	14
Partial Budgeting	15
CHAPTER TWO: REPRODUCTIVE AND ECONOMIC OUTCOMES FOLLOWING PRE-SYNCHRONIZATION OF DAIRY HEIFERS	
EXPERIMENTAL OBJECTIVES AND RATIONALE	17
MATERIALS AND METHODS	19
Animals	19
Treatments	19
Housing	21
Blood Collection	21
Pregnancy Diagnosis	22
Statistical Analysis	22
Calculation of Reproductive Metrics	22
RESULTS	24
Heifer Body Weight	24
Cyclic Status	24

Estrous Detection
Days to First Artificial Insemination
Days to Pregnancy
Proportion of Heifers Pregnant within First Week of Breeding Eligibility
First Service Conception Rate during the First Week of Breeding Eligibility
Days on Feed from Entrance to Breeding Pen to Projected Calving Date
DISCUSSION
Body Weight and Cyclic Status
Estrous Detection
Days to First Artificial Insemination
Proportion of Heifers Pregnant within First Week of Breeding Eligibility
First Service Conception Rate during the First Week of Breeding Eligibility
Days on Feed from Entrance to Breeding Pen to Projected Calving Date
Economic Implications
CONCLUSIONS
FIGURES
TABLES
LITERATURE CITED

# LIST OF FIGURES

Figure 2.1 Pre-synchronization and control treatments 1- 14 d CIDR (CIDR was inserted
on d -30 and removed on d -16, PG administered on d 0), 2- 2X PG (PG injections
administered on d -11 and d 0), 3- 1X PG (PG administered only on d 0)
Figure 2.2 Distribution of standing estrus within 5 d of CIDR removal for 14 d CIDR
treatment
Figure 2.3 Distribution of standing estrus within 5 d of PG administration for 14 d CIDR
treatment
Figure 2.4 Distribution of standing estrus within 5 d of PG administration for 2X PG
treatment

# LIST OF TABLES

<b>Table 2.1.</b> Reproductive performance of dairy heifers treated with progestin and	
prostaglandin $F_{2\alpha}$ based pre-synchronization protocols	41
Table 2.2. Costs of items included in economic evaluation	42
Table 2.3. Partial budget for dairy heifer pre-breeding protocols in comparison to the	1X PG
(control) protocol already implemented at M/M Feedlot, Parma, ID	42

# **ABBREVIATION KEY**

- AI- artificial insemination
- CIDR- controlled internal drug releasing insert
- CL- corpus luteum
- CR- conception rate
- DOF- days on feed
- E<sub>2</sub>- estradiol
- FSH- follicle stimulating hormone
- GnRH- gonadotropin releasing hormone
- LH- luteinizing hormone
- OXY- oxytocin
- P<sub>4</sub>- progesterone
- PG- prostaglandin  $F_{2\alpha}$

# **INTRODUCTION**

Raising replacement heifers, together with feed and labor costs associated with the lactating herd, is one of the largest expenses of a dairy farm (Frazer, 2013). Consequently, efficient pregnancy production in replacement heifers provides an earlier return on investment for dairy producers.

Feed is the single largest expense in the heifer raising enterprise. Consequently, time from birth to first calving is important. Days on feed is determined by age at first calving. Age at first calving is determined by age at conception; therefore, timely and efficient pregnancy production is important. When evaluating heifer raising programs, average age at conception should be the focus, as delays in age at conception result in prolonged rearing costs and a loss of income, primarily due to decreased productive life, milk yield, and reproductive performance (Keown and Everett, 1986; Gabler et al., 2000; Ettema and Santos, 2004).

To reach an age at conception goal, time from breeding program enrollment to first artificial insemination (AI) needs to be considered. This is because the sooner an animal receives AI, the sooner she can conceive, and consequently the sooner she can potentially calve. While there are numerous estrous synchronization programs used to efficiently facilitate reproduction in dairy heifers, there is not one that will fit the managerial practices on each individual farm. One of the most common programs used in heifer management involves an injection of prostaglandin  $F_{2\alpha}$  (PG) upon entry to the AI pen. Heifers detected in estrus after PG injection receive AI, whereas those not detected in estrus are not inseminated and are eligible to receive another injection of PG 11 to 14 d later.

1

Pre-synchronization during the voluntary waiting period in lactating dairy cows has become a common strategy to facilitate timely AI and generate pregnancies. The goal of managing the pre-breeding period of lactating dairy cows is to synchronize and prepare them to conceive to AI upon enrollment in the breeding program.

In an effort to manage the pre-breeding period similarly to the voluntary waiting period of lactating cows, we proposed the investigation of two pre-synchronization strategies in dairy heifers. The two strategies included 1) 14 d progesterone insert (controlled internal drug release inert, CIDR) followed by PG and then AI after detection of estrus, and 2) 2X PG and AI after detection of estrus. Despite the fact that approximately 65-70% of heifers will be in estrus within 5 d of the first PG injection (Louis et al., 1973; Lauderdale, 2009), a second injection of PG given 11 d later should result in a greater percentage of treated heifers in estrus within 5 d of the second injection (Escalante et al., 2013). Use of a 14 d CIDR protocol in beef heifers was reportedly effective in synchronizing both ovulation and corpus luteum (CL) formation (Leitman et al., 2008) so that PG could be administered 16 d after CIDR removal to facilitate AI. This strategy is expected to produce a tight synchrony of heifers detected in estrus and AI. The same 14 d CIDR protocol was also reported to be effective for timed AI in dairy heifers (Mallory et al.,

#### **CHAPTER ONE: A REVIEW OF LITERATURE**

#### **Reproductive Management**

Management is the sum of decisions and actions which drive a program toward success or failure (Dzuik and Bellows, 1983). Convenience, economics, and disease control are among many facets that have driven animal agriculture towards more intensive reproductive management (Dzuik and Bellows, 1983). Before a successful reproductive management program is set in place, it is important to understand the biological and physiological events that occur within the animal before, during, and after pregnancy.

# The Bovine Estrous Cycle

Females enter a period of reproductive cyclicity after achieving sexual maturity. Heifers will begin cycling once they have reached 40 -50% of their mature weight (Head, 1992, Chebel, 2010). The estrous cycle consists of a series of predictable reproductive events which allow the female to become pregnant (Senger, 2012). The bovine estrous cycle is a 21 d cycle on average (17-24 d in length depending on the animal) and is regulated by a plethora of hormones secreted from the hypothalamus, anterior pituitary gland, ovaries, and the uterus. The estrous cycle is comprised of two phases: the follicular phase and the luteal phase. The two phases are separated by the event of ovulation. Different hormones and structures dominate each of these phases (Senger, 2012).

### Follicular Phase

The follicular phase is characterized by follicular growth in a low progesterone (P<sub>4</sub>) environment. It is the shorter of the two phases that make up the bovine estrous cycle (Smith et al., 2010). The follicular phase begins with regression of the CL and ends with ovulation (Senger, 2012). This phase can be subdivided into two stages, proestrus and estrus. Proestrus is the period that precedes estrus and is characterized by the endocrine transition from progesterone dominance to estradiol dominance (Senger, 2012). Regression of the corpus luteum (CL), or luteolysis, is accompanied by a drop in P<sub>4</sub> which abolishes negative feedback on the tonic center of the hypothalamus, specifically affecting gonadotropin releasing hormone (GnRH). Consequently, GnRH is emitted in a high frequency, low amplitude fashion, which results in high frequency, low amplitude luteinizing hormone (LH) pulses to be released from the anterior pituitary. The increased pulsatility of LH stimulates the oocyte to resume meiosis and the dominant follicle to proceed towards ovulation (Senger, 2012).

An increase in the pulsatility of both GnRH and LH stimulates the dominant follicle to produce an increasing amount of estradiol ( $E_2$ ). Follicle stimulating hormone (FSH) is an important player in the production of follicular  $E_2$ , as described in the two cell, two gonadotropin model (Senger, 2102).

Cattle tend to have two or three follicular waves during an estrous cycle which are mediated by action of FSH and LH. A follicular wave begins with the recruitment of a cohort of small follicles. Recruitment occurs when FSH levels are high and there are lower levels of  $E_2$  and less frequent pulses of LH (Senger, 2012). Then as the FSH concentration decreases and LH concentration rises, one single follicle of the cohort is selected to continue growing and becomes the dominant follicle (Smith et al., 2010). As the dominant follicle matures, the number of LH receptors increase and the granulosa cells start to secrete inhibin. Inhibin is a glycoprotein hormone that aids in inhibition of FSH from the anterior pituitary, which ultimately causes the other follicles in the cohort undergo atresia (Smith et al., 2010). Atresia is an irreversible degenerative process that is the fate of over 90% of ovarian follicles (Senger, 2012). In the presence of high progesterone, the dominant follicle will not proceed towards ovulation, will undergo atresia, and another follicular wave will begin. In contrast, following luteolysis the dominant follicle will continue to grow and secrete  $E_2$  which, once at threshold level, feeds back to the surge center of the hypothalamus and causes a GnRH surge. This surge of GnRH is what prompts the preovulatory LH surge from the anterior pituitary. Elevated concentrations of  $E_2$  paired with low concentrations of  $P_4$  are the cause of profound behaviors in females during the time of estrus (Senger, 2012). Increased vocalization and activity, as well as standing to be mounted are behaviors influenced by hormone profile during estrus.

## Luteal Phase

The luteal phase is longer than the follicular phase as it spans about 80% of the estrous cycle (Senger, 2012). It is defined by the presence of a CL. The CL is a transient endocrine organ required for normal pregnancy (Niswender et al., 1994). The CL is one of the few tissues of a mature animal that exhibit regular periods of growth (Shams et al., 2004). Formation of the CL is initiated by a series of morphological and biochemical changes in the theca and granulosa cells of the preovulatory follicle (Shams et al., 2004). The lifespan of the CL starts

with its formation immediately after ovulation, then its maintenance, and its destruction during luteolysis.

The luteal phase is comprised of two sub-stages. The first of the two stages which immediately follows estrus is called metestrus. Metestrus can be identified as the period between ovulation and the formation of the CL (Senger, 2012). During metestrus, E<sub>2</sub> and P<sub>4</sub> are low.

Luteinization is the process by which CL formation occurs at the site of ovulation. During luteinization, structural remodeling of ovarian tissue gives rise to an intraovarian endocrine gland; the CL (Senger, 2012). This occurs following the pre-ovulatory LH surge, ultimately resulting in differentiation of follicular cells into luteal cells (Shams et al., 2004). The corpus luteum begins to secrete P<sub>4</sub> shortly after ovulation, but 2-5 days are needed before significant quantities are produced (Senger, 2012). Progesterone is arguably the most important hormone involved in the estrous cycle (Niswender et al., 2000). Progesterone ultimately controls the estrous cycle by suppressing estrus and preventing ovulation (Escalante et al., 2013). This hormone also primes the uterine glands to support the early development of the conceptus, and to inhibit muscle contractions of the myometrium.

The diestrus stage follows metestrus. It is the longest stage of the four that make up the estrous cycle, lasting 10-14 d (Senger, 2012). Diestrus begins when the CL is fully functional and high concentrations of P<sub>4</sub> are being secreted (Senger, 2012). If pregnancy does not occur, the CL regresses so that a new cycle may be initiated. Diestrus ends with luteolysis. Luteolysis occurs when the CL loses the capacity to synthesize and secrete P<sub>4</sub>. This is followed by a loss of cells that make up the CL (Niswender et al., 2000). Uterine prostaglandin  $F_{2\alpha}$  (PG) is considered to be the primary luteolytic hormone in mammals

(Niswender et al., 1994). Prostaglandin  $F_{2\alpha}$  reduces blood flow to the CL which aids in luteolysis by depriving the CL of nutrients, substrates for steroidogenesis, and luteotropic support (Niswender et al., 2000). Deprivation of blood flow to the CL is detrimental to luteal function because the CL is a highly vascularized structure. More than 80% of ovarian blood supply goes to the CL (Niswender et al., 1994).

There is evidence that oxytocin (OXY) and PG stimulate each other in a positive feedback manner during luteolysis. According to Senger (2012), injections of exogenous OXY cause secretion of PG by the uterus, and injections of PG lead to rapid release of ovarian OXY during the late luteal phase. In ruminants, action of OXY on the uterus promotes PG secretion which acts on the CL to begin luteolysis (McCracken et al., 1999). Consequently, large luteal cells release OXY which stimulates further PG secretion by the uterus, which results in continued luteolysis (McCracken et al., 1999).

# **Estrous Behavior and Detection of Estrus in Cattle**

Estrus can be defined as the period in which a female is sexually receptive to males (Senger, 2012). The primary sign of estrus in cattle is standing to be mounted by other individuals, which is termed lordosis. Secondary signs of estrus include frequent mounting, clear vulvar mucous, increased vocalization, and increased activity or restlessness (Senger, 2012). A study conducted by Kiddy (1977) confirmed that cows in estrus had approximately four times the activity of cows not observed in estrus. Amyot and Hurnik (1987) performed a study that monitored cows with time-lapse video recording and found that cows spend more

time walking and less time eating or resting when in estrus than when they were not in estrus.

Poor detection of estrus is arguably the most critical problem that limits reproductive efficiency in dairy herds. In 1994, Senger estimated that failure to detect estrus resulted in an annual loss of over \$300 million to the U.S. dairy industry. That figure is likely much higher in 2015 due to increased costs over the past 21 years.

There are many estrous detection aids that are commercially available to dairy cattle operations. Some of the most popular and less expensive detection aids are tail chalk or paint and heat mount patches. A variety of automated heat detection and activity monitoring systems are commercially available and may play a vital role when used in conjunction with visual observation and appropriate interpretation of records (Dalton, 2011).

#### **Estrous Synchronization**

The purpose of an estrous synchronization program in dairy herds is to manage reproduction more efficiently (Pursley et al., 1997). Estrous synchronization groups animals together, which is more convenient from a managerial standpoint since AI technicians can breed more animals on a given day or days. Today there is a wide variety of recognized methods used to synchronize estrus in dairy heifers (DCRC, 2014). Estrus can be synchronized by using various hormones. The primary hormones used are prostaglandins and progestins due to their convenience and effectiveness.

### Prostaglandin

The most common and inexpensive way to synchronize estrus is by treating animals with a luteolytic dose of PG. According to the National Animal Health Monitoring System Part IV published in 2007, 16.3% of dairy operations used hormones for synchronization of estrus in heifers for first AI. Of these herds, approximately 65% used PG injections to cause luteolysis (USDA, 2009). Approximately 65-70% of heifers injected with PG will show estrus within five days of treatment (Escalante et al., 2012). The injection of PG causes the regression of a mature CL and allows for the continued development of a dominant follicle. This is then followed by the development of a pre-ovulatory follicle, then behavioral estrus, and ovulation (Lucy et al., 2001, Stevenson et al., 2008). A CL that is less than 5 d old will not usually be regressed with a single injection of PG because it is not mature enough. To overcome this limitation, 2 injections of PG can be administered between 11 and 14 d apart. After the second injection of PG, a larger percentage of animals will exhibit estrus than if only one injection was administered (Escalante et al., 2013). Prostaglandin  $F_{2\alpha}$  based protocols are low cost and are effective in shortening the interval to first AI. They also improve economic return when compared to insemination on spontaneous estrus (Lopes et al., 2013).

# Progestins

An early method used to control the estrous cycle in beef cattle was by feeding a progestin such as melengestrol acetate (MGA) or administering medroxyprogesterone acetate (MPA) (Patterson et al., 1989, Odde, 1990, Lauderdale, 2009). Controlled internal drug release (CIDR) inserts are commonly used in synchronization protocols as a means of progestin treatment prior to PG treatment. The CIDR is a vaginal insert that releases P<sub>4</sub> in a controlled fashion and functions to delay estrus and ovulation until after removal, thereby facilitating synchronization of estrus and AI. Depending on the day of the estrous cycle when the CIDR is inserted and the duration of the treatment, a CL could be present and functional at the time of CIDR removal. If there is a functional CL present at the time of CIDR removal, it will take longer for those animals to show estrus after CIDR removal (Leitman et al., 2008; Escalante et al., 2012). If the CIDR treatment is started in the mid or late phase of the estrous cycle, a persistent dominant follicle is likely to develop upon CL regression during the treatment (Sirois and Fortune, 1990; Kinder et al., 1996). Fertility after long term progestin (MGA and CIDR) treatment is poor because the persistent dominant follicle contains an aged oocyte when ovulation occurs after progestin removal (Ahmad et al., 1995; Revah and Butler, 1996; Roche et al., 2009). Although fertility is decreased due to an aged oocyte, a fully functional CL can still be formed.

Studies done on long term CIDR use in beef cattle have shown promising results in regard to tight estrus synchronization (Mallory et al., 2012). However, few studies have been conducted on long term CIDR use in dairy cattle. One such study done by Escalante and associates (2013) examined follicular populations and luteal function in dairy heifers treated with a 14 d CIDR (n=57) vs. heifers treated with a simple one shot PG protocol (n=57). Escalante et al. (2013) reported more heifers pre-synchronized with the 14 d CIDR (89%) had CL that were greater than 20 mm in diameter compared with the control group (55%) on d 30 of the study. Escalante and co-workers (2013) also reported a higher percentage (75.4%) of dairy heifers treated with a 14 d CIDR and PG treatment (CIDR inserted d 0

through 14, PG administered on d 30) exhibited estrus as compared to the control group which employed a single PG injection on d 30 (22.8%). Conception rate was higher for heifers treated with the 14 d CIDR (61.2%) vs. heifers treated with a single injection of PG (40.6%). Escalante et al. (2013) also reported that heifers were first detected in estrus approximately two days after CIDR removal.

Escalante and associates (2012) conducted an earlier pre-synchronization study utilizing a 14 d CIDR protocol in postpartum dairy cows (n=1,021). The study assessed whether or not a luteolytic dose of PG at time of CIDR removal would enhance estrous synchrony and CR compared with no PG at the time of CIDR removal. Escalante et al. (2012) reported that an injection of PG upon CIDR removal, as compared to no PG at CIDR removal, increased the amount of estrus activity detected within 5 d (determined by visual observation of compete removal of tail paint; 68.9% vs. 58.1%, respectively) However, there was no effect on CR.

Another study that involved 14 d CIDR use in dairy heifers was conducted by Mallory and associates in 2013. This study compared the reproductive outcomes of heifers synchronized with a 14 d CIDR and then bred using conventional or sexed semen. All heifers (n=240) were synchronized using the Show-Me-Synch protocol (14 d CIDR followed by PG 16 d later on d 30, then GnRH and TAI 66 hours later). The results provide evidence that estrous response did not differ between groups (53% and 58%, respectively). Pregnancy per AI was greater for heifers that received conventional semen compared with those who received sexed semen (68% vs. 38%, respectively) (Mallory et al., 2012).

In a study done by Kojima and associates in 2004, distribution of estrus and AI was more synchronous among beef heifers treated with a 14 d CIDR than those fed MGA for 14 days. Conception rate following synchronization with a 14 d CIDR was 63% compared to 43% for

heifers treated with MGA for 14 d. It was theorized that differences between the two treatments may have resulted, in part, due to improved synchronization of follicular waves due to 14 d CIDR treatment (Kojima et al., 2004).

Busch and associates (2007) performed a study comparing two TAI protocols in beef heifers (n=217). The CIDR Select protocol consisted of a 14 d CIDR followed by GnRH 9 d after CIDR removal, an injection of PG 7 d after GnRH, and then GnRH and TAI 72 hours later. The Co-Synch+ CIDR protocol consisted of a 7 d CIDR (inserted d 23-30 of study), followed by an injection of PG (administered after CIDR removal on d 30) and then TAI 54 hours later. Busch et al. (2007) reported that pre-synchronization with a 14 d CIDR before GnRH (9 days after CIDR removal) and a PG injection (7 days after GnRH) enhanced the estrous response of heifers (87% detected in estrus compared with 67%) as well as improved the pregnancy rate to TAI (62% vs. 47%, respectively).

Progestins are also known to induce cyclicity in cattle that are not cycling at the start of treatment. This is because removal of exogenous P<sub>4</sub>, as in the case of removal of a CIDR, mimics luteolysis; the drop in P<sub>4</sub> leads to a lack of negative feedback on the tonic center of the hypothalamus. Consequently, GnRH and LH pulsatility changes to a high frequency, low amplitude fashion, leading to further follicular development, an increase in E<sub>2</sub>, and eventually behavioral estrus and ovulation. Lucy and associates (2001) found that the use of a CIDR and PG treatment improved synchronization of estrus and pregnancy rate in acyclic and cyclic beef and dairy heifers when compared to treatment groups that did not receive a CIDR.

#### **Artificial Insemination**

Since its adoption by the dairy industry, artificial insemination (AI) has been important in reducing disease transmission, allowing for genetic selection, and ultimately increasing the health and productive life of cows (Dransfield et al., 1998). Timing of AI has been studied in depth for more than 70 years (Trimberger and Davis, 1943) with the intent of trying to improve fertility of dairy cattle.

Due to the short lifespan of the oocyte in cattle (Chohan and Hunter, 2003), the interval from ovulation to AI is critical for optimizing fertility in cattle inseminated based on activity associated with estrus. An early study done by Trimberger and Davis (1943), in which intense visual observation of estrus was employed, indicated that the highest conception rates associated with AI were achieved from the middle of the estrus period until a few hours after the end of the expression of standing behavior. From this research, the A.M/P.M management guideline was developed, which states that cows observed in estrus in the morning should be bred in the evening and cows observed in estrus in the evening should be bred in the evening and Davis., 1943). Factors that affect fertility include the functional viable life of the gametes, transport time of viable sperm from site of AI to fertilization, in addition to the timing of AI in relation to ovulation (Dransfield et al., 1998). Sperm transport (from the site of deposition to the oviducts) of viable sperm takes roughly 6 hours (Hawk, 1987), while sperm are thought to have a lifespan of approximately 24 hours in the female bovine reproductive tract (Trimberger, 1948).

Proper semen handling ensures that sperm will not be compromised prior to insemination. Frozen semen is stored in a liquid nitrogen (LN<sub>2</sub>) tank at -320 degrees Fahrenheit. The tank should be handled with care, and should never be tipped over or dropped. Frost or freezing around the neck or body of the tank is indicative of vacuum loss which, if not caught early enough, could lead to premature thawing of sperm.

As described by Dalton et al. (2004), fertility following AI is most likely to be maximized when technicians a) accurately identify animals in estrus, b) thaw semen in warm water (95-98 degrees F) for a minimum of 45 seconds, c) use appropriate hygienic procedures, d) maintain thermal protection of straws during AI gun assembly and transport to the cow, and e) deposit semen in the uterus within approximately 15 minutes after thawing.

# **Economics of Heifer Rearing**

Raising replacement heifers is one of the most expensive aspects of a dairy business. The total cost of raising dairy replacements depends on two major factors, the costs directly associated with growing the heifers and the number of heifers grown. These costs include feed, labor, reproduction, health and housing (Tozer and Heinrichs, 2001). According to the Idaho Livestock Costs and Returns Estimate (Painter and Gray, 2012), the cost to raise heifers from 13-22.5 months of age is between \$1.80 and \$2.29 per head per day, and increases with age. The relationship between increased cost and increased age can be explained by the increase in feed consumption as the heifers continue to grow and mature. Larger animals consume more feed and the number of days on feed increases with age.

Consequently, heifers that calve at an older age result in overall higher feed costs. The opposite is true of animals that calve at a younger age. Tozer and Heinrichs (2001) conducted a study on the economics of raising replacement heifers in a closed 100 cow herd and reported that a lower age at first calving ultimately lowered total rearing costs and required fewer replacement heifers to be raised.

Synchronization strategy is also a factor in the expense of heifer rearing. In regard to breeding strategy expenses, Stevenson and associates (2008) conducted a study that compared costs of 4 different breeding protocols. The protocols assessed were 1) a single PG injection on day of breeding pen enrollment followed by estrus detection and AI; if not bred after 14 d, they were given a second injection of PG and observed for estrus and artificial insemination, 2) insertion of a CIDR for 7 d, followed by an injection of PG on day of CIDR removal, and then either breeding based on estrus detection or enrolled in TAI if they weren't bred by 72 hours post PG injection, 3) GnRH on d 0, followed by insertion of a CIDR for 7 d with an injection of PG and GnRH and TAI 48 hours later, and 4) a control group that was only observed and bred off of estrus. It was reported that overall, the single injection of PG on day 0 was the least expensive protocol and resulted in the lowest cost per pregnancy generated.

### **Partial Budgeting**

Partial budgeting is a planning and decision-making strategy used to compare the costs and benefits of alternative management strategies that are considered by a farm business (Penn State Agricultural Research and Cooperative Extension, 2002). A partial budget analysis focuses on associated changes in income and expenses as a result of implementing alternative management strategies, in comparison to those currently implemented on a farm. A partial budget allows producers to ask how proposed changes could impact profitability of an enterprise (Penn State Agricultural Research and Cooperative Extension, 2002).

Partial budgets assume that small business changes have effects in one of the following areas: increase in income, reduction or elimination of costs, increase in costs, or a reduction in income (Iowa State University Extension and Outreach, 2006). Taking added and reduced costs of implementing changes into account can help individuals make decisions when considering making changes on the farm (Penn State Agricultural Research and Cooperative Extension, 2002). It is important to recognize that the value of a partial budget depends on the quality of the data incorporated in the analysis. Accurate data is needed to ensure that the partial budget constructed provides an accurate analysis. It is also important to recognize that while partial budgeting can be a useful tool to aid decision making regarding potential changes on the farm, it only estimates possible financial impact and does not assure outcomes (Iowa State University Extension and Outreach, 2006).

# CHAPTER TWO: REPRODUCTIVE AND ECONOMIC OUTCOMES FOLLOWING PRE-SYNCHRONIZATION OF DAIRY HEIFERS

# EXPERIMENTAL OBJECTIVES AND RATIONALE

There are many costs associated with raising replacement dairy heifers. The approximate cost per heifer per year in the 12-22 month age bracket is \$860; this includes feed, labor and veterinary expenses (Painter and Gray, 2012). In order to provide an earlier return on these animals, it is crucial to have an effective breeding program established to efficiently produce pregnancies. Age at first calving is determined by age at conception; this is why timely and efficient pregnancy production in heifer raising facilities is important. Age at first conception should be more carefully considered than age at first calving; delays in age at conception ultimately lead to an overall loss of income and shorten both the animal's reproductive and productive life (Keown and Everett, 1986; Gabler et al., 2000; Ettema and Santos, 2004).

To reach an age at conception goal, time from entry to the breeding pen to first AI must be considered. The focus of this study involved the utilization of two pre-synchronization protocols to manage the pre-breeding period of virgin Holstein heifers similarly to the way the voluntary waiting period of lactating dairy cows is managed. The two treatments were 1) 14 d CIDR, and 2) 2X PG. Hypotheses were that 1) pre-synchronization of estrus would reduce the number of days to first AI compared with no pre-synchronization, and 2) use of pre-synchronization would result in an increased proportion of heifers pregnant within the first week of entry into the breeding program compared with no pre-synchronization.

Implementing pre-breeding strategies such as the two under investigation in this study may be economically beneficial to dairy replacement heifer programs. Therefore, an economic analysis was performed to allow for a better understanding of the costs and benefits, if any, associated with each pre-synchronization strategy implemented.

# **MATERIALS AND METHODS**

All procedures were approved by the University of Idaho's Animal Care and Use Committee.

# Animals

Holstein heifers at a heifer raising facility located in south western Idaho were used in this study (n=358). On day -35, treatment animals (n=237) were selected based on projected criteria on day of entry to AI pen (weight  $\geq$  390.1 kg, height at the withers  $\geq$  129.54 cm, and age  $\geq$  12.5 mo). Heifers were then randomly assigned one of two treatment groups. Control heifers were selected based on the same criteria.

The diets for all heifers used in this study included corn silage, alfalfa hay, mint silage, straw, dried distillers grains, steam flaked corn, UltraFerm (molasses by-product) and a vitamin, trace mineral pack with Rumensin. The diet was formulated to exceed the nutritional requirements of Holstein heifers gaining 0.8 kg/d (NRC, 2001).

# Treatments

Heifers in treatment group 1 (14 d CIDR; n=119) received an intravaginal progesterone insert (EAZI-BREED CIDR, Zoetis, Florham Park, NJ) on d -30 for 14 d (Figure 2.1). On d -16 of the study, CIDR removal occurred. On d 0 heifers in this group received a single injection of PG (25 mg i.m. Lutalyse Sterile Solution, Zoetis, Florham Park, NJ) and tail

paint (Rust-oleum Specialty Fluorescent Orange, Vernon Hills, IL) to facilitate detection of estrus.

Heifers in treatment group 2 (2X PG; n=118) received an injection of PG on d -11 and on d 0 (25 mg i.m. Lutalyse Sterile Solution, Zoetis, Florham Park, NJ) (Figure 2.1). On d 0 these heifers also received tail paint (Rust-oleum Specialty Fluorescent Orange, Vernon Hills, IL) to facilitate the detection of estrus.

Control heifers (1X PG; n=121) were administered a single injection of PG (25 mg i.m. Lutalyse Sterile Solution, Zoetis, Florham Park, NJ) on d 0 in accordance with the current management strategy at the heifer raising facility (Figure 2.1). Heifers also received tail paint (Rust-oleum Specialty Fluorescent Orange, Vernon Hills, IL) to facilitate detection of estrus. Heifer ranch personnel administered PG to control heifers, all of which were housed in a single pen.

All study heifers were restrained in headlocks once daily to facilitate tail paint application, detection of estrus based on removed tail paint, and AI. A single technician with > 40 yr experience performed AI on all heifers upon detection of estrus. Five straws of semen were thawed in warm water (95-98 degrees F) simultaneously. Straws were provided thermal and hygienic protection during AI gun assembly, transport, and insemination. The average elapsed time from initial thaw to completion of the fifth insemination in sequence was 11.8 minutes, which is within recommended semen handling guidelines (Dalton et al., 2004).

# Housing

All heifers were housed in dry lot pens with self-locking head stanchions. Heifers in treatments 1 and 2 (14 d CIDR and 2X PG, respectively) were housed in adjacent, identical pens. Due to on-farm managerial limitations, control heifers (1X PG) were housed in a similar dry lot pen separate from treatment animals.

#### **Blood Collection**

Blood samples were collected from a subset of heifers in the 14 d CIDR (n=30) and the 2X PG (n=29) treatments via coccygeal venipuncture into Monoject (Kendall Tyco Healthcare, Mansfield, MA) EDTA (K<sub>3</sub>) blood collection tubes. Samples were collected on d -30, -16, - 11, 0, and on the day of AI for progesterone analysis. On d -30 (d of CIDR insertion) blood was collected 1.5 hours prior to CIDR insertion. On d -16 (d of CIDR removal) blood samples were drawn 1.5-2 hours after removal. All samples were placed on ice prior to centrifugation (2500 × g for 12 min). Plasma was stored at -60°C until radioimmunoassay (Coat-A-Count progesterone (TKPG1), Siemens Healthcare Diagnostics, Los Angeles, CA) at the University of Idaho and Washington State University Center for Reproductive Biology. The intra-assay coefficient of variation was 3.28% and the inter-assay coefficient of variation was 3.47 %.

# **Pregnancy Diagnosis**

Pregnancy (open) status was determined by the herd veterinarian via transrectal palpation of uterine contents 35-42 d after AI.

# **Statistical Analysis**

SAS 9.4 was used to analyze the data from this experiment. Skewed discrete count data was analyzed using the Generalized Linear Mixed Model procedure (PROC GLIMMIX) (Stroup, 2014). PROC GLIMMIX was also used to interpret non-normal binomial data associated with days to pregnancy, conception rate (CR) for the first week of breeding eligibility, and proportion of heifers pregnant during the first week. Analysis of heifer body weight (BW) homogeneity was done using the Generalized Linear Model (PROC GLM). To test the potential effect of BW on first week pregnancy rate, additional statistical analysis using logistic regression (PROC LOGISTIC) was used. The model contained BW (low  $\leq$  395.5 kg) or high  $\geq$ 395.5 kg), treatment, and BW x treatment interaction.

# **Calculation of Reproductive Metrics**

Various parameters were used to assess the reproductive outcomes following presynchronization treatment. Some of these parameters required specific calculations. Pregnancy rate (proportion pregnant) was calculated by dividing the total number of animals pregnant to first week AI by the total number of animals in the group. Conception rate was defined as the proportion of animals that conceived to an AI in the first week of breeding eligibility. Days on feed were calculated by adding the number of days it took for each individual heifer to become pregnant and the approximated length of gestation (280 d).

#### **RESULTS**

# **Heifer Body Weight**

Treatment and control heifers were weighed upon entry to the AI pen. All body weights are expressed as LSM  $\pm$  SEM. The mean body weight (BW) of the control heifers was 398.6  $\pm$  1.5 kg, with a range from 376.5 to 444.5 kg. For the 2X PG group, the mean BW was 400.8  $\pm$  1.5 kg, with a range from 358.4 to 438.2 kg. The 14 d CIDR treatment group exhibited a mean BW of 393.2  $\pm$  1.5 kg. The BW range for this group was 352.8 to 453.6 kg. Although animals were randomly allotted to treatments, there was evidence of a difference in body weight between 14 d CIDR and 1X PG groups and 14 d CIDR and 2X PG groups (P=0.0019 and P=0.0138, respectively; Table 2.1).

### **Cyclic Status**

Blood samples collected from a subset of animals from the two treatment groups were monitored for progesterone concentration to assess cyclicity, response following CIDR removal, and response to PG injections. To be considered cyclic, one of the five blood samples collected must exhibit a P<sub>4</sub> concentration of >1.0 ng/ml. All heifers within the 14 d CIDR subset (100%; 30/30) had at least one blood sample with a concentration >1.0 ng/ml (Appendix 1, Table A). On day of CIDR removal, 83% of the subset had P<sub>4</sub> concentrations <1.0 ng/ml (25/30). Five days after CIDR removal, 73% had blood P<sub>4</sub> concentrations < 1.0 ng/ml (22/30). Based on a P<sub>4</sub> concentration of < 1.0 ng/ml, 100% (30/30) of heifers enrolled in the 14 d CIDR treatment responded to the injection of PG administered on the first day of AI eligibility. Similar to the 14 d CIDR treatment group, 100% (29/29) of the 2X PG animals were cyclic, based on at least one sample >1.0 ng/ml of progesterone (Appendix 1, Table B). For the 2X PG treatment group, 80% (25/29) responded to the second injection of PG administered on the day of AI eligibility. Samples from one heifer were compromised and therefore 29 heifers were included in the 2X PG blood subset.

## **Estrous Detection**

There was a treatment effect (P<0.05) on the proportion of animals detected in estrus during the first week of breeding eligibility. Following PG administration, 114/119 (95.8%  $\pm$ 1.8%) of heifers in the 14 d CIDR group were detected in estrus, as compared to 88/118 (74.6%  $\pm$  4.0%) and 81/121 (66.9%  $\pm$  4.2%) for the 2X PG and control groups, respectively. Furthermore, there was a difference (P<0.05) in percentage detected in estrus between the 14 d CIDR group and both the 2X PG and control groups. However, there was no difference between the 2X PG and control group (P=0.19) (Table 2.1). On d 3 after PG injection, nearly 58% of heifers in the 14 d CIDR were detected in estrus (Figure 2.3) whereas nearly 31% of heifers in the 2X PG group were detected in estrus on both d 2 and 3 after PG injection (Figure 2.4).

#### **Days to First Artificial Insemination**

Days to first AI following PG injection were affected by treatment (P<0.01). Mean days to first AI were (LSM  $\pm$  SEM) 3.6  $\pm$  0.4, 5.0  $\pm$  0.4, and 6.8  $\pm$  0.5, for heifers in the 14 d CIDR, 2X PG, and control groups, respectively (Table 2.1). Differences between the 14 d CIDR and control group (P<0.01), as well as between the 14 CIDR and 2X PG group (P=0.01) were evident. Further, heifers in the 2X PG group exhibited fewer days to first AI than the control (P=0.01) (Table 2.1).

## **Days to Pregnancy**

Days from entry to breeding pen to pregnancy was affected by treatment (P=0.0278). Days to pregnancy (LSM  $\pm$  SEM) for the 14 d CIDR group (15.1  $\pm$  2.3 d) were fewer (P<0.05) than heifers in the control group (25  $\pm$  2.8 d), and tended to be fewer (P=0.06) than heifers in the 2X PG group (21.8  $\pm$  2.7 d) (Table 2.1).

## Proportion of Heifers Pregnant within First Week of Breeding Eligibility

A treatment effect was observed for the proportion of pregnant heifers within the first week of breeding eligibility (P<0.05). Differences were detected (P<0.05) between 14 d CIDR and control groups as well as between 14 d CIDR and 2X PG groups , but not between the 2X PG and control groups (P=0.76). The 14 d CIDR treatment resulted in the highest proportion of pregnant heifers within the first week of breeding eligibility (68.9%  $\pm$  4.2%), whereas the 2X PG and control groups had  $43.2\% \pm 4.6\%$  and  $41.3\% \pm 4.5\%$ , respectively (Table 2.1).

## First Service Conception Rate during the First Week of Breeding Eligibility

First service conception rates to AI during the first week of breeding eligibility were (LSM  $\pm$  SEM) 71.9%  $\pm$  4.2% (14 d CIDR), 58%  $\pm$  5.3% (2X PG), and 61.7%  $\pm$  5.4% (1X PG), and were different (P<0.05) between 14 d CIDR and 2X PG heifers (P=0.03) (Table 2.1).

#### Days on Feed from Entrance to Breeding Pen to Projected Calving Date

There was a treatment effect observed for DOF from entrance to the breeding pen until projected calving date (P=0.0232). Days on feed from entrance to the breeding program to projected calving date were (LSM  $\pm$  SEM): 295  $\pm$  2.6 d (14 d CIDR), 302  $\pm$  2.6 d (2X PG), and 305  $\pm$  2.5 d (1X PG), and were different between 14 d CIDR and 1X PG heifers (P=0.01), and tended to differ between 14 d CIDR and 2X PG groups (P=0.07) (Table 2.1).

#### DISCUSSION

## **Body Weight and Cyclic Status**

Despite a difference in mean BW between 14 d CIDR ( $393.2 \pm 1.5$  kg) heifers and both 1X PG ( $398.6 \pm 1.5$  kg) and 2X PG heifers ( $400.8 \pm 1.5$  kg), all heifers appeared to be of sufficient weight, and were expected to be cyclic. Cyclicity was confirmed as 100% of heifers in both treatment subset groups had one or more of the five blood samples with P<sub>4</sub> concentrations >1.0 ng/ml. (Appendix I, Tables A and B). Unfortunately, blood samples could not be obtained from the control group due to managerial limitations; however, heifers in the control group were of sufficient size (BW) that cyclicity was to be expected. A previous report of heifers housed at the feedlot under the same management strategy confirms a high rate of cyclicity upon entry to the AI pen (Johnson et al., 2008).

#### **Estrous Detection**

The 14 d CIDR treatment group had the highest percentage of animals (95.8%) detected in estrus during the first week of breeding eligibility (Table 2.1, Figure 2.3) Escalante and associates (2013) reported similar results in a study that also used a 14 d CIDR protocol in dairy heifers. They detected 86.0% of treatment heifers in estrus compared to 56.1% of control heifers (1X PG) (Escalante et al., 2013). In beef heifers, Leitman and associates (2008) reported 88% of heifers subjected to long-term CIDR use (14 d) were detected in estrus following PG administration on d 30. Long term CIDR treatment may result in a high percentage detected in estrus 16 d after CIDR removal because the 14 d CIDR treatment is over half of the length of one full bovine estrus cycle (21 d), which likely results in a high

percentage of animals regressing a CL while the CIDR is in place, and the 16 d interval to the PG injection allows for a high percentage of heifers to have a CL capable of responding to PG on d 30 (Escalante et al., 2013).

The 14 d CIDR protocol appears to have led to a prolonged synchrony among heifers over a period of 16 d, from CIDR removal to PG administration. Evidence for this is shown as 96.63% (115/119) of heifers were detected in estrus following CIDR removal (Figure 2.2), and 16 d later, after all heifers received a single injection of PG, 95.8% (114/119) of heifers were detected in estrus (Figure 2.3). It appears most 14 d CIDR heifers were detected in estrus (55.5%  $\pm$  4.6%) on d 2 after CIDR removal. In contrast, it appears most heifers (59.7%  $\pm$  4.5%) were detected in estrus on d 3 after PG administration. The fact that the majority of the 14 d CIDR heifers responded to the PG injection within 4 d by exhibiting estrus is a good indication that the CIDR treatment effectively synchronized the animals so they had a CL of sufficient size to respond to a single injection of PG 16 d later. The high percentage of heifers detected in estrus following CIDR removal and after a single injection of PG 16 d later (Figure 2.2 and 2.3) provides compelling evidence that the 14 d CIDR protocol can be used to effectively pre-synchronize dairy heifers.

A smaller percentage of animals in the 2X PG group were detected in heat (74.6%) during the first week of breeding eligibility compared to the 14 d CIDR treatment group (95.8%; Table 2.1). The results for the 2X PG group agree with Fogwell and coworkers (1986), who investigated the use of PG as a means of estrous synchronization in dairy heifers, and reported a similar percentage detected in estrus (72.7%; 1,025/1,409 animals) after two injections of PG were administered 11 d apart. Patterson and associates (2008), however, reported that the 2X PG protocol yielded a 57% estrus response (241/422) in beef heifers,

which was likely influenced by a high percentage of pre-pubertal heifers. In the present study, the distribution of estrus for the 2X PG group showed that the greatest percentage of heifers were detected in estrus on d 2 and 3 (30.5% for both d 2 and 3) (Figure 2.4). Few heifers were detected in estrus on d 1 and 5 (1.70% for both days). There was also a larger percentage of 2X PG heifers that did not show estrus in the first 5 d compared with the 14 d CIDR group (27.1% vs. 4.1%, respectively) (Figure 2.3 and 2.4).

It was not surprising that the control heifers (1X PG) had the lowest percentage (66.9%) detected in estrus within the first seven days in the breeding pen, as these heifers were not pre-synchronized and likely in different stages of the estrous cycle at the time of PG injection. Nevertheless, the 1X PG injection upon entry to the AI pen is a common and effective management strategy to facilitate timely AI in dairy heifers (Stevenson et al., 2008).

#### **Days to First Artificial Insemination**

Heifers in the 14 d CIDR treatment had the fewest days to first AI (3.6 d) as compared to heifers in the 2X PG and control groups (5 d and 6.8 d, respectively, Table 2.1). Fewer days to first AI in the 14 d CIDR group is due to the high percentage of 14 d CIDR heifers that were detected in estrus after PG administration upon entrance to the breeding pen (Table 2.1), as all heifers detected in estrus received AI. The fact that the 14 d CIDR group averaged 3.6 d to first AI provides evidence that many heifers regressed a CL in response to PG on d 0 and therefore the 30-d (14 d CIDR and 16 d interval before PG) protocol was successful in synchronizing CL development.

The goal of pre-synchronizing dairy heifers prior to entry to the breeding pen is to decrease the number of days to first AI, and increase the proportion of heifers pregnant within the first week of eligibility (to be discussed in the next section), thereby controlling days on feed. Days on feed is an important economic factor when raising dairy heifers, as days on feed is determined by age at first calving, which is determined by age at conception (Dalton, 2012).

#### **Proportion of Heifers Pregnant within First Week of Breeding Eligibility**

Pre-synchronization with a 14 d CIDR program was an effective strategy to increase the proportion of heifers pregnant within the first week of eligibility, as the 14 d CIDR treatment resulted in the largest proportion of heifers pregnant in the first week following entry to the AI pen (68.9%) (Table 2.1). This was not surprising due to the fact that more animals showed heat in the 14 d CIDR group as compared to the 2X PG and 1X PG groups (95.8% vs. 74.6% and 66.9%, respectively) and were bred in the first week than the other groups. Consequently, an increased percentage of animals detected in estrus in the 14 d CIDR group led to an increased percentage receiving AI and becoming pregnant within the first week of eligibility. In contrast, the proportion of heifers pregnant within the first week for the 2X PG and control groups were 43.2% and 41.3%, respectively. As compared to the 14 d CIDR group, these percentages were likely lower because fewer animals were detected in estrus within the first week, leading to fewer animals receiving AI and having the opportunity to become pregnant within the first week of breeding eligibility.

It is unlikely that a small difference of 7.6 kg between the highest mean BW (2X PG) and the lowest mean BW (14 d CIDR) could have an effect on the proportion of heifers pregnant during the first week of eligibility, as 100% of heifers in each treatment subset (14 d CIDR and 2X PG) were cyclic based on progesterone concentrations (Appendix I, Table A and B). Further statistical analysis revealed there was no effect of BW, treatment, or BW X treatment interaction on proportion of heifers pregnant within the first week of eligibility (P<0.05).

## First Service Conception Rate during the First Week of Breeding Eligibility

There was a difference (P<0.05) in first service CR between the 14 d CIDR heifers and the 2X PG group, but not the control group (Table 2.1). A possible reason we observed a low CR for the 2X PG group (58%; Table 2.1) could have been that despite 74.6% of heifers were detected in estrus , they may not have had CL that were entirely regressed, perhaps leading to delayed or lack of ovulation. Possible evidence of incomplete luteolysis may be seen in that the reality that only 80% of the 2X PG heifers in the blood sample subset appeared to respond to PG on d 0 as indicated by blood P<sub>4</sub> levels <1.0 ng/ml (Appendix I, Table B). Nevertheless, the CR for the 2X PG and control heifers (58% and 61.7%, respectively) appears to be similar to a previous report on Holstein heifers in the United States which indicated the average CR to be 57% (Kuhn et al., 2006). Lastly, the CR for the 2X PG and 1X PG is within the lower range of CR achieved within the previous calendar year at the collaborating feedlot.

The 14 d CIDR heifers most likely had the highest CR because the protocol works well with the physiology of the heifer's estrous cycle. Based on the theoretical mechanism of the protocol's effect on the estrous cycle, most of the animals probably had a mature enough CL to respond to PG on d 0 of the study which resulted in 95.8% of the group showing heat and therefore being bred. This claim is supported by evidence as 100% of the 14 d CIDR blood subset group responded to the PG injection given on d 0 based on blood P<sub>4</sub> concentrations that were <1.0 ng/ml (Appendix I, Table A). The 14 d CIDR protocol appears to have had positive influence on fertility as more animals in this group responded to PG, exhibited estrus, ovulated and conceived within the first week of breeding eligibility. The results reported here for the 14 d CIDR heifers agree with Mallory et al. (2013) who used the "Show-Me-Synch" protocol and reported a CR of 68% with conventional semen in Holstein dairy heifers. Escalante and associates (2013) reported a CR of 61.2% vs. 40.6% (P < 0.10) for heifers inseminated with sexed semen in 14 d CIDR and 1X PG groups, respectively, both of which were inseminated upon detection of estrus. Both of these studies indicated that use of a 14 d CIDR resulted in acceptable CR.

#### Days on Feed from Entrance to AI Pen to Projected Calving Date

Days on feed from entrance to the AI pen to projected calving date were fewest for the 14 d CIDR heifers (295 d) as compared to the other two groups (2X PG= 302 d, Control= 305 d). These results are not surprising as the 14 d CIDR heifers had the highest percentage of animals detected in estrus, the fewest days to first AI, the highest first service CR during the first week of eligibility, and the fewest days to pregnancy. For this producer, fewer DOF is advantageous in the sense that animals will be returned to the home dairy sooner. If heifers

spend less time at the feedlot, there is more room for new animals to come in; therefore, more heifers can be raised at a faster rate, potentially leading to decreased rearing costs for the dairy producer and increased efficiency and income for the heifer enterprise.

#### **Economic Implications**

For this study, a partial budget was developed to describe potential economic implications of pre-synchronizing dairy heifers. There was no change in revenue as there was no change in value of animals throughout the study. It is important to recognize that although there was no statistical difference between the control and 2X PG groups, there was an overall effect of treatment on the DOF parameter. For completeness and clarity, a partial budget analysis of both pre-synchronization treatments was constructed so that the producer may see potential economic outcomes for both methods.

The assumption for treatment labor was estimated using a labor rate of \$15 per hour, with 5 minutes to process and insert each CIDR and 2 minutes to administer two doses of PG (1 minute per dose; Painter and Gray, 2012). The partial budget developed for this study provides evidence that reduced costs associated with feed and labor were associated with the treatment groups (Table 3). Specifically, the 14 d CIDR group had a reduced feed cost of \$23.50 per animal compared with the 2X PG group which had a reduced feed cost of \$7.05. Reduced feed costs were calculated by multiplying the number of days saved (10 d and 3 d) by the cost of feed per head per day (\$2.35). This is a direct result of the decreased days on feed as compared with the control (Table 3). Labor costs were also decreased for both treatment groups due to fewer DOF. The 14 d CIDR treatment resulted in a savings of \$4.10

whereas the 2X PG group resulted in \$1.23 savings. Decreased labor costs were calculated by multiplying \$.41 by the number of days saved on feed.

Overall, the 14 d CIDR treatment resulted in a total reduced cost of \$27.60 per head and the 2X PG treatment resulted in a total reduced cost of \$8.28 per head in comparison to the Control group. Total reduced costs for each treatment were calculated by adding reduced costs of labor and feed.

In contrast to reduced costs, there were increased costs associated with both presynchronization treatments. These increased costs were related to both materials and labor (Tables 2 and 3). Material related costs for the 14 d CIDR group were higher (\$10.50/head) due to the cost of the CIDR (Table 2) in comparison to the 2X PG (\$2.80). Labor costs were also higher for the 14 d CIDR group than the 2X PG group (\$1.25 vs. \$.25) since 14 d CIDR heifers were handled three times (CIDR insertion, CIDR removal, and PG injection upon entry to the AI pen) whereas the 2X PG heifers were handled twice for shorter periods of time. In comparison to the control, the 14 d CIDR treatment cost \$11.75 more per head, while the 2X PG treatment cost an additional \$3.05.

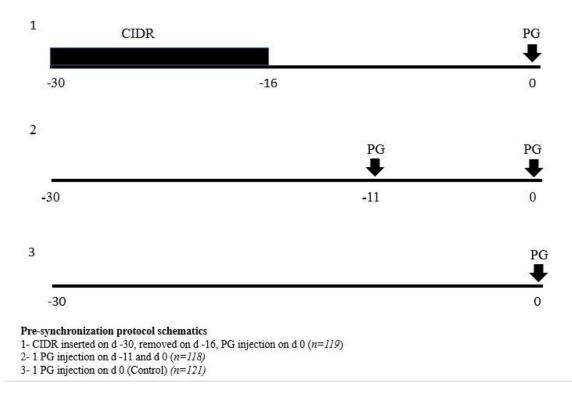
Pre-synchronization with the 14 d CIDR protocol resulted in an overall treatment balance of \$15.85 per heifer (Table 3), while the treatment balance for the 2X PG group was \$5.23 per heifer. Treatment balance is calculated by subtracting the total increased costs of a treatment from the total reduced costs of the treatment. Therefore, the treatment balance can be denoted as the overall savings seen by the producer for this feedlot operation. Since the 14 d CIDR treatment was significantly different from the 2X PG and control groups for DOF, the treatment balance calculated may be expected to provide an economic benefit to the producer. There was no statistical difference between 2X PG and control groups for DOF;

therefore the treatment balance calculated for the 2X PG group may not be realized. Nonetheless, the treatment balance has been mentioned for clarity and completeness. These results provide evidence that pre-synchronization with a 14 d CIDR may provide an economic benefit to producers raising replacement heifers under similar conditions.

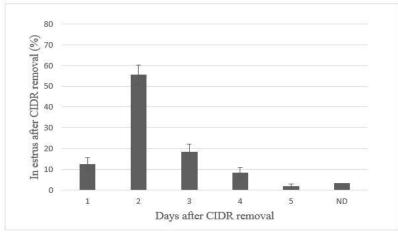
## CONCLUSIONS

The results of this study provide evidence that use of a 14 d CIDR as a pre-synchronization strategy in dairy heifers is an effective way to synchronize estrus as supported by the high percentage of heifers that responded to the PG injection on d 0 and were observed in estrus and therefore bred within the first week of breeding eligibility. Furthermore, fertility following AI was high, leading to an increased proportion of heifers pregnant within the first week of eligibility, and decreased days to pregnancy. Although material and labor costs associated with the 14 d CIDR treatment were increased compared with the other protocols used, the 14 d CIDR protocol ultimately may reduce producer expenses due to lesser DOF, as calculated from entrance to the breeding pen to projected calving date.

## **FIGURES**

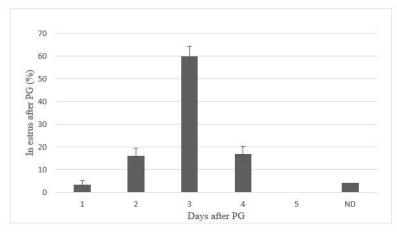


**Figure 2.1** Pre-synchronization and control treatments. 1- 14 d CIDR (CIDR was inserted on d -30 and removed on d -16, then PG administered on d 0), 2- 2X PG (PG injections administered on d -11 and d 0), 3- 1X PG (PG administered only on d 0).



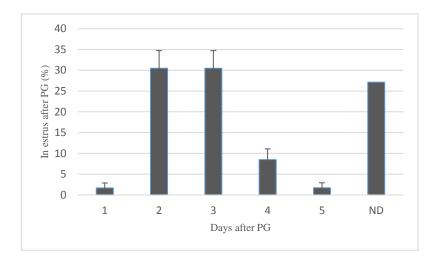
\* CIDR insertion occurred on d -30 and removed 14 d later on d-16

**Figure 2.2** Distribution of standing estrus within 5 d after CIDR removal. Two days after CIDR removal resulted in the highest percentage of heifers detected in estrus. ND= Not detected in estrus.



\*PG was administered 16 d after CIDR removal on d 0 upon entry to the breeding pen

**Figure 2.3** Distribution of standing estrus within 5 d after administration of PG on d 0. Three days after PG administration resulted in the highest percentage of heifers in estrus. ND= Not detected in estrus.



**Figure 2.4** Distribution of standing estrus within 5 d after administration of PG on d 0. Days 2 and 3 had the greatest percentage of heifers in estrus. ND= Not detected in estrus

## **TABLES**

Parameter		Treatment	
	14 d CIDR	2X PG	Control (1X PG
Number of animals (n)	119	118	121
Avg. BW upon entry to AI pen (kg)	393.2 ± 1.5ª	400.8 ± 1.5 b	398.6 ± 1.5 <sup>b</sup>
Heat detection (%) for 1 <sup>st</sup> week of breeding elig.	95.8 ±1.8ª	$74.6 \pm 4^{b}$	$66.9 \pm 4.2^{b}$
Days to 1 <sup>st</sup> service	$3.6\pm0.4^{a}$	$5 \pm 0.4^{b}$	6.8 ± 0.5 <sup>c</sup>
Days to pregnancy	15.1 ± 2.3ª*	$21.8 \pm 2.7^{b}$	25 ± 2.8 <sup>b</sup>
Proportion (%) pregnant in 1 <sup>st</sup> week of breeding elig.	$68.9 \pm 4.2^{a}$	43.2±4.6 <sup>b</sup>	41.3 ±4.5 <sup>b</sup>
CR (%) for 1 <sup>st</sup> week of breeding elig.	$71.9 \pm 4.2^{a}$	58 ± 5.3 <sup>b</sup>	61.7 ± 5.4ª,b
DOF (entrance to breeding pen to projected calving date)	$295 \pm 2.6^{a^*}$	302 ± 2.6 <sup>b</sup>	305 ± 2.5 <sup>b</sup>

**Table 2.1** Reproductive performance of dairy heifers treated with progestin and prostaglandin  $F_{2\alpha}$  based management strategies. 14 d CIDR + PG= CIDR inserted from d -30 to -16, single PG injection on d 0. 2X PG= injection of PG on d -11 and on d 0. Control= single injection of PG on d 0.

Item	Cost (\$)
	0000(0)
PG <sup>1</sup> , per dose	2.80
CIDR insert <sup>2</sup> , per insert	10.50
Labor <sup>3</sup> , per hour	15.00
Feed cost <sup>4</sup> , per heifer per day	2.35
<sup>1</sup> Lutalyse Sterile Solution, Zoetis, Florham Park,	
<sup>2</sup> Eazi-Breed controlled internal drug-release (CII Park, NJ	DR) insert, Zoetis,Florhar
<sup>3</sup> General labor from University of Idaho Replacer	ment Heifer budget

<sup>3</sup>General labor from University of Idaho Replacement Heifer budget <sup>4</sup>Cost per day for M&M Feedlot, Parma ID

Table 2.2 Predominant costs included in economic evaluation

	1X PG (Control)	14 d CIDR	2X PG
Days to calving	305	295	302
Time savings from treatment (days)		10	3
Feed Cost (\$/hd/day)		\$ 2.35	\$ 2.35
Labor (\$/hd/day) <sup>1</sup>		\$ 0.41	\$ 0.41
Increased Costs			
Treatment Materials		\$10.50	\$ 2.80
Treatment Labor <sup>2</sup>		\$ 1.25	\$ 0.25
Treatment Cost (\$/head)		\$ 11.75	\$ 3.05
Reduced Costs			
Feed		\$ 23.50	\$ 7.05
Labor		\$ 4.10	\$ 1.23
Total Reduced Costs (\$/hd)		\$ 27.60	\$ 8.28
Treatment Balance (\$/hd)		\$ 15.85	\$ 5.23

Assumptions:

<sup>1</sup>General Labor from UI Replacement Heifer Budget: EBB\_DRI\_2012. available online at:

http://web.cals.uidaho.edu/idahoagbiz/files/2014/10/EBBDR2\_12\_10000-hdHeiferRaisingFacility2.pdf

 $^2$ Treatment labor estimated using labor rate of \$15/hour, with 5 minutes to process and install CIDR and 2 minutes to process and administer two dosages of PG

**Table 2.3** Partial budget for dairy heifer pre-breeding protocols in comparison to the 1X PG (control) protocol already implemented at M&M feedlot, Parma, ID

## LITERATURE CITED

- Ahmad, N., F.N. Schrick, and R.L. Butcher. 1995. Effect of persistent follicles on early embryonic losses in beef cows. Biol. Reprod. 52:5:.1129-1135. .
- Amyot, E. and J. F. Hurnik. 1987. Diurnal patterns of estrous behavior of dairy cows housed in a free stall. Can. J. Anim. Sci. 67: 605-614.
- Busch, D.C., D.J. Wilson, D.J. Schafer, N.R. Leitman., J.K. Haden, M.R. Ellersieck, M.F. Smith, and D.J. Patterson. 2007. Comparison of progestin-based estrus synchronization protocols before fixed-time artificial insemination on pregnancy rate in beef heifers. J. Anim. Sci. 85: 1933-1939.
- Chebel, R.C. 2010. New strategies for synchronization of dairy heifers and economic considerations. In: Proc. Dairy Cattle Reproduction Council Annual Meeting, St. Paul, MN, pp. 25-32.
- Chohan, K.R., and A.G. Hunter. 2003. In vitro maturation, fertilization and early cleavage rates of bovine fetal oocytes. Theriogenology. 61: 373-380
- Dalton, J.C., A. Ahmadzadeh, B. Shafii, W.J. Price, and J.M. DeJarnette. 2004. Effect of thawing multiple 0.5-mL semen straws and sequential insemination number on conception rates in dairy cattle. J. Dairy Sci. 87:972-975.
- Dalton, J.C. 2011. Strategies for success in heat detection and artificial insemination. WCDS Advances in Dairy Technology. 23:215-229.
- Dalton, J.C. 2012. Synchronization programs for dairy heifers. In. Proc. Dairy Calf and Heifer Association Annual Meeting, Visalia, CA, pp. 86-96.
- DCRC. 2014. Dairy heifer protocol sheet. Dairy Cattle Reproduction Council, Champaign, IL.
- Dransfield, M.B.G., R.L. Nebel, R.E. Pearson, and L.D. Warnick. 1998. Timing of insemination of dairy cows identified in estrus by a radiotelemetric estrus detection system. J. Dairy Sci. 81: 1874-1882.
- Dzuik, P.J. and R. A. Bellows. 1983. Management of reproduction of beef cattle, sheep, and pigs. J. Anim. Sci. 57: 355-379.
- Escalante, R.C., S.E. Poock, D.J. Mathew, W.R. Martin, E.M. Newsom, S.A. Hamilton, K.G. Pohler, and M.C. Lucy. 2012. Short communication: Pre-synchronization for timed artificial insemination in grazing dairy cows by using progesterone for 14 days with or without prostaglandin  $F_{2\alpha}$  at the time of progesterone withdrawal. J. Dairy Sci. 95: 5102-5108.
- Escalante, R.C., S.E. Poock, and M.C. Lucy. 2013. Follicular populations and luteal function in dairy heifers treated with a controlled internal drug release insert for 14 days as a method to synchronize the estrous cycle before prostaglandin  $F_{2\alpha}$  treatment and artificial insemination. J. Dairy Sci. 96: 3806-3816.

- Ettema, J. F., and J. E. Santos. 2004. Impact of age at calving on lactation, reproduction, health, and income in first-parity Holsteins on commercial farms. J. Dairy Sci. 87:2730–2742.
- Fogwell, R. L., W.A. Reid, C.K. Thompson, M.J. Thome, and D. A. Morrow. 1986. Synchronization of estrus in dairy heifers: A field demonstration. J. Dairy Sci. 69: 1665-1672.
- Frazer, LLP. (Certified Public Accountants and Consultants). 2013. In: Dairy Farm Operating Trends, June 30, 2013, pp. 1-4.
- Gabler, M.T., P.R. Tozer, and A.J. Heinrichs. 2000. Development of a cost analysis spreadsheet for calculating the costs to raise a replacement dairy heifer. J. Dairy Sci. 83:1104-1109.
- Hawk, H.W. 1987. Transport and fate of spermatozoa after insemination of cattle. J. Dairy Sci. 70: 1487-1503.
- Head, H.H. 1992. Heifer performance standards: Rearing systems, growth rates, and lactation. In: Large Dairy Herd Management, H.H. Van Horn and C.J. Wilcox (Eds.), American Dairy Science Association, Champaign, IL p. 424.
- Iowa State University Extension and Outreach. 2006. Partial budgeting: A tool to analyze farm business changes. Iowa State University, Ames, IA 50011.
- Johnson, C.R., A. Ahmadzadeh, K.S. Jensen, S. Etter, R. Wilson, N. Dalton, and J.C. Dalton. 2008. Effect of duration of intravaginal progesterone insert (CIDR) treatment on pregnancy rate per AI in dairy heifers using a timed-AI protocol. In: Proc. Western Section, ASAS, Laramie, WY. 59: 33-36.
- Keown, J. F. and R.W. Everett. 1986. Effect of days carried calf, days dry, and weight of first calf heifers on yield. J. Dairy Sci. 69:1891-1896.
- Kiddy, C.A. 1977. Variation in physical activity as an indication of estrus in dairy cows. J. Dairy Sci. 60:235–243.
- Kinder, J.E., F.N. Kojima, E.G. Bergfeld, M E. Wehrman, and K.E. Fike. 1996. Progestin and estrogen regulation of pulsatile LH release and development of persistent ovarian follicles in cattle. J. Anim.Sci. 74: 1424-1440.
- Kojima, F.N., J.F. Bader, J.E. Stegner, D.J. Schafer, J.C. Clement, R.L. Eakins, M.F. Smith, and D.J. Patterson. 2004. Substituting EAZI-BREED CIDR inserts (CIDR) for melengestrol acetate (MGA) in the MGA Select protocol in beef heifers. J. Anim. Sci. 82(Suppl. 1):255.
- Kuhn, M.T., J.L. Hutchison, and G.R. Wiggans. 2006. Characterization of Holstein heifer fertility in the United States. J. Dairy Sci. 89: 4907-4920.
- Lauderdale, J.W. 2009. ASAS Centennial Paper: Contributions in the Journal of Animal Science to the development of protocols for breeding management of cattle through synchronization of estrus and ovulation. J. Anim. Sci. 87:801-12.

- Leitman, N.R., D.C. Busch, D.A. Mallory, D.J. Wilson, M.R. Ellersieck, M.F. Smith, and D.J. Patterson. 2008. Comparison of long-term CIDR-based protocols to synchronize estrus in beef heifers. Anim. Reprod. Sci. 114:345–355.
- Lopes, G., C.R. Johnson, L.G.D. Mendonca, P.R.B Silva, J.G.N Moraes, A. Ahmadzadeh, J.C. Dalton, and R.C. Chebel. 2013. Evaluation of reproductive and economic outcomes of dairy heifers inseminated at induced estrus or at fixed time after a 5-day or 7-day progesterone insert-based ovulation synchronization protocol. J. Dairy Sci. 96: 1612-1622.
- Louis, T.M., H.D. Hafs, and B.E. Sequin. 1973 Progesterone, LH, estrus and ovulation after prostaglandin  $F_{2\alpha}$  in heifers. Proc. Soc. Exp. Biol. Med. 43:152-155.
- Lucy, M.C., H.J. Billings, W.R. Butler, L.R. Ehnis, M.J. Fields, D.J. Kesler, J.E. Kinder, R.C. Mattos, R.E. Short, W.W. Thatcher, R.P. Wettemann, J.V. Yelich, and H.D. Hafs. 2001. Efficacy of an intravaginal progesterone insert and an injection of PGF2alpha for synchronizing estrus and shortening the interval to pregnancy in postpartum beef cows, peripubertal beef heifers, and dairy heifers. J. Anim. Sci.79: 982-995.
- Mallory, D.A., S.L. Lock, D.C., Woods, S.E. Poock, and D.J. Patterson. 2012. Comparison of sex-sorted and conventional semen within a fixed-time artificial insemination protocol designed for dairy heifers. J. Dairy Sci. 96: 854-856.
- McCracken, J. E. Custer, and J. Lamsa. 1999. Luteolysis: Neuroendocrine-mediated event. Physiological Reviews. 79:263-305.
- USDA. 2007. Dairy 2007, Part IV: Reference of dairy cattle health and management practices in the United States, 2007. USDA: APHIS: VS, CEAH. Fort Collins, CO. #N494.0209
- Niswender, G.D., J. L. Juengel, W.J. McGuire, C.J. Belfiore, and M.C. Wiltbank. 1994. Luteal function: The estrous cycle and early pregnancy. Biol.Reprod. 50: 239-247.
- Niswender, G.D., J. L. Juengel, P.J. Silva, M. K. Rollyson, and E.W. McIntush. 2000. Mechanisms controlling the function and life span of the corpus luteum. Physiological Reviews. 80: 1-20.
- NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th ed. Natl. Acad. Press, Washington, DC.
- Odde, K. G. 1990. A review of synchronization of estrus in postpartum cattle. J Anim Sci, 68:817-830.
- Painter, K. M., and Gray, C.W. 2012. Idaho livestock costs and returns estimate: Holstein small herd replacement enterprise. EBB-DR1-12. The University of Idaho, Department of Ag. Econ. And Rural Soc.
- Patterson, D. J., G.H. Kiracofe, and L.R. Corah. 1989. Control of the bovine estrous cycle with melengestrol acetate (MGA): A review. J. Anim. Sci. 67:1895-1906.

- Patterson, D.J., D.A. Mallory, J.M. Nash, and M.F. Smith. 2008. Estrus synchronization protocols for heifers. In Proc. Appl. Reprod. Strat. Beef Cattle, Nashville, TN, pp 76-114.
- Penn State Agricultural Research and Cooperative Extension. 2002. Partial budgeting for agricultural business. The Pennsylvania State University, University Park, PA 16802.
- Pursley, J.R., M.C. Wiltbank, J.S. Stevenson, J.S. Ottobre, A. Garverick, and L.L. Anderson. 1997. Pregnancy rates per artificial insemination for cows and heifers inseminated at a synchronized ovulation or synchronized estrus. J. Dairy Sci. 80:295-300.
- Revah, I, and W.R. Butler. 1996. Prolonged dominance of follicles and reduced viability of bovine oocytes. J. Reprod Fertil. 106:39-47.
- Roche, J.R., N.C. Friggens, J. Kay, M.W. Fisher, K.J. Stafford, and D.P. Berry. 2009. Invited review: Body condition score and its association with dairy cow productivity, health, and welfare. J. Dairy Sci. 92: 5769-5801.
- Senger, P.L. 1994. The estrus detection problem: new concepts, technologies, and possibilities. J. Dairy Sci. 77: 2745-2753.
- Senger, P.L. 2012.Pathways to pregnancy and parturition, third edition. Current Conceptions, Inc. Pullman, WA.
- Shams, D., and B. Berisha. 2004. Regulation of corpus luteum function in cattle-An overview. Reprod. Dom. Anim. 39: 241-251.
- Sirois, J., and J.E. Fortune.1990. Lengthening of the bovine estrous cycle with two levels of exogenous progesterone: A model for studying ovarian follicular dominance. Endocrinology. 127: 916-925.
- Smith, M.F., G.A. Perry, J.A. Atkins, E.M. Jinks, K.G. Pohler, and D.J. Patterson. 2010. Physiological principles underlying synchronization of estrus. In: Proc. Appl.Reprod. Strat. Beef Cattle, San Antonio, TX, pp. 29-52.
- Stevenson, J.L., J.A. Rodrigues, F.A. Braga, S. Bitente, J.C. Dalton, J.E.P. Santos, and R.C. Chebel. 2008. Effect of breeding protocols and reproductive tract score on reproductive performance of dairy heifers and economic outcome of breeding programs. J. Dairy Sci.91: 3424-3438.
- Stroup, W.W. 2014. Rethinking the analysis of non-normal data in plant and soil science. Agron. J. 106: 1-17.
- Tozer, P.R., and A. J. Heinrichs. 2001. What affects the cost of raising replacement dairy heifers: A multiple-component analysis. J. Dairy Sci. 84: 1836-1844.
- Trimberger, G.W., and H. P. Davis. 1943. Conception rate in dairy cattle from artificial insemination at various stages of estrus. Nebraska Agric. Exp. Stn. Res. Bull. 129:1-14.

Trimberger, G.W. 1948. Breeding efficiency in dairy cattle from artificial insemination at various intervals before and after ovulation. Nebraska Agric. Exp. Stn. Res. Bull. 153: 1-26.

# **APPENDIX I**

## Concentration of Blood Progesterone (ng/ml) for

## 14 d CIDR and 2X PG Treated Heifers

Table A- Concentration of blood progesterone for 14 d CIDR + PG treatment heifers (ng/ml)									
	Pre CIDR	Post CIDR		PG	Day of AI				
Heifer ID	6/28/2014	7/12/2014	7/17/2014	7/28/2014	7/29/2014-8/3/2014				
25541	2.95	4.31	<0.1	4.77	<0.10				
25978	5.77	0.14	0.91	6.41	0.11				
26263	3.69	0.51	1.06	8.92	0.18				
26472	0.18	4.05	0.5	5.3	< 0.1				
26475	8.57	0.39	< 0.1	10.75	< 0.1				
26539	7.38	0.52	0.18	5.08	<.1				
26545	0.2	5.27	0.39	7.76	0.27				
26547	7.63	0.34	0.77	8.08	0.16				
26587	0.24	8.53	0.16	6.3	0.18				
26642	1.78	0.7	0.74	5.98	0.14				
26698	9.25	0.28	1.11	6.59	< 0.1				
26755	6.04	0.57	0.4	6.33	< 0.1				
26775	5.51	0.38	5.08	< 0.1	< 0.1				
26805	9.38	0.41	1.02	8.29	< 0.1				
26806	0.81	0.56	0.76	5.15	< 0.1				
26823	4.2	0.51	< 0.1	7.07	0.18				
26826	7.36	0.38	< 0.1	5.3	< 0.1				
26833	9.63	0.25	1.14	6.86	< 0.1				
26838	6.71	0.51	0.77	6.7	< 0.1				
26841	0.14	3.15	0.13	5.2	< 0.1				
26880	6.02	0.3	1.19	6.91	0.16				
26883	10.91	0.2	0.91	8.02	0.77				
26906	10.89	0.28	< 0.1	5.34	< 0.1				
26911	5.02	< 0.1	0.61	5.34	< 0.1				
26943	4.7	0.3	0.57	8.05	< 0.1				
26950	7.48	0.9	< 0.1	4.33	0.11				
26969	5.4	0.43	0.97	9.3	0.21				
26985	< 0.1	0.3	0.37	7.1	< 0.1				
26986	5.85	0.51	1.24	9.37	0.17				
26999	7.16	0.33	1.05	7.1	0.25				

]	Table B- Concentration of blood progesterone for 2X prostaglandin $F_{2\alpha}$ treatment heifers (ng/ml)									
			1 <sup>st</sup> PG	$2^{nd} PG$	Day of AI					
Heifer ID	6/28/2014	7/12/2014	7/17/2014	7/28/2014	7/29/2014-8/3/2014					
26248*	0.11	13.65	<0.1	3.77	0.67					
26270	1.34	7.1	0.17	8	0.24					
26374*	0.85	2.46	1.3	8.3	0.68					
26389	7.99	2	6.47	5.78	0.2					
26402*	8.72	3.74	5.54	3.34	1.74					
26526	0.45	7.93	7.19	4.16	0.3					
26527*	1.08	7.78	0.18	3.98	1.58					
26562	< 0.1	9.1	0.32	5.1	< 0.1					
26603	0.54	8.53	< 0.1	4.5	< 0.1					
26606*	< 0.1	7.06	0.65	5.5	2.91					
26618*	6.57	< 0.1	4.46	3.43	1.04					
26643	0.16	8.78	0.76	5.66	< 0.1					
26646	6.12	0.25	1.12	6.23	0.11					
26656	2.85	< 0.1	3.35	5.65	0.22					
26663	15.61	3.49	6.82	8.36	0.35					
26706	< 0.1	10.39	< 0.1	5.92	0.17					
26762*	1.76	1.33	0.58	4.68	0.59					
26772	2.98	< 0.1	0.7	5.04	< 0.1					
26774	8.13	8.63	6.92	6.77	< 0.1					
26776	7.81	0.55	7.46	5.53	0.13					
26782*	6.72	8.91	< 0.1	6.32	0.12					
26789*	10.58	7.77	9.56	5.38	1.48					
26793	5.31	3.43	6.1	4.53	N/A					
26832	0.34	6.9	0.37	3.86	0.27					
26864	6.54	6.61	9.83	3.38	< 0.1					
26882	11.58	2.42	6.46	2.95	0.24					
26915*	6.69	1.56	7.37	7.18	1.43					
26932	1.57	0.53	1.53	7.04	0.5					
26933	9.72	5.74	8.08	6.69	0.19					
26936	< 0.1	8.81	1.07	3.49	0.63					

\* Indicates that heifer was not detected in estrus and did not receive AI during first week of eligibility

One heifer was removed from the subset due to missing samples, n=29

# **APPENDIX II**

## Breeding Data for 14 d CIDR and 2X PG Treated Heifers

	Ta	ble A: Bre	eding data f	or 14 d C	IDR treat	nent he	eifers <sup>1</sup>	
Heifer			AI	Batch	Seq. of	Start	End	Total time
ID	Trt	AI Date <sup>2</sup>	Technician	number	AI	Time	Time	(min)
26634	CIDR	7/29/2014	1	1	1	7:46	7:54	8
26780	CIDR	7/29/2014	1	1	3	7:46	7:57	11
26841	CIDR	7/29/2014	1	1	4	7:46	7:59	13
26883	CIDR	7/29/2014	1	1	2	7:46	7:56	10
26223	CIDR	7/30/2014	1	1	2	7:45	7:54	9
26278	CIDR	7/30/2014	1	1	3	7:45	7:54	9
26497	CIDR	7/30/2014	1	2	3	7:54	8:00	6
26509	CIDR	7/30/2014	1	1	5	7:45	7:55	10
26519	CIDR	7/30/2014	1	3	3	7:56	8:04	8
26545	CIDR	7/30/2014	1	3	5	7:56	8:06	10
26584	CIDR	7/30/2014	1	4	2	8:04	8:11	7
26614	CIDR	7/30/2014	1	2	1	7:54	7:58	4
26626	CIDR	7/30/2014	1	4	4	8:04	8:13	9
26669	CIDR	7/30/2014	1	4	3	8:04	8:12	8
26726	CIDR	7/30/2014	1	2	4	7:54	8:01	7
26750	CIDR	7/30/2014	1	4	1	8:04	8:10	6
26804	CIDR	7/30/2014	1	3	4	7:56	8:05	9
26818	CIDR	7/30/2014	1	4	5	8:04	8:14	10
26838	CIDR	7/30/2014	1	6	3	8:14	8:25	11
26839	CIDR	7/30/2014	1	3	2	7:56	8:04	8
26842	CIDR	7/30/2014	1	2	5	7:54	8:03	9
26847	CIDR	7/30/2014	1	1	1	7:45	7:53	8
26872	CIDR	7/30/2014	1	1	4	7:45	7:55	10
25541	CIDR	7/31/2014	1	10	2	8:44	8:55	11
25978	CIDR	7/31/2014	1	9	4	8:41	8:51	10
26080	CIDR	7/31/2014	1	7	2	8:30	8:39	9
26260	CIDR	7/31/2014	1	15	1	9:12	9:21	9
26263	CIDR	7/31/2014	1	10	1	8:44	8:54	10
26359	CIDR	7/31/2014	1	13	5	9:03	9:14	11
26363	CIDR	7/31/2014	1	8	1	8:36	8:43	7
26382	CIDR	7/31/2014	1	12	3	8:57	9:06	9
26391	CIDR	7/31/2014	1	7	4	8:30	8:40	10
26440	CIDR	7/31/2014	1	11	3	8:51	9:01	10
26472	CIDR	7/31/2014	1	4	1	8:16	8:22	6
26473	CIDR	7/31/2014	1	2	4	8:07	8:15	8
26475	CIDR	7/31/2014	1	3	5	8:11	8:20	9

26481	CIDR	7/31/2014	1	11	5	8:51	9:03	12
26494	CIDR	7/31/2014	1	15	2	9:12	9:22	10
26496	CIDR	7/31/2014	1	4	5	8:16	8:26	10
26507	CIDR	7/31/2014	1	14	4	9:08	9:18	10
26518	CIDR	7/31/2014	1	10	4	8:44	8:56	12
26534	CIDR	7/31/2014	1	5	3	8:20	8:29	9
26539	CIDR	7/31/2014	1	12	1	8:57	9:05	8
26542	CIDR	7/31/2014	1	5	4	8:20	8:30	10
26564	CIDR	7/31/2014	1	10	5	8:44	8:57	13
26566	CIDR	7/31/2014	1	11	2	8:51	9:00	9
26567	CIDR	7/31/2014	1	1	3	7:43	7:51	8
26575	CIDR	7/31/2014	1	3	3	8:11	8:19	8
26583	CIDR	7/31/2014	1	12	4	8:57	9:07	10
26587	CIDR	7/31/2014	1	8	3	8:36	8:44	8
26589	CIDR	7/31/2014	1	12	2	8:57	9:06	9
26592	CIDR	7/31/2014	1	7	5	8:30	8:41	11
26596	CIDR	7/31/2014	1	14	2	9:08	9:17	9
26601	CIDR	7/31/2014	1	4	2	8:16	8:23	7
26612	CIDR	7/31/2014	1	13	2	9:03	9:11	8
26627	CIDR	7/31/2014	1	5	1	8:20	8:28	8
26640	CIDR	7/31/2014	1	11	4	8:51	9:02	11
26642	CIDR	7/31/2014	1	11	1	8:51	8:59	8
26655	CIDR	7/31/2014	1	8	5	8:36	8:46	10
26660	CIDR	7/31/2014	1	6	4	8:25	8:36	11
26672	CIDR	7/31/2014	1	13	4	9:03	9:12	9
26676	CIDR	7/31/2014	1	2	3	8:07	8:14	7
26698	CIDR	7/31/2014	1	10	3	8:44	8:56	12
26701	CIDR	7/31/2014	1	1	4	7:43	7:52	9
26704	CIDR	7/31/2014	1	4	4	8:16	8:25	9
26732	CIDR	7/31/2014	1	7	3	8:30	8:39	9
26739	CIDR	7/31/2014	1	1	1	7:43	7:49	6
26755	CIDR	7/31/2014	1	5	5	8:20	8:31	11
26760	CIDR	7/31/2014	1	9	1	8:41	8:48	7
26766	CIDR	7/31/2014	1	3	4	8:11	8:20	9
26769	CIDR	7/31/2014	1	8	2	8:36	8:44	8
26781	CIDR	7/31/2014	1	1	2	7:43	7:50	7
26783	CIDR	7/31/2014	1	13	3	9:03	9:12	9
26786	CIDR	7/31/2014	1	9	3	8:41	8:50	9
26788	CIDR	7/31/2014	1	9	5	8:41	8:52	11
26805	CIDR	7/31/2014	1	14	3	9:08	9:17	9
26806	CIDR	7/31/2014	1	12	5	8:57	9:08	11
26813	CIDR	7/31/2014	1	6	5	8:25	8:36	11
26817	CIDR	7/31/2014	1	1	5	7:43	7:52	9

26833	CIDR	7/31/2014	1	2	2	8:07	8:13	6
26840	CIDR	7/31/2014	1	3	2	8:11	8:18	7
26843	CIDR	7/31/2014	1	6	3	8:25	8:35	10
26849	CIDR	7/31/2014	1	3	1	8:11	8:18	7
26857	CIDR	7/31/2014	1	5	2	8:20	8:28	8
26865	CIDR	7/31/2014	1	9	2	8:41	8:49	8
26878	CIDR	7/31/2014	1	14	5	9:08	9:19	11
26880	CIDR	7/31/2014	1	7	1	8:30	8:38	8
26911	CIDR	7/31/2014	1	13	1	9:03	9:10	7
26941	CIDR	7/31/2014	1	14	1	9:08	9:16	8
26943	CIDR	7/31/2014	1	8	4	8:36	8:45	9
26946	CIDR	7/31/2014	1	4	3	8:16	8:24	8
26985	CIDR	7/31/2014	1	6	2	8:25	8:34	9
26986	CIDR	7/31/2014	1	6	1	8:25	8:33	8
26991	CIDR	7/31/2014	1	2	1	8:07	8:12	5
26194	CIDR	8/1/2014	1	4	5	7:57	8:11	14
26378	CIDR	8/1/2014	1	3	4	7:51	8:05	14
26427	CIDR	8/1/2014	1	4	2	7:57	8:09	12
26547	CIDR	8/1/2014	1	4	4	7:57	8:10	13
26590	CIDR	8/1/2014	1	1	2	7:41	7:49	8
26602	CIDR	8/1/2014	1	1	4	7:41	7:51	10
26775	CIDR	8/1/2014	1	2	2	7:47	7:55	8
26784	CIDR	8/1/2014	1	3	2	7:51	8:03	12
26823	CIDR	8/1/2014	1	4	1	7:57	8:08	11
26824	CIDR	8/1/2014	1	2	4	7:47	7:58	11
26826	CIDR	8/1/2014	1	2	1	7:47	7:54	7
26844	CIDR	8/1/2014	1	3	3	7:51	8:04	13
26855	CIDR	8/1/2014	1	2	3	7:47	7:56	9
26863	CIDR	8/1/2014	1	3	1	7:51	8:02	11
26906	CIDR	8/1/2014	1	1	3	7:41	7:50	9
26950	CIDR	8/1/2014	1	2	5	7:47	8:00	13
26969	CIDR	8/1/2014	1	3	5	7:51	8:06	15
26980	CIDR	8/1/2014	1	1	1	7:41	7:48	7
26998	CIDR	8/1/2014	1	4	3	7:57	8:10	13
26999	CIDR	8/1/2014	1	1	5	7:41	7:52	11

<sup>1</sup>All data shown was collected from animals that received AI within the first week of breeding eligibility

<sup>2</sup>Heifers were bred if identified in estrus by the breeder in the morning (AM) hours of the day

	Table B: Breeding data for 2X PG treatment heifers <sup>1</sup>										
				Batch	Seq. of	Start	End	Total time			
Heifer ID	Trt	AI Date <sup>2</sup>	AI Tech	Number	AI	Time	Time	(min)			
26609	PG	7/29/2015	1	1	5	7:46	8:02	6			
26928	PG	7/29/2014	1	2	1	7:53	8:05	12			
26526	PG	7/30/2014	1	5	1	8:08	8:17	9			
26964	PG	7/30/2014	1	5	2	8:08	8:18	10			
26803	PG	7/30/2014	1	5	3	8:08	8:19	11			
26558	PG	7/30/2014	1	5	4	8:08	8:20	12			
26945	PG	7/30/2014	1	5	5	8:08	8:21	13			
26984	PG	7/30/2014	1	6	1	8:14	8:22	8			
26552	PG	7/30/2014	1	6	2	8:14	8:23	9			
26838	PG	7/30/2014	1	6	3	8:14	8:25	11			
26656	PG	7/30/2014	1	6	4	8:14	8:26	12			
26793	PG	7/30/2014	1	6	5	8:14	8:27	13			
26936	PG	7/30/2014	1	7	1	8:19	8:29	10			
26663	PG	7/30/2014	1	7	2	8:19	8:30	11			
26415	PG	7/30/2014	1	7	3	8:19	8:31	12			
26535	PG	7/30/2014	1	7	4	8:19	8:32	13			
26951	PG	7/30/2014	1	7	5	8:19	8:33	14			
26389	PG	7/30/2014	1	8	1	8:27	8:35	8			
26478	PG	7/30/2014	1	8	2	8:27	8:36	9			
26900	PG	7/30/2014	1	8	3	8:27	8:37	10			
26319	PG	7/30/2014	1	8	4	8:27	8:38	11			
26581	PG	7/30/2014	1	8	5	8:27	8:39	12			
26384	PG	7/30/2014	1	9	1	8:32	8:41	9			
26551	PG	7/30/2014	1	9	2	8:32	8:42	10			
26888	PG	7/30/2014	1	9	3	8:32	8:43	11			
26882	PG	7/30/2014	1	9	4	8:32	8:44	12			
26918	PG	7/30/2014	1	9	5	8:32	8:45	13			
26932	PG	7/30/2014	1	10	1	8:41	8:47	6			
26751	PG	7/30/2014	1	10	2	8:41	8:48	7			
26914	PG	7/30/2014	1	10	3	8:41	8:49	8			
26982	PG	7/30/2014	1	10	4	8:41	8:50	9			
26312	PG	7/30/2014	1	10	5	8:41	8:50	9			
26690	PG	7/30/2014	1	11	1	8:44	8:55	11			
26827	PG	7/30/2014	1	11	2	8:44	8:55	11			
26955	PG	7/30/2014	1	11	3	8:44	8:56	12			
26819	PG	7/30/2014	1	11	4	8:44	8:57	13			
26645	PG	7/30/2014	1	11	5	8:44	8:58	14			
26939	PG	7/30/2014	1	12	1	8:51	8:58	9			

26471	PG	7/31/2014	1	15	3	9:12	9:23	11
26292	PG	7/31/2014	1	15	4	9:12	9:24	12
26768	PG	7/31/2014	1	15	5	9:12	9:25	13
26603	PG	7/31/2014	1	16	1	9:19	9:27	8
26738	PG	7/31/2014	1	16	2	9:19	9:28	9
26743	PG	7/31/2014	1	16	3	9:19	9:28	9
26643	PG	7/31/2014	1	16	4	9:19	9:30	11
26776	PG	7/31/2014	1	16	5	9:19	9:31	12
26703	PG	7/31/2014	1	17	1	9:31	9:34	3
26772	PG	7/31/2014	1	17	2	9:31	9:38	7
26591	PG	7/31/2014	1	17	3	9:31	9:39	8
26495	PG	7/31/2014	1	17	4	9:31	9:40	9
26940	PG	7/31/2014	1	17	5	9:31	9:41	10
26646	PG	7/31/2014	1	18	1	9:36	9:43	7
26868	PG	7/31/2014	1	18	2	9:36	9:44	8
26613	PG	7/31/2014	1	18	3	9:36	9:45	9
26933	PG	7/31/2014	1	18	4	9:36	9:46	10
26700	PG	7/31/2014	1	18	5	9:36	9:46	10
26450	PG	7/31/2014	1	19	1	9:40	9:50	10
26992	PG	7/31/2014	1	19	2	9:40	9:50	10
26795	PG	7/31/2014	1	19	3	9:40	9:52	12
26132	PG	7/31/2014	1	20	1	9:45	9:54	9
26453	PG	7/31/2014	1	20	2	9:45	9:56	11
26455	PG	7/31/2014	1	20	3	9:45	9:57	12
26767	PG	7/31/2014	1	20	4	9:45	9:58	13
26864	PG	7/31/2014	1	20	5	9:45	9:59	14
26774	PG	7/31/2014	1	21	1	9:50	10:01	11
26630	PG	7/31/2014	1	21	2	9:50	10:02	12
26659	PG	7/31/2014	1	21	3	9:50	10:02	12
26632	PG	7/31/2014	1	21	4	9:50	10:05	15
26798	PG	7/31/2014	1	21	5	9:50	10:06	16
26832	PG	7/31/2014	1	22	1	9:58	10:08	10
26623	PG	7/31/2014	1	22	2	9:58	10:09	11
26695	PG	7/31/2014	1	22	3	9:58	10:11	13
26727	PG	7/31/2014	1	22	4	9:58	10:12	14
26989	PG	7/31/2014	1	22	5	9:58	10:13	15
26942	PG	8/1/2014	1	5	1	8:04	8:14	10
26869	PG	8/1/2014	1	5	2	8:04	8:14	10
26538	PG	8/1/2014	1	5	3	8:04	8:15	11
26706	PG	8/1/2014	1	5	4	8:04	8:16	12
26679	PG	8/1/2014	1	5	5	8:04	8:17	13

26641	PG	8/1/2014	1	6	1	8:10	8:19	9
26270	PG	8/1/2014	1	6	2	8:10	8:20	10
26712	PG	8/1/2014	1	6	3	8:10	8:21	11
26913	PG	8/1/2014	1	6	4	8:10	8:22	12
26686	PG	8/1/2014	1	6	5	8:10	8:23	13
26562	PG	8/2/2014	1	1	1	7:26	7:31	5
26489	PG	8/2/2014	1	1	2	7:26	7:32	6
26948	PG	8/3/2014	1	1	1	7:28	7:33	5
26400	PG	8/3/2014	1	1	2	7:28	7:35	7

<sup>1</sup> All data shown was collected from animals that received AI within the first week of breeding eligibility <sup>2</sup>Heifers were bred if identified in estrus by the breeder in the morning (AM) hours of the day

## **APPENDIX III**

#### **IACUC** Authorization of Procedures

## University of Idaho Institutional Animal Care and Use Committee

Date: Monday, June 02, 2014

To: Joseph Dalton

From: University of Idaho

Institutional Animal Care and Use Committee

#### Re: Protocol 2014-68

Reproductive and Economic Outcomes of Two Pre-breeding Strategies in Dairy Heifers

Your animal care and use protocol for the project shown above was reviewed and approved by the Institutional Animal Care and Use Committee on Monday, June 02, 2014. This protocol was originally submitted for review on: Saturday, May 10, 2014 The original approval date for this protocol is: Monday, June 02, 2014 This approval will remain in affect until: Tuesday, June 02, 2015 The protocol may be continued by annual updates until: Friday, June 02, 2017

Federal laws and guidelines require that institutional animal care and use committees review ongoing projects annually. For the first two years after initial approval of the protocol you will be asked to submit an annual update form describing any changes in procedures or personnel. The committee may, at its discretion, extend approval for the project in yearly increments until the third anniversary of the original approval of the project. At that time, the protocol must be replaced by an entirely new submission. Brad Williams, DVM Campus Veterinarian University of Idaho 208-885-8958