

**EFFECT OF TIMING OF INSEMINATION AND SYNCHRONIZATION OF
ESTRUS METHOD ON ARTIFICIAL INSEMINATION (AI) PREGNANCY
RATES IN BEEF HEIFERS**

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ABSTRACT

Objectives were to evaluate time of insemination relative to estrus and synchronization with melengestrol acetate (MGA) plus prostaglandin (PG) or progesterone insert (CIDR) plus PG on AI pregnancy rate in beef heifers ($n = 662$) during Fall or Spring. Fall heifers ($n = 349$) received MGA-PG (MGA for 14 d followed by PG on d 18) or CIDR-PG (CIDR for 7 d, PG administered 1 d before CIDR removal). Estrus was monitored by HeatWatch® ($n = 200$) or visually ($n = 149$). Spring heifers ($n = 313$) underwent CIDR-PG with detection of estrus by HeatWatch®. Heifers not in estrus by 96-100 h after PG were bred AI as non-responsive AI (NRAI). Across seasons, 548 heifers were bred following estrus (EAI). Heifers synchronized during the Fall with MGA received more ($P < 0.05$) mounts than Fall CIDR heifers (76.8 ± 6.7 and 47.6 ± 7.4 , respectively), but duration of estrus was similar. Fall CIDR heifers had greater ($P < 0.05$) mounting activity and duration of estrus (47.9 ± 5.2 mounts and 15.5 ± 1.1 h) compared to Spring CIDR heifers (34.5 ± 3.1 mounts and 12.7 ± 0.6 h). Heifers grouped in 4 h blocks from 0 to 24 h had no difference ($P > 0.05$) in pregnancy rates (mean 62.5 %). Treatment had no effect ($P > 0.05$) on EAI pregnancy rates. Pregnancy rates across seasons for EAI, NRAI and overall was 61.0 %, 26.3 %, and 54.5%. In conclusion, a 24 h window may exist to successfully AI heifers.

Key Words: Heifers, Synchronization of estrus, AI, CIDR

Dedication

I dedicate this work to my Heavenly Father and Savior the Lord Jesus Christ for blessing me with the health and ability to accomplish this thesis and Master of Science degree. I also want to thank Him for blessing me with the opportunity to attend veterinary school at The Ohio State University.

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Table of Contents

Chapter 1 Introduction.....	1
Impact of reproduction on the cow/calf enterprise.....	1
Use of artificial insemination in the United States.....	3
Barriers to artificial insemination.....	4
Chapter 2 Review of Literature.....	5
Bovine estrous cycle.....	5
Development of artificial insemination in the United States-Beginning to present.....	9
Timing of insemination in cattle and mechanisms of action for fertilization.....	15
Semen effects on fertility.....	21
Synchronization of estrus: Mechanisms of action of progesterone and Prostaglandin F ₂ α.....	22
MGA-PG.....	24
CIDR-PG.....	28
Experimental Objectives.....	30
Chapter 3 Materials and Methods.....	31
Experiment 1.....	31
Experiment 2.....	35
Statistical methods.....	38
Chapter 4 Results.....	39
Characteristics of onset of estrus.....	39
Characteristics of estrus.....	43
Synchronization of estrus method and pregnancy rate.....	46
Timing of AI and pregnancy rate.....	48
Chapter 5 Discussion.....	52
Chapter 6 Implications.....	62
Literature Cited.....	64
Vita.....	74

List of Tables

Table 1. HeatWatch detected mounts, mount intensity, and duration of estrus by synchronization of estrus method.....	43
Table 2. HeatWatch detected mounts, mount intensity, and duration of estrus by season.....	44
Table 3. Mount characteristics and duration of estrus (mean \pm SE) in heifers bred at different times after onset of estrus.....	45
Table 4. Pregnancy rates in heifers as affected by total number of mounts as detected by HeatWatch by season and synchronization of estrus.....	46
Table 5. Effect of type of AI breeding on pregnancy rates in beef heifers by season and method of synchronization of estrus.....	47

List of Figures

Figure 1. Experiment 1 CIDR-PG and MGA-PG synchronization of estrus system protocols conducted in November.....	34
Figure 2. Experiment 2 CIDR-PG synchronization of estrus system protocol conducted in April.....	36
Figure 3. Numbers of heifers in estrus relative to prostaglandin injection for MGA and CIDR heifers.....	41
Figure 4. Numbers of heifers in estrus by time of day by season of experiment.....	42
Figure 5. Effect of timing of insemination relative to onset of estrus (4 h blocks) on pregnancy rates in beef heifers.....	49
Figure 6. Effect of timing of insemination relative to onset of estrus (8 h blocks) on pregnancy rates in beef heifers.....	50
Figure 7. Regression of timing of insemination relative to the onset of estrus (4 h blocks) on pregnancy rate.....	51

Chapter 1 Introduction

Impact of reproduction on the cow/calf enterprise

The success of the cow/calf enterprise is dependent on good reproductive performance. Reproductive performance is the single most important economic trait in a beef cow herd (Trenkle and Willham, 1977). As an economic trait to the cow/calf enterprise, reproduction is 3.24 times more important than production and production is 2.87 times more important than consumption (Melton, 1995). In order for high profitability to be achieved, cows must have a high reproductive rate and resulting calves must have good feed efficiency and acceptable weaning weights. This is difficult to achieve with selection alone. Just 10% of the variation in reproduction will respond to selection (Willham, 1973). Because most components of fertility that influence calving and subsequent reproductive performance are not highly heritable, it is logical to assume that the majority of factors related to reproductive performance in cattle are influenced almost entirely by management (Patterson et al., 1999).

Reproductive failure and (or) loss within a herd occurs primarily as a result of cows failing to become pregnant or the loss of calves at or near birth (Wiltbank, 1990). Puberty in the heifer and resumption of estrous cyclicity following calving in the postpartum cow are the critical reproductive events that determine whether and when pregnancy will occur (Patterson et al., 1999). The initiation of puberty and at what age, will determine the lifetime productivity of that heifer for the beef herd. Heifers bred to calve as two yr olds should be exposed for breeding before mature herd mates, and early calving periods can be used as a means of increasing production efficiency (Wiltbank, 1970). However, breeding heifers on their first (pubertal) or second estrus should be

avoided because of reduced fertility. Fertility of heifers bred at the pubertal estrus was 21% lower than for those bred on their third estrus (Byerley et al., 1987). Therefore, to increase conception rates of heifers on their first breeding, they should be bred on their third or greater estrus.

Management of replacement heifers during the post-weaning-to-breeding period influences to a large extent when puberty, pregnancy, and parturition will occur (Patterson et al., 1999). Higher lifetime production results when heifers calve early during their first calving season (Lesmeister et al., 1973). Because most calves are weaned at a particular time rather than on a weight-constant or age-constant basis, calves born late in the normal calving season are usually lighter than those born early, decreasing lifetime productivity of their dams (Lesmeister et al., 1973). Cows that calve late one year tend to calve late the next year or not at all (Burris and Priode, 1958).

In order for heifers to calve early, they must reach puberty as soon as possible. Hall et al. (1995) found that heifers on a high gain diet reached puberty sooner than heifers on a moderate gain diet. Adequate nutrition for the growing heifer is also needed to alleviate dystocia problems at calving. The target weight principle calls for feeding heifers to a pre-breeding target weight that represents 65% of the heifer's projected mature weight (Patterson et al., 1999). As a result, when heifers are ready to calve, they are almost full grown. Therefore, if heifers do not reach their target pre-breeding weight, reproduction will suffer. To achieve this goal, adequate nutrition from weaning to puberty is essential. If the weaning weights of heifer calves selected for potential replacements is known as well as the weight needed for those heifers to attain puberty, gains per day needed to reach that target weight can be calculated (Dziuk and Bellows, 1983).

Gestation represents the lowest losses incurred to the cow calf producer. During a 14 yr summary, reduction in net calf crop from calf losses during gestation in disease free herds was 2.3% (Dziuk and Bellows, 1983). This data suggests that once conception occurs and pregnancy has been diagnosed, the animal will likely sustain the pregnancy. Accurate pregnancy diagnosis can aid in the reproductive efficiency of the cow/calf operation. Age estimates of the embryo-fetus can allow dams to be divided into predicted early, late calving date or non-pregnant groups and nutrition level can be adjusted accordingly or culling done (Dziuk and Bellows, 1983). Selection of females that conceived early can also be done based on age of the embryo.

Use of artificial insemination in the United States

Once the pre-pubertal heifer has reached puberty and is cycling, and the mature cow has calved, it is the goal of the producer to get the animals bred. Today, an efficient method of establishing pregnancy is through artificial insemination. Benefits of AI range from greater genetic selection, reduced disease transmission, use of old or crippled bulls, and elimination of danger from handling unruly bulls (Webb, 1992; Nebel et al., 2000). Artificial insemination also allows the producer to choose bulls that have been proven to sire smaller calves for reduced dystocia problems. However, management of beef cattle does not facilitate the use of AI extensively in the beef industry. Most beef cattle are in extensive range environments where detection of estrus and rounding up animals in estrus for insemination is not cost effective (Foote, 2002). Many beef producers are reluctant to invest in necessary management practices and added labor needed to develop an AI program. The majority of beef cattle operations (91.9 %) only use natural service for breeding cows and heifers (NAHMS, 1997). Artificial insemination is practiced by 7.1 %

of beef cattle operations (NAHMS, 1997). The remaining 1 % of operations use only pre-bred females.

The majority of producers in the dairy industry have adopted the use of AI. Approximately 70 to 80 % of the dairy farms in the U.S. currently use AI (Pursley et al., 1997). In North America there are 69 semen collection centers that house 8322 dairy bulls compared to 1305 beef bulls (Thibier and Wagner, 2002). The nature of the dairy operation facilitates the use of AI. Animals are housed in smaller confines compared to the range environment of many beef operations, making estrus detection easier. Dairy cattle are also handled daily for milking, thus there is greater access to each animal for breeding by an AI technician.

Barriers to artificial insemination

Cows in estrus usually stand to be mounted by herd mates, and, therefore, observations of this primary behavior can be an effective method for estrus detection (Rankin et al., 1992). However, the greatest stumbling block to the adoption of AI programs for both beef and dairy operations is the detection of estrus. Estrus detection is cited many times as the most common and costly failure of AI programs (Walker et al., 1996). Inefficient detection of estrus results in lost lifetime milk yield, decreased number of calves born per lifetime, excessive days open, and increased reproductive culling (Walker et al., 1996). The goal of an estrus detection program should be to identify estrus positively and accurately in all cycling animals and consequently to identify animals not cycling (Nebel et al., 2000). Insufficient time allocation for detection of estrus contributes to lower efficiency and missed periods of estrus, particularly in cattle in which estrus is of lesser intensity and shorter duration (Stevenson et al., 1996). Adequate

labor for the detection of estrus is often cost prohibitive. The use of electronic systems for estrus detection has been shown to reduce the need for labor to perform visual observation of estrus. The HeatWatch® (HW; CowChips, LLC Denver, CO) remote pressure sensing system for detection of standing to be mounted activity has shown to be a helpful tool for improving efficiency and accuracy of estrus detection and timing of AI (Nebel et al., 1994; Stevenson et al., 1996; Dransfield et al., 1998).

A possible solution to the problem of detection of estrus could be the adoption of synchronization of estrus programs by the beef industry. Synchronization of estrus can be an effective aid to an AI program by concentrating the period when heifers and cows are in estrus into an abbreviated time span. Synchronization of estrus improves time management for producers that use AI by concentrating the breeding and resulting calving periods (Patterson et al., 1999). However, only 11.9 % of all beef operations use an estrus synchronization program (NAHMS, 1997).

Chapter 2 Review of Literature

Bovine estrous cycle

The bovine estrous cycle consists of a 21 day period that ranges from 18 to 24 days in length. The cycle consists of a follicular phase that is made up of proestrus and estrus and a luteal phase that is made up of metestrus and diestrus. The follicular phase is governed by the hypothalamus, the anterior pituitary, and the ovary through the production of estradiol and the absence of progesterone (Senger, 1999). Proestrus is a transition period from progesterone dominance to estrogen dominance. During proestrus gonadotropin releasing hormone (GnRH) secretions start to increase from the hypothalamus. Release of GnRH is controlled by the ventromedial nucleus and arcuate

nucleus of the tonic center and the preoptic nucleus, anterior hypothalamic area and suprachiasmatic nucleus of the surge center (Senger, 1999). The tonic center releases GnRH in small quantities throughout the entire estrous cycle, whereas the preovulatory surge of GnRH from the surge center occurs only once during each cycle (Senger, 1999). Release of GnRH causes the adenohypophysis to release follicle stimulating hormone (FSH) and luteinizing hormone (LH). Follicle stimulating hormone initiates follicular growth. Resultant growth of the follicles produces estradiol and follicular fluid. Increasing estradiol levels cause growth of the tubular genitalia, swelling of the vulva, enlargement of the cervix, an increase in uterine mucosa vascularity and secretion of mucus by the cervical canal (Salisbury et al., 1978). Proestrus lasts for two to three days (Bearden and Fuquay, 2000).

In preparation for ovulation, follicles undergo recruitment, selection, and dominance (Senger, 1999). During recruitment, the adenohypophysis is releasing large quantities of FSH and the neurohypophysis is releasing a smaller amount of LH. At this time, estrogen release is increasing from the follicles. Selection marks a decrease in FSH release because of inhibin production by the follicles (Senger, 1999). This period marks a reduction in the role of FSH and an increase in LH importance. When FSH production decreases below a critical level, follicular recruitment stops. All but the largest follicle that was recruited undergo atresia. The stage of dominance is characterized by continued decreasing FSH levels and increasing LH levels (Senger, 1999). Growth of the dominant follicle continues and it reaches its maximum size. During this time, estradiol output from the dominant follicle will reach threshold, causing the LH surge from the

adenohypophysis, resulting in ovulation. In beef cattle, ovulation occurs an average of 31.1 hrs after the onset of estrus (White et al., 2002).

The luteal phase lasts from the time of ovulation until luteolysis of the CL near the end of the estrous cycle (Senger, 1999). Metestrus makes up the early luteal phase and lasts three to four days of the cycle (Bearden and Fuquay, 2000). Metestrus is marked by the process of luteinization. Luteinization involves the transition of a preovulatory follicle into a highly vascular CL capable of secreting large quantities of progesterone (Smith et al., 1994). During this time, a corpus hemorrhagicum forms from the ruptured follicle due to the destruction of blood vessels within the follicular wall. This process is controlled by LH. The walls of the follicle collapse into many folds allowing thecal cells and the granulosal cells to mix, thus forming a gland consisting of connective tissue cells, thecal cells, and granulosal cells (Senger, 1999). At about day three to five of the estrous cycle, the CL begins to increase in size and lose its hemorrhagic appearance (Senger, 1999).

Metestrus signals the start of progesterone synthesis by the CL. The presence of basal LH and cholesterol is necessary for progesterone to be produced by the luteal cells (Senger, 1999). Large luteal cells undergo hypertrophy, while small luteal cells undergo hyperplasia as they progress toward complete functionality of the CL (Senger, 1999). The CL becomes fully functional at diestrus which occurs at day five of the cycle and ends on day 16 or 17 with regression of the CL (Bearden and Fuquay, 2000). Progesterone is of major importance in the endocrine control of reproduction because it exerts a strong negative feedback on the hypothalamus (Senger, 1999). The tonic center of the hypothalamus reduces its secretion of GnRH under conditions of high

progesterone. This in turn reduces production of FSH and LH but still allows follicular waves to occur during the luteal phase. High progesterone therefore prevents development of preovulatory follicles, production of estrogen, behavioral estrus and the preovulatory surge of GnRH and LH (Senger, 1999). Progesterone also prepares the uterus for pregnancy if conception occurs. The endometrium thickens, and the glands and muscles of the uterus develop, thus preparing the uterus for the nourishment of the embryo and the formation of the placenta (Salisbury et al., 1978).

At the end of diestrus, the CL undergoes luteolysis resulting in a precipitous drop in progesterone levels in the blood. The two main hormones controlling luteolysis are oxytocin from the CL and prostaglandin F₂α (PGF₂α) produced by the uterine endometrium (Senger, 1999). The animal will remain in a sustained luteal phase if luteolysis does not occur. The uterus must be exposed to elevated progesterone for a period of days before it can synthesize and release PGF₂α in sufficient quantities to cause luteolysis (Senger, 1999). There is no PGF₂α production by the uterine endometrium during the early luteal phase. However, during the late luteal phase oxytocin released from the CL binds to oxytocin receptors located in the uterine endometrium. This results in the pulsatile release of PGF₂α. The pulses increase in frequency and amplitude as the end of the luteal phase approaches (Senger, 1999). Prostaglandin F₂α binds to specific receptors on the plasma membrane of large luteal cells which causes calcium channels to open resulting in apoptotic effects (Senger, 1999). Progesterone production is stopped by protein kinase-C which is initiated by the PGF₂α receptor complex. The decrease in progesterone production removes the progesterone block on the hypothalamus, thus

paving the way for increased GnRH secretions and the beginning of a new estrous cycle starting over again with proestrus.

Development of Artificial Insemination in the United States: Beginning to present

The practice of AI for domestic animals traces its beginnings back to the fourteenth century. Undocumented tales exist of Arabs obtaining sperm from mated mares belonging to rival groups and using the sperm to inseminate their own mares (Foote, 2002). The first person to see sperm was Anton Van Leeuwenhoek which he called animalcules by using his early microscope in 1678 (Foote, 2002). It was not until the next century that the first scientific studies using AI were performed. Lazzaro Spallanzani, the Italian physiologist, in 1780 inseminated a confined dog in heat, using fresh semen, and introduced it directly into the uterus with a syringe (Herman, 1981). The experiment resulted in the successful birth of three puppies that closely resembled the female and male semen donor. Spallanzani discovered that the fertilizing power of semen was due to the spermatozoa carried in the seminal fluids and that freezing stallion semen did not kill the sperm, but held them in a dormant state until exposed to heat, after which they resumed motility (Herman, 1981). It was not until the late 1800s that AI was practiced regularly. English dog breeders used the technique between 1884 and 1896, finding that several females could be bred with one ejaculate and that it is a convenient way to cross dog breeds (Herman, 1981). In 1890, the French veterinarian Repiquet used AI in horses as a means of improving fertility which resulted in the development of horse breeding centers in several European countries that practiced AI (Herman, 1981).

Practical use of AI for farm animals was developed in Russia. Under the direction of the Russian scientist E.I. Ivanov in 1899, AI was practiced on numerous government

horse breeding farms that produced conception rates higher than by natural service (Herman, 1981). Ivanov's laboratory produced improved methods for the collection and dilution of semen and practical inseminating techniques for cattle, sheep, and horses. In 1938, 40,000 mares, 1.2 million cows, and 15 million sheep in the Soviet Union were serviced by means of AI (Herman, 1981). The first artificial vagina for large animals was also developed in Russia. The Russian scientist V. R. Milovanov and others are credited for constructing artificial vaginas suitable for collecting semen from bulls, rams, and stallions (Herman, 1981). This was an enormous improvement over the earlier method of collecting semen from sponges placed in the vagina of mount animals and resulted in the widespread use of AI in the field (Foote, 2002; Herman, 1981).

The rectovaginal method of artificially inseminating cattle was developed in Denmark. In 1937, Danish veterinarians developed the rectovaginal or cervical fixation method of AI for cows (Perry, 1968). The cervix is manually manipulated from the rectum, which allows the inseminating rod to be guided through the cervix into the uterus. This technique provided a tremendous advantage because fewer sperm were required for insemination of each cow and fertility was improved by approximately 10 percent (Foote, 2002).

The first documented use of AI in the United States was with horses. In 1906 L. L. Lewis, of the Oklahoma Agricultural Experiment Station, described the use of the impregnator and in 1911 published a bulletin on AI in horses (Herman, 1981). Before the use of the impregnator, horse breeders collected semen in a pan after the stallion dismounted, placed it in a flask, kept it warm, transferred the semen to a capsule, and introduced it into the vagina of mares to be bred (Herman, 1981). A similar method was

used by cattle breeders before the artificial vagina was invented. In 1907, a “whiteface” calf was born in the herd of R. L. Hughey, Alva, Oklahoma, as the result of the dam being artificially inseminated by collecting semen from the vagina of another cow that had just been bred naturally and inserting the semen into the dam by way of a gelatin capsule (Herman, 1981). The first documented usage of AI in dairy cattle occurred in Washington State. Thomas C. Webster, of Fort Steilacoom, Washington, while in charge of the fort’s dairy herd, began using AI in 1926 and later in 1930 went to Wisconsin where he was involved in the use of AI on Wisconsin State Institution cattle herds (Herman, 1981).

Producers in the U.S. had begun using AI on their private herds for more than 30 yrs before the first organized AI programs formed. Beginning in 1937, C. L. Cole, at the University of Minnesota, North Central Station, set up an experiment to test the practicality of AI in dairy herds under ordinary farm conditions that resulted in 98 cows becoming pregnant (Herman, 1981; Perry, 1968). The first cattle breeding organizations to use AI in the United States began operations in New Jersey on May 17th, 1938, as Cooperative Artificial Breeding Association No. 1 (Perry, 1968). This system was modeled after an AI cooperative established in Denmark. The organization started with 102 members, and 1,050 dairy cows were enrolled by the owners (Herman, 1981). In 1938 other AI organizations formed in Hughesville in Missouri at the Missouri College of Agriculture and the Farm Security Administration and in New York with the development of the New York Artificial Breeders, Cooperative Inc. in Ithaca (Herman, 1981; Foote, 2002). The New York Cooperative formed a collaboration with researchers and extension personnel at Cornell University that resulted in the experimental

insemination of hundreds of thousands of cows and publication of more than 100 research papers on sire selection, testicular evaluation, semen collection, evaluation and processing; and fertility testing (Foote, 2002). During 1939, there were seven AI businesses with 33 sires involving 7,359 cows in 646 herds enrolled in the program (Herman, 1981). Later, in 1947 there were 608 artificial breeding associations located in 34 states that consisted of 140,571 herds containing 1,125,040 dairy cows (Herman, 1981). By 1950, the number had increased to 97 AI businesses, 2,104 sires, and services in 409,300 herds, with 2,619,555 cows involved (Herman et al., 1994).

Beef producers were less enthusiastic over the adoption of AI than dairy farmers during the infancy of AI development. They developed registration rules that prevented calves from being registered if the dam and sire did not belong to the same owner. It was argued that since beef cattle ranged over wide areas and were not handled like dairy cows, AI would be of little value to the beef producer, but some beef producers turned to AI in the early years as a means of controlling the spread of venereal disease (Herman, 1981). Starting in the late 1930's, research was conducted on the use of AI in beef cattle. From 1939 to 1942, research workers from the University of Missouri with the cooperation of the Bureau of Animal Industry, USDA, and the Indian Service, U. S. Department of the Interior, demonstrated the successful field use of AI for beef cattle (Herman, 1981). The study was conducted over four breeding seasons using cattle from the San Carlos Apache Indian Reservation in San Carlos, Arizona. Over a three year period, 79 percent of the cows inseminated dropped calves, requiring 1.63 inseminations per calf (Herman, 1981). Some dairy bull studs began using beef sires for their AI programs. Starting in 1942, the purpose was, first, to service milk cows for dairymen

who were converting to beef, and second to breed first calf heifers so as to obtain a smaller calf and reduce calving difficulties (Herman, 1968).

Technical developments in the dilution, storage, and shipping of semen allowed the use of AI to expand rapidly. Only by the extension of semen, anywhere from ten to 200 times its original volume, can many cows be inseminated by a single ejaculate (Herman, 1981). Sperm numbers per insemination with liquid semen were reduced from more than 100×10^6 sperm per insemination to 4×10^6 sperm per insemination (Foote, 2002). The first major improvement in the AI procedure initiated in the United States was the development of a yolk phosphate semen extender by P. H. Phillips and H. A. Lardy in 1940 to protect sperm cells during cooling and storage (Herman, 1981; Foote, 2002). In 1941, G. W. Salisbury and coworkers improved the extender by the addition of a sodium citrate buffer that dispersed the fat globules in egg yolk, making sperm visible for microscopic examination (Herman, 1981; Foote, 2002). Researchers at Cornell added the antibiotic mixture of penicillin, streptomycin, and polymyxin B in 1950 to semen extender to prevent disease transmission from bulls (Foote, 2002).

From 1936 to 1954, liquid semen was in universal use (Herman, 1981). For shipment, sperm metabolism was reduced by cooling semen to 35^0 F to 40^0 F with ice, water or electric refrigerators which worked well if semen was to be used within two days (Herman, 1981). Long distance deliveries required a fast mode of transportation and in the late 1940s many AI organizations used airplanes for semen delivery. Artificial Breeding Association in Springfield, Missouri, perfected a shipping container with a rubber pad on the bottom to prevent breakage and utilized parachutes to drop the semen container to technicians (Herman, 1981).

The advent of frozen semen no longer perpetuated the need for a quick delivery system for semen because semen viability could now be maintained for months. The first successful effort in freezing bull spermatozoa came in 1950 at Cambridge University, when C. Polge and others added glycerol to semen diluent that permitted freezing at -79°C without destroying all fertility (Herman, 1981). Frozen semen was used in field trials in 1952 by several AI organizations with the first calf resulting from the use of frozen semen being born in the United States in 1953 (Herman, 1981). By the late 1950's liquid nitrogen had replaced alcohol and dry ice as the preferred method for freezing semen. It allowed semen to be stored at -196°C and allowed shipment of semen anywhere in the world, giving semen a viable shelf life of many years (Herman, 1981). Liquid nitrogen also provides conception rates that are equal to those of fresh semen (Nishikawa, 1964).

By the early 1960's, all AI organizations in the United States were using frozen semen. The use of frozen semen made it possible to transport semen to more distant places at larger intervals of time which resulted in the expansion of the service range for each bull stud (Nishikawa, 1964). As a result, the number of bull studs has decreased. From 1950 to 1963, the number of bull studs in operation decreased from 97 to 51 (Nishikawa, 1964). As a consequence, bull studs became larger through resulting mergers, with fewer studs housing more bulls.

Throughout the 1950's and 1960's the number of cattle artificially inseminated steadily increased. In 1963 a total of 7,673,582 cattle were artificially inseminated in the United States, 235,289 of which were beef cattle (Herman et al., 1994). The use of AI in the U. S. was second only to the amount performed in the Soviet Union which artificially

inseminated over 18 million cattle (Nishikawa, 1964). By 1971, 8,643,089 cattle were bred by AI in the U. S. including 1,357,918 beef cattle (Herman et al., 1994). That year there were 24 AI centers that housed 96 bulls each that inseminated on average 3,620 cows per sire (Herman et al., 1994). Since 1971, the volume of AI business in the U. S. has been estimated in terms of “units of semen” which is designated as the amount needed for one insemination (Herman et al., 1994). This method came into use because of discrepancies in the usage of semen. Much semen is sold directly to herd operators through distributors, who may or may not provide insemination services which make it impossible to determine the number of first services because in many cases, semen is stored for future use or it is resold (Herman et al., 1994).

In order to determine how many dairy and beef cattle are bred by AI, calculations are made based on how many units of semen are needed to produce a pregnancy. In general, it requires 1.5 to 2.0 units of semen to get a dairy cow in calf and in beef AI, where clean up bulls are used, it takes 1.3 to 1.5 units of semen per cow settled (Herman et al., 1994). By 1992, over eight million dairy cows and heifers and two million beef cows and heifers were serviced by AI accounting for 65 % of the dairy cattle population and 6% of the beef cattle population in the U. S. (Herman et al., 1994). Current data indicates that 70 to 80 % of all dairy farms in the U. S. use AI and 7.1 % of all beef cattle operations use AI (NAHMS, 1997; Pursley et al., 1997). Therefore, continuing efforts are needed in the beef industry to increase the adoption of AI by producers.

Timing of insemination in cattle and mechanisms of action for fertilization

The most effective use of AI in the breeding of livestock is possible only if accurate knowledge of the time of ovulation is available (Nalbandov and Casida, 1942). With

reduced sperm numbers per dose in AI compared to natural breeding, the time of deposition of semen in relation to estrus onset and ovulation is exceedingly important (Gwazdauskas, 1978). Ovulation is the key reproductive marker in the pathway to successful fertilization. However, in order for viable sperm to have time to travel to the site of fertilization in the oviduct at the time of ovulation, the animal must be artificially inseminated at a time that allows the optimum lifespan of both the sperm and ovum to coincide. Ovulation is a silent event with no external signs. Initiation of estrus is the best external sign to estimate time of ovulation and when to inseminate cows (White et al., 2002). The rise in estradiol-17 β concentrations which occurs almost simultaneously with the onset of estrus, is responsible for the initiation of behavioral estrus (Stevenson et al., 1998). Estradiol-17 β is also indirectly responsible for the release of LH by the modification in the amplitude and frequency of release of GnRH in the absence of progesterone, thus, estradiol-17 β is ultimately responsible for ovulation (Nebel et al., 2000). If the CL does not regress and progesterone remains elevated some or all of the subsequent events (LH surge, estrus, ovulation) do not occur even when estradiol is elevated (Wiltbank et al., 2000).

Once ovulation does occur, the ovum in the cumulus is expelled to the surface of the ovary in close proximity to the fimbriated end of the oviduct where it is then swept into the infundibulum (Salisbury et al., 1978). Cilia lining the oviducts of the cow continuously thrash toward the uterus, thus transporting the ovum through the infundibulum into the ampulla where fertilization will take place (Lombard et al., 1950; Salisbury et al., 1978; Senger, 1999). After the ovum has ovulated, it has a fertile lifespan of 6-10 h, thus sufficient numbers of fertile spermatozoa must be present in the

ampulla waiting on the ovum to arrive in order for successful fertilization to occur (Brackett et al., 1980).

Artificial insemination deposits sperm in the body of the uterus. Transport of spermatozoa is primarily the result of elevated tone and motility of the muscularis of the female reproductive tract (Senger, 1999). Estradiol is high during the follicular phase when insemination occurs, causing contractions of the muscularis, particularly the myometrium, and PGs in semen ($\text{PGF2}\alpha$ and PGF_1) cause increased tone and motility of the uterus and oviduct (Senger, 1999). In order for a population of sperm to reach the oviducts that is sizable enough to cause fertilization, a transport time of at least 6 h is needed with sperm numbers steadily increasing thereafter from 8 to 18 h (Thibault, 1973; Wilmut and Hunter, 1984; Hawk, 1987; Dransfield et al., 1998). For maximum fertility to be achieved, spermatozoa must reside in the female reproductive tract for a minimum period of time (Senger, 1999). This phenomenon, termed “capacitation,” endows the sperm with the ability to undergo the acrosome reaction, exhibit hyperactivated motility and fuse with the oocyte (Yanagimachi, 1981; Bedford, 1983; Herz et al., 1985). In the bovine, the time required *in vivo* for capacitation of ejaculated sperm has been estimated at 6 h based on first penetration of ova after insemination (Hunter, 1980; Herz et al., 1985). Sperm have been found to retain their fertility in the female bovine reproductive tract from 28-50 h (Hamner, 1973; Dukelow and Riegle, 1974). Saacke et al. (2000) examined embryo quality in relation to time of insemination and found a shift from high quality embryos achieved by inseminations at onset of estrus to low quality embryos resulting from inseminations at 24 h following estrus onset. Success in breeding early

appeared to be limited by sperm life leading to fertilization failure and breeding late was limited by declining embryonic quality (Saacke et al., 2000).

In studies using the HW system on dairy cattle, duration of estrus averaged seven to 10 h, and mounting activity averaged 8-14 times per cow (Walker et al., 1996; Dransfield et al., 1998). In beef heifers the average duration of estrus was 14 h with 50 standing events per heifer (Stevenson et al., 1996). The duration of estrus for beef cows ranged from 13.9 h to 17.6 h (White et al., 2002). Ovulation in dairy cattle occurs 27.6 h after the onset of estrus and 31.1 h after the onset of estrus in beef cattle (Walker et al., 1996; White et al., 2002).

Early studies found a narrow window of time for optimum results of fertilization by AI. Trimberger and Davis, (1943) found the optimum time for AI to be from the middle of estrus up to six h after the end of estrus. Poor conception rates occurred at the start of estrus and 12 h after the end of estrus. These findings resulted in the development of the AM-PM rule for the timing of AI. Barrett and Casida, (1946) collected data from 3,841 inseminations of dairy cattle and grouped them into blocks of time based on when they were bred in relation to onset of estrus determined by visual observation. Average pregnancy rate was 52.4% with the worst pregnancy rates occurring after the estimated time of ovulation. Aschbacher et al., (1956), bred 50 dairy cattle by AI at three different intervals during the same estrus to determine optimum time for insemination. Time of breeding on pregnancy rate was not significant with only 50 cows; however, higher pregnancy rates occurred when inseminations were done before the expected time of ovulation. Early research was most consistent with the idea that cows should not be bred

after ovulation and did not convincingly show an optimal time for AI (Wiltbank et al., 2000).

Several studies have been performed to determine the effectiveness of breeding once per day versus the A.M.-P.M. rule. Nebel et al., (1994) collected results from 3,659 inseminations performed once daily and 3,581 inseminations performed using the A.M.-P.M. rule. Non-return rate results were not significantly different at 60d with 64.6% using once daily inseminations and 65.6% using the A.M.-P.M. rule.

Graves et al, (1997) conducted a study using 337 Jersey cows and heifers using once daily AI versus the AM-PM rule and produced conception rates of 57.6% and 60.5% which were not significantly different. However, slightly lower conception rates occurred in cows detected in estrus in the morning and bred later the same morning compared with cows that were not bred until 12 h after estrus onset. Artificial inseminations performed once daily in dairy cattle have proven to be as effective under certain conditions as the AM-PM rule. Under routine field conditions of less frequent observation for estrus, cows can be submitted for AI shortly after detection of estrus with nearly optimal results with respect to timing (Nebel et al., 1994). It has been reported that onset of estrus occurs more frequently at night in dairy cattle, thus by inseminating at mid morning cows have been in estrus between 12 to 18 h, yielding the highest probability for conception (Hurnik et al., 1975; Nebel et al., 1994).

Maatje et al., (1997) used pedometers on 121 Holstein cows and heifers to predict the optimal time of insemination. Highest chances for conception occurred 6 to 17 h after pedometer activity heightened which coincides with estrus onset. From the data, it was determined that 11.8 h after elevated pedometer activity was the optimum time for AI.

Dransfield et al., (1998) studied the interval from estrus onset to AI on 2,661 breedings with dairy cattle. Using the HW system, continuous estrus surveillance was conducted. Optimum conception rates (46-51%) occurred from 4-16 h after estrus onset. Mathematical modeling to predict the optimal time for AI using activity pedometers and visual signs of estrus, estimated 11.8 h from onset (Maatje et al., 1997), which coincides with the approximate midpoint of 4 to 16 h as optimum using HW (Dransfield et al., 1998).

Information available on the duration of elapsed time required from estrus detection to insemination to achieve optimal fertility in beef cattle is sparse and inconclusive (Laster, 1974; Fields et al., 1975; Robbins et al., 1978). Robbins et al., (1978), determined calving rates of 2,091 beef cows from first inseminations at various intervals from estrus. Visual detection of estrus was conducted between dawn and noon, and then from 3-4 pm until dark. All cows were bred the following day after being observed in estrus resulting in insemination times of 12 to 29 h after estrus observation. Optimal calving rates occurred when inseminations occurred between 16 and 25 h after estrus observation with fertility not being lowered precipitously when inseminations were made 4 h earlier or 4 h later (Robbins et al., 1978).

The greatest deficiency in most studies designed to examine the optimum time for AI has been the lack of an effective method to accurately determine onset of estrus in cattle. Timing of AI based on visual detection is handicapped by the inability to provide 24 h surveillance of estrus onset. Cattle observed visually in the A.M. could be in early, mid, or late estrus. Studies using pedometers, and more recently the HW system have been

able to provide around the clock detection for estrus onset resulting in optimal times for AI in cattle (Maatje et al., 1997; Dransfield et al., 1998).

Semen Effects on Fertility

Success of mating is dependent upon both quality and quantity of semen delivered to the female (Saacke et al., 1994). Determinates of good semen fertility are based on compensable and non-compensable traits of the semen. Compensable traits of semen are those believed to be associated with the inability of sperm to reach the site of fertilization or to initiate the block to polyspermy by penetration of the ovum vestments; however, these reductions in fertility can be overcome by using high numbers of spermatozoa during insemination (Saacke et al., 1994; Saacke et al., 2000). Both sperm viability and morphology are important to the compensable traits because aberrations in either result in complete or partial exclusion at several barriers in the female tract of which the zona pellucida may be the most formidable (Saacke et al., 2000).

Uncompensable traits are those that cannot be overcome by increasing the number of spermatozoa in the inseminate because these defects affect the function of spermatozoa during the later stages of fertilization and embryonic development (Braundmeier and Miller, 2001). Chromatin aberrations in morphologically normal or near normal spermatozoa from abnormal semen samples appear to be the best candidates for uncompensable deficiency (Saacke et al., 2000). Defects in spermatogenesis and spermatozoal maturation that cause aberrant spermatozoal chromatin structure may not be manifested until spermatozoa-oocyte fusion, egg activation, or early development (Evenson, 1999; Braundmeier and Miller, 2001).

*Synchronization of Estrus:
Mechanisms of action of progesterone and prostaglandin F2 α*

Progesterone (P4) plays a key role in regulating the length of the bovine estrous cycle, thus that ability has made it a major player in synchronization of estrus. The physiological basis for synchronization of estrus followed the discovery that progesterone inhibited maturation of Graafian follicles (Nellor and Cole, 1956; Hansel et al., 1961; Lamond, 1964; Patterson et al., 1989). During the follicular phase, levels of P4 are low. During this time, rising concentrations of estradiol act on the hypothalamus and pituitary to stimulate low amplitude, high-frequency pulses of LH, which result in elevated circulating concentrations of LH that drive follicular development to the point of ovulation (Niswender, et al., 2000). After ovulation, as the CL develops, its P4 production increases, inhibiting LH production. This effect of P4 is the result of actions on both the hypothalamus and pituitary mediated through the P4 negative feedback which prevents the surge of GnRH from the hypothalamus, preventing estrus and ovulation until CL regression (Attardi and Happe, 1986; Kasa-vubu et al., 1992).

With P4's known capabilities, it was used in an effort to prolong the luteal phase of the estrous cycle or to establish an artificial luteal phase allowing an entire group of animals to have a synchronized estrus together after removal of the P4 (Hansel and Malven, 1960; Zimbelman, 1963; Hansel et al., 1966; Zimbelman and Smith, 1966; Patterson et al., 1989).

Prostaglandin F2 α , a member of the biologically active family of lipids, is a primary prostaglandin with two double bonds (5-6 and 13-14) and three hydroxyl groups (9,11, and 15), and has become well known in the field of reproduction as a result of the discovery that it is the primary luteolytic agent in cattle (Inskeep, 1973). Luteolytic

agents are important for estrous cycle control. The lifespan of the CL dictates when ovulation will occur through its production of P4 inhibiting the ovulatory surge of LH, and when the estrous cycle will end. Before the adoption of luteolytic agents into synchronization of estrus protocols, progestins alone were used to control ovulation by preventing acute release of LH until the CL regressed in each animal within a group naturally (Inskeep, 1973). Wiltbank et al., (1965) synchronized estrus in 20 dairy heifers using progesterone alone that resulted in a 40% conception rate. The only alternative was accomplished by manual removal of the CL by digital pressure per rectum, inducing cows into an early estrus, however this method was time consuming and somewhat dangerous to the animal so it found limited use (Inskeep, 1973; Bridges et al., 1999).

Research conducted from the late 1950's through the 1970's explored the usefulness of PG, estrogens and oxytocin in causing regression of the CL, however they had little effect on prevention of ovulation (Armstrong and Hansel, 1959; Hansel et al., 1961; Wiltbank et al., 1961; Hendricks et al., 1974; Lauderdale et al., 1974; Louis et al., 1974; Delatang, 1975; Lemon, 1975; Thimonier et al., 1975; Patterson et al., 1989). Based on these observations, Heersche et al. (1979) concluded that luteolytic compounds needed to be used more than once or combined with progestogens to obtain a maximal synchronization response (Patterson et al., 1989).

Wiltbank and Casida, (1956) found that the life span of the CL was extended when hysterectomies were performed in cattle and sheep. Since that time, it has been demonstrated clearly and unequivocally that the uterus is responsible for regression of the CL of the estrous cycle in sheep, cattle, hogs, and horses (Inskeep, 1973). The mode of action the uterus uses in causing luteolysis of the CL is mediated through PG release.

Studies using both dairy and beef cattle have found that intrauterine infusion of PG in the uterine horn ipsilateral to the CL has resulted in lysis of the CL, decreases in blood P4, decrease in estrous cycle length, and estrus and ovulation (Liehr et al., 1972; Louis et al., 1972). Lauderdale et al., (1974) observed fertility in 69 crossbred beef heifers after a single injection of PGF_{2α} that resulted in pregnancy rates of 52.2% compared to 53.3% for controls. Prostaglandin F_{2α} is transported to the ipsilateral ovary through a vascular countercurrent exchange mechanism (Senger, 1999). The PG produced by the uterine endometrium enters the uterine vein in high concentrations, and then enters the ovarian artery by countercurrent exchange which is closely intertwined with the uterine vein to travel on to the CL (Senger, 1999). Exogenous PGF_{2α} is limited by the stage of the cycle the animal is in. A fully functional CL is needed for PGF_{2α} to act on, which is only present in the mid to late luteal phase of the estrous cycle.

MGA-PG

The MGA-PG system consists of feeding cattle melengestrol acetate (MGA); (0.5 mg/hd/d) for 14d followed by injection of PG 19d after cessation of MGA feeding (Lamb et al., 2000). Melengestrol acetate, an orally active progestational steroid (6-methyl-17-alpha-acetoxy-16 methylene-pregn-4, 6-diene-3, 20-Dione), was developed in 1962 (Patterson et al., 1989). It is biologically characterized as an analog of medroxyprogesterone acetate (MAP) but it is 300 to 900 times more potent (Patterson et al., 1989; Odde, 1990).

Development of oral progestogens was originally designed for the inhibition of ovulation in women but later was found to reduce chances of fertilization and implantation (Lamond, 1964; Cooper et al., 1967). Zimbelman and Smith, (1966) found

that 4 mg/d of MGA effectively maintained pregnancy in bilaterally ovarioectomized dairy heifers and 0.2 to 0.5 mg/d effectively prevented ovulation. Melengestrol acetate was first marketed for use in feedlot heifers to improve feed efficiency and rate of gain by allowing ovarian follicular development while inhibiting estrus and ovulation when daily intake was 0.5 mg per cow (Bloss et al., 1966; Newland and Henderson, 1966; Zimbelman, 1966; Zimbelman and Smith, 1966; O'Brien et al., 1968; Young et al., 1969; Purchas et al., 1971). Bloss et al., (1966) conducted three trials of feeding MGA to feedlot heifers and detected weight gains of 6.2%, 9.5% and 18.1% above controls. Effectiveness of MGA administered either orally or intramuscularly was found to be equal with MGA being absorbed and not degraded when given orally (Zimbelman and Smith, 1966).

In 1963, MGA was first used to synchronize estrus of normal cycling cattle by administering it daily for 14 to 18d at a dosage of 0.5 to 1.0mg (Zimbelman and Smith, 1966; Patterson et al., 1989). Synchronized estrus occurred 3 to 7d after cessation of MGA administration with longer time periods to estrus when dosages exceeded 1mg/d (Zimbelman and Smith, 1966; Rousell et al., 1969; Patterson et al., 1989). Treatment of cattle with MGA resulted in high percentages of animals displaying estrus (66 to 93%), however fertility was reduced on the first estrus after MGA withdrawal (Boyd, 1970; Roussel et al., 1969; Britt et al., 1972). Low fertility was a common characteristic of the first estrus after the withdrawal of all progestogens, but average to above average fertility resulted in all subsequent displays of estrus (Trimberger and Hansel, 1955; Britt et al., 1972; Patterson et al., 1989). Despite lower conception that occurred after withdrawal of MGA, pregnancy rates at the end of a regular breeding season were greater than or equal

to controls (Patterson et al, 1989). Treatment with MGA is associated with increased frequency of LH pulses in the absence of a CL resulting in the development of persistent follicles and the ovulation of abnormal oocytes, accounting for the reduction in fertility observed in cattle bred at the synchronized estrus (Kojima et al., 1995).

In an effort to alleviate the problem of low fertility after long term MGA treatment, MGA was combined with estradiol- 17β , but this resulted in lower conception at the synchronized estrus and increased numbers of short estrous cycles (Smith and Zimbelman, 1968; Patterson et al., 1989). Wiltbank and Kasson, (1968) fed a progestational compound to cattle for nine days and gave an injection of estradiol valerate on day two of feeding. Estrus was synchronized in 95% of the 66 treated heifers and 54% of these heifers conceived when bred at the synchronized estrus, while 52% of the 33 control heifers conceived at first service (Wiltbank and Kasson, 1968). The theory behind combined progestagen-PG treatment was that animals beginning treatment with progestogens early in their estrous cycles had normal luteal development, and estrus occurred after administration of PG, but when treatment with a progestogen began late in the estrous cycle, the CL regressed spontaneously during treatment, and females were held out of estrus until the source of the progestogen was removed (Patterson et al., 1989). Beal et al. (1988) administered beef cattle MGA for 7d with PG injection given on the last day of MGA feeding. Synchronized estrus occurred in 72% of cattle treated, however, fertility on the synchronized estrus was reduced (55%) compared to (73%) for untreated controls.

Longer feeding periods of MGA (14, 15, or 16d) with PG injected 16 or 17 d after MGA withdrawal were developed simultaneously with the 7d program (Brown et al.,

1986; Patterson et al., 1989). The longer MGA-PG system utilizes two approaches to control duration of the estrous cycle and to synchronize estrus: 1) feeding the oral progestin (MGA) to prolong estrus after naturally occurring luteolysis, and 2) subsequent administration of PG to regress the CL before natural luteolysis, thereby shorten the estrous cycle (Lamb et al., 2000). Jaeger et al. (1992) administered MGA for 14 d to 304 beef heifers with PG given 17d after MGA withdrawal. Synchronized estrus after PG injection was 77% with a pregnancy rate of 50%. Funston et al. (2002) used the same 14d MGA system in 394 beef heifers and obtained similar results. Synchronized estrus was 77% with a 47% conception rate.

Currently the MGA-PG system has been modified so that MGA is administered for 14d and PG is now given 19d after MGA cessation instead of 17d. With an injection of PG 17d after MGA treatment, heifers need to be grouped into the late luteal phase (Day 10 to 15) of a normal estrous cycle; however, delaying the PG injection to d19 groups heifers even later in the luteal phase at d 12 to 17 of the estrous cycle (Lamb et al., 2000). Heifers treated for estrous synchronization after d 12 of their estrous cycle had the greatest response to treatment (>91%); (Beal, 1998). Heifers with a smaller preovulatory follicle at the beginning of proestrus have a longer interval to estrus than heifers with a larger preovulatory follicle (Lamb et al., 2000). Heifers injected with PG 19d after the cessation of MGA treatment and grouped into d 12 to 17 of the estrous cycle have a larger preovulatory follicle at the time of PG induced luteolysis resulting in shorter intervals to estrus and AI (Lamb et al., 2000). First service AI conception rate was 81.4% for heifers on the MGA-PG on d 19 compared to 75.9% for heifers on MGA-PG on d 17 (Lamb et al., 2000).

CIDR-PG

The controlled internal drug release (CIDR) device is a recent development in the synchronization of estrus being approved by the U.S. Food and Drug Administration in 2002 for use in beef cattle and dairy heifers (Mapletoft et al., 2003). Developed in New Zealand, the CIDR is a T-shaped vaginal insert containing 1.38 g of P4 in silicon molded over a nylon spine (Mapletoft et al., 2003). The CIDR is inserted into the vagina by a specialized applicator that collapses the wings of the CIDR for insertion; expulsion of the CIDR causes the wings to straighten, which confers retention by pressure on the vaginal wall (Macmillan et al., 1991; Mapletoft et al., 2003). Retention of the CIDR by the animal has been found to be 100% (Tjondronegro et al., 1987). Label directions for AI indicate that the device should be in the vagina for 7 d; PG is given 24 h before device removal and estrus detection begins 48 h after device removal (Mapletoft et al., 2003).

The CIDR acts like an artificial CL releasing P4 during the treatment period, which blocks GnRH release from the hypothalamus and subsequent LH and FSH release from the anterior pituitary. Martinez (2002), found P4 concentrations spiked at or near luteal levels (5 to 7 ng/ml) by 24 h in ovariectomized cows, then decreased to concentrations of 2 to 3 ng/ml after 2 to 3 d, where they remained until CIDR removal on d 7 (Mapletoft et al., 2003). The PG injection causes regression of the CL. Regression of the CL is followed by the development of a preovulatory follicle, behavioral estrus, and ovulation (Lauderdale et al., 1974; Roche, 1974; Lucy et al., 2001). Prostaglandin F_{2α} is not able to regress any developing CL on the ovary younger than d 5 of the estrous cycle (Lauderdale, 1972; Lucy et al., 2001). Therefore, administration of the progestin in the form of a CIDR for 7 d before PG injection ensures that the CL will regress in response

to PG because all cattle will have a CL that has developed for at least 7 d (Lucy et al., 2001).

Lucy et al. (2001) performed research using the CIDR-PG system in beef cows and heifers and dairy heifers. In 851 cows used, 59% of the CIDR-PG cows displayed estrus in the first 3d of the breeding period while only 33% of PG treated cows displayed estrus and 15% of control cows displayed estrus. Breeding by AI was performed over a 31d period. Pregnancy rates for the CIDR-PG treated cows were highest (58%) followed by PG treated cows (55%) and controls (50%). Of the 724 heifers used, 65% of the CIDR-PG treated group displayed estrus during the first 3d of the 31 d breeding period compared with 27% and 13% for PG and control heifers. Pregnancy rates for heifers in the entire breeding period were 60%, 43% and 50% for the CIDR-PG, PG, and control group respectively.

Lamb et al. (2001) included the use of a CIDR in the CO-Synch protocol in 273 beef cows. The CO-Synch protocol consists of 100 µg of GnRH (i.m.) followed in 7 d with 25 mg of PG, which is then followed 48 h later by a second injection of GnRH and one fixed-time insemination. Control cattle received the CO-Synch protocol without a CIDR. Pregnancy rates were similar between CO-Synch and CO-Synch+CIDR treatments when cycling cows had elevated concentrations of P4 at d 0, but pregnancy rates were greater in the CO-Synch+CIDR (79%) than in the CO-Synch (43%) treatment when cycling cows had low concentrations of P4 on d 0 at PG injection (Lamb et al., 2001). Similarly, among noncycling cows, pregnancy rates were greater in the CO-Synch+CIDR (59%) treatment than in the CO-Synch (39%) treatment (Lamb et al., 2001).

Perry et al. (2004) compared the ability of CIDRs against MGA to induce estrous cycles in postpartum beef cows. Cows were divided into four treatment groups consisting of a CIDR group (1.9g P4); inserted for 6 d, normal MGA, (0.55mg/kg/d), and high MGA, (4.41mg/kg/d) fed for 7 d or control. The percentage of CIDR treated cows that had ovulated was greater than the percentage of normal MGA treated, high MGA treated, or control cows (Perry et al., 2004). The percentage of cows that exhibited standing estrus before the first postpartum ovulation was also highest for the CIDR group (65%), followed by normal MGA (57%), high MGA (35%) and control (30%) (Perry et al., 2004).

There is need of further adoption of AI by the beef industry. This could be accomplished by developing reliable systems for synchronization of estrus, complemented by efficient estrus detection methods that aid in the use of AI. Knowledge of the optimum time to inseminate beef cattle would enhance the reproductive efficiency of the beef producer. Improvements in detection of estrus through the use of HW, has allowed a better understanding of mount behavior and duration of estrus. Mounting activity is greater in beef cattle compared to dairy cattle; therefore, the HW system could be more efficient in detecting heats in beef cattle. As a result, producers could inseminate animals at the optimum time relative to the onset of estrus to achieve acceptable pregnancy rates.

Experimental Objectives

The objectives of the present study were twofold: determine the effect of timing of insemination relative to the onset of estrus on AI pregnancy in beef heifers; and compare

the distribution of estrus, degree of synchrony, and pregnancy rates of heifers synchronized with MGA-PG or CIDR-PG systems.

Chapter 3 Materials and Methods

Experiment 1

Crossbred Angus sired beef heifers, that ranged in age from 1 to 1.5 yr, were used in this study conducted in November 2003, (Experiment 1, Fall; n = 349) and April 2004 (Experiment 2, Spring; n = 313) at the Southampton Correctional Facility, located in Capron, Va (latitude N36.7 and longitude W77.5). November and April heifers had an average prebreeding wt of 381.5 ± 2.3 kg and 359.6 ± 1.7 kg, recorded 2 to 6 wks before inseminations. Both groups of heifers had an average BCS of 6. In Experiment 1, heifers were blocked by weight (heavy >307 kg, light > 261 kg) recorded 3 mo before initiation of treatment. Heifers were grouped to evenly distribute heavy and light weights for assignment to treatment. Reproductive fitness was evaluated by assessing reproductive tract scores (RTS) via palpation (Anderson et al., 1991) and pelvic area measurements using a Rice pelvemeter on d -9 (Deutscher, 1991). Heifers received an RTS of 1 to 5. The best score, RTS 5 meant there was a functional CL present, with good uterine tone. An RTS 4 had ovarian follicles > 10 mm in diameter, a CL possibly, and good uterine tone. An RTS of 3 had ovarian follicles 8-10 mm in diameter, no CL, and slight uterine tone. An RTS of 2 had follicles 8 mm in diameter, no CL, and no uterine tone. An RTS of 1 had no ovarian structures and no uterine tone. Any heifer that received an RTS of 1 was removed from the study (n = 2).

In Experiment 1, heifers were randomly assigned to one of two synchronization treatments: MGA-PG (n = 176) or CIDR-PG (n = 173), (Figure 1). Heifers were fed a

silage based diet supplemented with corn and soybean meal. The diet was designed to meet NRC (1996) requirements for growing beef heifers for protein and energy, and was calculated to produce gains of 0.75 kg/d. On d 0, both treatment protocols were initiated. Heifers were housed in dry lots and group fed (two pens per treatment). For 14 d, from d 0 to d 13, heifers were fed either 0.5 mg melengestrol acetate (MGA, Pfizer, New York, NY) mixed with 1.4 kg of ground corn carrier (MGA-PG heifers) or 1.4 kg of ground corn carrier without MGA (CIDR-PG heifers) in feed bunks before daily rations were given. Treatment feeding began at 0900 every morning and each heifer was provided 0.5 m of feed bunk space. Any heifer not eating the MGA-PG carrier or CIDR-PG carrier was noted. The MGA-PG group received MGA (0.5 mg/hd/d for 14 d) followed by i.m. injection of PG (25mg Lutalyse®, Pfizer, New York, NY) 18 d after last feeding of MGA on d 31. Controlled internal drug releasing devices (CIDR; 1.38g progesterone; Pfizer, New York, NY) were inserted into the vagina of heifers for 7 d (beginning on d 25 of experiment) and PG was administered 1 d before CIDR removal on d 31. Time of PG injection and CIDR removal were recorded for each heifer.

Detection of estrus and AI for both treatment groups were performed from d 31 to d 35 of the study with first inseminations performed on d 32. Heifers were fitted with HeatWatch® transmitters (HW; CowChips LLC Denver, CO) (n=200) or Kamar® device (Kamar Inc., Steamboat Springs, CO) (n=149). The HW transmitters were placed on the MGA-PG heifers on d 31 and the CIDR-PG heifers on d 32. The radio telemetric device, HeatWatch®, that was attached to each heifer consisted of a miniaturized radio wave transmitter powered by a lithium 3-V battery and linked to a pressure sensor enclosed in a hard plastic case 5.3 × 8.1 cm and 1.8 cm in height (Dransfield et al., 1998). Each

transmitter was placed in a weather proof container with nylon mesh. This was attached just caudal to the sacral region of each heifer with adhesive. Activation of the pressure sensor by weight of a mounting herd mate for a minimum of 2 s produced a radio wave transmission (0.4-km range); (Dransfield et al., 1998). When a mount occurred, the HW system recorded transmitter and heifer ID, mount duration, and date and time. Transmitted data were sent to a signal receiver that stored them in a buffer to be downloaded by a personal computer operating the HW system. In both Experiment 1 and 2, HW transmitters were removed at the time of insemination. Visual observation for standing estrus was also employed three times daily for heifers equipped with HW transmitters and Kamar® devices. Observation was conducted daily at 0630, 1200 and 1630 and performed for 30 to 60 min at a time. Heifers detected in estrus before 0800 from the previous 24 h were randomly bred by estrus artificial insemination (EAI) once daily starting at 0900. Time of first visual recorded standing estrus or HW identified onset of estrus was recorded for each heifer.

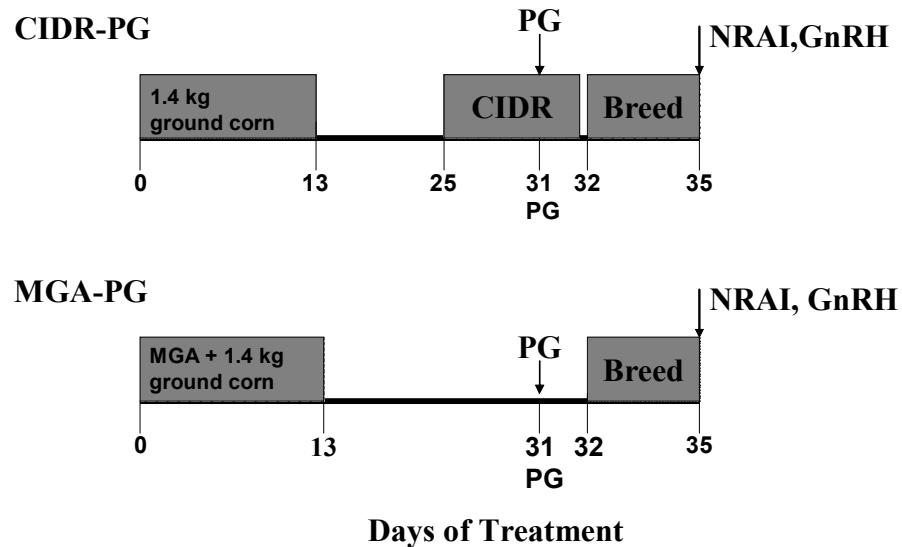


Figure 1. Experiment 1 CIDR-PG and MGA-PG synchronization of estrus system protocols conducted in November.

Any heifer detected in estrus after 0800 was bred the following morning. Breeding date and times were recorded for all heifers. Heifers not detected in estrus by 96 h after PG were bred as non responsive AI (NRAI) and received GnRH (100 μ g Cystorelin[®], Merial, Duluth, GA) at AI on d 35. Clean up bulls were placed with all heifers two weeks after AI on d 49 for 46 d and removed the day before initial pregnancy diagnosis. Pregnancy was determined via ultrasonography at 60 d post AI and via palpation on day 110-120 after AI.

Experiment 2

In Experiment 2, all heifers (n=313) received the CIDR-PG treatment, (Figure 2). Heifers were reproductive tract scored, weighed, and had pelvic areas measured using a Rice pelvemeter, before synchronization on d -12. Heifers had to have a minimum RTS of 2 and a minimum pelvic area of 150 sq. cm to be used in the study. All detection of estrus in Experiment 2 was performed by HW. HeatWatch[®] transmitters were fitted on all heifers on d 7. Detection of estrus began on d 8. All heifers detected in standing estrus with HW in the 24 h preceding 0800 on breeding days were bred by AI beginning at 0900. All heifers were inseminated on d 9 through 11. Time of PG injection, CIDR removal, onset of estrus, and insemination for each heifer was recorded. All heifers not detected in estrus by an average of 96 h after CIDR removal were inseminated as non responsive AI (NRAI) and received GnRH (100 μ g Cystorelin[®]) at NRAI on d 11. On d 21, heifers were placed with clean up bulls for 35 d. Pregnancy status was determined via ultrasonography on d 60 after AI, and via palpation on day 110-120 after AI.

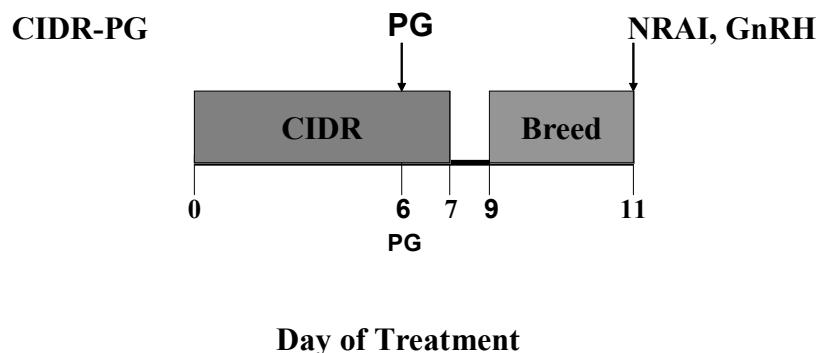


Figure 2. Experiment 2 CIDR-PG synchronization of estrus system protocol conducted in April.

Onset of estrus was determined by HW ($n = 146$) or visual observation ($n = 103$). The HW system defined onset of estrus as when a heifer stood to be mounted for greater than 3 mounts in a 4 h period with each mount lasting greater than 2 s. Visual observation noted time of estrus occurring when a heifer was first observed standing to be mounted without walking away when other heifers attempted to mount. Time of AI in relation to onset of estrus was determined for each heifer by subtracting date and time of onset of estrus from date and time of insemination. For analysis of effect of timing of insemination on pregnancy rate, heifers were assigned to one of the following 4 h blocks of time based on time from onset of estrus to insemination: 0-4, 4-8, 8-12, 12-16, 16-20, 20-24, or >24 h. Heifers were then placed into 8 h blocks of time: 0-8, 8-16, and > 16 h. Again, time of AI in relation to onset of estrus determined in which 8 h block of time heifers were placed. Onset of estrus represented h 0 for both groupings. Experiment 1 heifers were detected in estrus by either HW, by visual observation three times daily, or a red Kamar[®] device. Some heifers were detected in estrus both by HW and by visual observation. In those cases, the HW time was used as the determinate for onset of estrus for those heifers. For heifers that did not have a HW transmitter but were detected by visual observation, the visual time of onset of estrus was used. Heifers that had a red Kamar[®] were not used for timing of AI in relation to onset of estrus because time of onset of estrus was unknown. These heifers were bred upon visual inspection of an activated Kamar. In Experiment 2, no visual detection of estrus was performed because all heifers had HW transmitters.

Statistical methods

The Experiment 1 data was analyzed by the frequency procedure (SAS, 2003) to obtain percentages and proportions, and the logistic procedure of SAS was used to test for significance. The original full model used for analysis of experiment 1 was:

Artificial insemination pregnancy rate = Hour block + AI technician + AI technician × Hour block + Pre-breeding weight + error. Stepwise elimination of non-significant factors was performed to produce the reduced model: Artificial insemination pregnancy rate = Hour block + AI technician + Pre-breeding weight + error. The GLM procedure of SAS (ANOVA) was also used to analyze the effect of synchronization treatment on time from PG injection to onset of estrus, time from HW detected onset of estrus to insemination, and time from visual detected estrus to insemination. The GLM procedure (ANOVA) was used to analyze the effect of synchronization treatment on number of estrual mounts, estrus mount intensity, and duration of estrus.

In experiment 2, to analyze the effect of timing of insemination on pregnancy rate, the Frequency Procedure of SAS was used to obtain percentages and proportions of heifer pregnancy, whereas the Logistic Procedure of SAS was used to test for significance. The original full model used for analysis of April data was: Artificial insemination pregnancy rate = AI technician + Hour block + Pre-breeding weight + AI technician × Pre-breeding weight + AI technician × Hour block + Hour block × Pre-breeding weight + error. Stepwise elimination of non-significant interactions was performed to create the reduced model: Artificial insemination pregnancy rate = AI technician + Hour block + error. Pre-breeding weight was included as a covariate in the final model. Analysis of variance using the GLM procedure was used to analyze the effect of season on time from PG

injection to onset of estrus, time from HW detected estrus to insemination, and time from CIDR removal to onset of estrus. The GLM procedure (ANOVA) was used to analyze the number of mounts, mount intensity, and duration of estrus. Level of significance for both Fall and Spring data sets was set at >95% confidence interval (i.e. $P < 0.05$). Timing of AI (combined Fall and Spring data set into 4 hour blocks) was regressed using Regression procedures of SAS.

Chapter 4 Results

Characteristics of onset of estrus

Characteristics of the time of onset of estrus and time of day of onset of estrus were analyzed for synchronization method and season of breeding. Using data for heifers detected in estrus by both HW and visual observation, time from PG injection to onset of estrus was greater ($P < 0.05$) for CIDR heifers at 73 ± 1.5 h compared to MGA heifers at 60 ± 1.5 h respectively (Figure 3). Time from PG injection to onset of estrus was similar ($P = 0.12$) for CIDR heifers bred in the Fall (68.3 ± 1.4 h) compared to CIDR heifers bred in the Spring (70.7 ± 0.7 h) using data for heifers detected in estrus by HW because HW provided the most accurate method of onset of estrus. Distribution of estrus ranged from 6 h after PG injection to 102 h after PG injection for MGA heifers and 42 h after PG injection to 102 h after PG injection for CIDR heifers. Mean time of day of onset of estrus was later ($P < 0.05$) for Fall heifers (1054) compared to Spring heifers (0836); a difference of 2 h, 18 min (Figure 4). However, season did not alter ($P = 0.64$) hours from CIDR removal to onset of estrus with time from CIDR removal to estrus averaging 46.9 h and 47.6 h for Fall and Spring, respectively.

The amount of time from HW detected estrus to insemination was calculated for both synchronization methods conducted for Experiment 1 in the Fall. Time from HW detected estrus to insemination was similar ($P = 0.51$) for CIDR and MGA heifers (17.2 ± 1.4 h and 16.1 ± 1.1 h, respectively). Time from visual detection of estrus to insemination was similar ($P = 0.68$) between synchronization methods and averaged 10.1 ± 1.6 h and 9.3 ± 1.2 h for CIDR and MGA heifers, respectively. For the 77 heifers that were detected in estrus both by HW and visual observation, the interval from observed estrus to insemination was 8 h greater ($P < 0.05$) for HW (17.1 ± 0.9 h) than visual observation (9.2 ± 0.9 h), respectively.

Reproductive tract score ($P = 0.61$) and weight ($P = 0.90$) had no effect on onset of estrus to insemination time in the Spring heifers. However, heifers with lower RTS scores had numerically shorter lengths of time from onset of estrus to insemination: RTS 2 = 13.6 ± 2.0 h, RTS 3 = 14.6 ± 1.3 h, RTS 4 = 15.3 ± 1.0 h, RTS 5 = 16 ± 0.8 h. Heifer RTS had no effect ($P = 0.11$) on onset of estrus after PG injection. Heifers with an RTS of 2 (44.4 ± 2.9 h) or 5 (46.4 ± 1.1 h) came into estrus sooner after CIDR removal ($P < 0.05$) compared with heifers with an RTS of 4 (48.1 ± 1.3 h) or 3 (51.7 ± 1.8 h).

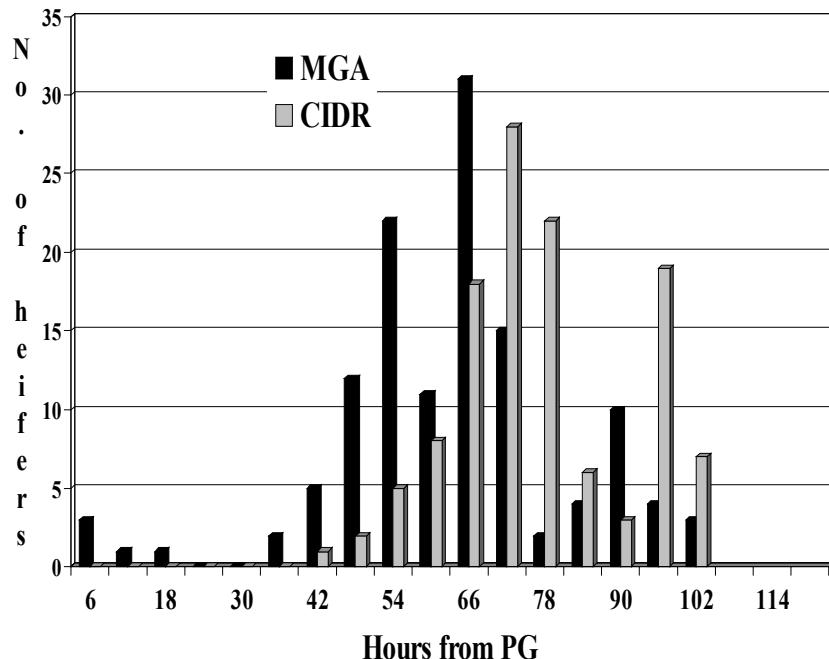


Figure 3. Numbers of heifers in estrus relative to prostaglandin injection for MGA and CIDR heifers. (Both HeatWatch® and visually observed heifers) Hour 0 = injection of prostaglandin (25 mg Lutalyse®) Effect of synchronization of estrus method ($P < 0.05$).

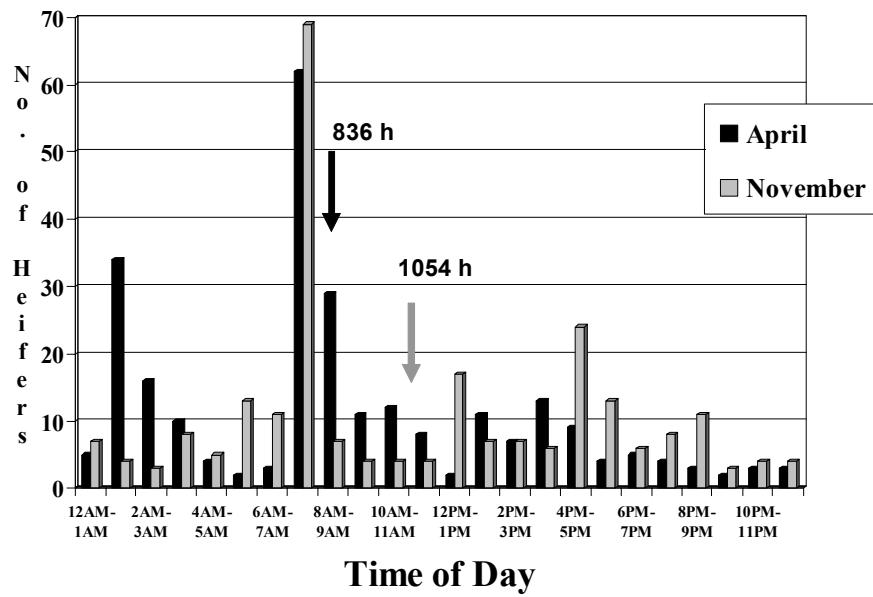


Figure 4. Numbers of heifers in estrus by time of day by season of experiment.
Effect of season ($P < 0.05$). (Both HeatWatch® and visually observed heifers).

Characteristics of estrus

All the mount data recorded by HW was analyzed for both Experiment 1 and Experiment 2 for heifers inseminated \geq 16 h after onset of estrus (Tables 1-4). Greater than or equal to 16 h was chosen because the reported mean duration of estrus in beef heifers is 14 h (Stevenson et al., 1996). Therefore, heifers should have gone out of estrus by 16 h and stopped all mounting activity. In addition, HW transmitters were removed at time of breeding. Duration of estrus for all heifers ranged from 0.7 to 35.3 h and number of mounts ranged from 3 to 270.

In Experiment 1, synchronization of estrus method did not ($P = 0.42$) affect duration of estrus (16.0 ± 0.6 h, Table 1). Mean number of mounts and mounting intensity were increased ($P < 0.05$) for heifers treated with MGA compared to CIDR treated heifers.

Experiment 2 conducted in the Spring used only the CIDR-PG protocol. Therefore the mount data from CIDR-PG heifers used in Experiment 1 and 2 were compared to examine effects of season of breeding (Table 2). Duration of estrus was longer and mean number of mounts was greater ($P < 0.05$) for Fall heifers compared to Spring heifers. However, there was no difference ($P = 0.87$) in mount intensity for Fall heifers compared to Spring heifers.

Table 1. HeatWatch detected mounts, mount intensity, and duration of estrus by synchronization of estrus system.

Synchronization of Estrus System	Mounts		Mount Intensity		Duration of Estrus	
	Number	P Value	(Mounts/Hour)	P Value	(Hours)	P Value
Fall MGA-PG	76.8 ± 6.7	0.005	6.1 ± 1.0	0.03	16.5 ± 0.8	0.42
Fall CIDR-PG	47.6 ± 7.4		3.0 ± 1.1		15.5 ± 0.9	

Table 2. HeatWatch detected mounts, mount intensity, and duration of estrus by season.

Season	Mounts		Mount Intensity		Duration of Estrus	
	Number	P Value	(Mounts/Hour)	P Value	(Hours)	P Value
Fall CIDR-PG	47.9 ± 5.2	0.04	3.1 ± 0.3	0.9	15.5 ± 1.1	0.03
Spring CIDR-PG	34.5 ± 3.1		3.0 ± 0.2		12.7 ± 0.6	

To determine if timing of insemination relative to the onset of estrus altered mounting characteristics, heifers were grouped by timing of insemination into three groups: 16-20, 20-24, and 24-28. Recorded mount activity could have been terminated due to the removal of HW transmitters at breeding. Heifers grouped into 16-20 h after onset of estrus received fewer ($P < 0.05$) mounts than heifers bred between 20 and 24 h post estrus (Table 3). No difference in number of mounts existed ($P = 0.20$) for heifers in the 20-24 h group that received 50.5 mounts compared to heifers in the 24-28 h group that received 40.1 mounts. Mount intensity ($P < 0.05$) was also lower for heifers in the 16-20 h group compared to heifers in the 20-24 h group. No difference in mount intensity existed ($P = 0.20$) for heifers in the 20-24 h group compared to heifers in the 24-28 h group. However, duration of estrus was similar ($P = 0.84$) among all three groups. Number of mounts had no effect on pregnancy rates by synchronization of estrus method or season (Table 4).

Table 3. Mount characteristics and duration of estrus (mean \pm SE) in heifers bred at different times after onset of estrus.

Hours from onset of estrus to AI	Number of Mounts	Mount Intensity (mounts/hour)	Duration of Estrus (hours)
16-20	33.0 \pm 5.2 ^a	2.5 \pm 0.3 ^a	13.6 \pm 1.1
20-24	50.5 \pm 4.8 ^b	3.7 \pm 0.3 ^b	14.3 \pm 1.0
24-28	40.1 \pm 4.4 ^{a,b}	2.9 \pm 0.3 ^{a,b}	14.3 \pm 1.0

^{a,b}Values with different superscripts within column differ ($P < 0.05$).

Table 4. Pregnancy rates in heifers as affected by total number of mounts as detected by HeatWatch by season and synchronization of estrus method.

Pregnancy rate mount data						
Season of Treatment	N	3-25 Mounts	26-60 Mounts	>60 Mounts	P value	
		Percent Pregnant				
Fall MGA	46	50%	69.2%	51.9%	0.54	
Fall CIDR	37	61.5%	53.9%	72.7%	0.63	
Spring CIDR	106	54%	63.2%	72.2%	0.36	
Combined Total	189	55.1%	62.5%	62.5%	0.60	

Synchronization of estrus method and pregnancy rate

In the Fall, a majority of heifers 78.8% (275/349) were bred by AI following estrus. HeatWatch detected 146 heifers, 180 were detected by visual observation, 77 were detected by both methods and 26 were bred based on detection of a red Kamar patch. The 77 heifers detected by both HW and visual detection were placed in the HW group and subtracted from the 180 detected by visual observation because the HW time of onset of estrus was more accurate than onset of estrus times derived from visual detection. Therefore, there were 146 heifers bred on estrus detected by HW, 103 bred on estrus detected visually and 26 bred based on a red Kamar for a total of 275 heifers bred on estrus. Pregnancy rates were grouped according to how heifers were bred: Estrus AI (EAI), Non responsive AI (NRAI), and combined overall AI. Heifers bred by EAI compared to NRAI were more likely to become pregnant ($P < 0.05$; Table 5).

Synchronization of estrus method did not ($P > 0.05$) affect overall AI, EAI or NRAI pregnancy rates (Table 5). Overall pregnancy rates for MGA-PG ($n = 176$) compared to

CIDR-PG heifers ($n = 173$) were similar ($P = 0.69$). Therefore, data were pooled across synchronization of estrus method ($n = 249$) to determine effect of timing of AI relative to estrus on AI pregnancy rate. When data from both HW and visual detection were used, time of AI relative to estrus did not ($P = 0.53$) affect EAI pregnancy rate. Neither did time of AI relative to estrus ($P = 0.68$) affect pregnancy rate of heifers detected in estrus by HW only or visual only ($P = 0.99$). Insemination technician had no effect on AI pregnancy rate ($P = 0.76$) and there was no technician by hour block interaction ($P = 0.72$) for Fall.

In the Spring data set, a majority of heifers 87.2% (273/313) were bred by AI following estrus. A total of 16 heifers were removed from Experiment 2 because their data sets were incomplete.

Table 5. Effect of type of AI breeding on pregnancy rates in beef heifers by season and method of synchronization of estrus.

Fall (Experiment 1) Pregnancy Rates			
Method	EAI	NRAI	Overall AI
CIDR-PG	63.6% (82/129) ^a	29.6% (13/44) ^b	54.9% (95/173) ^a
MGA-PG	58.2% (85/146) ^a	26.7% (8/30) ^b	52.8% (93/176) ^a
Spring (Experiment 2) Pregnancy Rates			
Method	EAI	NRAI	Overall AI
CIDR-PG	61.1% (157/257) ^x	22.5% (9/40) ^y	55.9% (166/297) ^x

EAI = Artificial insemination after detected estrus.

NRAI = Artificial insemination of non responsive heifers.

Overall AI = Artificial insemination of all heifers.

Fall Experiment 1: ^{a,b} Values within rows or columns with different superscripts differ.

Effect of type of AI breeding ($P < 0.05$). Effect of method ($P = 0.36$).

Spring Experiment 2: ^{x,y} Values within row differ ($P < 0.05$)

In Experiment 2, pregnancy rate was not affected ($P = 0.11$) by time of AI relative to onset of estrus. Technician tended to influence EAI pregnancy rate ($P = 0.06$). However there was no technician by timing of AI interaction ($P = 0.98$) on pregnancy rate for Spring.

Timing of AI and pregnancy rate

To analyze effect of season of breeding on pregnancy rate, all heifers that received HW from both Fall and Spring were combined. This analysis revealed no season of breeding effect ($P = 0.17$) on pregnancy rate. In addition, time of AI in relation to onset of estrus did not ($P > 0.3$) affect EAI pregnancy rate.

An analysis of the combined Fall and Spring data was then performed by adding all heifers observed in estrus visually to all the heifers with HW (Figure 5). This resulted in no effect ($P = 0.49$) of time of AI in relation to onset of estrus on pregnancy rate. However, there was a tendency ($P = 0.06$) for a season by timing of AI interaction on pregnancy rate.

The data was then grouped into early (0-8 h), recommended (8-16 h), or late (> 16 h) timing of insemination blocks. The increased number of heifers in the blocks would enhance the ability to detect differences if they existed. Analysis grouped in this manner, again revealed that time of AI in relation to onset of estrus did not ($P > 0.3$) affect EAI pregnancy rate (Figure 6).

Timing of insemination relative to estrus was regressed on pregnancy rate using the combined Fall and Spring heifers that received HW transmitters and visual detection. This curve was different ($P < 0.05$) from the straight line (Figure 7), with an R^2 value of 0.95. Both quadratic and linear functions contributed to the equation ($P < 0.003$).

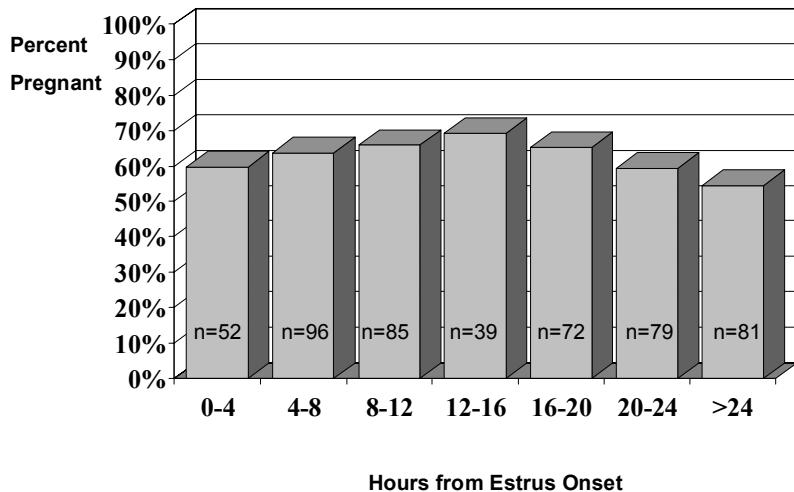


Figure 5. Effect of timing of insemination relative to onset of estrus (4 h blocks) on pregnancy rates in beef heifers (n = 504). Effect of time of AI in relation to onset of estrus ($P = 0.49$) Combined Fall and Spring heifers detected by HeatWatch® and visual observation.

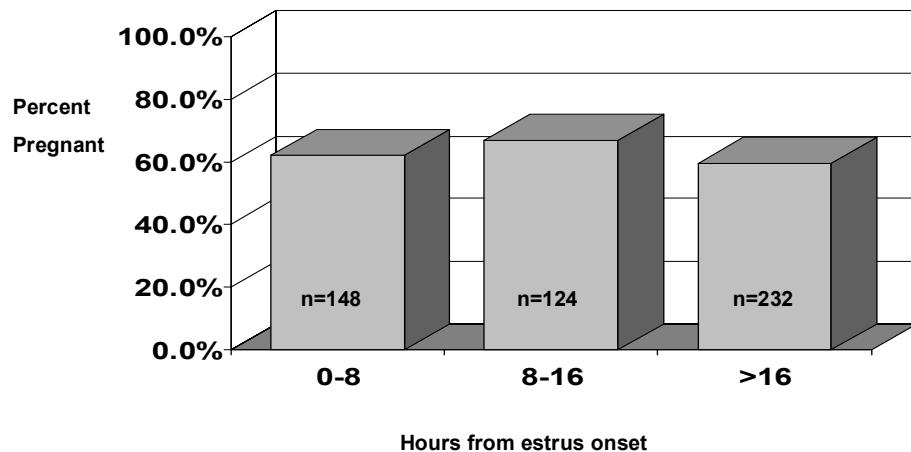


Figure 6. Effect of timing of insemination relative to onset of estrus (8 h blocks) on pregnancy rates in beef heifers (n = 504). Effect of time of AI in relation to onset of estrus ($P > 0.3$). Combined Fall and Spring heifers detected by HeatWatch® and visual observation.

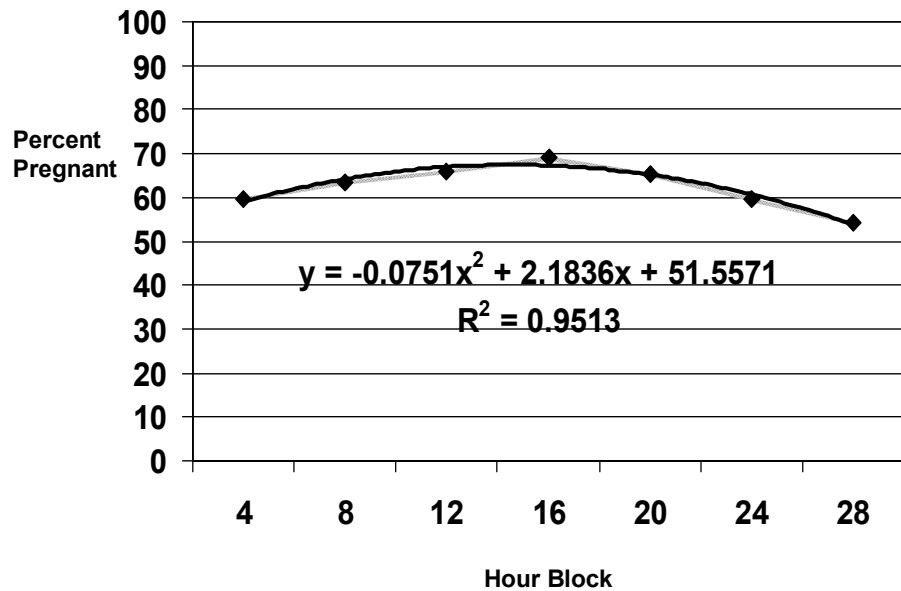


Figure 7. Regression of timing of insemination relative to the onset of estrus (4 h blocks) on pregnancy rate. The quadratic equation is significant ($P < 0.002$). (All heifers)

Chapter 5 Discussion

The majority of studies performed in the past concerning the timing of insemination for optimum pregnancy rates involved dairy cattle. This was only logical because of the dairy industry's early embrace of artificial insemination. This contrasts with the beef industry which has been slow to adopt AI and subsequently a lesser volume of research has been reported on timing of AI in beef cattle. The results of this study indicate that beef heifers are in estrus for a longer duration of time than previously reported, and that there is a large window of time during which to artificially inseminate beef heifers. The window during which it is possible to achieve acceptable pregnancy rates lasts up to 24 h in length starting from onset of estrus.

Earlier onset of estrus in MGA treated heifers, compared to CIDR treated heifers may be a result of MGA treated heifers having a more mature preovulatory follicle than the CIDR treated heifers. Lamb et al., (2000) found that beef heifers given PG 19 d after MGA withdrawal instead of 17 d, had larger, and more mature follicles that may have accounted for the shorter intervals to estrus. Utt et al., (2003) found that beef cows given CIDRs for 9 d instead of 7 d had larger dominant preovulatory follicles. Thus, onset of estrus after CIDR removal would be longer in cows that received CIDRs for 7 d instead of 9 d, because they possessed smaller, less mature dominant preovulatory follicles.

Fall heifers' average onset of estrus time was later in the day compared to Spring heifers, which may be an artifact of visual detection of estrus and HW detection of estrus both being performed in November when daylight hours are decreased compared to April. Only HW detection of estrus was performed in the Spring. Visual detection of estrus only occurred in daylight hours.

HeatWatch® may have measured fewer mounts in CIDR heifers than the MGA heifers because the degree of synchrony of estrus was tighter for CIDR heifers than MGA heifers. Tighter synchrony of estrus would mean more mounting activity occurring in an abbreviated period of time. When this occurs, HW may not be able to detect all mounting activity. With increasing frequency of mounts the likelihood that some signals would be competitively ignored and not processed would increase (Cavalieri et al., 2002). Cavalieri et al., (2002) found that if signals from two or more HW transmitters are sent in a short period of time, the signal that is received first will be processed. The other signals will not be processed unless processing of the first signal has been completed. In some cases, heifers that received MGA came into estrus in higher numbers per time group than the CIDR heifers. However, if HW was not processing signals from some of the CIDR heifers due to the concentrated number of mounts, it would be plausible to assume HW would not also process some of the MGA mounts. This does not seem to be the case. HeatWatch® picked up more MGA mounts than CIDR mounts even though a higher number of MGA heifers came into estrus at the same time compared to the CIDR heifers. Mount intensity was greater for MGA heifers compared to CIDR heifers because MGA heifers received more mounts than CIDR heifers in the same duration of estrus. Number of mounts (35) and mount intensity (2.9) found by Richardson et al., (2002) in dairy heifers, is lower than the mount results obtained in this study.

Duration of estrus was longer and mounting activity was greater in the Fall compared to the Spring. These results are in agreement with White et al., (2002) who found a seasonal effect on the duration of estrus. In that study, crossbred beef cows were in estrus longer in summer (17.6 h) than in winter (15.5 h) or spring (13.9 h). White et al.,

(2002) also found greater mounting activity in winter compared to spring, perhaps associated with warmer temperatures in the spring, agreeing with the results in the current study. In the current study, heifers were in estrus longer (16.0 ± 0.6 h) compared to Stevenson et al., (1996) that found beef heifers in estrus for $14\text{ h} \pm 0.8$ h using HW. Differences in age, herd size, management conditions, frequency of observation and definition of onset of estrus may account for much of the variation in duration of estrus among studies (Nebel et al., 2002). Mount intensity was not different between Fall and Spring heifers even though Fall heifers received more mounts than Spring heifers. This can be accounted for by the longer duration of estrus which occurred in the Fall compared to the Spring.

It is possible that the number of mounts is lower for the heifers inseminated 16 to 20 h after onset of estrus because these heifers may still have been in estrus when HW transmitters were removed at the time of insemination. Had HW transmitters been left in place it is possible some heifers would have recorded more mount activity in the 16-20 h group.

In this study, pregnancy rates achieved were approximately 60% or greater from 0 to 24 h for most of the 4 h long hour blocks. These observations are in disagreement with earlier studies that looked at optimal time of AI in dairy cattle. These experiments found a smaller window of fertility for insemination of cattle. Trimberger and Davis, (1943) concluded that cattle should be bred from the middle to end of estrus up to six h after the end of estrus. Trimberger, (1948) concluded that best rates of conception for dairy heifers ($n = 46$) occurred in an 18 h window that began 1.5 h after onset of estrus and ended 6 h before ovulation. A similar 18 h window was found for dairy cows ($n = 86$).

This window began 4.5 h after onset of estrus and ended 6 h before ovulation. This study led to the am-pm rule for inseminating dairy cattle. The fertile window of time for inseminating cattle was large enough that cattle detected in estrus in the morning could be successfully bred that evening. Likewise, cattle detected in estrus in the evening could be successfully inseminated the next morning. This rule was developed at a time when dairy herds were small in relation to herd sizes today and thus the average dairyman had more time to observe estrus and inseminate more than once a day if necessary. This rule was also convenient for the dairy operation since estrus could be observed in the morning and evening when animals were brought in for milking. The dairy cow was also physiologically different during this time than it is today. Today's dairy cow has continuously been bred for increased milk production since the 1940s. In addition to this factor, increased housing of cattle on concrete compared to pasture in the 1940s, could contribute to a decrease in mounting activity and thus a decrease in detection of estrus by HW in today's dairy cow. In today's beef and dairy industry of fewer and larger herds, it would be cost effective to operators if acceptable pregnancy rates could be achieved with once daily AI.

Based on the results of this and earlier studies, once a day breeding of heifers may be a viable option for the beef cattle producer. Gonzalez et al., (1985) compared pregnancy rates in dairy heifers ($n = 261$) bred AI either once daily in the morning or by the am-pm rule. Pregnancy rates for once daily AI were 62% and by the am-pm rule 62.9%. Eighty-five cattle entered estrus naturally and the rest were synchronized with a single injection of PG. Nebel et al., (1994) found no statistical difference between once a day inseminations in the morning compared with inseminations by the am-pm rule for dairy

cows ($n = 7240$) detected in estrus by visual observation. Non-return rates for once daily and am-pm AI were 64.6 and 65.6% at 60 d, 60.1 and 60.6% at 75 d, and 58.4 and 57.8% for non-return periods (Nebel et al., 1994). They also found that movement of cattle before observation of estrus behavior increased mounting activity, and that length of observation of estrus should be greater than 15 min at a time. This is in agreement with procedures used in this study for the visual observation of estrus performed in Fall. Similar results were found by Graves et al., (1997) who inseminated dairy cows and heifers by once a day insemination in the morning or by the am-pm rule. The am-pm rule produced pregnancy rates of 60.5% compared with 57.6% for once a day insemination in the morning. Like the current study's Fall heifers, visual detection of estrus was performed three times daily in that study. Therefore, Gonzalez et al., (1985), Nebel et al., (1994), and Graves et al., (1997) are in agreement with this current study that once daily AI performed in the morning produces acceptable pregnancy rates.

Robbins et al., (1978) performed once a day AI on beef cows ($n = 2091$). Visual estrus detection was performed twice a day with cows detected in estrus bred the following morning. Optimal calving rates occurred from 12 to 29 h after detected estrus and averaged 63.4%. That study and the current study both concluded that a wide window of time is available to inseminate beef cattle and still achieve acceptable pregnancy rates. However, the current study's use of HW should provide a more accurate account of onset of estrus compared to visual observation. Also, these calving rates were generated using postpartum cows compared with the current study's use of heifers.

One common deficiency may exist for all of the above studies mentioned. All of these studies inseminated cattle based on visual observation of estrus. These observations were made two to three times a day at various intervals. The current study's use of HW and visual observation has detected a lack of accuracy in visual observation of onset of estrus. Of the 77 heifers in this study detected in estrus by both methods, HW detected these heifers in estrus 7.9 h before they were detected visually. Therefore, this data suggests that earlier studies that have relied on visual detection of estrus for timing of insemination may be inseminating cattle up to 8 h later than the actual onset of estrus, due to the inability of visual observation to continuously detect estrus 24 h a day because of cost and labor constraints.

More recent studies performed on the optimum time to inseminate cattle have had the advantage of electronic devices for detection of estrus. Dransfield et al., (1998) using HW in dairy cows ($n = 2661$) concluded that highest conception rates occurred from AI performed from 4 to 12 h after onset of estrus. This 8 h window of time is smaller than the 18 h window of time that Trimberger (1948) suggests is available. With the use of HW, Dransfield et al., (1998) was able to accurately detect onset of estrus and detect small differences ($\leq 5\%$) between conception rates due to a large sample size. Trimberger (1948) may have been hindered by a low sample size in the ability to detect differences in conception rate among the groups bred before ovulation (Trimberger, 1948).

The current study's use of HW for onset of estrus suggests a 24 h window of time to inseminate beef heifers, which is in conflict with Dransfield et al., (1998). This conflict may be explained by some differences between these studies. Dairy cows and beef

heifers differ in their time length in estrus. Dairy cows are in estrus an average of 7.1 ± 5.4 h and ovulate 27.6 ± 5.4 h after onset of estrus (Walker et al., 1996; Dransfield et al., 1998) compared with beef heifers that are in estrus 16.0 ± 0.6 h according to this study, and ovulate 31.1 ± 0.6 h after onset of estrus (Stevenson et al., 1996; White et al., 2002). These differences in length of estrus may be due in part to housing conditions. The majority of cows used by Dransfield et al., (1998) were housed on concrete compared to dry lot for the heifers in the current study. Slick concrete floors may possibly decrease the period of time that dairy cows are willing to mount each other. In contrast, beef heifers may display estrus for a longer period of time due to better footing on pasture or dry lot.

Another factor that should be considered is ovulation time. It is roughly 4 h earlier in the dairy cow compared to the beef heifer. This later time in beef cattle permits greater play in allowing the optimum lifespan of the sperm and egg to coincide for the best chance of fertilization. If dairy cows are bred early, before 4 h after onset of estrus, spermatozoa are unlikely to live long enough to fertilize the ovum because their viable lifespan is 24 to 30 h (Trimberger, 1948). Ovulation occurs at 27.6 ± 5.4 h (Walker et al., 1996), thus making it possible for the dairy cow to ovulate as far out as 33 h after onset of estrus when all spermatozoa have died. Inseminating late in the dairy cow has similar results. Inseminating at 24 h after onset of estrus presents no problems for sperm viability but does possibly for ovum viability (Saacke et al., 2000). Ovulation of the ovum can occur as early as 22.2 h after onset of estrus. Since it requires up to 8 h for sperm to reach the site of fertilization (Wilmut and Hunter, 1984), the ovum would be

aged by the time sperm would reach it with a fertile life of 6 – 10 h (Brackett et al., 1980).

In contrast, the beef heifer may possibly allow early and late inseminations.

Inseminating early, before 4 h after onset of estrus allows spermatozoa to still be viable when ovulation occurs at 31.1 ± 0.6 h because they have a viable life of up to 30 h.

Inseminating late in the beef heifer has similar results. Inseminating at 24 h after onset of estrus allows spermatozoa to reach the site of fertilization by 32 h which would be within the lifespan of the ovum that would have ovulated 31.1 ± 0.6 h after onset of estrus.

Two factors that may have increased the pregnancy rates in the current study as well as created the 24 h window of time to inseminate are the low number and high quality of bulls from which semen was collected, and the number of heifers in the study. Only three highly proven bulls from Select Sires Inc., Plain City, OH were used in the study ($n = 2$ Experiment 1 and $n = 1$ Experiment 2). Perhaps, the good pregnancy rates and 24 h window in which to inseminate were due to the exceptional quality of the bull semen used in the study. If a greater number of bulls of lesser quality had been used, pregnancy rates may have been lower, and the window of opportunity to inseminate heifers could narrow.

The *a priori* analysis of effect of time of AI relative to the onset of estrus was performed using four h time blocks. Pregnancy rates ranged from a low of 54.6 % at greater than 24 h after onset of estrus ($n = 81$) to a high of 69.2 % at 12 to 16 h after onset of estrus ($n = 39$). No difference in pregnancy rate was detected due to low statistical power caused by a limited number of heifers in each 4 h time block. A difference in pregnancy rate of 15 % or greater would be needed in order to detect a difference.

Therefore, in order to increase statistical power, the data was divided into eight h blocks of time as more heifers in each hour block would enhance the ability to detect differences if they existed. Duration of estrus in this study was 16.0 ± 0.6 h. Therefore, if a difference in insemination time relative to onset of estrus was detected, a conclusion could be drawn about the best time to AI beef heifers. This would be either early estrus (0-8 h), late estrus (8-16 h), or after the end of estrus (>16 h). However, this analysis failed to detect a difference.

In order to better understand the timing of AI in relation to onset of estrus, timing of insemination relative to onset of estrus was regressed on pregnancy rate using the combined Experiment 1 and 2 heifers that were detected in estrus by HW or visual observation. The seven average pregnancy rates for each 4 h time block were used from the data. When the data was fit into a curve, it was statistically different ($P < 0.002$) from a flat line. It may be possible to imply that the peak of the curve represents the optimum time to inseminate beef heifers, although, the curve may not be dramatic enough to conclude this definitely. It should also be noted that the data used in the regression were analyzed as continuous data and the pregnancy rate data from each of the seven hour blocks was averaged into seven single pregnancy rates and used in the regression analysis in this manner.

Experimental conditions prevented pre-assigning heifers to hour blocks to get an even distribution of heifers in each hour block like Dransfield et al., (1998). The insemination period in this study lasted less than five d. Dransfield et al., (1998) inseminated cattle over a year's time.

Another difference between the current study and Dransfield et al., (1998) is effect of time of AI in relation to onset of estrus and effect of season. When Fall and Spring heifers with CIDRs were combined, there was no effect of AI in relation to onset of estrus or effect of season on pregnancy rates which is in conflict with Dransfield et al., (1998) which did have a time of AI in relation to onset of estrus effect and a season effect. Optimum conception rates occurred 4 to 12 h after onset of estrus. Dransfield et al., (1998) performed inseminations year round with summer inseminations having greatest negative effect on pregnancy rate due to heat stress. The current study only performed inseminations during November and April.

In the Fall there was no technician effect or technician by time of insemination effect. However, in the Spring there was a tendency for a technician effect but no technician by time of insemination effect. This implies that one of the technicians had above average ability. In spite of a tendency for a technician effect, no technician by time of insemination effect occurred because technicians were distributed across hour blocks.

Chapter 6 Implications

In summary, MGA-PG and CIDR-PG were equally effective in heifers when inseminated after detected estrus. The use of HW and visual observation of estrus in combination revealed that HW may be more precise at detection of estrus compared to visual observation of estrus. This may warrant a change in thinking on the effectiveness of visual observation of estrus. Heifers visually observed in estrus for the first time in the morning may have been in estrus several hours. The duration of estrus was longer in this study compared to what earlier studies have found on duration of estrus in beef heifers; however a high variation in mounting activity was detected. With over 500 heifers, no differences in pregnancy rates were detected from 0 to 24 h after onset of estrus. This may suggest that once a day breeding may be a viable option for the beef producer.

Some further avenues of research may be warranted. The regression analysis suggests that a larger sample size would be needed in order to detect the best time to AI beef heifers in relation to onset of estrus. Examining heifers from different locations to increase the genetic variation in each heifer's background may also prove useful. Another aspect that may deem further research would be to increase the number of bulls from which semen was collected. These bulls should be of lesser quality than the three used in the current study. If such a study were performed using more bulls of a lesser quality, it may determine that the high pregnancy rates maintained from 0 to 24 h after onset of estrus were due to the high quality semen used.

This study provides the beef industry with a good impetus to adopt the use of AI once a day after detection of estrus with HW. Pregnancy rates of 60 % or better in beef heifers is not a bad trade off for the time and labor saved by once a day AI. It also increases the

incentive to not rely solely on visual observation for detection of estrus due to the possible imprecise nature of visual observation uncovered in this study.

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Vita

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