USING CONTROLLED INTERNAL DRUG RELEASE (CIDR®) INSERTS FOR ESTRUS SYNCHRONIZATION IN DAIRY HEIFERS

by

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Under the Direction of William M. Graves

ABSTRACT

In Experiment 1, 164 heifers were assigned to two treatment groups. Each received a CIDR insert for 7 days. Treatment 1 received prostaglandin (PGF2α), 5 mg; i.m., on day 6 and Treatment 2 received PGF2α on day 7. 86.6% of the heifers (142 of 164) exhibited signs of estrus and were inseminated. 47% of the 142 inseminated were pregnant (33 in Treatment 1 and 34 in 2). In Experiment 2, 71 heifers were assigned to three treatment groups and received a CIDR insert for 7 days and PGF2α on day 7. Treatment 1 received a CIDR and PGF2α. Treatment 2 were injected with ECP, 0.5 mg; i.m., on day 8. Treatment 3 animals were injected with GnRH, 2 mg; i.m., on day 9. All heifers were bred by TAI on Day 10. 50.7 % of the 69 heifers were pregnant (13 in Treatment 1, 10 in 2, and 12 in 3).

INDEX WORDS: Controlled Internal Drug Release (CIDR), Dairy heifer, Estrus synchronization, Progestin, Estradiol cypionate (ECP), Gonadotropin releasing hormone (GnRH), Prostaglandin (PGF2α), and Timed artificial insemination (TAI)

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TABLE OF CONTENTS

LIST OF TABLES

CHAPTER I
INTRODUCTION

Inefficient detection of estrus limits the economic success of many Southeastern dairy operations. Average herd size continues to grow and making it even more challenging to accurately detect estrus (Xu et al., 1999). The average estrus detection rate for producers is between 40-65% (Poole and Mabey 1996), but in Georgia the yearly average for 2002 was much lower, about 30% according to state DHIA averages. However, in some countries such as New Zealand, the average estrus detection rate is as high as 90% (Macmillan and Curnow, 1981). Countries such as New Zealand breed all cattle at the same time of the year. This method is referred to as compact calving (Crosse et al., 1994).

An effective estrous detection program is important for producers regardless of their geographical location. Estrous detection is the first step to conception. Estrous synchronization improves estrous detection efficiency by narrowing the window when cattle are expressing signs of estrus. An effective program can help reduce culling rates, improve management efficiency and increase producers' net profits.

Estrus synchronization is an integral part to many systematic breeding procedures including fixed-timed artificial insemination and embryo transfer. Some of the earliest methods of synchronization included progestins.

Heersche et al. (1979) implanted beef heifers with a progestin known as norgestomet for 7 days and injected PGF on day 6 or day 7. This progestin and prostaglandin treatment resulted in 93% of the heifers exhibiting estrus within 5 days and a 62% conception rate. Norgestomet is no longer available for use, however other progestins such as Melengestrol acetate (MGA) and the newer Controlled Internal Drug Release (CIDR's) can be used to successfully synchronize heat (Meyer et al., 2001).

A recent approval in 2002 by the Food and Drug Administration allowed a new technology called a CIDR insert (Eazi-Breed CIDR®, Pfizer-Pharmacia Animal Health, Kalamazoo, MI) on the market. A CIDR is an intravaginal progesterone insert used to synchronize prepubertal beef and dairy heifers as well as anestrous beef and dairy cows. It is a simple device that is easily inserted intravaginally into the animal and removed traditionally seven days later. Hormonal injections can be administered along with the CIDR treatment to increase efficiency (Medina-Britos et al. 2001). For example, the hormone prostaglandin can be used as part of the synchronization protocol when using the CIDR insert. The CIDR suppresses the onset of estrus but an injection of prostaglandin can be given to bring the heifer into estrus. Other hormones can also be used including gonadotropin releasing hormone or estrogen, which induce ovulation along with supplemented progesterone from the CIDR insert.

Using an intravaginal progesterone releasing device for seven days followed by an injection of prostaglandin F2α (PGF2α) near the time of insert withdrawal inserted into a group of heifers would be ready for breeding about forty-eight hours later (Lemaster et al., 1999). It is beneficial to producers breeding heifers to know an exact time of estrus and when to breed. Currently the label (Eazi-Breed CIDR®, Pfizer-

Pharmacia Animal Health, Kalamazoo, MI) recommends injecting PGF2α on day 6 and removing CIDR's on day 7. Injection on day 7 would be more practical for producers. Injecting PGF on the same day as CIDR removal decreases labor as well as stress on the animals because less handling is required. Furthermore, an injection of GnRH on day nine or an injection of ECP on day eight may help regulate estrus and ovulatory activity as well as allow for breeding at a designated time. Current programs developed to synchronize ovulation do not work as well in heifers (Lammoglia et al., 1998). More effective programs must be researched and developed.

CHAPTER II

LITERATURE REVIEW

Synchronization of estrus in cows and heifers is an effective management tool, especially when using artificial insemination. A major limitation to the success of a dairy producer's estrus synchronization program is the presence of prepubertal heifers in the breeding herd. The use of prostaglandin, estrogens, or gonadotropin hormones will not work when attempting to synchronize prepubertal heifers due to the fact these heifers have yet to reach puberty (Senger, 1999). If the heifer has not reached puberty she will not ovulate, show signs of estrus, nor will she conceive. She will not ovulate or exhibit signs of estrus due to lack of adequate quantities of gonadotropin releasing hormone (GnRH) and lack of GnRH which will not signal the LH surge needed for ovulation (Senger, 1999). However, the recent availability of the new technology, Controlled Internal Releasing Device (CIDR), provides an alternative strategy in synchronizing prepubertal heifers and anestrous cows. The CIDR contains 1.38 grams of progesterone, which is released intravaginally in the heifer from the time of insertion until removed. Early onset of estrus will be prevented if a heifer is exposed to progesterone and at the same time no alteration of the stages of follicular development occurs (Thatcher, 2001).

The normal estrous cycle of bovine is approximately 21 days but can range from 17-24 days. The estrous cycle is divided into four phases; Proestrus, Day 18-20, ovulatory follicles form and estrogen (E2) is found at high concentration; Estrus, Day 21 or 0, when cattle display sexual activity or receptivity and E2 peaks; Metestrus, Day 1-5, when LH surges and ovulation occurs, CL begins formation after ovulation; and Diestrus, Day 6-17, when CL is functioning and progesterone (P4) is at it's highest concentration preventing ovulation and expression of estrus. Proestrus and Estrus are considered the follicular stages of the estrous cycle. Progesterone is at a low concentration and E2 is at a high concentration and peaks during estrus. Metestrus and Diestrus are part of the luteal phase, when P4 is at a high concentration and E2 is at a low (Senger, 1999).

Most synchronization methods control the estrous cycle by manipulating the luteal phase, by using PGF2 to regress the corpus luteum (CL) and additional injections of gonadotropin-releasing hormone (GnRH) to activate preovulatory follicles to develop and possibly interfere with intrafollicular growth factors (Ireland et al., 2000). Prepubertal heifers normally do not have lutenizing hormone surges or a CL. Progesterone mimics the CL that is found in the luteal phase and improves initiation of estrus and ovulation in prepubertal heifers (Thatcher, 2001).

Improved synchronization will improve conception rates and some synchronization protocols decrease the need for estrus detection by improving the synchronization of ovulation. The use of CIDR's in heifers can increase the effectiveness of fixed-time AI (TAI). A recent study was conducted in New Zealand (Xu et al., 1999) using 1,123 heifers to be synchronized and bred by TAI or not treated. Each heifer was given a CIDR insert (1.38 g of progesterone) for 10 days, a 10 mg estradiol benzoate (EB) capsule delivered at the time of CIDR insert and an injection of 25 mg of PGF2α on day 6. Pregnancy rate (PR) was higher in the synchronized group (72.4%) versus the untreated heifers (67.8%). Their study reports the synchrony was efficient enough for AI to be carried out at a single fixed time, 50 to 54 hours after CIDR removal, without the need for estrus detection (Xu, et al., 1999).

A similar study by Lucy et al. (2000) reported a higher incidence of estrus in the cows during the first 3 days after insert removal as well as a higher pregnancy rate (58%) in cows treated with CIDR (1.38 g of progesterone) plus PGF2α (25 mg). Prostagladin F2α was administered on day 6 and the CIDR insert was removed on day 7. Improvement in estrus synchrony and pregnancy rates was observed in the beef heifers, but the results were different in the dairy heifers. Dairy heifers treated with CIDR and PGF2α had a greater incidence of estrus (84%) during the first 3 days of breeding compared with heifers only given PGF2α (57%) on day 6 (without CIDR insert). Unlike the beef heifers, the pregnancy rates for the first 3 days of breeding were not improved in the dairy heifers treated with CIDR plus PGF2 (Lucy et al., 2000). Administration of PGF2α on day 6 may have resulted in some dominant follicles being maintained for a longer than normal period and this may have been associated with a reduced conception rate reported in the dairy heifers (Xu, et al., 1999).

Progestins have been used successfully to synchronize prepubertal heifers and anestrous cattle. Miksch et al., 1978 reported that the use of progestins in anestrous cattle induces estrous cycles. Enhanced methods of estrus synchronization such as the use of CIDR's and other hormonal treatments can help maintain a yearly calving interval and maximize reproductive efficiency, especially since herds contain cystic, anestrous, and prepubertal animals. Estrus synchronization can prevent long calving intervals and decrease culling rates, and improve net income to producers (Bellows et al., 1979).

Estrus synchronization improves estrus detection rates (Ryan et al., 1999 and Xu, et al., 1999). Most producers have low estrus detection rates, leading to longer calving intervals and economic losses (Larson et al., 1992).

Many studies including Fike et al. (1997) concentrated on the use of CIDR's and various applications of estradiol treatments to achieve the highest synchronization results possible. Estradiol benzoate (EB) has been used in many studies with CIDR's (Fike et al., 1997, Lammoglia et al., 1998, Kinder et al., 1996, Bo et al., 1994, and Meyer et al., 2001). Estradiol benzoate promotes a new follicular wave after the insert is removed and ovulation is initiated (Meyer et al., 2001).

Estrus synchronization in anestrous cows was the main focus of a study conducted by Fike et al. (1997). Four groups of anestrous cows (n=362) were treated using the CIDR with and without EB. Estradiol benzoate was injected at 1 mg on day 7, 24 to 30 hours after removal of insert. The intravaginal progesterone insert was used to induce estrus and develop a functional CL after insert removal and the 1 mg of EB was injected in theory of enhancing estrus responses after CIDR removal. In this study, the insert combined with EB resulted in a greater number of cows that formed short-lived or typical lifespan CL's as compared to cows not treated. The cows that received the CIDR only had a greater incidence of estrus. These anestrous cows formed a CL with a typical lifespan. After application of the insert and injecting EB, anestrous cows returned to normal luteal function.

Various dosages of EB have been used. Lammoglia et al. (1998) used beef heifers at puberty to determine the optimal amount of EB needed when used with CIDR's to optimize estrus synchronization. Fifty-seven heifers were treated with an intravaginal progesterone insert for 7 days along with an injection of PGF2α on day 6. The heifers according to assigned group received 0, 0.2, 0.38, or 0.75 mg of EB 24 to 30 hours after removal of the insert. Seven heifers were chosen randomly and bled every 4 hours for 76 hours to monitor for lutenizing hormone (LH) and estradiol-17β (E2) levels after injection with EB. Heifers that received 0.38 grams of EB had the highest peak of LH. These heifers exhibited an increase in E2 and LH in the blood serum. Progesterone suppresses LH secretion to threshold to prevent ovulation, but it also increases the amplitude of LH pulses (Kinder et al., 1996). Lammoglia et al. (1998) found that these heifers had a higher percentage (86%) of estrous behavior when compared to the other treatment groups. This study supported other studies that suggested a single injection of 0.38 mg of EB can be used effectively with CIDR's in heifers by inducing estrous behavior and ovulation without decreasing pregnancy rates (Fike, et al., 1997; Johnson et al., 1997; Lemaster et al., 1999).

Some estrus synchronization programs have been associated with low conception rates or poor fertility. Beale et al. (1988) reported that progestin treatments in cattle beginning later than day 13 of the estrous cycle resulted in lower fertility. However, Macmillan et al. (1991) reported this to be associated with ovulation of persistent follicles, which can be overcome by the use of other treatments along with a progestin. Other researchers such as Fike et al. (1997) have concentrated on the use of CIDR's and various applications of estradiol treatments to achieve the highest synchronization results possible. Other treatments that can be used in conjunction with a CIDR include PGF2α, gonadotropin releasing hormone (GnRH), estradiol cypionate (ECP), or estradiol benzoate (EB) to induce new follicular waves (Bo et al., 1994). Stevenson et al. (2002) reported that the use of a CIDR for 7 days along with ECP (1 mg, i.m.) substituted for the second GnRH injection given 24 hours after PGF injection resulted in more dairy cows in estrus with little effect on fertility (greater conception rates). Fewer ovulations, (59% of 59 cows) were recorded when compared with CIDR plus GnRH, (100 µg, i.m, 83% of 46 cows).

The ultimate goal of estrus synchronization is to synchronize estrus of groups of animals in a defined period of time for insemination. This should induce ovulation and improve outcome of the insemination. The effectiveness of a synchronization treatment can be determined by its ability to induce ovulation at a certain desired time so the heifer or cow can be artificially inseminated (TAI) at a predictable time (Odde, 1990).

Melengestrol acetate (MGA) has commonly been used in heifers as a synchronization method and MGA works like CIDR's as a progestin to suppress estrus. However, many times this method is not effective due to the small amount of MGA (0.5 mg) that must be fed individually to each heifer daily for 14 days (Odde, 1990). Most prepubertal heifers go through a sub-fertile estrus after withdrawal (after one stops feeding the MGA for 14 days) and must then be resynchronized and brought back into estrus. This can lengthen the breeding season, requires special feeding facilities and more labor (Lemaster et al., 1999).

The use of the CIDR in heifers should shorten the breeding season and make the procedure more effective and less time consuming (Lemaster et al., 1999). Occasionally, a CIDR maybe lost. Ryan et al. (1994) reported a loss rate of 3.6% for 1,559 CIDR's inserts for 8 days. When compared with MGA treatment the chances of the heifer not receiving the progesterone from the CIDR is unlikely. More importantly, synchronization by the use of CIDR's is less time consuming and more precise (Meyer et al., 2001).

In some countries such as Ireland and New Zealand, dairy cows must calve early in the spring before they are turned out on grass pastures. This is referred to as compact calving (Crosse et al., 1994). Compact calving operations must have a high rate of estrus detection and pregnancy rates on first time service. Macmillan (1983) reported that PGF2α treatment in early diestrus resulted in the greatest rate of synchrony. Prostaglandin specifically causes regression of the corpus luteum (CL). Prepubertal heifers have not cycled and will not respond to administration of PGF2α. Producers could improve estrus synchrony in heifers by using a CIDR along with PGF2α injection. Day (1998) has shown that injecting PGF2α on day 7 at the time of CIDR removal instead of day 6, a day before insert removal, induces ovulation of a dominant follicle after PGF2α injection. Prior to the injection the progesterone increases the amplitude of LH pulses, contributing to the growth of the dominant follicle (Kinder et al., 1996). Researchers have also looked at the incidence of LH surges when cattle are treated with CIDR's and ECP and GnRH. Stevenson et al. (2002) reported that the incidences of LH surges was decreased when treating dairy cows with CIDR insert for 7 days along with an injection of 1 mg of ECP (82%) compared with CIDR plus GnRH (100 µg, i.m 24 hours after PGF2α injection on day 7) were greater (90%). Ovulation was also similar for both groups, a lower incidence of ovulation in CIDR plus ECP treated cows compared to CIDR plus GnRH cows.

The synchronization method described by Day et al. (1999), using a CIDR insert for 7 days and an injection of PGF on day 7 before insert removal, is ideal for a producer with a compact calving operation or a producer performing TAI because the suggested protocol enhances the ability to control time of ovulation and insemination. Similar studies giving PGF2α on day 7 versus day 6 along with other injections at various times have been observed. Lemaster et al. (1999) used a similar protocol, but with a group of crossbred Brahman heifers (n=60). These heifers were assigned to three groups according to body weight and body condition score. All three groups were treated with a CIDR insert for 7 days and received an injection of PGF2α on day 7. Group one only received the CIDR and an injection of PGF2α. However, group two and three both received an injection of EB. Group two was injected with EB 24 hours after insert removal and group three received EB 48 hours after insert removal. At the end of the study all groups had similar sized ovulatory follicles. This study suggested that the heifers that received a CIDR insert for 7 days and a PG injection on day 7 after removal, along with an injection of EB 24 hours after removal, were reported to have “the tightest synchrony of estrus.” Statistically the interval from CIDR insert to removal on day 7 to ovulation decreased in the heifers treated with EB. The heifers that received EB 24 hours instead of 48 hours after insert removal showed the highest percent of estrus (83%).

Lemaster et al. (1999) has suggested that in this treatment not only estrus is synchronized effectively but also more importantly ovulation is more effectively synchronized. Bridges et al. (2002) reported on giving EB at time of CIDR insert and after removal. They concluded that TAI pregnancy rates (PR) were increased in Angus and crossbred *Bos indicus* cattle receiving EB at time of CIDR insertion.

Controlled Internal Drug Release (CIDR's) can also be used successfully with other treatments such as Estradiol Cypionate (ECP) and Gonadotropin Releasing Hormone (GnRH) in TAI situations but little research has been done in this area. Medina-Britos et al. (2001) used a variety of synchronization applications for resynchronizing cattle that did not conceive on the first service in a TAI protocol. The authors reported that the use of CIDR plus ECP increased the return rate to estrus without affecting conception. Also, this treatment increased the 23-day pregnancy rate. Other studies have confirmed that resynchronization with CIDR's after TAI increased pregnancy rate (El-Zarkouny et al., 2001, Meyer et al., 2001 and Xu et al., 1999).

A common ovulation synchronization protocol such as Ovsynch® employs the use of GnRH on day 0 followed by an injection of PGF on day 7 and a final injection of GnRH on day 9. Baitis et al. (2003) used CIDR's in an Ovsynch protocol with crossbreed beef cows. A total of 379 cows received one of the two treatments. Animals in Treatment 1 received the following treatment: 50 µg of GnRH on day 0, PGF on day 7 (25 mg) and 100 µg of GnRH on day 9. Cows in the second treatment group received a similar Ovsynch® protocol, but with a CIDR insert and 50 µg of GnRH on day 0, PGF on day 7 when the CIDR insert was removed and 100 µg of GnRH on day 9. Cows were fitted with a Kamar device to check for premature estrus (PE) and regular estrus activity.

Cows that were considered to show signs of PE were bred 12-16 hours after onset of heat. All other cows were bred 12-16 hours after PGF injection. Pregnancy rates were higher for the cows that received a CIDR insert along with the injections of GnRH (Ovsynch protocol with CIDR) when compared to cows that received the Ovsynch protocol.

Kojima et al. (2003) compared two TAI protocols. One protocol employed the 7-11 Synch treatment, cows were fed MGA for 7 days, on day 7 received an injection of PGF (25 mg, i.m) and GnRH on day 11 (100µg, i.m., Cystorelin®) and on day 18 were injected with PGF (25 mg, i.m.). The second treatment group used the CO-Synch protocol plus the CIDR (GnRH day 0 and 9, CIDR insert for 7 days, and PGF on day 7). Cows were inseminated in the 7-11 Synch 60 hours after injection of PGF on day 18 and were bred 48 hours after injection of PGF (day 9) in CO-Synch protocol. Pregnancy rate was not different for the two synchronization methods. The CO-Synch plus CIDR insert provides yet another alternative for TAI that does not affect fertility while eliminating the need to detect estrus.

Meyer et al. (2001) also reported that CIDR's have no adverse effects on conception including recipient cows or heifers in a TAI protocol. They reported that the use of CIDR's in a TAI program for Brangus cows along with an injection of EB 24 hours after removal improved first service pregnancy rate and with a higher synchrony rate in recipient cows receiving one embryo. Conception rates were also improved in pubertal dairy heifers treated with CIDR insert for 7 days and an injection of PG on day 6. Estrus activity was decreased after once TAI (Richardson et al., 2001). This study supports that CIDR's can be used to manipulate the estrous cycle of cows in different stages of estrous, as well as prepubertal heifers.

However in some breeds and treatments the use of CIDR's has not proven to be as effective (Tatman et al., 2001). Normal estrus behavior was reduced in a group of dry, multiparous Brahman cows treated with CIDR inserts for 7 days, along with an injection of PGF on day 7 when compared with cows who just received one injection of PGF on day 14. Interestingly, a major difference the study of Tatman et al. (2001) was that heat detection included using four sterile bulls instead of Heatwatch or tail head chalk used to monitor heat in other studies. However follicle size remained consistent in terms of size for both groups in the study.

Richardson et al. (2001) also reported low estrus detection rates in a group of dairy heifers treated with CIDR inserts for 7 days and injected with 100µg of GnRH on day 1 and 25 mg of PG on day 7. Pregnancy rates were lower for this treatment group when compared with a group of heifers treated with CIDR insert for 7 days and injected with PGF upon CIDR removal on day 7. In a similar scenario, Barnett et al. (1999), reported a decrease in pregnancy rates in a study using a group of Brangus cows. The cows were treated with CIDR plus PGF for 7 days and the pregnancy rate was reduced when compared with other groups (Barnett et al., 1999). An adverse effect occurred when Richardson et al. (2001) used this same treatment CIDR insert for 7 days and followed by

PG on day 7 on dairy heifers instead of cows. The heifers showed an increase in pregnancy rates unlike the Brangus cows reported by Barnett et al. (1999).

In conclusion, these studies have shown that CIDR inserts can be used to initiate estrus in prepubertal heifers, anestrous cows, and induce ovulation in a short amount of time and more efficiently when compared with other progestin treatments traditionally used such as MGA. The objective of this study is to evaluate the effectiveness of various methods of estrus synchronization in dairy heifers using an intravaginal progesterone insert for 7 days and prostaglandin F2α injection on day 6 or 7. Both estrus detection and fixed timed AI will be evaluated. GnRH and ECP will be compared for timed AI procedures.

CHAPTER III

MATERIALS AND EXPERIMENTAL PROCEDURES

Experiment 1

Hypothesis. There will be no significant difference in estrus synchronization and conception rates when an injection of 25 milligrams of prostaglandin F2α administered on day 7 before removal of CIDR insert is compared with an injection of 25 milligrams of prostaglandin F2α administered on day 6 when used with a CIDR insert for 7 days.

Objective: To compare the effectiveness of day 6 versus day 7 prostaglandin (PGF2α) injections used in-conjunction with CIDR inserts placed intravaginally for seven days.

Materials & Procedure. One hundred sixty-four dairy heifers from two farms (Briarpatch Farms, Eatonton, GA and Laranda Farms, Lyons, GA) were treated for seven days with an intravaginal progestin insert (Eazi-Breed CIDR®, Pfizer-Pharmacia, Kalamazoo, MI) containing 1.38 grams of progesterone. The heifers were randomly assigned to one of two treatment groups. A 25-milligram intramuscular (i.m.) injection of prostaglandin (Lutalyse, Pfizer-Pharmacia, Kalamazoo, MI) was administered on day 6 (Treatment 1) or day 7 (Treatment 2) to each heifer before the CIDR insert was removed on day 7.

Two different lubricants were also applied randomly to both treatment groups.

One lubricant contained an antiseptic (Nolvalube®, FortDodge Animal Health, Fort Dodge, Iowa) and the other did not (SAFE-LUBE, H&W Products, Inc., Salem, OH). The CIDR insert applicator was disinfected after each CIDR was inserted in all heifers treated with SAFE-LUBE by washing it with a disinfectant solution (Nolvasan® Solution, FortDodge Animal Health, Fort Dodge, Iowa). An effort was made to shake and dry applicators between animals after disinfecting. The applicator was not washed in the disinfectant solution when heifers were treated with Nolvalube® but was wiped clean with paper towels after each use.

All CIDR tails (the plastic string on the end of the CIDR insert that remains on the external side of the animal so the device can be removed) were altered in length. Two different tail lengths were used. Tail length was randomly assigned. The tail was cut at the tip of the vulva or was cut 2 ½" below the tip of the vulva. All heifers' weight was recorded on day 0 before CIDR's were inserted. Heifers' weight was estimated at Briarpatch Farms, Eatonton, GA using a weight tape which measures the circumference of the hearth girth and is correlated to estimated pounds of body weight. Heifers at Laranda Farms, Lyons, GA were weighed using electronic scales. At time of CIDR removal, the number of CIDR's retained was recorded. After the CIDR was removed heifers were individually examined for vaginal discharge and all discharges were recorded.

Estrus detection devices were applied to all heifers. At time of CIDR removal from heifers at Briarpatch Farms either a KaMar® (KaMar® Heat mount Detectors, KaMar®, Inc., Steamboat Springs, CO) or Estrus Alert (Estrus Alert, Universal Cooperatives, Inc., Eagan, MN) heat detection device was applied. Heat was observed and recorded when KaMar® was bright red or Estrus Alert was scratched or rubbed mostly red. All heifers at Laranda Farms were fitted with an electronic HeatWatch estrus detection system mount detector (DDx, Denver, CO) on day 6 before CIDR removal on day 7. The HeatWatch data was transmitted to HeatWatch software on the farm's computer and was recorded when heifers were mounted one or more times for more than 3 seconds during a 12-hour period. Number of mounts, hours from prostaglandin injection to onset of standing heat and hours from standing heat to time of insemination was recorded. Heifers were bred approximately six to twelve hours after standing heat was observed at both locations.

There were two inseminators at Briarpatch Farms and three at Laranda Farms. Pregnancy was determined at Briarpatch Farms on approximately day 35 by ultrasonography and on approximately day 45 at Laranda Farms by rectal palpation. Pregnancy status was recorded for all heifers. All data was analyzed by Chi-Square in a 2x2 or 2x3 contingency tables (Steel and Torrie, 1960).

Experiment 2

Hypothesis. The administration of 0.5 milligrams of estradiol cypionate (ECP) when used with a CIDR insert for 7 days and an injection of 25 milligrams of prostaglandin F2α (PGF2α) on day 7 or an injection of 100 micrograms of gonadotropin releasing hormone (GnRH) on day 9 will improve conception rates in dairy heifers timed artificially inseminated approximately 72 hours after insert removal when compared with heifers injected with 25 milligrams of PGF2α on day 7 along with a CIDR insert for 7 days also timed artificially inseminated approximately 72 hours after insert removal.

Objective: To evaluate the use of estradiol cypionate (ECP) or gonadotropin releasing Hormone (GnRH) in a timed artificial insemination (TAI) protocol using CIDR insert for 7 days followed by PGF2α.

Materials & Procedure. A total of seventy-two dairy heifers were treated and randomly assigned to three treatment groups. Heifers were located at Embry Farms in Eatonton, GA. Heifers in all three-treatment groups received a CIDR insert on day 0, which contained 1.38 grams of progesterone and were injected with 25 mg, i.m. of prostaglandin (Lutalyse, Pfizer-Pharmacia Animal Health, Kalamazoo, MI) on day 7. Inserts were removed on day 7. All heifers were treated with the same lubricant (SAFE-LUBE). The insert applicator was washed with disinfectant solution (Nolvasan® Solution) after each insert. Heifers' weights were estimated using a weight tape and recorded. In addition, insert tail length was modified by cutting the tail at the tip of the vulva or adjusted to 2 1/2" below the vulva and randomly applied within each treatment group.

Treatment 1 served as the control and received a CIDR insert for 7 days and an injection of 25 milligrams i.m. of prostaglandin on day 7. Heifers in treatment 2 were treated with the control protocol and were administered an injection of 0.5 mg i.m. of estradiol cypionate (ECP®, Pfizer-Pharmacia Animal Health, Kalamazoo, MI) on day 8 to induce estrus and aid in ovulation (Day, 1998). Treatment 3 heifers received the control protocol and were injected with 100 micrograms i.m. of GnRH (Factrel®, Fort Dodge Animal Health, Fort Dodge, Iowa) on day 9 to assist in improving ovulation (Meyer et al., 2001). At time of insert removal the number of CIDR's retained was recorded and each individual was examined for vaginal discharge, which was recorded. All heifers were timed artificially inseminated by the inseminator on day 10, approximately 72 hours after prostaglandin injection and insert removal. Pregnancy status was diagnosed by rectal palpation on day 45 and results were recorded. All data was analyzed by Chi-Square in a 2x2 or 2x3 contingency tables (Steel and Torrie, 1960).

CHAPTER IV

RESULTS & DISCUSSION

CIDR Retention Rates

In Experiment 1 and 2, 100% of the 233 CIDR's inserted were retained (Table 4.1). Ryan et al. (1995) reported over 5% of 1,559 inserts were lost. Lucy et al. (2001) reported losing from 1% to 5% of the inserts per treatment group. Neither of the studies compared or reported methods used in altering the tail length to maintain retention rates. However, in this study, unaltered CIDR tails (unaltered meaning no tails were left intact without alterations) were not evaluated and should be compared to altered CIDR tails in future studies.

Table 4.1 CIDR Tail Length Treatment Results. CIDR tails were cut to two different lengths, the tip of the vulva (Tip) or 2 ½ inches from the tip of the vulva (2 ½"), number of CIDR's cut, number of CIDR's retained by heifers, and percent of CIDR's retained.

CIDR Treatment	**No. Cut**	**No. Retained**	**% Retained**
Tip	116	116	100
2 ½"	117	117	100
Total	233	233	100

Characteristics of Heifers in Studies

Heifers in Experiment I and II were similar in weight. The lightest group averaged 734 pounds and the heaviest averaged 834. Heifers in Experiment 2 (mean weight=824.63, Standard Error= 0.8622) weighed more than heifers in Experiment 1 (mean weight= 762.95, Standard Error=4.7558).

All heifers were determined to be of adequate weight and age for breeding (heifers 12 months of age and older). Most heifers used at all three farms in both experiments were Holsteins. All the heifers located at Briarpatch Farms were of Holstein breed. However at Embry Farms (location of Experiment 2) and Laranda Farms (second location of Experiment 1) there were several Holstein and Jersey crosses. Also, at Laranda Farms there were two Brown Swiss heifers and one Milking Shorthorn heifer that was part of the study (Experiment 1). In addition a total of 7 free martins were found in this study. Five free martins were discovered at Laranda Farms and two at Embry Farms. These heifers were excluded from the study and could not be used.

Experiment 1

Lubricant Evaluation. A total of 164 heifers were treated with one of two types of lubricants, one without an antiseptic (SAFE-LUBE) and the other containing an antiseptic (NolvaLube). Twenty-nine out of 81 (35.80%) heifers treated with SAFE-LUBE exhibited a discharge at insert removal and 18 out 83 (21.69%) heifers treated with NolvaLube displayed a discharge. A total of 47 of 164 heifers showed signs of discharge when the CIDR insert was removed. A higher number of heifers treated with SAFE-LUBE (n=29) showed signs of vaginal discharge at CIDR removal when compared to heifers treated with antiseptic lube (NolvaLube) (n=18). The results, (Table 4.2) indicate there was no significant difference ($P>0.05$) the percent of heifers having a discharge upon CIDR removal between the two groups.

Table 4.2 Antiseptic Lubricant vs. Non-antiseptic Lubricant Treatment Results. Lubricant treatment SAFE-LUBE (no antiseptic) or NolvaLube (lube with antiseptic), number of heifers treated, number of heifers with discharge at insert removal, and % of heifers with vaginal discharge.

Treatment	**No. Treated**	**No. of Discharges***	**% with Discharge**
SAFE-LUBE	81	29	35.80
NolvaLube	83	18	21.69
TOTAL	164	47	28.66

NS= Not Significant
** P=3.335, NS*

A total of 142 of the 164 heifers treated were inseminated once after treatment and sixty-nine were pregnant. Of the 142 who were inseminated, 47 of them had a discharge at CIDR removal. A total of 35.8% percent discharges were recorded in heifers treated with Safe-Lube compared to 21.69% discharges in NolvaLube treatment group. The number in estrus and inseminated was more in the Nolvalube group (76 of 83) versus the SAFE-LUBE group (66 of 81). There were more pregnant heifers (n= 40) in the NolvaLube group when compared with the number of pregnant heifers in the Safe-Lube group (n=29) as shown in Table 4.3. None of the differences were significant ($P>0.05$).

Table 4.3 Lubricant Treatment Discharges & Pregnant Heifer Results. Lubricant treatment SAFE-LUBE (no antiseptic), NolvaLube (antiseptic), number of heifers treated, number of heifers in estrus and inseminated, number with Discharge at time of CIDR removal, and number of heifers pregnant.

Treatment	**No. Treated**	**No. Discharges***	**No. In Estrus & Inseminated**	**No. Pregnant****
SAFE-LUBE	81	29	66	29
NolvaLube	83	18	76	40
TOTAL	164	47	142	69

NS= Not Significant
**P=3.335, NS*
***P=0.749,NS*

CIDR and Prostaglandin Results. For both treatments combined a total of 142 out of 164 (86.59%) heifers were in estrus after the CIDR and PGF treatment. A total of 67 out of 164 (47.18% conception rate) conceived as shown in Table 4.4. A total of 71 of 82

(86.59%) heifers in Treatment 1 (injected with prostaglandin on day 6) exhibited estrus and 71 of 82 (86.59%) heifers in Treatment 2 (injected with prostaglandin on day 7) as shown in Table 4.4. For Treatment 1, 33 of the 71 heifers (46.47%) who were in estrus conceived and 34 of 71 (47.89%) conceived for Treatment 2. For both treatments (Trt) 1 and 2, the same number of heifers was in estrus (Trt 1= 71 vs. Trt 2= 71), with the same synchrony rate (86.59% vs. 86.59%) and the number of heifers who conceived (33 vs. 34) were similar. There was no significant difference for number of heifers in estrus and conception rate between Treatment 1 compared with Treatment 2 ($P<0.05$) as shown in Table 4.4.

Table 4.4 Day 6 versus Day 7 PGF Estrus Results. PGF day 6 (Treatment 1) compared to day 7 (Treatment 2), CIDR's inserted per treatment group, number of heifers in estrus, synchrony rate, number pregnant, and conception rate (CR).

PGF Treatment	**No. CIDR's Inserted**	**No. In Estrus***	**Synchrony %**	**No. Pregnant**	**CR%****
Treatment 1	82	71	86.59	33	46.47
Treatment 2	82	71	86.59	34	47.89
TOTAL	164	142	86.59	67	47.18

*$P=0.055$
**$P=0.000$

Six heifers from Laranda Farms were omitted from the estrous data because they came into estrus over a week after the CIDR removal. At the beginning of this experiment at Laranda Farms, one heifer was omitted from Treatment 1 and four were omitted from Treatment 2 due to being diagnosed as free martins.

On day 9, approximately 48 hours after the CIDR insert was removed (day 7), a majority of the heifers in both treatments were in estrus (Trt 1=72.22% vs. Trt 2=63.38 %) and inseminated (52.78% vs. 57.75%). Lemaster et al. (1999) reported finding similar to those in the present study when treating Brahman heifers with a CIDR insert for 7 days and injected PGF on day 7. Lemaster et al. (1999) reported a majority (85%) of the heifers exhibited signs of visual estrus 48 to 71 hours (day 9 and 10) after insert removal. A small group of heifers (n=4) in Treatment 1 were in heat on day 8 (Table 4.4), but none in Treatment 2. Lucy et al. (2001) reported that dairy heifers treated with the same protocol (CIDR insert for 7 days and injected with prostaglandin on day 6 before insert removal), began showing signs of estrus 24 hours after insert removal. In contrast, Lemaster et al. (1999) reported no visual signs of estrus 24 hours after CIDR insert removal when Brahman heifers were injected with prostaglandin on the same day as insert removal. Other work (Roche, 1976, Hansel and Beal, 1979) validated that a 7 to 9 day progesterone treatment with prostaglandin administered at or before termination of progesterone increased the synchrony of estrus.

In the present study, Treatment 2 had 5.63% more heifers in estrus for one day longer, day 11, when compared with Treatment 1 where only one heifer (1.39%) was in heat past day 10. This suggests that heifers receiving Treatment 1 had a slightly tighter synchrony of estrus when comparing the percentage of heifers in estrus on the first three days (Trt 1= 98.61% vs. Trt 2 = 94.36%). The lowest percentages of heifers (5.63%) were in heat on day 8 and 11 for Treatment 2 (Table 4.6). There were no heifers in estrus on day 11 for Treatment 1 (Table 4.5) but four heifers were in heat in Treatment 2 (Table 4.6). Lucy et al. (2001) reported that approximately 10% of the heifers treated with CIDR inserts for 7 days and injected with PGF2α on day 6 were in estrus on day 11-14. In the present study there was one heifer in Treatment 1 in estrus on day 14 and no heifers in estrus past day 11 for Treatment 2 (Table 4.6).

Table 4.5 Day 6 PGF Estrus and Insemination Results. Treatment 1 (injection of PGF2α on day 6), number of heifers in estrus each day after insert removal (Total number of heifers in estrus 72), the percent in estrus per day, the number artificially inseminated per day, and the percent inseminated each day of the total number treated.

Treatment 1	**No. In Estrus**	**% In Estrus**	**No. Inseminated**	**% Inseminated**
Day 8	4	5.56	4	5.56
Day 9	52	72.22	38	52.78
Day 10	15	20.83	23	31.94
Day 11	0	0	6	6.94
Day 12	0	0	0	0
TOTAL	71	98.61%	71	97.22%

Table 4.6 Day 7 PGF Estrus and Insemination Results. Treatment 2 (injection of PG F2α on day 7), number of heifers in estrus each day after insert removal (Total number of heifers in estrus 71), the percent in estrus per day, the number artificially inseminated per day, and the percent inseminated each day of the total number treated.

Treatment 2	**No. In Estrus**	**% In Estrus**	**No. Inseminated**	**% Inseminated**
Day 8	4	5.63	4	5.63
Day 9	45	63.38	41	57.75
Day 10	18	25.35	17	23.94
Day 11	4	5.63	7	9.86
Day 12	0	0	2	2.82
TOTAL	71	99.99%	71	100%

Evidence of mounting activity was determined by the aid of heat detectors. KaMars and Estrus Alert patches were used to aid in heat detection at Briarpatch Farms. Seven of the 28 KaMars applied remained white. Twenty-one of the 28 KaMars were recorded red and indicated the heifers were in heat. Eight of the 29 Estrus Alert detectors were lost. This loss may have been improved if the patches had been applied differently. Recent literature provided by the manufacturer of Estrus Alert showed the patches being placed horizontally across the tail head and not vertically placed as done with traditional patches. All Estrus Alert patches in the present study were placed like the Kamar on the tail head vertically located. However, 20 of the 21 intact Estrus Alert patches were rubbed red, indicating these heifers were in estrus.

Heifers located at Laranda Farms were monitored electronically for mounting activity by computer using the HeatWatch system. The number of mounts and time of onset of estrus were recorded. The average number of mounts was more for Treatment 1, 37.30 mounts (Standard Error= ±4.945) compared with 21.52 mounts (Standard Error= ±3.745) in Treatment 2 (Table 4.7). In Treatment 1 the average number of mounts may be higher because of two heifers with records of over 100 mounts each (120 mounts and 135 mounts), but there were no heifers for Treatment 2 with more than 58 mounts. Both heifers that recorded over 100 mounts in Treatment 1 were in estrus on day 8 and were inseminated on day 9. One of these heifers was confirmed pregnant.

The average number of hours from injection of prostaglandin to onset of heat was longer for Treatment 1 heifers (over 20 hours longer, SE= ±2.272) when compared with Treatment 2 (69 hours and 10 minutes, SE= ±3.164) as shown in Table 4.7. This is to be expected because heifers in Treatment 1 were injected with prostaglandin 24 hours before the animals in Treatment 2. The average hours from standing (onset of estrus) to time of artificial insemination was approximately two hours more for Treatment 1 (11 hours and 12 minutes, SE= ±0.443), compared with Treatment 2 (9 hours and 25 minutes, SE= ±0.395) (Table 4.7). Other studies have reported similar findings to the present study where heifers injected with prostaglandin on day 6 during a 7 day progesterone insert treatment came in heat on average earlier than the heifers injected with PG F2α on day 7 (Lemaster et al., 1999 and Lucy et al., 2001). There was no difference (*$P>0.05$)* in conception rate (CR) among treatment groups (Trt 1= 41.02% vs. Trt 2= 44.44%) as shown in Table 4.7.

Table 4.7 HeatWatch Data Results. PGF Treatment Groups, Average number of mounts, Average hours from PGF injection to Estrus, Average hours from Standing to Insemination, and Conception Rate at Laranda Farms.

PGF Treatment	**Avg. No. of Mounts**	**Avg. Hrs. from PGF injection to Estrus**	**Avg. Hrs. from Standing to Insemination**	**Conception Rate***
Treatment 1	37.30 STD=32.052 SE=4.945	89h 11m STD= 14.189 SE=2.272	11h 12m STD= 2.771 SE=0.443	41.02
Treatment 2	21.52 STD= 15.563 SE=3.745	69h 10 m STD=17.903 SE=3.164	9h 25m STD=0.791 SE=0.395	44.44

Standard Deviation= STD
Standard Error= (SE)
*$P=0.157$

Three inseminators (A, B, and C) were used at Laranda Farms for inseminating heifers receiving both Treatments 1 and 2 (Table 4.8). Inseminator B had the highest conception rate for Treatment 1 (75%) and overall (60%). Inseminator B inseminated the fewest number of heifers (n= 4) for treatment 1 and overall (n=15). Inseminator C had the highest conception rate for Treatment 2 (63.64%) and second highest conception rate overall. Inseminator A had the lowest conception rate for combined treatment groups (32.14% of 28) inseminations compared with Inseminator B, 60% (n=15) and Inseminator C, 48.57% (n=35). Inseminator C inseminated the most animals (n=35) (Table 4.8). There was no significant difference ($P>0.05$) in each inseminators' conception rates in individual treatment groups (Table 4.8).

Table 4.8 Inseminators Conception Rate Results. Inseminators, Treatment 1 number of pregnant heifers, Treatment 1 Conception Rate (CR %), Treatment 2 number of pregnant heifers, Treatment 2 Conception Rate (CR %), Total animals inseminated by each inseminator, and Total Conception Rate (CR %) for each inseminator at Laranda Farms.

Inseminator	**Trt 1 No. Pregnant***	**Trt 1 CR%**	**Trt 2 No. Pregnant****	**Trt 2 CR%**	**Total No. Inseminated*****	**Total CR %**
A	6	42.68	3	21.34	28	32.14
B	3	75.00	6	54.55	15	60.00
C	10	41.67	7	63.64	35	48.57

*P=1.582
** P=5.095
***P=3.415

Further research should be conducted to better monitor the cyclicity and to determine the heifers' status of maturity (prepubertal or not) by taking blood samples 7 days before the start of the experiment (day –7), on day 0 and day 7. Lemaster et al. 1999 collected blood samples at 8 hours after insert removal and at 4-hour intervals following the onset of estrus that was determined by HeatWatch, until ovulation occurred. Blood should be examined for progesterone (P4), estrogen (E2) and lutenizing hormone (LH) concentrations to determine the effects of the CIDR insert as well as other hormone treatments used in the protocol. It is possible that prepubertal heifers were present in both experiments but weight did not indicate prepubertal heifers were among the treatment groups. Hip height could have also been used to better determine maturity status of heifers. If prepubertal heifers were among the study this could account for some of the heifers who did not show signs of estrus. Handling facilities and methods of working cattle prior to synchronizing and inseminating should be evaluated and performed so that the cattle are exposed to the least amount of stress to prevent an increase in cortisol concentrations. Ultrasonography of ovaries would provide information about follicular development and a more precise time and day of ovulation.

Experiment 2

Lubricant Evaluation. A total of 17 discharges were recorded in 69 heifers. All were treated with one lubricant (SAFE-LUBE). The number of heifers with discharge was similar for all treatment groups (Table 4.9). The control (CIDR plus PGF) had the lowest number of heifers (n=5) with signs of discharges (21.74%). Six of 24 heifers (25%) of the CIDR plus GnRH group exhibited a discharge, and 6 out of 24 (27.27%) in the CIDR plus ECP group (Table 4.9). Both lubricants were not evaluated in this study due to the working facilities that were available, as well as the time constraints in this experiment. Results for all three groups were slightly higher in Experiment 2, with 24.64% (n=17 with discharge out of 69), when compared with the number of heifers treated in Experiment 1(n= 27 with discharge out of 82) 26.99%.

Table 4.9 Timed Artificial Insemination Discharge Results. Timed Artificial Insemination treatments: CIDR+PGF (control), CIDR plus GnRH, CIDR+ECP, number of heifers treated, number of heifers with vaginal discharge at time of CIDR removal, and the percent of heifers with vaginal discharge at time of CIDR removal.

TAI Treatments	**No. Treated**	**No. with Discharge***	**% with Discharge**
Control	24	5	21.74
GnRH	24	6	25.0
ECP	23	6	27.27
TOTAL	71	17	24.64

**P=0.199*

CIDR and TAI Results. Seventy-one heifers received CIDR inserts and were timed inseminated. A total of 35 were diagnosed as pregnant (Table 4.10). In the control group (CIDR plus PGF), 13 of 23 (56.52%) conceived, 12 of 24 (50%) heifers in the GnRH treatment conceived and 10 of 22 (45.45%) in the ECP group. The control group had the highest conception rate (56.52%) when compared with the GnRH treatment (50.00%) and the ECP treatment (45.45%) conception rates. In the present study there was no significant difference (*P>0.05*) among the three treatment groups in terms of conception rates (Table 4.10). Two heifers (one heifer in the control and one from the ECP treatment group) were not available at time of pregnancy check and were omitted from the pregnancy data.

Table 4.10 Timed Artificial Insemination Conception Results. Timed Artificial Insemination treatments CIDR+PGF (control), CIDR plus GnRH, and CIDR+ECP, number of CIDR's inserted, number of heifers pregnant, and conception rate (CR).

TAI Treatment	**No. of CIDR's Inserted**	**No. Pregnant***	**Conception Rate**
Control	24	13	56.52
GnRH	24	12	50.00
ECP	23	10	45.45
TOTAL	71	35	50.72

**P=0.544*

Bridges et al. (2002) reported an increase in TAI conception rates when an injection of estradiol benzoate (EB) which is very similar to ECP is given at time of CIDR insertion in Angus and crossbred cows of *Bos indicus* breeding. Lammoglia et al. (1998) reported that an injection of 0.38 mg of EB (given 24 hours after removal of insert) and a CIDR insert produced a larger percentage (86%) of heifers in estrus. They reported no differences in pregnancy rate, and related this to EB causing "growth and dominance of follicles which may have resulted in a new follicular wave caused by a premature release of LH and this may have interfered with pregnancy by lutenizing the dominant follicle and preventing ovulation." Ryan et al. (1995) used several synchronization methods including a protocol using a CIDR insert and an injection of 1 mg of EB 48 hours after CIDR removal. This treatment resulted in higher pregnancy rates when compared with dairy cows treated with a CIDR insert and an injection of PGF day before the CIDR removal.

Other studies have suggested injecting estradiol treatments at the beginning of progesterone insert treatment because the use of an estradiol at the onset of the treatment with a CIDR increases follicle turnover with recruitment of new dominant follicles (Thatcher et al., 1989). Stevenson et al. (2002) reported fewer ovulations in dairy cows treated with ECP (59%) compared with GnRH (83%) along with a CIDR insert for 7 days. However, he did report higher conception rates in cows treated with ECP plus CIDR (39%) compared with GnRH plus CIDR (5%).

Conception rates could be better evaluated by taking blood samples to monitor LH and surges of LH, as well as plasma progesterone, and E2. Ultrasonography would be beneficial in observing the ovaries and follicular growth and responses to the hormone treatments throughout the duration of the synchronization treatment. There was no evidence in Experiment II that GnRH or ECP improved fertility of a TAI program using a PGF and CIDR inserts breeding scheme.

CHAPTER V

CONCLUSIONS

In conclusion, the CIDR inserts were very effective in estrus synchronization (87.20% of 164). CIDR retention rates were 100%. No inserts were lost in this study; other work has reported the loss rate to be as high as a 5% (Lucy et al., 2001 and Ryan et al., 1995). Altering the length of the CIDR tail does appear to have helped improve retention rates when comparing this present study's retention rate (100%) to other studies such as, Lucy et al. (2001) and Ryan et al.(1995).

Discharges at the time of CIDR insert removal were similar for both treatment groups in Experiment 1, but there was a higher incidence of discharges in Experiment 2. Experiment 2 animals were only treated with a lubricant lacking an antiseptic (SAFE-LUBE). It does appear that there may be an advantage to using an antiseptic lubricant, but further studies are needed.

No significant difference ($P>0.05$) was seen in Experiment 1 between administering prostaglandin on day 6 (Trt. 1 at 86.59%) and prostaglandin on day 7 (Trt. 2 at 86.59%). However, the estrus synchrony rate was the same. The average hours for heifers in Treatment 1 from time of prostaglandin injection to time of onset of estrus and displaying signs of estrus was 20 hours longer than for animals in Treatment 2, but there was no difference in the first service conception rates ($P>0.05$).

Heifers in Treatment 1 were in estrus within three days of CIDR removal whereas heifers in Treatment 2 were in estrus within four days of CIDR removal. The tight synchrony of Treatment 1 and Treatment 2 suggests that an injection of 25 mg of prostaglandin could be administered on day 7 versus day 6 along with a CIDR insert for 7 days and still prove to be effective. This would eliminate an extra day of handling and would benefit producers in daily operations.

There was no significant difference ($P<0.05$) in conception rates when comparing Treatment 1 (50.00%) to Treatment 2 (47.89%). Overall a total of 70 of 164 heifers in both treatments were diagnosed as pregnant resulting in a combined conception rate of 48.95%.

The CIDR and injection of prostaglandin proved to be effective in synchronizing the estrous cycle of mostly Holstein or Holstein crossed heifers. Conception rates could be possibly improved by improving methods in semen placement, semen handling and closely monitoring time of breeding. Monitoring the AI technician's techniques as well as looking at the fertility of the bulls being used on the heifers can also improve conception rates. Less stressful environments and improved ways of working the cattle through the handling facilities could also improve synchronization and conception results.

Blood analysis could be done to better observe the affects of the hormones being administered to the animals throughout the seven-day procedure. Taking blood samples could better indicate the time of ovulation. Ultrasonography could also be employed to better observe follicular growth and ovulation between different treatment groups.

In conclusion, the use of CIDR's in a Timed Artificial Insemination protocol was effective. However, there was no significant difference (*P*>0.05) among treatment groups, the overall combined conception rate was slightly higher (50.72%) in this experiment when compared to Experiment 1 (48.95%).

More research should be dedicated to developing a more effective Timed Artificial Insemination protocol. Further studies should concentrate on possible alternative times of administering hormone injections of ECP or GnRH. As suggested earlier, blood analysis and ultrasonography could also be incorporated into this timed artificial insemination protocol to enhance evaluation of responses to the hormonal treatment and conception rates.

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