SYNCHRONIZATION AND RESYNCHRONIZATION OF OVULATION AND TIMED INSEMINATION IN LACTATING DAIRY COWS AND HEIFERS

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ABSTRACT

This study was conducted to evaluate the efficacy of intravaginal progesterone (P_4) inserts (CIDR) in synchronization protocols combined with timed artificial insemination (TAI) as related reproductive performance. In the first study, heifers were synchronized with CIDR inserts followed by TAI. Heifers in the estradiol cypionate (ECP) group were synchronized with a combination of ECP, CIDR, prostaglandin ($PGF_{2\alpha}$), and ECP (CIDR-ECP), while the gonadotropin-releasing hormone (GnRH) group was synchronized using a combination of ECP, CIDR, $PGF_{2\alpha}$, and GnRH (CIDR-GnRH). All heifers were bred at either 48, 56, or 72 hours (h) after CIDR removal. Overall pregnancy rate (PR) for synchronized heifers was 60.1%, and embryo survival rate (ESR) was 98%. Pregnancy rate for CIDR-ECP treated heifers was influenced by artificial insemination (AI) time. In conclusion, ECP or GnRH may be used effectively in a CIDR-based TAI program in heifers. In the second study, cows were synchronized with CIDR devices or Ovsynch. The CIDR group received a combination of ECP, CIDR, $PGF_{2\alpha}$, and GnRH, while the Ovsynch group was synchronized using a combination of GnRH and $PGF_{2\alpha}$. Cows were bred at either 0, 8, or 24 h after the final GnRH injection. Overall PR for first service was 30.5% with ESR of 82.8%. Overall resynchronization PR was 35.1% with an ESR of 84.8%. In conclusion, Ovsynch and CIDR-based protocols are equally effective in synchronizing ovulation in a TAI program and resulted in comparable PR.

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LIST OF ABBREVIATIONS

AI: artificial insemination
CIDR: controlled internal drug release device or intravaginal progesterone inserts
CIDR-B: CIDR-Bovine
CL: corpus luteum
CR: conception rate
d: day(s)
DRMS: Dairy Records Management System
E_2: estrogen
EB: estradiol benzoate
ECP: estradiol cypionate
ESR: embryo survival rate
FSH: follicle stimulating hormone
GnRH: gonadotropin-releasing hormone
h: hour(s)
IGF1: insulin-like growth factor 1
i.m.: intramuscular
FSH: follicle stimulating hormone
LH: luteinizing hormone
ml: milliliter(s)
mg: milligram(s)
mm: millimeter(s)
ng: nangram(s)
P_4: progesterone or progestins
$PGF_{2\alpha}$: prostaglandin
PR: pregnancy rate
TAI: timed artificial insemination

INTRODUCTION

The dairy industry has entered a new era in which rapid progress in genetics and management have allowed a smaller number of dairy cows to meet the growing demands for dairy products. To meet supply demands, the dairy cow in 2004 is producing 16% more milk than the average dairy cow in 1994 (USDA, 2002). Moreover, in 2001, dairy cows were housed on 21% fewer farms, and 39% of the dairy operations housed 500+ head compared with 1997 (USDA, 2002). Therefore, in the last 10 years the dairy cow has adapted to rapid changes in management and production. With these changes, problems such as infertility and diseases induced by stress have followed.

Reproductive efficiency is a significant constituent of overall herd profitability in today's dairy operations. There are many factors that lead to efficient reproductive performance that include herd health, nutrition, cow comfort, management decisions, and labor. Estrus detection and proper timing of insemination, however, are the lead components hindering reproductive efficiency in many dairies. Larger herds manage more cows; therefore, more time is required for estrus detection, identification, and insemination, record keeping, and increased labor is needed to perform the work. In January 2004, Dairy Records Management Systems (DRMS, Raleigh, NC) reported that 38% of expected estrus events were detected (n = 13,892). While, this low percentage demonstrates that detection of estrus is a problem area in dairy operations, several factors including insufficient time and labor contribute to this inefficiency.

Many methods for the detection of estrus have been developed. In some herds, estrus detection aids have been implemented such as tail chalk and pressure sensitive patches. Other dairy operations have implemented technology that uses a pressure sensor that transmits mounting activity to a computer where the information is stored, interpreted, and accurate information about the onset of estrus is retrieved (HeatWatch®, DDx, Inc., Denver, CO). Research has also been conducted to control the estrous cycle of the cow with the administration of hormones. Through research, a number of estrus and ovulation synchronization protocols have been developed; however, they differ in required hormones, hormone administration time, timing of insemination, and cost. The following hormones have been in various synchronization protocols: prostaglandin ($PGF_{2\alpha}$), progestins (P_4), estrogens (E_2), and gonadotropin-releasing hormone (GnRH). Synchronization programs were developed to ensure that all cows receive first service consistently following the voluntary waiting period, to concentrate or eliminate visual observation, and most importantly, to increase labor efficiency. Many larger herds are implementing new methods to monitor reproduction. Timed AI (TAI) programs offer acceptable pregnancy rates (PR) without visual detection of estrus. A great deal of research has been conducted evaluating the effectiveness of synchronization protocols that combine the use of $PGF_{2\alpha}$, GnRH, P_4, and E_2 in dairy cattle. While abundant research has been conducted on estrous synchronization using intravaginal P_4 inserts also known as controlled internal drug release devices (CIDR), little research has been conducted using the CIDR insert with TAI.

The objectives of the first study were to investigate the effect of estradiol cypionate (ECP) administered at the time of a CIDR device insertion on synchronization of follicular wave emergence and to compare the response of ECP given 24 h or GnRH 48 h following CIDR removal on ovulation rate, time of ovulation, diameter of the ovulatory follicle, and PR after TAI at either 48, 56, or 72 h following CIDR removal in dairy heifers. The objectives of the second study were to determine the PR and embryo survival rate (ESR) for lactating dairy cows synchronized using a CIDR-based protocol or the Ovsynch protocol followed by TAI at either 0, 8, or 24 h after the final GnRH injection. In addition, cows that were diagnosed not pregnant at ultrasonography on d 31 (±1) were re-inseminated using the same initial protocol, and PR and ESR for both protocols were compared after second and third services.

LITERATURE REVIEW

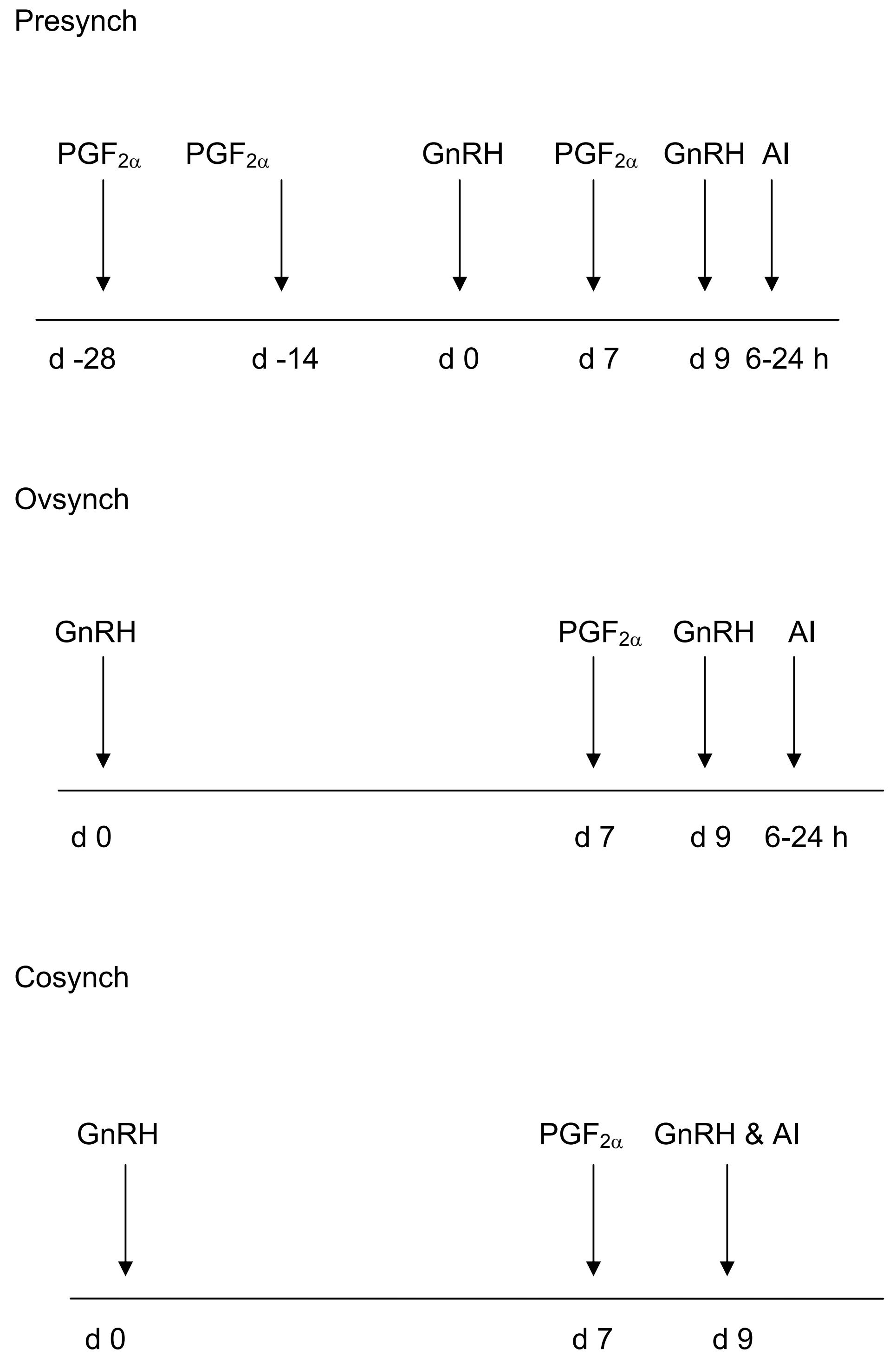
SYNCHRONIZATION OF ESTRUS OR OVULATION

Fricke (2003) explained that there are three strategies that must be implemented early in the breeding period of lactating dairy cows to establish an aggressive reproductive management program. First, all cows must have their initial service at the end of the predetermined voluntary waiting period. Next, pregnancy status must be determined soon after insemination. Ultrasonography allows for early pregnancy diagnosis and detection of nonpregnant cows (Pierson et al., 1984; Kastelic et al., 1988). Finally, cows failing to conceive need to be submitted immediately for the next service. Today, dairy producers have numerous protocols to synchronize estrus for ovulation, which aid in the timely submission of cattle for artificial insemination (AI). Synchronization programs were developed to 1) ensure that all cows receive first service consistently following the voluntary waiting period, 2) concentrate or eliminate visual observation for the detection of estrus, and 3) increase labor efficiency. There are currently several synchronization protocols and they all use a combination of $PGF_{2\alpha}$, P_4, E_2, and GnRH.

The motive behind synchronization protocols is to control both luteal and follicular growth. There are two methods to control the length of the estrous cycle: 1) administer $PGF_{2\alpha}$, causing a regression of the corpus luteum (CL) thus shortening the cycle and 2) administer P_4 to delay the onset of estrus following either natural or induced luteolysis. The next approach in ovulation control has been to manipulate the ovulatory follicle by administering GnRH, which should cause ovulation of the dominant follicle.

Initially, synchronization protocols focused on controlling the luteal phase of the estrous cycle by the regression of the CL with exogenous $PGF_{2\alpha}$ followed by visual observation for signs of estrus 18 to 80 h later. Lauderdale et al. (1974) found that cows could be in detectable estrus for 5 days (d) following a single injection of $PGF_{2\alpha}$. In addition, cows receiving TAI 72 to 80 h following the second subsequent administration of $PGF_{2\alpha}$ had a lower PR than cows receiving AI following a detected estrus (Stevenson et al., 1987). The single injection protocol was a success when cows were inseminated after a detected estrus; however, protocols were introduced that called for multiple $PGF_{2\alpha}$ injections that were administered 14 d apart, which would further reduce the time required for detection of estrus (Seguin et al., 1978; Lauderdale et al., 1974). It should be noted that 15% of cows receiving multiple administrations of $PGF_{2\alpha}$ lacked luteal function (Stevenson et al., 1987). Thus, use of $PGF_{2\alpha}$ as a synchronization protocol required visual observation of estrus for 5 d and did not produce acceptable PR with TAI because of inconsistent follicular development and ovulation.

Synchronization protocols evolved and required the administration of GnRH to manipulate follicular dynamics. Gonadotropin-releasing hormone initiates the release of luteinizing hormone (LH) and therefore causes ovulation of the dominant follicle. This allows the release of follicle stimulating hormone (FSH), which allows recruitment of a new follicular wave. There are several synchronization protocols that use GnRH for TAI, they include: Presynch, Ovsynch, and Cosynch. Figure 1 illustrates these protocols.



Presynch

Ovsynch

Cosynch

Figure 1. Gonadotropin-releasing hormone (GnRH) synchronization protocols.

Results from studies performed by Vasconcelos et al. (1999) and Moreira et al. (2000) in lactating dairy cows and dairy heifers, respectively, suggest that the initiation of Ovsynch between days 5 to 12 of the estrous cycle may result in improved conception rate (CR) over the Ovsynch protocol started on a random day of the estrous cycle. This can be accomplished by using the Presynch protocol, which requires administering two $PGF_{2\alpha}$ injections 12 to 14 d apart preceding the standard Ovsynch protocol. Moreira et al. (2001) conducted a study using lactating dairy cows (n = 543) to determine reproductive performance when estrus was synchronized using Ovsynch or Presynch. Cows were assigned to one of two treatments for their first postpartum TAI: 1) standard Ovsynch protocol or 2) Presynch protocol containing two $PGF_{2\alpha}$ injections 14 d apart followed 12 d later by the standard Ovsynch protocol. All cows were bred using TAI 16 h following the final GnRH injection. The CR for the Presynch treatment group was higher than the Ovsynch group (43 versus 29%). Therefore, the use of Presynch for synchronizing estrus improved first service CR in dairy cows.

Cosynch has the identical time intervals for the administration of $PGF_{2\alpha}$ and GnRH as Ovsynch, but cows are inseminated immediately following the final injection of GnRH to eliminate additional animal handling. Although this may be advantageous from a management standpoint, optimal CR was not achieved using this protocol. In well-managed operation expected CR should be 37% using the Cosynch program (Pursley et al., 1998).

Most recently, estrus synchronization protocols have advanced to use P_4, which prevents estrus while in use. The use of P_4 was implemented to delay the time of estrus following natural or induced luteolysis and to subsequently extend the length of the estrous cycle. Currently, synchronization programs have the ability to control the timing of ovulation and not just the expression of estrus. This advantage led to the development of TAI protocols that would allow dairyman to inseminate cows successfully without the detection of estrus. The CIDR® was developed jointly at the Ruakura Agricultural Research Center by the Agricultural Division of the Carter Holt Harvey Plastic Products Group Ltd., both located in Hamilton, New Zealand during the early 1990s. In May of 2002, the United States Food and Drug Administration approved the use of CIDR inserts for synchronization of estrus in dairy heifers and beef cattle, and in July of 2003, approval was granted for use in lactating dairy cows. These devices are used to induce estrous activity in anestrous cows and prepubertal heifers (Lucy et al., 2001), and to synchronize the return to estrus in previously inseminated cattle (Macmillan and Peterson, 1993; Chenault et al., 2003b).

OVSYNCH SYNCHRONIZATION

Ovsynch synchronizes follicular development, luteal regression, and time of ovulation allowing for TAI after the second GnRH injection (Pursley et al., 1995). Ovsynch is usually initiated without regard to the stage of the estrous cycle (Vasconcelos et al., 1999). The Ovsynch protocol requires a 100-μg intramuscular (i.m.) injection of GnRH, which stimulates luteinization or ovulation of the largest follicle and initiation of a new follicular wave. Seven d later, a 25-mg i.m. injection of $PGF_{2\alpha}$ is administered, resulting in regression of the CL and removal of the negative feedback of P_4, thus allowing the development of a new follicular wave. A second 100-

μg i.m. injection of GnRH is given 48 h later causing ovulation of the dominant follicle (Pursley et al. 1995).

THE USE OF OVSYNCH IN COWS

Pursley et al. (1995) developed the Ovsynch protocol for ovulation synchronization 9 years ago, and today, it is a common tool used on many dairy farms. Initially, Pursley et al. (1995) wanted to determine if ovulation could be synchronized and cows bred using TAI; therefore, eliminating the detection of estrus. Cows (n = 20) were administered the Ovsynch protocol at a random stage of the estrous cycle, and TAI occurred 20 to 24 h following the final GnRH administration. Five days prior to synchronization, ovarian structures were monitored daily by transrectal ultrasonography and continued until ovulation. Cows were diagnosed for pregnancy at 32 d post insemination via rectal ultrasonography. Pursley et al. (1995) reported that 90% (18/20) of cows responded to the first GnRH injection by ovulation of the dominant follicle (14.21 ± 0.75 mm), while 100% (20/20) responded to the $PGF_{2\alpha}$ injection by regression of the CL. All cows (20/20) ovulated 24 to 32 h after the second GnRH injection and 10 of 20 cows were pregnant at the 32-d ultrasound procedure. The study concluded that the synchronization of ovulation could be achieved in lactating dairy cattle and an acceptable CR was obtained using a TAI protocol. This study had a major impact on managing reproduction in cattle because it eliminated the need for detection of estrus and allowed for a successful TAI protocol.

Pursley et al. (1995) found that the Ovsynch protocol could be initiated without regard to the stage of the estrous cycle and would synchronize ovulation in a narrow time interval. At any given stage of the estrous cycle, different structures (follicles and

CL) can be present on the ovaries; therefore, it was suggested that cows may have different responses to synchronization due to the stage of the estrous cycle at initiation of the protocol. Vasconcelos et al. (1999) used the Ovsynch protocol in 156 lactating dairy cows at a known stage of the estrous cycle, and determined whether the CL was present by monitoring P_4 serum levels. Cows were pregnancy diagnosed 28 d following AI. Beginning on the day of hormone administration and continuing 48 h after the final GnRH injection, ovarian structures were monitored by transrectal ultrasonography to determine if ovulation had occurred. The overall synchronization rate was 87%. Vasconcelos et al. (1999) reported that only 64% of the cows responded to the first GnRH injection. In addition, cows ovulating after the first GnRH injection had a 91% overall synchronization rate, while cows not responding to the hormone had a final synchronization rate of 79%. The success of the synchronization response did depend on the stage of the estrous cycle at initiation of the protocol. Cows in the metestrous stage (d 1 to 4) of the estrous cycle had a higher synchronization rate (91%) but a lower PR (25%) which was most likely due to the presence of large follicles. Cows in the diestrous stage (d 5 to16) of the estrous cycle had the highest PR (42%), but had the smallest ovulatory follicles during the hormone administration. Seventy-seven percent of the cows initiated during the proestrous stage of the estrous cycle (d 17 to 21) ovulated after the initial GnRH injection. It was suggested that this could be due to increased frequency of cows with three follicular waves. Furthermore, 18% of cows in the proestrous stage expressed estrus and ovulated prior to the final GnRH injection. Subsequent studies reported that 5 to12% of cows in the proestrous stage of the estrous cycle prematurely ovulated between the initial GnRH and $PGF_{2\alpha}$ injections of the Ovsynch protocol (Roy et al., 1999; DeJarnette et al., 2001). Vasconcelos et al. (1999) concluded that cows should be started on the Ovsynch protocol during d 5 to12 of the estrous cycle to achieve the highest synchronization and CR.

Studies demonstrated that ovulation could be synchronized and cows bred using TAI without detection of estrus; however, the optimal insemination time had not been determined. In 1998, Pursley et al. (1998) conducted a study to assess the optimal time for AI to occur relative to the synchronized ovulation in an Ovsynch protocol. Lactating dairy cows (n = 732) were assigned to five groups by stage of lactation and parity. Synchronized ovulation was achieved using the Ovsynch protocol and cows were TAI at 0, 8, 16, 24, or 32 h after the second injection of GnRH. Pregnancy status was determined by ultrasound 25 to 35 d after AI. Pregnancy rates tended to be greater for the 0 (37%), 8 (41%), 16 (45%), and 24 h (41%) groups compared with the 32 h (32%) group. Calving rates were greater ($P < 0.10$) for the 0 (31%), 8 (31%), 16 (33%), and 24 h (29%) groups compared with the 32 h (20%) group. The pregnancy loss rate for the 0 h group (9%) was lower ($P < 0.10$) compared with the 8 (21%), 16 (21%), and 24 h (21%) groups, while there was a tendency for greater pregnancy loss rate in the 32 h (32%) group. The overall pregnancy loss rate was 22%. In conclusion, there was no evidence that CR differed from 0 to 24 h after the second GnRH injection; however, insemination at 32 h decreased PR and calving rates.

Numerous studies have been conducted to determine the reproductive performance of cows that were synchronized using the Ovsynch protocol. Several studies (Burke et al., 1996; Pursley et al., 1997a; Cordoba and Fricke, 2001) found the Ovsynch system to be an effective method for achieving high reproductive performance and reducing the negative economic factors associated with visual detection of estrus.

Burke et al. (1996) investigated PR and CR in lactating dairy cows (n = 171) that were subjected to the Ovsynch protocol and TAI. The study compared cows synchronized using the Ovsynch protocol with TAI 16 h following synchronization to cows inseminated at detected estrus. Pregnancy status was determined 42 d post-insemination by rectal palpation of the uterus. Blood samples were collected immediately before the initial GnRH and $PGF_{2\alpha}$ administrations to determine P_4 concentrations. Body condition scores (BCS) were determined between 0 and 10 d postpartum. The proportion of cows detected in estrus prior to synchronization was 44.8%; therefore, a majority of the cows were cycling when they began the protocol. Synchronized cows had a PR of 29% and a CR of 26.5%; both were slightly lower for synchronized cows than for cows detected in estrus (30.5 and 41.5%, respectively). Cows receiving the Ovsynch protocol had more consistent CR and PR over time compared with cows that were detected in estrus and inseminated. The CR and PR at first synchronization were influenced positively by body condition score ($P < 0.06$ and $P < 0.01$, respectively) and plasma levels of P_4 ($P < 0.005$ and $P < 0.001$, respectively). Thus, as the BCS increased (1.7 to 4.3) at 65 d postpartum, the CR and PR increased 13 and 16%, respectively. Furthermore, plasma P_4 increased by 2.2 nangrams/mililter (ng/ml) for every increase in BCS. Therefore, cows with higher BCS and plasma P_4 were more likely to conceive.

Pursley et al. (1997a) conducted a study to compare the effectiveness of the Ovsynch protocol combined with TAI to AI following detected estrus and the occasional use of $PGF_{2\alpha}$ as reproductive management tools. Lactating dairy cows (n = 333) housed in three different Wisconsin dairy farms received the Ovsynch protocol or were detected in estrus and bred 12 h after the detection of estrus. When determined by the veterinarian and herd manager to be appropriate, $PGF_{2\alpha}$ and GnRH were used. Synchronized cows received the standard Ovsynch protocol and were bred 20 to 24 h after the final GnRH administration. Pregnancy diagnosis was performed 32 and 38 d post AI using transrectal ultrasonography. Cows failing to conceive were resynchronized using the same protocol until diagnosed pregnant or culled from the herd. Median days to first service (54 versus 83) and days open (99 versus 118) were lower for the Ovsynch group compared with the group detected in estrus ($P < 0.001$). Pregnancy rates for the first AI were similar (37 versus 39%) for both groups. Pregnancy rates for the second and third service AI at 100 d in milk averaged 53% for the Ovsynch treated cows compared with 35% for cows detected in estrus. In conclusion, the Ovsynch protocol with TAI 20 to 24 h after the last GnRH injection allowed for efficient reproductive management of lactating dairy cows.

Cordoba and Fricke (2001) conducted a study to compare the standard Ovsynch protocol to the Presynch protocol as related to synchronization, CR, and PR in lactating dairy cows (n= 142). Cows received the standard Ovsynch protocol, or the standard Presynch protocol. The researchers hypothesized that administering $PGF_{2\alpha}$ 12 d prior to the initiation of Ovsynch would induce luteal regression in cows with a responsive CL, thereby, decreasing the number of cows in the metestrous or proestrous stages of the estrous cycle. Body condition scores were determined 30 d prior to synchronization and the day synchronization began. Pregnancy status was determined by ultrasound 32 d post AI and then again 60 d post AI. Synchronization rates (86 versus 78.8%), CR (51.2 versus 48.8%), and PR (44 versus 38.5%) did not differ between the Ovsynch group and Presynch groups, respectively.

In comparing literature reports from the field, there have been slight differences in the reproductive performance for the Ovsynch protocol. Fricke (2003) concluded that the number of anovluar cows, follicular dynamics, and the ability of the farm personnel to implement the protocol all contribute to different success rates when implementing the Ovsynch protocol. In addition, physiologic factors associated with milk production could affect response to the Ovsynch protocol (Cordoba and Fricke, 2001).

THE USE OF OVSYNCH IN HEIFERS

While the Ovsynch protocol is an effective reproductive management tool for lactating dairy cows, the protocol does not appear to be effective for dairy heifers. Pursley et al. (1995) evaluated the use of Ovsynch in the synchronization of ovulation in 24 dairy heifers 14 to16 months of age. The Ovsynch protocol was initiated at a random stage of the estrous cycle combined with TAI 24 h following the last GnRH injection. Ovarian structures were monitored daily during the Ovsynch protocol by ultrasonography for 12 heifers and every other day for 12 heifers. Heifers were pregnancy checked using ultrasound 32 d post AI. A total of 54% (13/24) of the heifers ovulated a dominant follicle (15.2 ± 0.87 millimeters (mm)), therefore, demonstrating a response to the initial GnRH injection. In a subset of heifers, ovulation occurred in 67%

(8/12) and a new follicular wave emerged in the eight heifers 2.5 ± 0.26 d after the GnRH injection. The average day of emergence in the subset of heifers was 1.5 ± 0.47 d from the injection of GnRH. Thus, the Ovsynch protocol was not an effective method for the synchronization of heifers.

In a second study, Pursley et al. (1997b) again demonstrated that the Ovsynch protocol does not produce acceptable reproductive performance in dairy heifers. Heifers (n = 155) ranging from 13 to 23 months of age were used to determine the reproductive effectiveness of GnRH and $PGF_{2\alpha}$ in synchronizing ovulation. Heifers received either the Ovsynch protocol or a series of three injections of $PGF_{2\alpha}$ given 14 d apart until estrus was detected or TAI 72 to 80 h following the third $PGF_{2\alpha}$ injection. Approximately 80% of the heifers were diagnosed pregnant via ultrasound 25 to 30 d following AI, while 20% were rectally palpated 35 to 49 d following AI. The PR for heifers detected in estrus (74.4%) was greater than heifers in the Ovsynch group (35.1%).

Heifers have a low synchronization rate and PR when Ovsynch is implemented as the synchronization protocol. The reason for this low reproductive performance has not been well characterized. Pursley et al. (1997b) concluded that lactation might produce differences in oocyte quality and uterine environment, which may reduce fertility. Nebel and Jobst (1998) suggested that the reason virgin heifers respond poorly to Ovsynch could be possibly due to inconsistent follicular waves.

Overall, for lactating dairy cows, the Ovsynch protocol offers a method to achieve timely pregnancies without detection of estrus. In addition, it increases the rate of insemination and allows control over time to first AI, while having minimal impact on CR.

CIDR® INSERT SYNCHRONIZATION

A new reproductive tool, the CIDR insert, was developed jointly at the Ruakura Agricultural Research Center and the Agricultural Division of the Carter Holt Harvey Plastic Products Group Ltd., both located in Hamilton, New Zealand during the early 1990s. There are several reasons to use the inserts 1) to induce estrus activity in anestrous cows and prepubertal heifers (Lucy et al., 2001), 2) to synchronize the return to estrus in previously inseminated cattle (Chenault et al., 2003b; Macmillan and Peterson, 1993), and 3) as a component of treatment regimens administered to synchronize estrus in cattle (Lucy et al., 2001; Macmillan and Peterson, 1993). The device achieves this by the slow delivery of P_4 into the blood circulation of the animal. Progesterone provides a potent suppression of estrus and ovulation (Macmillan et al., 1996) and following removal of the CIDR, the rapid drop in circulating P_4 concentrations promotes a synchronized estrus (Rathbone et al., 2002; Macmillan and Peterson, 1993).

Rathbone et al. (2002) re-engineered the CIDR-Bovine (CIDR-B®; 1.9 mg P_4) protocol for a 7-d insertion period. The study resulted in a reduction in the initial drug load, which led to the development of the CIDR (CIDR®; 1.38 mg P_4) insert which is equivalent to the CIDR-B insert. The study reported a rapid rise in P_4 levels after CIDR insertion. Maximal P_4 levels are reached within 1 h after CIDR insertion. Levels of P_4 are maintained while the insert is in the vagina, and immediately upon CIDR insert removal, P_4 is lowered to basal levels.

In May 2002, the Food and Drug Administration approved the use of CIDR inserts for synchronization of estrus in dairy heifers and beef cattle and in July 2003 for lactating dairy cows. Globally, two intravaginal P_4 inserts containing either 1.9 g (CIDR-B® inserts) or 1.38 g of P_4 (CIDR inserts; EAZI-BREED™ CIDR® Cattle Insert) are marketed (Pfizer Animal Health, New York, NY). The CIDR is an intravaginal P_4 insert that is “T” shaped. The wings of the device fold in to form a rod that can be placed in the vagina with an applicator. Caudal to the wings is a tail, which facilitates the removal of the insert. The backbone of the CIDR is a nylon spine, which is covered by a P_4 impregnated silicone skin. The label recommendations for dairy heifers and beef cattle require a CIDR insertion on d 0, which prevents ovulation and estrus activity. Twenty-four hours prior to the CIDR removal on d 6, a 25-mg i.m. injection of $PGF_{2\alpha}$ is administered to regress the CL, if present. Finally, after CIDR removal, females are observed for estrus (3 d) and AI is recommended approximately 12 h after the onset of standing activity. Retention rates for CIDR inserts were reported to be 97 to 98% (Kesler et al., 2002). The label recommends the use of CIDR inserts for synchronization of the return to estrus in lactating dairy cows previously inseminated. The CIDR insert should be administered 14 ($\pm$1) d after the insemination and removed 7 d later. Cows should be observed for estrus 72 h after CIDR removal and inseminated 12 h after onset of estrus. Macmillan and Peterson (1993) conducted a series of trials in several herds to evaluate the CIDR-B device for cattle. The effectiveness of the CIDR-B was dependent on the cycle stage at insertion, duration of treatment, and interactions with other administered hormones. The study demonstrated the desirability of treatment regimes that require a shorter duration (<10

d) of CIDR insertion. Pregnancy rates after the first insemination increased if the CIDR-B device was inserted 6 to 8 d after AI. Furthermore, anestrus dairy cows treated with P_4 combined with pregnant mare serum gonadotropin at CIDR-B removal resulted in estrus with ovulation in 68% of animals; 14% ovulated with no estrus and 18% did not ovulate. While these rates did vary among herds, Macmillan and Peterson (1993) contributed the variation to differences in the nutritional status or energy balance of cows during early lactation. In conclusion, the study introduced the use of CIDR-B devices as research tools to explore factors dealing with hormone metabolism, ovarian activity, and PR. Macmillan and Peterson (1993) hypothesized that the use of CIDR-B in future research either alone or in combination with other hormones, would be effective in estrous cycle stimulation, and return to service regulation.

THE USE OF CIDR® INSERTS IN DAIRY HEIFERS

Research evaluating the effectiveness of CIDR protocols in dairy heifers has shown that they are effective in synchronizing estrus. Lucy et al. (2001) evaluated the use of CIDR inserts and $PGF_{2\alpha}$ for estrus synchronization and shortening the interval to pregnancy in dairy heifers. Holstein heifers (n = 260) housed in four locations (NY, IL, MS, and FL) were 14.5 ± 0.15 months of age at the beginning of the treatment. Heifers were assigned to one of two treatment groups: 1) a single injection of $PGF_{2\alpha}$ for the control group or 2) a CIDR for 7 d and $PGF_{2\alpha}$ administered on d 6 for the treatment group. The percentage of heifers in estrus during the first 3 d was significantly higher for the treated heifers (84 versus 57%). However, the control heifers demonstrated that the CIDR treatment did not improve PR for first AI (58 versus 64%) or the entire 31-d AI period (54 versus 50%). Among heifers (n = 136) given a CIDR insert, 14 heifers (10.3%) lost their insert prior to scheduled day of removal. Mild vaginitis was observed at the time of CIDR removal in nearly all heifers at one location and no evidence of vaginitis was detected at time of AI.

As with other methods of synchronization using exogenous P_4, the synchronization rate is enhanced when E_2 is administered during the treatment period (Hanlon et al., 1997). Thundathil et al. (1998) studied the effect of ECP on ovarian follicular development and ovulation in dairy cattle. In contrast to other studies, Thundathil et al. (1998) and Hanlon et al. (1997) reported ECP had limited efficacy for synchronizing follicular development and ovulation in dairy cattle when given at random stages of the estrous cycle. Many studies (Colazo et al., 2004; Colazo et al., 2003; Hanlon et al., 1997; Hanlon et al., 1996) demonstrated that exogenous E_2 is effective when used at the beginning and end of a CIDR synchronization protocol. In 1996, Hanlon et al. explored the effects of estradiol benzoate (EB) administered i.m. 24 h after the removal of the CIDR-B device in dairy heifers. The study was conducted in 13 herds and included 750 heifers randomly assigned to one of two protocols. All heifers received a CIDR-B insert containing a 10-mg EB capsule for 12 d. Twenty-four h after CIDR-B removal, heifers received either 0.5-mg EB or a placebo injection. Visual observation for estrus occurred at 48 and 72 h following the CIDR-B removal and cows were inseminated immediately following detection of estrus. The EB treatment significantly increased the number of heifers exhibiting estrus during the given time periods compared with the placebo (96 versus 90.5%). The onset of estrus was so precise that 86.6% of the EB treated heifers had exhibited estrus and conceived within 48 h, while only 72.3% of the placebo heifers exhibited estrus and conceived within the same time. As a result of this study, recommendations were formulated that heifers may be inseminated using TAI 48 h following the removal of CIDR-B inserts if exogenous E_2 is administered 24 h after insert removal.

THE USE OF CIDR® INSERTS IN LACTATING COWS

The approval of CIDR inserts for lactating dairy cows was not granted until the concerns pertaining to the concentration of P_4 in milk following the administration of CIDR inserts was examined. Milk from pregnant cows contains concentrations of P_4 that have been deemed safe for human consumption. Therefore, Chenault et al. (2003a) conducted a study to determine if concentrations of P_4 in milk during CIDR insert treatment was less than P_4 milk associated with pregnancy. Holstein cows (n = 64) received a 25-mg i.m. injection of $PGF_{2\alpha}$ and were assigned to one of two treatments: 1) controls, no further treatment, or 2) administration of a CIDR insert 14 d after estrus and removal 7 d later. Composite milk samples were collected from each of the cows, in addition to milk samples from 10 pregnant cows was collected twice daily for 10 d. Daily mean logs for concentrations of P_4 were 3.05 ng/ml for control cows, 3.33 ng/ml for CIDR insert cows, and 3.81 ng/ml for pregnant cows. Therefore, increased P_4 due to pregnancy was 0.76 ng d/ml, while increased P_4 due to CIDR insert was only 0.23 ng/ml. In conclusion, milk from pregnant cows is safe for human consumption; therefore, milk from CIDR insert treated cows should be safe based on P_4 concentration.

The label recommends the use of CIDR inserts for synchronization of the return to estrus in lactating dairy cows previously inseminated. The CIDR insert should be administered 14 (±1) d after the insemination and removed 7 d later. Cows should be observed for estrus 72 h after CIDR removal and inseminated 12 h after onset of estrus. There has been work conducted to evaluate this protocol (Chenault et al., 2003b), as well as studies conducted to evaluate alternative protocols (Kim et al., 2003).

Chenault et al. (2003b) evaluated the synchronization of the return to estrus, CR, and PR in Holstein dairy cows (n=1,893) that were previously inseminated on eight farms located in MI, NY, FL, IL, or CA that were 40 to 150 d open. Cows that were detected in estrus and AI within 96 h after a single injection of 25-mg i.m. injection of $PGF_{2\alpha}$ were assigned to one of two treatments: 1) controls, no further treatment, or 2) administration of a CIDR insert 14 d after insemination and removed 7 d later. Cows were observed for signs of estrus 72 h prior and 4 d after CIDR insert removal. The CIDR insert increased service rate (34 versus 19% in 3 d) and overall estrus detection (43% in 4 d versus 36% in 9 d) compared with controls. The PR to initial AI was lower for cows subsequently receiving a CIDR insert (32.7 versus 36.7%). For the 9 d resynchronization period, CR and PR for the CIDR treatment (26.7 and 12.2%) and control (30.9 and 11.1%) cows did not differ significantly. Chenault et al. (2003b) concluded that CIDR inserts improved synchronization of the return to estrus, slightly reduced PR to initial AI, but did not affect CR or PR to AI during the resynchronization period.

Kim et al. (2003) compared the Ovsynch protocol to the Ovsynch protocol combined with a CIDR in lactating Holstein dairy cows (n = 68). Cows were assigned one of two treatments: 1) the standard Ovsynch protocol, or 2) the standard Ovsynch protocol with the addition of a CIDR insert (OV+C) and an EB capsule. The PR for the OV+C treatment group was higher than the Ovsynch group (41.2 versus 20.0%). The OV+C treatment exhibited a smaller proportion of cows with premature estrus prior to the $PGF_{2ά}$.

TIMING OF ARTIFICIAL INSEMINATION FOLLOWING REMOVAL OF INTRAVAGINAL PROGESTERONE INSERTS IN DAIRY HEIFERS

ABSTRACT

The reproductive performance of dairy heifers (n = 110) after synchronization with CIDR and TAI was compared. Heifers were randomly assigned to one of two synchronization protocols. Heifers in the ECP group (n = 54) were synchronized with a combination of ECP, CIDR, $PGF_{2\alpha}$, and ECP (CIDR-ECP). Heifers in the GnRH group (n = 56) were synchronized using a combination of ECP, CIDR, $PGF_{2\alpha}$, and GnRH (CIDR-GnRH). Heifers were bred at either 48, 56, or 72 h after CIDR removal. Pregnancy diagnosis was conducted by ultrasonography 32 (±1) d post AI to confirm pregnancy and 60 (±1) d post AI to determine embryo survival rate (ESR). Ovarian follicular development was monitored with ultrasonography daily from d 0 to 7 and twice daily from d 8 to ovulation to examine emergence of a new wave of follicular development, size of the ovulatory follicle, and timing of ovulation on 15 heifers per protocol. New follicular development was detected 3.7 ± 0.22 d after CIDR insertion. Heifers receiving CIDR-ECP had a shorter interval from CIDR removal to ovulation than heifers receiving CIDR-GnRH (63.8 ± 3.0 and 71.6 ± 2.27 h, respectively). However, relative to the hormone injection after CIDR removal, ovulation occurred 39.8 ± 3.0 h in heifers given ECP and 23.6 ± 2.27 h for heifers given GnRH. Diameter of the ovulatory follicle did not differ among protocols. The PR for CIDR-ECP synchronized heifers (63.0%) was not different than the CIDR-GnRH synchronized heifers (57.1%). The ESR for CIDR-ECP treated heifers was 98% while all CIDR-GnRH treated heifers pregnant at d 32 were pregnant at d 60. The PR was not influenced by breed or AI technician. Heifers inseminated in February, 2003 had lower odds for pregnancy for first service. The PR for CIDR-ECP treated heifers was influenced by AI time. The CIDR-ECP heifers inseminated 56 h after CIDR removal had a higher PR (81.0%) compared with heifers inseminated 48 h (66.7%) or 72 h (50.0%) after CIDR removal. Heifers in the CIDR-GnRH group did not differ in the first service PR as related to AI time. In conclusion, either ECP or GnRH may be used in a CIDR-based TAI program in dairy heifers with acceptable reproductive performance achieved. The recommended AI time for heifers synchronized using CIDR-ECP is 56 h after CIDR device removal (32 h after injection), while CIDR-GnRH treated heifers can be inseminated at either the time of GnRH injection (48 h after CIDR removal) or at later times (8 or 24 h after GnRH injection) without compromising PR to TAI.

Key words: CIDR inserts, timed AI, reproductive performance, dairy heifer

INTRODUCTION

On many dairy operations, heifers are not bred using AI due to their location on remote pastures and the extra effort that must be made for detection of estrus and AI by farm personnel. This has limited the use of AI in dairy heifers. Synchronization protocols that include TAI and achieve acceptable PR could allow for increased use of AI in heifers. Using the Ovsynch protocol for TAI in dairy heifers decreased PR by 39% compared with AI following observed estrus (Pursley et al., 1997b; Pursley et al., 1995). Decreased PR of virgin heifers on Ovsynch protocols could be due to the number of follicular waves (Pursley et al., 1997b) or inconsistent follicular wave emergence (Nebel and Jobst, 1998). While, the Ovsynch protocol has not produced acceptable PR in heifers, little research has been conducted to evaluate other methods of synchronization for TAI in dairy heifers.

Initial research was conducted using dairy heifers to evaluate the effectiveness of the CIDR inserts reported an overall synchronization rate of 84% and a 45% PR for the first 3 d of the AI period after CIDR removal and detected estrus (Lucy et al., 2001). Synchronization rate is enhanced when E_2 is administered during P_4 period (Hanlon et al., 1997; Hanlon et al., 1996). Many studies (Colazo, et al., 2004; Colazo et al., 2003; Hanlon et al., 1997; Hanlon et al., 1996) have demonstrated that exogenous E_2 is effective when used at the beginning or end of a CIDR synchronization protocol. While abundant research has been published on the subject of estrus synchronization using CIDR inserts, little research has been conducted using the CIDR insert with TAI in dairy heifers.

The objectives of this study were to determine follicular wave emergence following ECP (ECP®; Pfizer Inc., New York, NY) injection at the time of a CIDR insertion and to compare the response of ECP given 24 h or GnRH 48 h following CIDR removal on ovulation rate, time of ovulation, diameter of the ovulatory follicle, and PR after TAI at either 48, 56, or 72 h following CIDR removal in dairy heifers.

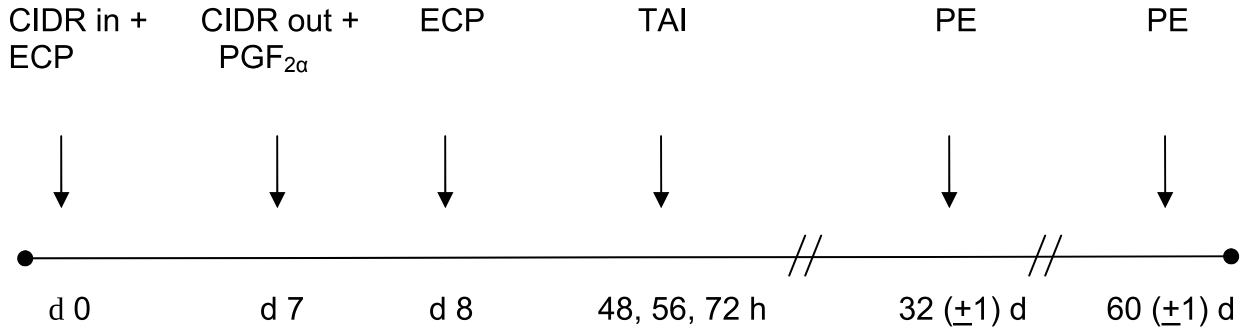
MATERIALS AND METHODS

HEIFERS AND TREATMENT PROTOCOLS

The trial was conducted from August 2002 to March 2004 using heifers (n = 110) from the Virginia Tech Dairy Center. Heifers were between 14 and 19 months of age at the start of the synchronization regimes. Both Jersey (n = 39) and Holstein (n = 71) heifers were managed together on pastures of orchard grass and fescue. Heifers were fed corn silage, concentrate, 2:1 mineral, and mixed orchard grass hay during the winter.

Heifers were randomly assigned to a synchronization protocol without regard to the stage of the estrous cycle at initiation of synchronization (Figure 2). Within the two synchronization protocols, heifers were randomly assigned to either 48 or 72 h TAI; however, a subset of heifers was assigned randomly to a synchronization protocol but bred 56 h after CIDR removal. All heifers (n = 110) received an intravaginal P_4 insert containing 1.38 g of P_4 (Eazi-Bred™ CIDR®; Pfizer Inc., New York, NY) and a 1-mg i.m. injection of ECP at time of device insertion (day 0). On d 7, the CIDR device was removed and a 25-mg i.m. injection of $PGF_{2\alpha}$ (Lutalyse®; Pfizer Inc.) was administered. Heifers in the CIDR-ECP synchronized group (n = 54) were given 0.5-mg i.m. injection of ECP 24 h after CIDR removal. Heifers (n = 56) assigned to the CIDR-GnRH

CIDR-ECP protocol



CIDR-GnRH protocol

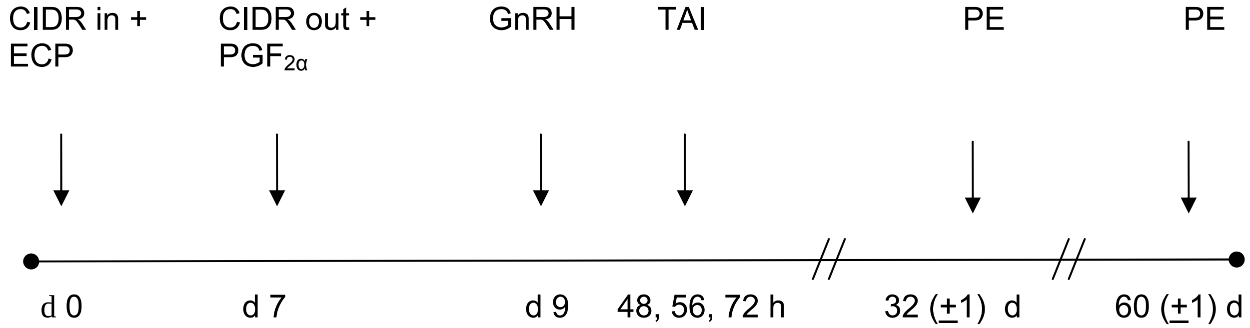


Figure 2. Experimental protocol used for dairy heifers synchronized with intravaginal progesterone inserts (CIDR) combined with either estradiol cypionate (ECP) at 24 h or with gonadotropin-releasing hormone (GnRH) at 48 h after CIDR removal followed by AI at either 48, 56, or 72 h after CIDR removal. Following timed AI (TAI), pregnancy exams (PE) were preformed 32 ±1 d and 60 ±1 d post AI to determine pregnancy rate and embryo survival rate.

synchronization group were given 100-μg i.m. injection of GnRH (Cystorelin®; Merial Ltd., Iselin, NJ.) 48 h after CIDR device removal. Heifers were not observed for estrus activity at any time during the study. Proven sires of known fertility were obtained from a single AI organization and inseminations were preformed by experienced AI technicians.

In a subset of heifers (n = 30), ovarian ultrasonographic examinations were performed daily from d 0 to 7 and twice daily from d 8 to ovulation to monitor follicular development, emergence of a new wave of follicular development, the size of the ovulatory follicle, and ovulation.

Pregnancy diagnosis was performed by ultrasonography with a 7.4 MHz broadband curved-array transducer (Sonosite 180PLUS; SonoSite, Inc., Bothell, WA) at 32 (±1) d following AI. A second pregnancy diagnosis was performed 60 (±1) d post AI to confirm pregnancy and determine embryo survival rate (ESR). The PR was defined as the number of heifers pregnant divided by the number of heifers synchronized. The ESR was the number of heifers diagnosed open 60 (±1) d post AI divided by the total number of heifers diagnosed pregnant 32 (±1) d post AI.

Data and Statistical Analysis

Interval from CIDR removal to ovulation and diameter of the largest or dominant follicle were measured on the initial 30 heifers. One-way analysis of variance was used to determine differences in the means for heifers treated with CIDR-ECP (n = 15) and CIDR-GnRH (n = 15).

First service PR was analyzed using logistic regression (SAS® Institute; Cary, NC) in a model that included month by year insemination, AI technician, breed, and AI time. Months were combined to avoid extremely small categories. Time of AI subclasses were evaluated using linear contrasts to test treatment, insemination at 56 h versus the average of 48 h and 72 h insemination times within each treatment, and 48 h versus 72 h within treatment. Results of the logistic analyses were presented as odds ratios and 95% confidence intervals. Odds ratios, which are a measure of the strength of association between explanatory and response variables, were interpreted as odds of pregnancy occurring for a particular explanatory variable category relative to the baseline category for that variable when the other explanatory variables were controlled in the model: 1, no effect on pregnancy; >1, increased probability of pregnancy; and <1, decreased probability of pregnancy compared with the baseline category. The 95% confidence intervals show the precision of the odds ratio estimates. A confidence interval that contained the value of 1.0 suggested no significant difference between the category and the baseline category for that variable. Significance was declared at the $P \leq 0.05$ level and tendencies at the $P \leq 0.1$ level.

RESULTS AND DISCUSSION

Follicular Development and Ovulation

A new wave of follicular development was detected on average 3.7 ± 0.22 d after ECP injection at CIDR insertion. No difference in follicular development between the two synchronization protocols was detected, or expected, since treatments were identical to that point (Figure 3). Heifers given ECP 24 h after CIDR removal had a shorter ($P \leq 0.05$) interval from CIDR removal to ovulation than heifers given GnRH (63.8 ± 3.0 h and 71.6 ± 2.27, respectively); however, relative to the hormone injection

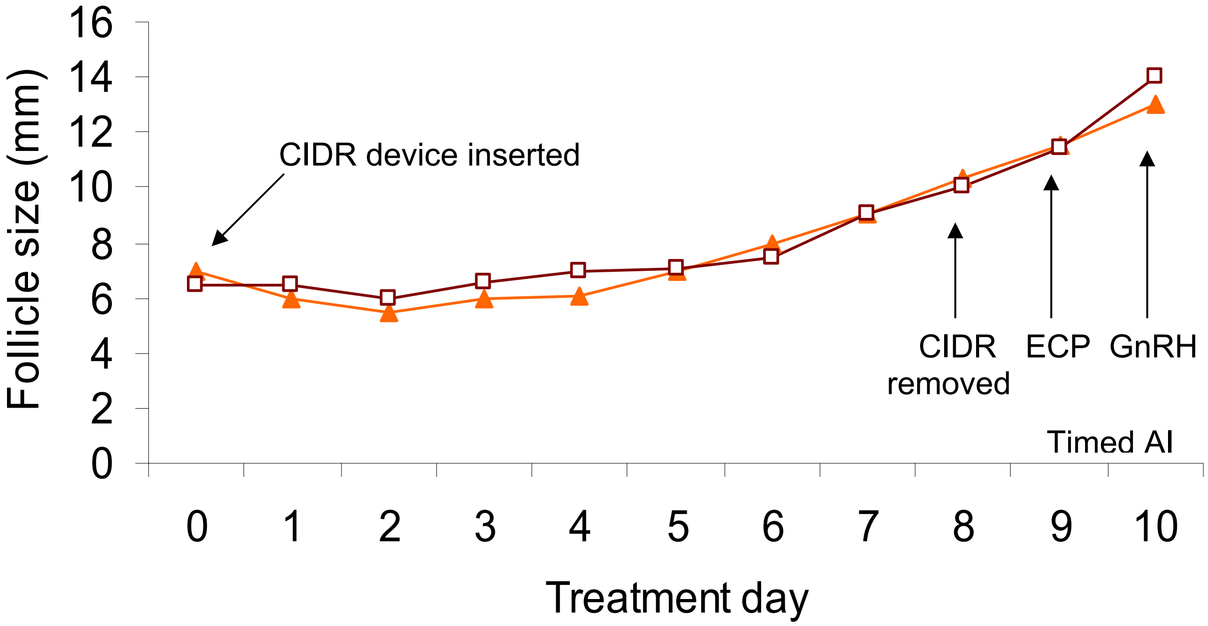


Figure 3. Average follicle size of largest or dominant follicle present for dairy heifers synchronized with intravaginal progesterone inserts (CIDR) combined with either estradiol cypionate (ECP) (▲) at 24 h with or gonadotropin-releasing hormone (GnRH) (□) at 48 h after CIDR removal followed by AI at either 48, 56, or 72 h after CIDR removal. Standard error for ECP group is ± 0.45 mm and ± 0.43 mm for the GnRH group.

after CIDR removal, ovulation occurred 39.8 ± 3.0 h in heifers given ECP and 23.6 ± 2.27 h for heifers given GnRH. Ovulation rate (100%) and diameter of ovulatory follicle (13.0 ± 0.45 and 14.0 ± 1.9 mm) did not differ ($P > 0.05$) between CIDR-ECP and CIDR-GnRH treatment groups, respectively (Table 1).

All heifers (30/30) in the subset that was subjected to daily ultrasound scanning regardless of treatment with either CIDR-ECP or CIDR-GnRH ovulated. Treatment with GnRH induces acute release of both LH and FSH, and if the dominant follicle present at GnRH injection has expressed LH receptors, it will ovulate (Colazo et al., 2004) and ovulation occurred 23.6 ± 10.1 h for CIDR-GnRH treated heifers. In contrast, administration of exogenous E_2 has been shown to synchronize follicular wave emergence regardless of stage of the dominant follicle when treatment was initiated (Adams et al., 1995; Bó et al., 1994). Mechanisms responsible for E_2 induced synchronization of follicular growth appear to involve suppression of plasma FSH concentrations by inhibition from the dominant follicle, followed by synchronous resurgence of FSH after atresia or removal of the dominant follicle (Bó et al., 2000; Bó et al., 1994). Therefore, the time from ECP injection to ovulation was much longer (39.8 ± 12.4 h) in heifers given CIDR-ECP and was the reason for administration of ECP 24 h prior to GnRH in the protocol design.

Table 1. Mean (±SE) of follicular activity for dairy heifers synchronized with intravaginal progesterone inserts (CIDR) combined with either estradiol cypionate (ECP) at 24 h or with gonadotropin-releasing hormone (GnRH) at 48 h after CIDR removal followed by AI at either 48, 56, or 72 h after CIDR removal.

	ECP treatment (n = 15)	GnRH treatment (n = 15)
Days to new follicular wave	3.94 ± 0.90	3.60 ± 0.99
Interval from CIDR device removal to ovulation [a], h	63.82 ± 3.00	71.60 ± 2.27
Diameter of dominant follicle, mm	13.00 ± 0.45	14.05 ± 1.90
Ovulation rate, %	100	100

[a] Means are statistically different ($P < 0.05$).

Pregnancy Rate at First Insemination

The effects of synchronization treatment, breed, or AI technician did not significantly influence first service PR (Table 2). The first service PR for Jersey heifers (64.1%) tended ($P < 0.1$) to be higher than the PR of Holstein heifers (57.7%). A majority of the month by year of AI for first service PR did not differ ($P > 0.1$); however, heifers bred in February 2003 had a lower probability of pregnancy ($P < 0.05$). Heifers bred in February 2003 had a 0.07 lower odds of pregnancy than heifers inseminated during August and September 2002, which was the baseline comparison group. The low PR for heifers bred during February 2003 was most likely due to extreme cold and wet environmental conditions during the period. All heifers were maintained on a dry lot without housing throughout out the study. The overall PR for heifers synchronized was 60.0%. The first service PR for CIDR-ECP synchronized heifers (63.0%) tended ($P < 0.1$) to be higher than the PR of CIDR-GnRH synchronized heifers (57.1%). The first service PR differed ($P < 0.05$) by AI time for heifers in the CIDR-ECP synchronized group. Heifers inseminated 56 h following the CIDR removal had a higher PR (81.8%) than heifers receiving inseminated 48 h or 72 h (66.7 vs. 50.0%, respectively) after CIDR removal; however, differences in PR for heifers AI at 48 or 72 h following CIDR removal were not significantly different. The first service PR did not differ among AI times in the CIDR-GnRH group (Figure 4). The ESR for CIDR-ECP treated heifers was 98% while all CIDR-GnRH treated heifers pregnant at d 32 were pregnant at d 60.

In a recent study (Lucy et al., 2001), the synchronization rate measured by a 3-d estrus detection period was higher ($P < 0.05$) in dairy heifers that received a

Table 2. Logistic binomial regression for effects of month and year of AI, breed, and AI technician on pregnancy rate (PR) of dairy heifers synchronized with intravaginal progesterone inserts (CIDR) combined with either estradiol cypionate at 24 h or with gonadotropin-releasing hormone at 48 h after CIDR removal followed by AI at either 48, 56, or 72 h after CIDR removal.

Category	AI	PR	Odds ratio[1]	95% Confidence interval
	(no.)	(%)		
Month and year of AI				
August-September 02	15	73.3	1.00	….
October-November 02	15	73.3	0.56	(0.07, 4.25)
February 03[2]	10	30.0	0.07	(0.01, 0.69)
March 03	10	80.0	2.08	(0.25, 17.02)
May 03	18	66.7	0.72	(0.14, 3.84)
August-September 03	15	53.3	0.34	(0.06, 1.90)
November 03	14	57.1	0.45	(0.08, 2.52)
January 04	13	38.5	0.20	(0.02, 1.98)
Breed				
Jersey	39	64.1	1.00	….
Holstein	71	57.7	0.73	(0.56, 2.09)
AI Technician				
1	60	56.7	1.00	….
2	37	70.3	2.02	(0.67, 6.07)
3	6	33.3	0.62	(0.04, 10.14)

[1] Odds ratio is the estimated odds of becoming pregnant for a heifer AI in a particular category relative to the baseline category for that variable for the effects of the other explanatory variables shown. Odds ratio: 1, no effect of pregnancy; >1, increased probability of pregnancy; and <1, decreased probability of pregnancy compared with the baseline category.

[2] $P < 0.05$.

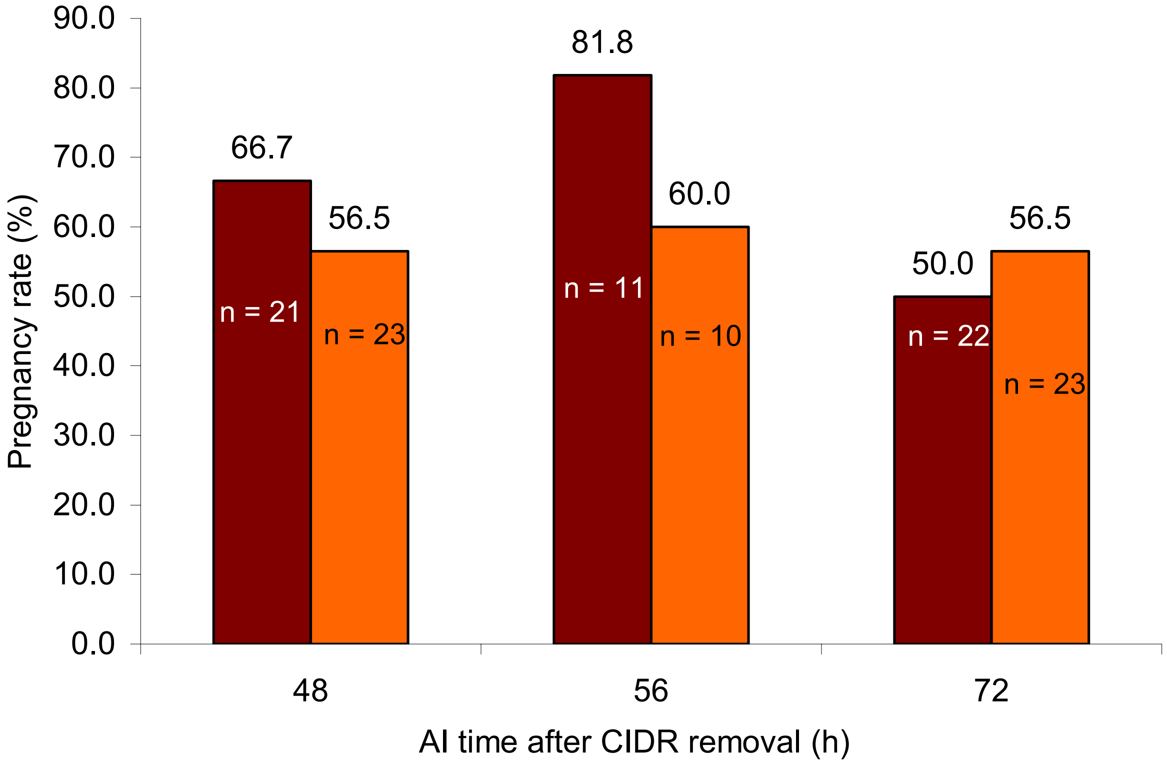


Figure 4. First service pregnancy rate for dairy heifers synchronized with intravaginal progesterone inserts (CIDR) combined with either estradiol cypionate (🟥) at 24 h or with gonadotropin-releasing hormone (GnRH) (🟧) at 48 h after CIDR removal followed by AI at either 48, 56, or 72 h after CIDR removal.

combination of CIDR inserts and $PGF_{2α}$ (84%) compared with heifers receiving only $PGF_{2α}$ (57%). The CR was higher in heifers receiving only $PGF_{2α}$ (65%) versus heifers receiving CIDR inserts and $PGF_{2α}$ (54%). The PR; however, was highest for heifers receiving CIDR inserts and $PGF_{2α}$ (45%) compared with heifers inseminated following $PGF_{2α}$ and visual estrus detection (37%). Another study (Macmillan et al., 1993) observed that 49% of heifers treated with a CIDR-based synchronization protocol combined with TAI were visually detected in estrus within 48 h after CIDR removal, while another 41% were visually detected in estrus 49 to 72 h following CIDR removal.

Summary

Timing of insemination markedly influenced ($P < 0.05$) PR for heifers in the CIDR-ECP treatment group. Heifers in the CIDR-ECP treatment group inseminated 56 h after CIDR removal had a higher PR (81.0%) compared with heifers inseminated at either 48 h (66.7%) or 72 h (50.0%) after CIDR removal. Timing of insemination did not influence ($P<0.1$) PR for heifers in the CIDR-GnRH treatment group. The interval from CIDR removal to ovulation was approximately 8 h shorter for heifers receiving ECP (63.8 versus 71.6 h). Therefore, using the difference in ovulation time after CIDR removal as the reference for TAI, the ideal AI time of ECP-treated heifers would be 52 h after CIDR removal compared with 60 h for heifers receiving GnRH 48 h following CIDR removal. In conclusion, ECP administration 24 h after CIDR removal resulted in PR comparable to those obtained in heifers administered GnRH 48 h after CIDR removal. Therefore, either CIDR-ECP or CIDR-GnRH may be used to synchronize ovulation in a CIDR-based TAI program in dairy heifers. To obtain the highest PR with TAI in heifers, the use of CIDR inserts and ECP 24 h after CIDR removal is recommended, with AI occurring approximately 52 h following CIDR removal or 32 h after ECP administration. In contrast, because of the later ovulation with GnRH, AI is recommended at approximately 60 h after CIDR removal or 12 h after GnRH administration.

Synchronization and Resynchronization of Ovulation and Timed Insemination in Lactating Dairy Cows

ABSTRACT

The reproductive performance of cows (n = 223) after synchronization with either CIDR inserts or Ovsynch followed by TAI was compared. Cows were randomly assigned to one of two synchronization protocols. Cows in the CIDR group (n = 110) received a combination of ECP, CIDR, $PGF_{2\alpha}$, and GnRH. Cows in the Ovsynch group (n = 113) were synchronized using a combination of GnRH, and $PGF_{2\alpha}$. Cows were bred at either 0, 8, or 24 h after the final GnRH injection. Pregnancy was diagnosed by ultrasonography 32 (±1) and 60 (±1) d post AI. The first service PR for Ovsynch synchronized cows was 33.6% and 27.3% for CIDR synchronized cows. However, no difference among AI times or synchronization protocols was revealed. The overall PR for first service was 30.5%. The average days open was 145. The first service embryo survival rate (ESR) for d 32 (±1) to 60 (±1) was 86.7% for cows synchronized with the CIDR protocol, while the ESR was 78.9% for cows receiving the Ovsynch protocol. The cumulative PR following second service for Ovsynch synchronized cows was 60.8% and was not significantly different from CIDR synchronized cows (55.3%). The second service PR for Ovsynch synchronized cows was 35.6%, and 37% for cows receiving the CIDR protocol. Cows with clinical mastitis prior to pregnancy had a tendency for lower odds of pregnancy. The ESR following second service was 88.9% for cows synchronized with the CIDR protocol and 71.4% for cows receiving the Ovsynch protocol. The cumulative PR following third service for Ovsynch synchronized cows was 71.1% and was not different ($P > 0.1$) from CIDR synchronized cows (72.8%). The third service PR for Ovsynch synchronized cows was 27.8% and 40% for

CIDR synchronized cows. Cows that had no structures on their ovaries during postpartum evaluation had lower odds of pregnancy. The ESR following third service was 88.9% for cows synchronized with the CIDR protocol and 90% for cows receiving the Ovsynch protocol. In conclusion, cows receiving the Ovsynch protocol resulted in PR comparable to that obtained in cows administered a CIDR insert. Therefore, Ovsynch or CIDR protocols may be used to synchronize ovulation in a TAI program in dairy cows.

Key words: synchronization, timed AI, reproductive performance, dairy cows

INTRODUCTION

Reproductive efficiency is a significant constituent of overall herd profitability of dairy operations. Factors that lead to efficient reproductive performance include herd health, nutrition, cow comfort, management decisions, and labor. Detection of estrus and timing of insemination; however, hinder reproductive performance on many dairy farms. Dairy herds enrolled in DRMS (Raleigh, NC) in January, 2004 reported that 38% of the expected estrus events were detected (n = 13,892).

Many factors influence reproductive performance; however, Fricke (2003) proposed that three strategies must be implemented early in the breeding period of lactating dairy cows to maintain reproductive efficiency. First, all cows must be bred by the end of the predetermined voluntary waiting period. Next, pregnancy status must be determined soon after insemination. Ultrasonography allows for early pregnancy diagnosis and detection of nonpregnant cows (Kastelic et al., 1988; Pierson et al., 1984). Finally, nonpregnant cows need to be quickly submitted for the next service. To achieve these goals, synchronization and resynchronization protocols need to be in standard practice.

Synchronization of estrus behavior through pharmacological control has been practice in dairy herds since the late 1970s. Methods of synchronizing estrus were originally devised to decrease the time spent detecting estrus; however, management strategies are now being used for convenience and efficiency in reproductive management. Moreover, the incorporation of a synchronization protocol should be based on a systematic approach for the entire herd rather than the individual cows to effectively influence reproductive performance and impact the income of the herd.

Basic research on the nature of follicular growth and atresia in cattle using transrectal ultrasonography resulted in the discovery of follicular waves (Savio et al., 1988; Sirois and Fortune, 1988), each wave culminating with the formation of a dominant follicle. The discovery of follicular waves made it clear that both follicular and luteal function must be controlled to accurately synchronize the estrous cycle and was the first step in the development of effective reproductive management strategies. Estrus and ovulation synchronization that regulate follicular development and luteal regression with GnRH and $PGF_{2\alpha}$ administered 7 d apart, respectively were established in the early 1990s (Thatcher et al., 1993; Wolfenson et al., 1994; Twagiramungu et al., 1995). The first synchronization protocol used as a management strategy that resulted in CR similar to those achieved with AI after a detected estrus was developed at the University of Wisconsin in 1995 (Pursley et al., 1995) and is commonly referred to as Ovsynch because ovulation rather than estrus is synchronized.

In an attempt to increase CR, Vasconcelos et al. (1999), using lactating cows, manipulated the estrous cycle such that the majority of cows were between d 5 to 12 of the estrous cycle when the Ovsynch protocol was initiated and compared it to Ovsynch that was initiated at a random stage of the estrous cycle. The Heatsynch protocol was developed to utilize a relatively low cost commercially available E_2 (ECP; Pfizer Animal Health, New York, NY) and required heat detection in combination with TAI of individuals not detected in estrus by the TAI period (Pancarci et al., 2002). Several researchers have evaluated the effectiveness of synchronization protocols that combine the use of $PGF_{2\alpha}$, GnRH, P_4, and E_2 in dairy cattle. Synchronization of dairy heifers with CIDR inserts has been evaluated in multiple studies (Lucy et al., 2001; Xu et al., 1999; Hanlon et al., 1997; Hanlon et al., 1996; Colazo et al., 2003; Colazo et al., 2004); however, little research has been conducted using the CIDR insert with TAI in lactating dairy cows.

The objectives of this study were to determine the PR and ESR for lactating dairy cows synchronized using a CIDR-based protocol or the Ovsynch protocol followed by TAI at either 0, 8, or 24 h after the final GnRH injection. In addition, cows that were diagnosed not pregnant at ultrasonography on d 31 (±1) were re-inseminated using the same initial protocol and PR and ESR for both protocols were compared after second and third services.

MATERIALS AND METHODS

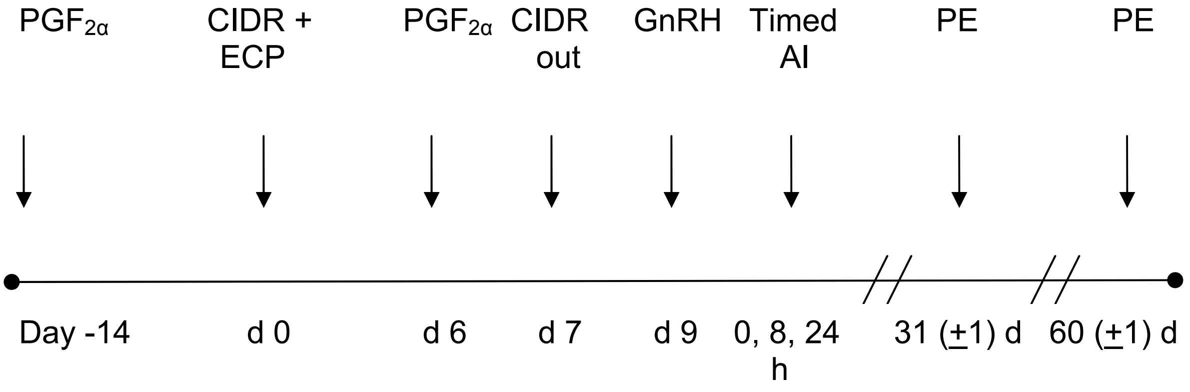
Cows and Treatment Protocols

The trial was conducted from August 2002 to March 2004 using both primiparous and multiparous lactating dairy cows (n = 223) from the Virginia Tech Dairy Center. The average days to first service was 79. Both Jersey (n = 68) and Holstein (n = 155) cows were managed in free stall barns and dry lots and milked twice a day. Cows were fed a total mixed ration twice daily consisting of corn silage, alfalfa haylage, soybean meal, high moisture corn, and a custom mineral mix to meet or exceed requirements for lactation (National Research Council, 2001).

Cows were randomly assigned to a synchronization protocol without regard to the stage of the estrous cycle at initiation of synchronization (Figure 5). Within the two synchronization protocols cows were randomly assigned to either 0 or 24 h TAI; however, a subset of cows was assigned randomly to a synchronization protocol but bred 8 h after the final GnRH (Cystorelin®; Merial Ltd., Iselin, NJ) injection. Cows in both synchronization treatment groups received a 25-mg i.m. injection of $PGF_{2\alpha}$ (Lutalyse®; Pfizer Inc.) 14 d prior to initiation of synchronization protocol. Cows (n = 110) synchronized using a CIDR received an intravaginal P_4 releasing device containing 1.38 g of P_4 (Eazi-Bred™ CIDR®; Pfizer Inc.) and 1-mg i.m. injection of ECP (ECP®; Pfizer Inc., New York, NY) was administered at device insertion (d 0). On d 6, a 25-mg i.m. injection of $PGF_{2\alpha}$ was administered and the CIDR was removed on d 7. On d 9, a 100-μg i.m. injection of GnRH was administered. Cows (n = 113) assigned the Ovsynch protocol received a 100-μg i.m. injection of GnRH on d 0 followed by a 25-mg i.m. injection of $PGF_{2\alpha}$ 7 d later. On d 9, a 100-μg i.m. injection of GnRH was administered. Visual estrus detection was not conducted at any time during the research. Proven sires of known fertility were obtained from a single AI organization and experienced AI technicians performed inseminations.

Pregnancy diagnosis was performed by ultrasonography with a 7.4-MHz broadband curved-array transducer (Sonosite 180PLUS; SonoSite, Inc., Bothell, WA) 32 (±1) d following AI. Nonpregnant cows were resynchronized using the same protocol for a maximum of three inseminations. A second pregnancy diagnosis was performed 60 (±1) d post AI to confirm pregnancy and determine ESR.

CIDR Treatment



Ovsynch Treatment

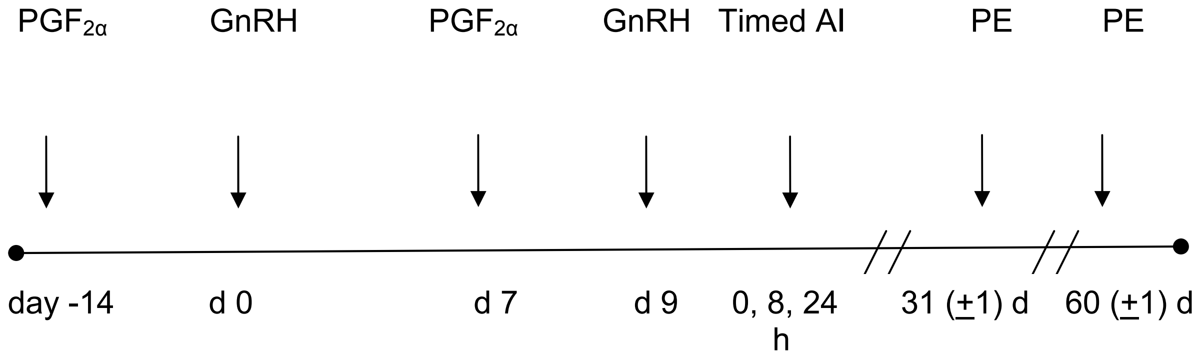


Figure 5. Experimental protocol used for lactating dairy cows synchronized with intravaginal progesterone inserts (CIDR) combined with estradiol cypionate (ECP) and gonadotropin-releasing hormone (GnRH) at 48 h after CIDR removal or Ovsynch followed by AI at either 0, 8, or 24 h after the final GnRH injection. Following timed AI, pregnancy exams (PE) were preformed 31 (±1) d and 60 (±1) d post AI to determine pregnancy rate and embryo survival rate.

Pregnancy rate was defined as the number of cows pregnant divided by the number of cows synchronized. Embryo survival rate was defined as the number of cows diagnosed open 60 (±1) d post AI divided by the total number cows diagnosed pregnant 32 (±1) d post AI. Second service cumulative PR was defined as the number of cows pregnant in both first and second services divided by the number of cows synchronized in both services. Third service cumulative PR was defined as the number of cows pregnant in all services divided by the number of cows synchronized in all services.

Information regarding postpartum evaluation, cases of clinical mastitis prior to first service or between first service and pregnancy exam, previous calving information, milk yield, and parity were collected using PCDART 7 management software (Dairy Record Management Systems, Raleigh, NC). Information from postpartum evaluations (20 to 60 d) for involution and measures reproductive soundness such as cases of metritis, enlarged uterus, and absences of ovarian structures (CL, follicles, cysts, and adhesions) were recorded. Similarly, clinical cases of mastitis or other diseases such as displaced abomasum, laminitis, clinical ketosis, hypocalcemia, and phenomena occurring prior to pregnancy exam (30±1) d post AI were entered.

Data and Statistical Analysis

First service PR was analyzed using logistic regression (SAS® Institute; Cary, NC) and a model that included breed, parity, summit milk, synchronization treatment, AI time, AI technician, season of AI, incidences of mastitis, metritis, and other disorders, and absences of structures on the ovaries. Summit milk was categorized by breed, divided into subclasses by production level, and was removed from the model as it was not significant. Months were grouped into seasons as follows: Season 1 = November, December, and January; Season 2 = February, March, and April; Season 3 = May, June, and July; and Season 4 = August, September, and October. Treatment-AI time subclasses were evaluated using linear contrasts to evaluate treatment, insemination at 8 h versus the average of 0 h and 24 h insemination times within each treatment, and 0 h versus 24 h within treatment. Resynchronizations were analyzed using logistic regression (SAS® Institute) in a model that included breed, lactation number, synchronization treatment, AI time, AI technician, season of AI, incidences of mastitis, metritis, and other disorders and absences of structures on the ovaries. Twenty-three cows were removed from the resynchronization models because they were not diagnosed pregnant after the first service and did not receive a second or third service. Results of the logistic analyses were presented as odds ratios and 95% confidence intervals. Odds ratios, which are a measure of the strength of association between explanatory and response variables, were interpreted as odds of pregnancy occurring for a particular explanatory variable category relative to the baseline category for that variable when the other explanatory variables were controlled in the model: 1, no effect on pregnancy; >1, increased probability of pregnancy; and <1, decreased probability of pregnancy compared with the baseline category. The 95% confidence intervals show the precision of the odds ratio estimates. A confidence interval that contains the value of 1.0 suggests no significant difference between the category and the baseline category for that variable. Significance was declared at the $P \leq 0.05$ level and tendencies at the $P \leq 0.1$ level.

RESULTS AND DISCUSSION

Pregnancy Rate at First Service

Effects of synchronization treatment, AI time, breed, lactation number, and AI technician did not influence first service PR (Table 3). Additionally, the presence of mastitis, metritis, and other diseases and absences of structures on the ovaries lowered first service PR, but not significantly. The overall PR for first service was 30.5%. The first service PR for Ovsynch synchronized cows (n = 113) was 33.6% and ranged from 26.7% for 0 h to 38.5% for 8 h, and 38.1% for 24 h, while the first service PR for CIDR synchronized cows (n = 110) was 27.3% and ranged from 23.7% for 0 h to 29.4% for 8 h and, 28.9% for 24 h. No difference ($P > 0.1$) among AI times or synchronization protocols was revealed (Figure 6). The first service PR for Jersey cows (38.2%) was not significantly different ($P > 0.1$) than the PR of Holstein cows (27.1%). The first service PR for primiparous cows (33.0%) was not significantly different ($P > 0.1$) than the PR of multiparous cows (28.7%). The average days open was 145. The first service ESR for d 32 (±1) to 60 (±1) was 86.7% for cows synchronized with the CIDR protocol, while the ESR was 78.9% for cows receiving the Ovsynch protocol (Figure 7).

Abundant research has been conducted using the Ovsynch protocol for synchronization (Burke et al., 1996; Pursley et al., 1995; Pursley et al., 1998; Cordoba and Fricke, 2001). Timing of AI following the Ovsynch protocol has ranged from 0 to 24 h (Pursley et al., 1998; Burke et al., 1996). First service PR ranged from 29% (Burke et al., 1996) to 41% (Pursley et al., 1998), which is similar to the PR revealed in

Table 3. Logistic binomial regression for effects of month and year of AI, breed, and AI technician on pregnancy rate (PR) following first service of lactating dairy cows synchronized with intravaginal progesterone inserts (CIDR) combined with gonadotropin-releasing hormone (GnRH) at 48 h after CIDR removal or the Ovsynch protocol followed by AI at either 0, 8, or 24 h after the final GnRH injection.

Category	AI	PR	Odds ratio[1]	95% Confidence interval
	(no.)	(%)		
Season of AI				
November - January	89	30.3	1.00	….
February - April	23	39.1	1.39	(0.47, 3.71)
May - July	46	32.6	1.10	(0.44, 2.46)
August - October	65	26.2	0.83	(0.37, 1.87)
Breed				
Jersey	68	38.2	1.00	….
Holstein	155	27.1	0.61	(0.31, 1.20)
Lactation number				
2+	129	28.7	1.00	….
1	94	33.0	1.24	(0.66, 2.35)
AI technician				
1	20	35.0		
2	112	27.7	0.77	(0.25, 2.33)
3	91	33.0	0.86	(0.28, 2.64)
Diseases				
No mastitis	183	32.8	1.00	….
Mastitis[2]	40	20	0.52	(0.21, 1.25)
No uterine problems	201	31.3	1.00	….
Uterine problems[3]	22	22.7	0.49	(0.16, 1.53)
Structures on ovaries	208	31.3	1.00	….
No structures on ovaries[4]	15	20	0.49	(0.13, 1.91)
No diseases	171	31	1.00	….
Other diseases[5]	52	28.9	0.79	(0.38, 1.64)

[1] Odds ratio is the estimated odds of becoming pregnant for a cow inseminated in a particular category relative to the baseline category for that variable for the effects of the other explanatory variables shown. Odds ratio: 1, no effect of pregnancy; >1, increased probability of pregnancy; and <1, decreased probability of pregnancy compared with the baseline category.

[2] Clinical mastitis prior to pregnancy exam (30±1 d).

[3] Enlarged uterus or metritis during postpartum evaluation (20 to 60 d).

[4] No structures on the ovaries during postpartum evaluation (20 to 60 d).

[5] Other diseases prior to pregnancy exam (30±1 d).

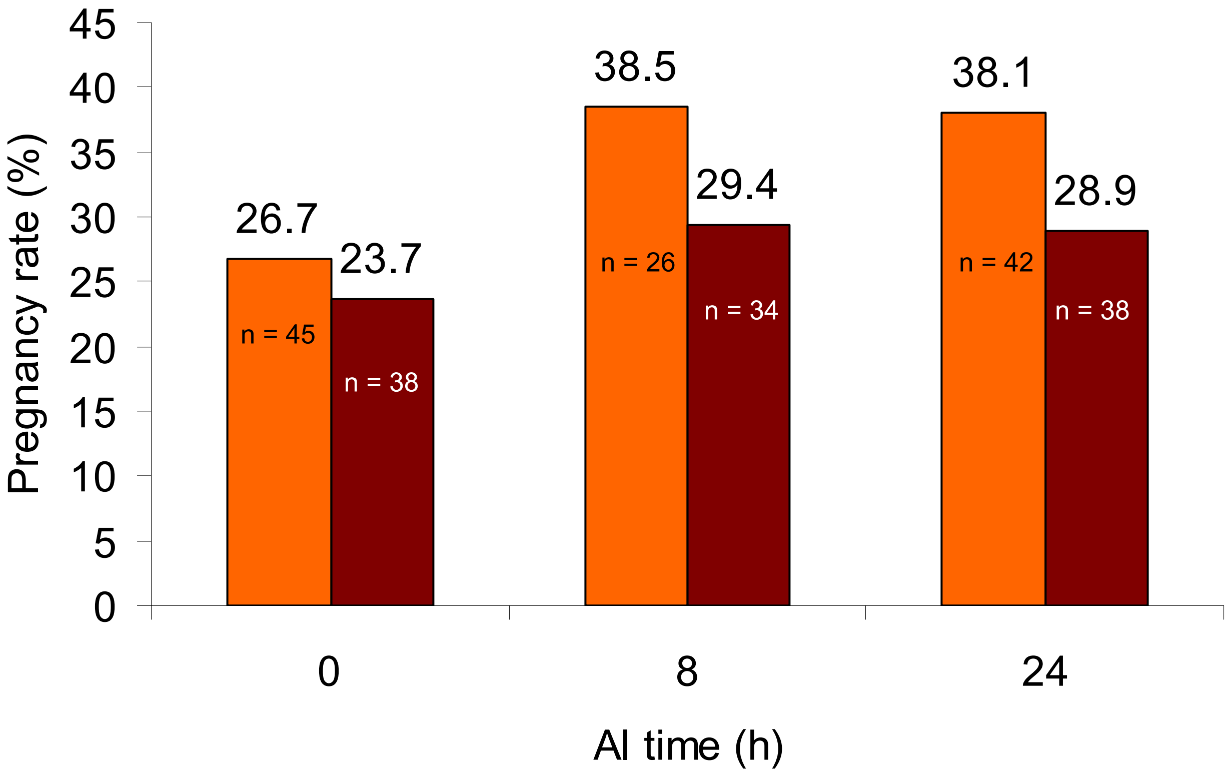


Figure 6. First service pregnancy rate for lactating dairy cows synchronized with Ovsynch (🟧) or intravaginal progesterone inserts (CIDR) combined with gonadotropin-releasing hormone after CIDR removal (GnRH) (🟥) followed by AI either at 0, 8, or 24 h after the final GnRH injection.

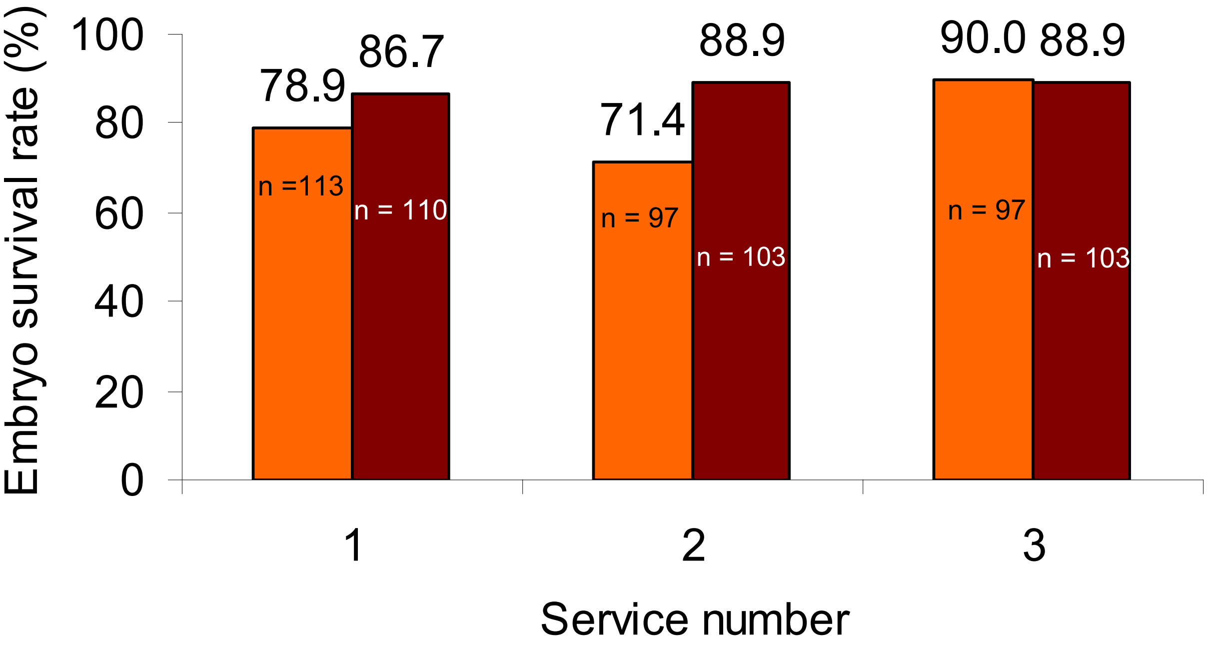


Figure 7. Embryo survival rate for lactating dairy cows synchronized and resynchronized with Ovsynch (🟧) or intravaginal progesterone inserts (CIDR) combined with gonadotropin-releasing hormone (GnRH) at 48 h after CIDR removal (🟥) followed by AI at either 0, 8, or 24 h after the final GnRH injection.

the current study. A 22% pregnancy loss (88%, ESR) for d 25 to calving or subsequent insemination using the Ovsynch protocol has been reported (Pursley et al., 1998), which is one percentage point higher than the current pregnancy loss in cows synchronized using the Ovsynch protocol. Researchers have reported slight differences in the reproductive performance for the Ovsynch protocol. Fricke (2003) concluded that the number of anovulatory cows, follicular dynamics, and the ability of the farm personnel to implement the protocol, all contribute to different success rates when implementing the Ovsynch protocol. In addition, physiologic factors associated with milk production could affect response to the Ovsynch protocol (Cordoba and Fricke, 2001).

Improved fertility at first service using CIDR inserts and TAI was reported in dairy cows (El-Zarkouny et al., 2004; Kim et al., 2003). These researchers found that first service PR was higher in cows synchronized using a combination of Ovsynch and CIDR inserts compared with cows receiving only Ovsynch. In the current study, there was no difference between the PR for cows synchronized using the Ovsynch protocol (33.6%) or the CIDR protocol (27.3%). Researchers have speculated that higher PR is obtained using CIDR-based protocols, because of the increase in circulating P_4 (Adams et al., 1992; Smith and Stevenson, 1995); therefore, improved fertility using Ovsynch combined with CIDR inserts could occur (El-Zarkouny et al., 2004). There have been slight differences in the reproductive performance among CIDR-based protocols, which could be due to differences in the nutritional status or energy balance of cows during early lactation (Macmillan and Peterson, 1993).

Resynchronization

The effects of resynchronization treatment, AI time, breed, parity, AI technician, incidences of mastitis, metritis, and other diseases or absences of structures on the ovaries did not significantly influence the cumulative PR following second service (Table 4). The cumulative PR following second service (Figure 8) for Ovsynch synchronized cows (n = 97) was 60.8% and was not different ($P > 0.1$) than CIDR synchronized cows (n = 103) (55.3%). The second service PR for Ovsynch synchronized cows was 35.6% and the PR for CIDR synchronized cows was 37% (Figure 9). Cows that had clinical mastitis prior to pregnancy had a tendency for lower odds ($P < 0.06$) of pregnancy than cows with no mastitis. Cows with clinical mastitis had 0.45 lower odds of pregnancy than cows inseminated without clinical mastitis, which was the baseline comparison group. Clinical mastitis was observed prior to first service and between first service and pregnancy exam (30±1) d post AI. The ESR following second service was 88.9% for cows synchronized with the CIDR protocol and 71.4% for cows receiving the Ovsynch protocol (Figure 7).

Mastitis has been related to a decrease in reproductive performance (Moore et al., 1991; Barker et al., 1998). Moore et al. (1991) reported a negative correlation between clinical mastitis and reproduction due to altered interestrus intervals and decreased length of the luteal phase in cows with clinical mastitis caused by gram-negative mastitis pathogens. Recent research (Barker et al., 1998) demonstrated that onset of

Table 4. Logistic binomial regression for effects of month and year of AI, breed, and AI technician on cumulative percent pregnant following second service of lactating dairy cows diagnosed not pregnant after first insemination and resynchronized with intravaginal progesterone inserts (CIDR) combined with gonadotropin-releasing hormone (GnRH) at 48 h after CIDR removal or the Ovsynch protocol followed by AI at either 0, 8, or 24 h after the final GnRH injection.

Category	AI	Cumulative percent pregnant	Odds ratio[1]	95% Confidence interval
	(no.)	(%)		
Season of AI				
November - January	89	56.9	1.00	….
February - April	23	61.1	0.92	(0.29, 2.92)
May - July	46	60.0	0.98	(0.41, 2.32)
August - October	65	56.9	0.90	(0.41, 1.95)
Breed				
Jersey	64	60.9	1.00	….
Holstein	136	56.6	0.89	(0.45, 1.77)
AI technician				
1	12	75.0	1.00	….
2	98	61.2	0.92	(0.13, 2.44)
3	90	52.2	0.56	(0.08, 1.51)
Diseases				
No mastitis	167	60.5	1.00	….
Mastitis[2,3]	33	45.5	0.45	(0.20, 1.02)
No uterine problems	180	56.7	1.00	….
Uterine problems[4]	20	70.0	1.40	(0.47, 4.19)
Structures on ovaries	185	59.5	1.00	….
No Structures on ovaries[5]	15	40.0	0.38	(0.12, 1.21)
No diseases	155	60.7	1.00	….
Other diseases[6]	45	48.9	0.55	(0.27, 1.13)

[1] Odds ratio is the estimated odds of becoming pregnant for a heifer AI in a particular category relative to the baseline category for that variable for the effects of the other explanatory variables shown. Odds ratio: 1, no effect of pregnancy; >1, increased probability of pregnancy; and <1, decreased probability of pregnancy compared with the baseline category.

[2] $P < 0.10$.

[3] Clinical mastitis prior to pregnancy exam (30±1 d).

[4] Enlarged uterus or metritis during postpartum evaluation (20 to 60 d).

[5] No structures on the ovaries during postpartum evaluation (20 to 60 d).

[6] Other diseases prior to pregnancy exam (30±1 d).

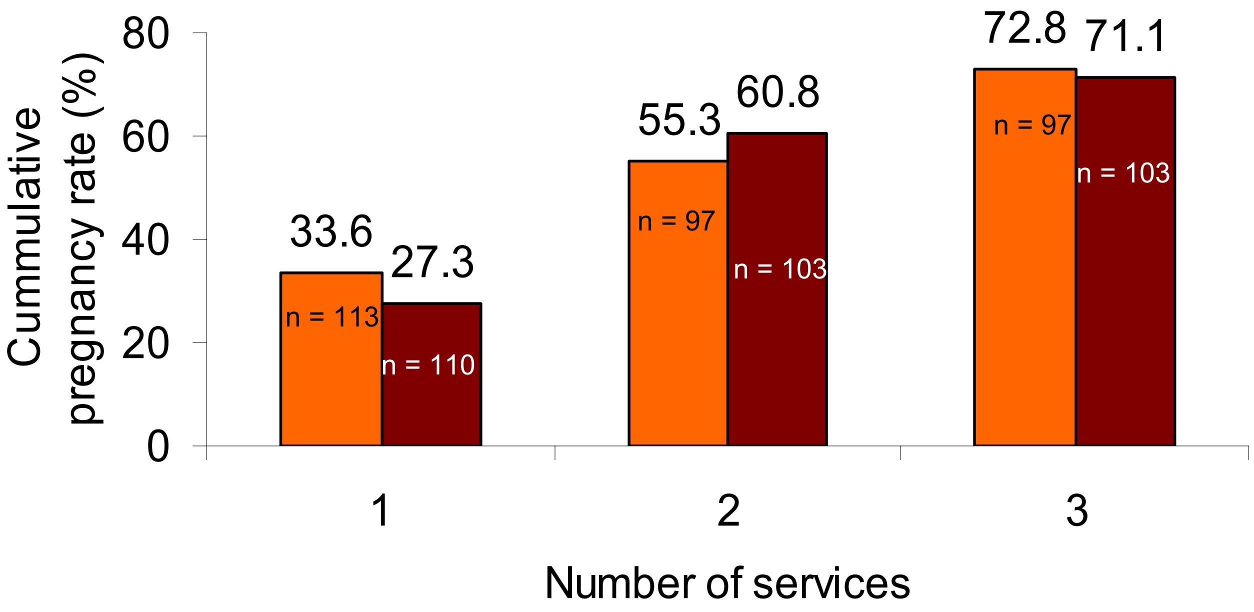


Figure 8. Cumulative percent pregnant for lactating dairy cows synchronized and resynchronized with Ovsynch (🟧) or intravaginal progesterone inserts (CIDR) combined with gonadotropin-releasing hormone (GnRH) at 48 h after CIDR removal (🟥) followed by AI at either 0, 8, or 24 h after the final GnRH injection.

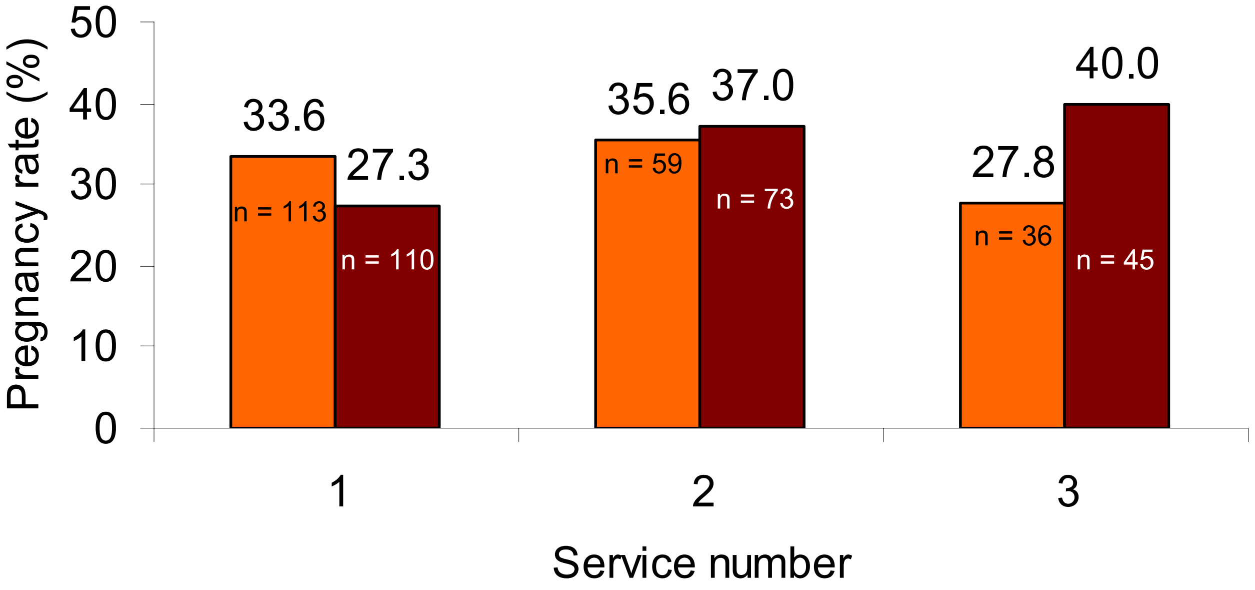


Figure 9. Pregnancy rate for lactating dairy cows synchronized and resynchronized with Ovsynch (🟧) or intravaginal progesterone inserts (CIDR) combined with gonadotropin-releasing hormone (GnRH) at 48 h after CIDR removal (🟥) followed by AI at either 0, 8, or 24 h after the final GnRH injection.

clinical mastitis before first service increased the number of days to first service and days open. Decreased reproductive performance due to mastitis before first service could be contributed to insufficient follicular development, anovulation resulting from blockage of the luteinizing hormone surge, or decreased E_2 synthesis, resulting in loss of behavioral estrus (Barker et al., 1998). Clinical mastitis after first service leads to early embryonic death from luteolysis and loss of P_4 dominance (Barker et al., 1998).

The effects of resynchronization treatment, AI time, breed, parity, AI technician, and incidences of metritis or mastitis did not significantly influence cumulative PR following third service (Table 5). The cumulative PR (Figure 80) following third service for Ovsynch synchronized cows (n = 97) was 71.1% and was not significantly different ($P > 0.1$) than CIDR synchronized cows (n = 103) (72.8%). The third service PR for Ovsynch synchronized cows was 27.8% and that for CIDR synchronized cows was 40% (Figure 9). Cows with no structures on their ovaries during postpartum evaluation (20 to 60 d) had lower odds ($P < 0.05$) of pregnancy than cows with structures on the ovaries, which was the baseline comparison group.. Cows with no structures on the ovaries had 0.65 lower odds of pregnancy than cows inseminated with structures on the ovaries. The ESR following third service was 88.9% for cows synchronized with the CIDR protocol and 90% for cows receiving the Ovsynch protocol (Figure 7).

Prolonged luteal phase and delayed first ovulation prior to breeding has been reported to decrease reproductive performance in dairy cows (Shrestha et al., 2004a). A recent study (Shrestha et al., 2004b) in Japan reported that 46% of cows failed to

Table 5. Logistic binomial regression for effects of month and year of AI, breed, and AI technician on cumulative percent pregnant following third service of lactating dairy cows diagnosed not pregnant after first and second insemination and resynchronized with intravaginal progesterone inserts (CIDR) combined with gonadotropin-releasing hormone (GnRH) at 48 h after CIDR removal or the Ovsynch protocol followed by AI at either 0, 8, or 24 h after the final GnRH injection.

Category	AI	Cumulative percent pregnant	Odds ratio[1]	95% Confidence interval
	(no.)	(%)		
Season of AI				
November - January	72	76.4	1.00	….
February - April	18	77.8	1.11	(0.28, 4.46)
May - July	45	66.7	0.67	(0.26, 1.71)
August - October	65	69.2	0.63	(0.27, 1.51)
Breed				
Jersey	64	75	1.00	….
Holstein	136	70	0.89	(0.41, 1.90)
AI technician				
1	12	91.7	1.00	….
2	98	74.5	0.36	(0.04, 3.15)
3	90	66.7	0.22	(0.03, 1.90)
Diseases				
No mastitis	167	73.1	1.00	….
Mastitis[3]	33	66.7	0.55	(0.23, 1.32)
No uterine problems	180	72.2	1.00	….
Uterine problems[4]	20	70.0	0.75	(0.24, 2.29)
Structures on ovaries	185	74.1	1.00	….
No structures on ovaries[2,5]	15	46.7	0.24	(0.08, 0.79)
No diseases	155	72.9	1.00	….
Other diseases[6]	45	68.9	0.65	(0.29, 1.43)

[1] Odds ratio is the estimated odds of becoming pregnant for a heifer insemination in a particular category relative to the baseline category for that variable for the effects of the other explanatory variables shown. Odds ratio: 1, no effect of pregnancy; >1, increased probability of pregnancy; and <1, decreased probability of pregnancy compared with the baseline category.

[2] $P < 0.05$.

[3] Clinical mastitis prior to pregnancy exam (30±1 d).

[4] Enlarged uterus or metritis during postpartum evaluation (20 to 60 d).

[5] No structures on the ovaries during postpartum evaluation (20 to 60 d).

[6] Other diseases prior to pregnancy exam (30±1 d).

resume ovarian cyclicity within 65 d postpartum. This delay adversely affects CR and ultimately calving interval in a TAI protocol. In addition, negative energy balance indicated by lower BCS in the early postpartum period can cause anovulatory ovaries or ovarian cysts. It was suggested that cows with greater negative energy balance have lower levels of insulin-like growth factor one (IGF-I) and LH, which promote ovarian follicular development (Lucy, 2000). The reduction in IGF-1 and LH may compromise ovarian follicular growth and development, which may lead to anovulatory ovaries, ovarian cysts, and nonfunctioning CL in postpartum cows (Shrestha et al., 2004b). Therefore, early resumption of ovarian activity will improve reproductive efficiency in dairy cows (Thatcher et al., 1973; Stevenson and Call, 1983).

Summary

First service PR for cows synchronized using a CIDR insert was not different from cows synchronized using the Ovsynch protocol. The PR following first service for all synchronized cows was 30.5%, and the ESR was 82.8%. The cumulative PR following second service cows synchronized using a CIDR insert was not different than cows synchronized using the Ovsynch protocol. The cumulative PR following second service for all synchronized cows was 58.1% and the ESR was 80.1%. Cows that had clinical mastitis prior to pregnancy exam (30±1) d post AI had a tendency for lower odds ($P < 0.06$) for pregnancy than cows with no mastitis in the second service. The cumulative PR following third service for cows synchronized using a CIDR insert was not different than cows synchronized using the Ovsynch protocol with one presynchronization injection. The cumulative PR following third service for all synchronized cows was 33.5% and the ESR was 89.4%. Cows with no structures on their ovaries during postpartum evaluation had lower odds of pregnancy than cows with structures on the ovaries. In conclusion, cows administered the Ovsynch protocol at first service or upon resynchronization resulted in PR comparable to that obtained in cows administered a CIDR insert. Cumulative PR (~72%) following third service was not different for Ovsynch or CIDR synchronized cows. Therefore, Ovsynch or CIDR protocols may be used to synchronize ovulation in a TAI program in dairy cows.

CONCLUSIONS

In the first study, CIDR-based synchronization protocols combined with TAI were used in dairy heifers and reproductive performance was evaluated. Heifers in the CIDR-ECP treatment group inseminated 56 h after CIDR removal had the highest PR. Heifers in the CIDR-GnRH treatment group did not differ in the PR as related to TAI. The interval from CIDR removal to ovulation was approximately 8 h shorter for heifers receiving ECP. Therefore, using the difference in ovulation time after CIDR removal as the reference for TAI, the ideal AI time for ECP-treated heifers would be 52 h after CIDR removal compared with 60 h for heifers receiving GnRH 48 h following CIDR removal. In conclusion, ECP administration 24 h after CIDR removal resulted in PR comparable to that obtained in heifers administered GnRH 48 h after CIDR removal. Therefore, either CIDR-ECP or CIDR-GnRH may be used to synchronize ovulation in a CIDR-based TAI program in dairy heifers. To obtain the highest PR with TAI in heifers, the use of CIDR inserts and ECP 24 h after CIDR removal is recommended with AI occurring approximately 52 h following CIDR removal or 32 h after ECP administration. In contrast, because of the later ovulation with GnRH, AI is recommended at approximately 60 h after CIDR removal or 12 h after GnRH administration.

In early 2004, the United States Food and Drug Administration removed ECP from the marketplace; therefore, ECP can no longer be used in synchronization protocols. The CIDR-GnRH protocol allows heifers to be synchronized and TAI with animal handling only occurring twice a week. This is advantageous for most dairy operations as heifers are normally housed in remote locations with minimal working facilities.

In the second study, CIDR-based synchronization protocols combined with TAI were compared to the Ovsynch protocol combined with TAI. First service PR for cows synchronized using a CIDR insert was not different than cows synchronized using the Ovsynch protocol. The PR following first service for all synchronized cows was 30.5% and the ESR was 82.8%. The PR after second service for all synchronized cows was 36.3% and the ESR was 80.1%. After two services the cumulative PR for Ovsynch synchronized cows was 60.8% and the PR for CIDR synchronized cows was 55.3%. Cows having clinical mastitis prior to pregnancy exam (30±1) d post AI tended to have lower PR. The PR after third service for all synchronized cows was 33.9% and the ESR was 89.4%. After three services the cumulative PR for Ovsynch synchronized cows was 71.1% and the PR for CIDR synchronized cows was 72.8%.Cows with no structures on their ovaries during postpartum evaluation had lower odds of pregnancy. In conclusion, cows administered the Ovsynch protocol resulted in PR comparable to that obtained in cows administered a CIDR insert. Therefore, Ovsynch or CIDR protocols may be used to synchronize ovulation in a TAI program in dairy cows. The Ovsynch protocol continues to achieve acceptable reproductive performance in lactating dairy cows. In this study, the CIDR-based protocol was not superior to the Ovsynch protocol, and by implementing a CIDR insert into a synchronization protocol, the cost of synchronization increases. Therefore, based on economics it is recommended that Ovsynch be used on 1st and 2nd services and CIDR for 3rd service.

The recommended AI time for both Ovsynch and CIDR-based synchronization protocols is 8 to 24 h following the final GnRH injection.

Synchronization protocols combined with TAI that obtain acceptable reproductive performance (i.e., higher PR and ESR) will play a major role in the reproductive management of dairy cattle in the ever changing dairy industry. Managers on today's dairy operations are searching for tools that allow them to decrease labor and operating cost without sacrificing herd health, milk yield, or reproduction. Future studies should be conducted to determine the effectiveness of the current protocols in beef cattle. Synchronization studies with larger numbers may provide additional information about the affects of mastitis or lack of ovarian structures on reproductive performance.

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