

**THE INTERACTION OF SIRE FERTILITY AND TIMING OF AI IN A SYNCHRONIZATION
PROTOCOL**

Jeffrey Max Cornwell

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Committee:

Dr. Raymond L. Nebel, Chairman
Dr. Ramanathan Kasimanickam
Dr. Michael L. McGilliard
Dr. R. Michael Akers, Department Head

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ABSTRACT

The objectives of this study were to determine if fixed-timed artificial insemination (FTAI) at two different times, 0 or 24 h after GnRH administration, in a Presynch-Ovsynch protocol influenced the pregnancy rate (PR) when average and high fertility sires were used. Additionally, a second experiment was conducted to determine the effectiveness of CIDR inserts to allow for resynchronization of estrus in cows that did not conceive or maintain the conceptus at FTAI. Lactating Holstein cows (n = 1,457) from two well-managed dairy herds located in the piedmont region of North Carolina were utilized for 12 mo. First artificial insemination (AI) PR differed for fertility group and was 24.1 and 29.2% for average and high fertility group, respectively. Timing of AI did not influence first AI PR and there was no interaction of fertility group and timing of AI. Cows that received a CIDR insert were detected more frequently in estrus during a 4 d period, d 21 to 24, than control cows, 92.5 and 62.0%, respectively. However, the CIDR insert did not increase the detection of estrus compared to control cows over a normal estrus return interval of 7 d, 18 to 24 d after GnRH administration of a FTAI protocol, 28.8 and 34.2% respectively. In conclusion, the use of high fertility sires is a practical recommendation for improving first AI PR and CIDR inserts allowed more cows to be detected in estrus during a shorter interval, but did not increase the estrus detection rate during a normal estrus return interval.

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LIST OF ABBREVIATIONS

AI: artificial insemination

ATA: Agritech Analytics, LLC.

CIDR: controlled internal drug release

CL: corpus luteum

CR: conception rate

d: day(s)

DHI: Dairy Herd Improvement

DRMS: Dairy Records Management Systems

ERCR: estimated relative conception rate

FDA: Food and Drug Administration

FTAI: fixed time artificial insemination

h: hour(s)

mo: month(s)

NEBAL: negative energy balance

NR70: 70 day non-return rate

PGF_{2α}: Prostaglandin F_{2α}

PR: pregnancy rate

RE: resynchronization of estrus

TMR: total mixed ration

yr: year(s)

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INTRODUCTION

Genetic selection for greater milk yields in dairy cows has compromised endocrine profiles to favor lactation at the expense of reproductive performance (Nebel and McGilliard, 1993; Lopez et al., 2004). An inverse relationship between milk yield and reproductive performance has been documented (Butler, 2000; Washburn et al., 2002). Increasing confinement, labor, and capital issues for dairy managers have also caused the decrease in estrus detection rates on farms, decreasing the reproductive efficiency of a dairy herd (Britt et al., 1986; Lucy, 2001; Washburn et al., 2002). Development of a fixed time artificial insemination (FTAI) allowed for elimination of estrus detection because all cows were inseminated at a specific time relative to ovulation (Pursley et al., 1995). Subsequent studies have investigated various hormone administration protocols for improving conception rates (CR) obtained at a first FTAI protocol (Vasconcelos et al., 1999; Portaluppi and Stevenson, 2005).

Research studies have resulted in an extensive body of literature aimed at increasing CR from a cow management standpoint. Research investigating sire fertility and timing of AI interactions on CR is limited. Estimated relative conception rate (ERCR) is a measure of the fertility of an individual sire and is predictable and repeatable over the productive life of an AI sire if ample data have been collected (Clay and McDaniel, 2001). An increase in the ERCR value of a sire has economic benefit to a dairy manager and is through a probable CR advantage at artificial insemination (AI; Pecsok et al., 1994; Stevenson, 2004). A high fertility sire may have the advantage of providing a greater number of accessory sperm to an ovum, increasing the fertilization rate and selection of a competent spermatozoon capable of sustaining embryogenesis. Accessory sperm and timing of AI affect the embryo quality (Dalton et al., 2001). Saacke et al. (1994) reported that the number of accessory sperm able to reach the site of fertilization is unique to an individual sire. The CR for sires of different fertility inseminated at varying stages of estrus was investigated (MacMillan and Watson, 1975). Low fertility sires suffered a decreased CR when AI occurred in early or mid-estrus and there was no difference in CR among sire fertility groups when AI occurred in late or post-estrus.

The approval by the Food and Drug Administration (FDA) of the United States (Washington, D. C.) of a controlled internal drug release (CIDR[®]) insert in 2003 provided an alternative method of reproductive management in dairy cows. Studies have investigated the reliability of a CIDR insert to identify a greater number of non-pregnant cows in estrus after AI (Chenault et al., 2003; El-Zarkouny and Stevenson, 2004). Chenault et al. (2003) reported that a greater number of cows were detected in estrus during a 4 d period beginning 2 d after removal of the CIDR insert than cows without a CIDR insert over the normal 9 d period of estrus detection, 18 to 26 d after AI, 42.8 and 36.4%, respectively. El Zarkouny and Stevenson (2004) reported that a once-used CIDR insert failed to return a greater proportion of non-pregnant cows to estrus over a 6 d period compared to control cows that did not receive a CIDR insert, 33.9 and 28.7%, respectively.

LITERATURE REVIEW

REPRODUCTIVE CHALLENGES

Reproductive efficiency measured by average days open and services per conception in lactating dairy cows has decreased over the past half decade (Washburn et al., 2002). Average days open represents the average days cows in a herd remain in lactation before becoming pregnant and services per conception represent the average number of inseminations to achieve a pregnancy. Increases in both measures represent a loss in capital, labor, and milk yield during the productive life of the cow for dairy managers (Washburn et al., 2002). Reproductive efficiency measures among dairy herds are inconsistent because of changing farm management decisions. Butler et al. (1989) documented a 32% decrease in CR among lactating dairy cows from 1951 until 1973 while milk production in the same population of New York dairy cows increased by 33%. Washburn et al. (2002) examined 561 (532 Holstein and 29 Jersey) herds in the southeastern US. Using DHI information from Dairy Records Management Systems (DRMS) at Raleigh, NC, data were included for herds with records for 23 yr from 1976 to 1999. Rolling herd averages for milk production increased linearly for Jersey and Holstein herds and herd size increased more for Jersey herds, 66% compared to 55% for Holstein herds during the same period. Days open and services per conception were 122 ± 2.8 d and 1.91 ± 0.08 , respectively for Jerseys and 124 ± 0.7 d and 1.91 ± 0.02 ,

respectively for Holsteins in 1976 to 1978. Reproductive measures increased nonlinearly for both breeds. Days open increased to 152 ± 2.8 for Jerseys and 168 ± 0.7 for Holsteins in 1997 to 1999. Services per conception reached 2.94 ± 0.04 for both breeds in 1994 to 1996. The reciprocal of services per conception is an estimate of CR. Conception rates decreased 35% among these southeastern herds from the late 1970s to the late 1990s. Butler (2000) concluded that these decreases were due to lactation demands and not genetic selection because heifer CR remained at 1951 levels, greater than 55%. Butler (2000) reviewed the role of nutritional deficiency in postpartum dairy cattle leading to severe negative energy balance (NEBAL). At parturition, the nutritional requirements of a dairy cow increase as milk yield increases. As the lactational demands increase and become greatest around 60 d after parturition the lactating cow is unable to consume the energy needed for milk production, maintenance, growth, and reproduction. The severity and duration of the time a dairy cow remains in NEBAL depends on these demands. Nebel and McGilliard (1993) suggested that the selection for greater milk yields have compromised endocrine profiles to favor milk yield while compromising reproductive function. Lopez et al. (2004) provided data confirming estradiol concentrations were lower for higher producers than those for lower producers 1 d prior to estrus. Estradiol concentrations for high and low groups were 7.7 and 9.6 pg/ml, respectively ($P = 0.04$) despite high producers having a larger follicle size than low producers, 18.0 and 16.7 mm ($P = 0.01$). Hageman et al. (1991) reported that genetic selection for high milk yield in a research herd at the University of Wisconsin-Madison increased days open and calving interval approximately 10 d per 1,000 kg increase in milk production (305 d) for first and second parity compared to cows selected for average production. Nebel and McGilliard (1993) reviewed data from 4550 Holstein herds processed by DRMS. Herds were categorized into one of six production groups (kg/yr). As milk production increased, estrus detection efficiency also increased, from 29% for the lowest production group to 51% for the second highest production group. Higher estrus detection rates that suggest better management of higher producing herds leads to reduced CR. Lower producing herds had the highest CR at first AI of 52% while herds in the highest production group had the lowest CR at first AI of 38% suggesting an antagonistic relationship between milk yield and CR at first AI. Management of high producing herds must be able to

detect a greater percentage of cows in estrus in order to overcome lower CR. The number of cows that become pregnant in any 21 d cycle is a function of the number of cows determined to be in estrus and inseminated and the percentage of inseminated cows that conceive. The first AI pregnancy rate (PR) for the lowest production herds summarized by Nebel and McGilliard (1993) had an estrus detection rate of 29% and a CR of 52%. Thus, 15% of the cows available for AI at first AI became pregnant in these low producing herds compared to 20% for the highest producing herds.

The key for implementing new AI protocols revolves around the detection of estrus. Increased confinement on larger dairy farms decreases mounting activity and requires greater labor, capital, and time to manage reproductive efficiency (Britt et al. 1986; Lucy, 2001; Washburn et al. 2002). Britt et al. (1986) reported differences for cows confined to concrete and dirt lots. Cows located on dirt lots displayed estrus for a longer duration, 13.8 and 9.4 h, respectively and were mounted more frequently, 3.8 and 2.7, respectively than cows on concrete allowing for easier visual detection of estrus. Confining cows on concrete has compromised estrus detection rates. Lopez et al. (2004) divided concrete confined cows into a low production or high production group based on milk yield of 39.5 kg/ d. After 50 d postpartum cows received a radiotelemetric transmitter to allow for continuous monitoring of estrus with the Heatwatch[®] system. Estrus is defined by the time between the first and last mounting event (8.7 h) and the number of mounting events recorded per hour (2.7 mounts per h). A greater percentage of high producing cows had a shorter duration and lower intensity estrus than low producing cows, 53.4 and 32.2%, ($P < 0.0001$) respectively and were not different across parity. Estrus detection has become an increasingly crucial part of reproductive management because the higher producing cow display estrus for a short duration and low intensity. Currently more than 10% of US dairy herds utilize a FTAI protocol (Lucy 2001). Visual estrus detection is a challenge for these farms and PR has suffered because of poor rates of estrus detection. Overall, greater lactation demands and increasing dairy herd sizes have spurred the need for bovine reproductive research to better understand the estrous cycle and increase PR among dairy herds.

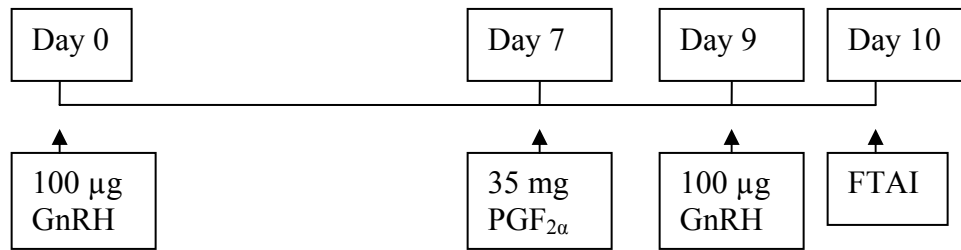


Figure 1: The hormone administration schedule and dosage rate for the Ovsynch protocol (Pursley et al., 1995).

OVSYNCH

Development of the Ovsynch protocol by Pursley et al. (1995) allows all cows to receive a specific FTAI (Figure 1). The protocol utilized one administration of 35 mg of prostaglandin ($\text{PGF}_{2\alpha}$) 7 d after and 2 d prior to 100 μg of gonadotropin releasing hormone (GnRH).

The first GnRH administration will initiate or start a new follicular wave when administered at a random stage of the estrous cycle. The second administration of the protocol $\text{PGF}_{2\alpha}$, will cause regression of the corpus luteum (CL) to allow emergence of a dominant follicle. The second administration of GnRH 48 h after $\text{PGF}_{2\alpha}$ will cause ovulation of the dominant follicle releasing an ovum for fertilization (Pursley et al., 1995). In the initial study, 100% of lactating cows ovulated within an 8 h period, 26 to 32 h after the last GnRH administration. All cows receive FTAI 24 h after the last GnRH administration because of synchronized ovulation of the dominant follicle. Many studies have investigated the Ovsynch protocol comparing CR to AI following the a.m. / p.m. guideline of a spontaneous estrus or $\text{PGF}_{2\alpha}$ induced estrus (Stevenson 1999, Jobst 2000, Pursley 1997a, Pursley 1997b). First AI PR of cows synchronized with Ovsynch is equal or greater than PR of cows bred either following spontaneous or following a $\text{PGF}_{2\alpha}$ induced estrus (Cartmill 2001a, Stevenson 1999, Pursley 1997a, Pursley 1997b). The advantage of a FTAI protocol at first AI is that 100% of eligible cows receive AI at a targeted period after calving. The herd manager for a dairy herd usually establishes a voluntary waiting period (VWP). Following the VWP, all cows should receive AI as soon as possible. Typically, programs that rely on estrus detection for first AI have a low PR due to low estrus detection rates. The decisive reproductive measure for a dairy herd is calving interval that represents how often the average cow is in peak lactation. A cow

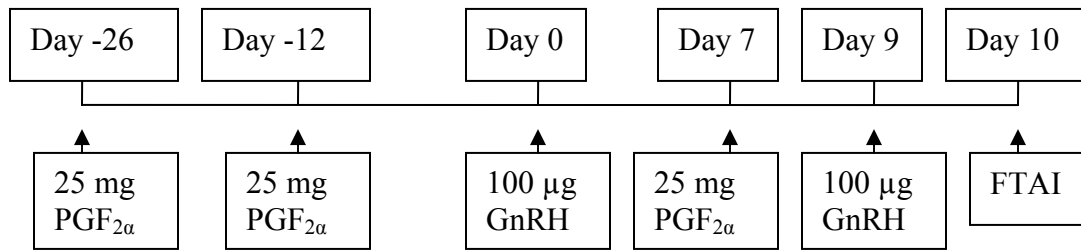


Figure 2: The hormone administration schedule and dosage rate for the Presynch-Ovsynch protocol (Vasconcelos et al., 1999).

that becomes pregnant immediately after the VWP will calve sooner and return to peak milk yield resulting in greater income for the producer.

PRESYNCH

As the Ovsynch protocol became accepted as a successful tool for first AI attempts were made to increase the resulting CR. Two administrations PGF_{2α} administrations 14 d apart and 12 d prior to the first administration of the Ovsynch protocol improved CR (Figure 2). Vasconcelos et al. (1999) examined the effects of beginning the Ovsynch protocol at varying stages of the estrous cycle. They reported that cows initiating the Ovsynch protocol in early diestrus, 5 to 9 d of the estrous cycle, experienced higher rates of ovulation at the subsequent GnRH administration than cows in metestrus, late diestrus, and proestrus leading to higher PR. In any random group of cycling cows, 62% will be in diestrus and possess a CL. Administration of PGF_{2α} will cause luteolysis of the CL. A second administration of PGF_{2α} 14 d later will cause luteolysis in 100% of the cows enrolled. These two PGF_{2α} administrations allow all cows to begin the Ovsynch protocol in early diestrus increasing the reproductive responsiveness to the initial administration of GnRH of the Ovsynch protocol.

Initial studies using the Presynch protocol administered PFG_{2α} 26 and 12 d prior to initiation of the Ovsynch protocol (Cartmill et al., 2001; Moreira et al., 2000; Moreira et al., 2001). A recent study examined the effects of giving the PGF_{2α} 14 d apart beginning 28 d before the first GnRH administration in order to set 1 d per wk where cows could receive four of the five administrations required for the Presynch protocol (Navanukraw et al., 2004) (Figure 3). The PR obtained for this modified Presynch protocol was similar to previous studies when compared to the Ovsynch protocol, 49.6

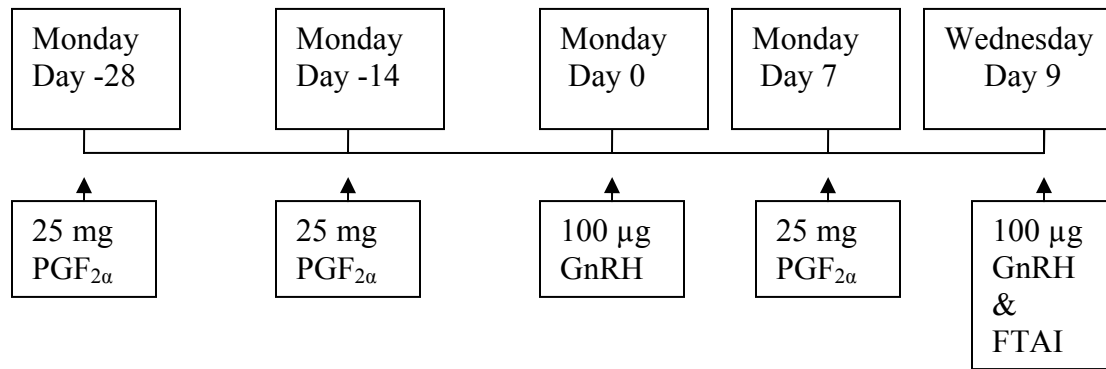


Figure 3: The Presynch-Ovsynch hormone administration schedule with FTAI using only two specific days of the week to minimize labor (Navanukraw et al.,

and 37.3%, ($P < 0.05$) respectively, while improving the protocol from a time management standpoint.

TIMING OF FINAL GnRH RELATIVE TO PGF_{2α}

In order for FTAI programs to be successful, ovulation of a dominant follicle must take place in a relatively tight timeline because AI takes place at the same time for all cows. Peters and Pursley (2003) examined four modified Ovsynch protocols with different times of the final GnRH administration after PGF_{2α}. Researchers injected GnRH 0, 12, 24, and 36 h after PGF_{2α}. Significant linear effects were found among the average ovulatory follicle size ($P < 0.05$) and the PR per insemination ($P < 0.0001$) as the amount of time increased between PGF_{2α} and GnRH. The PR per AI for GnRH times 0, 12, 24, and 36 were 8.8, 13.2, 21.4, and 28.0%, respectively. In the Ovsynch study by Pursley et al. (1995), the researchers investigated the administrations of PGF_{2α} and GnRH using a different method. Cows received GnRH on d 1 and 9. The researchers injected PGF_{2α} at times 0, 24, and 48 h before the last administration of GnRH. The CR for AI at time PGF_{2α} 0 was significantly lower than 24 and 48 h, 11, 46, and 55%, respectively ($P < 0.01$). After regression of the CL induced by PGF_{2α} the follicle must have sufficient time to mature. Studies suggest that the average ovulatory follicle of 14 mm is large enough for consistently higher CR (Peters and Pursley 2003), but it would be important to allow sufficient time for all cows to develop a mature dominant follicle of this size. Walker et al. (1996) used the Heatwatch[®] system to determine the onset of estrus. Walker et al. (1996) defined estrus as the first standing mount with a minimum of 3 standing mounts in a 4 h period, after administration of 25 mg of PGF_{2α} to be 73.1 ± 2.8 h. The

average ovulation time from the onset of estrus activity was 27.6 ± 5.4 h. Within a group of cows, 66% will experience spontaneous ovulation between 92 and 109 h after administration of PGF_{2 α} . Successful FTAI could occur with the last GnRH administration occurring at 72 h after PGF_{2 α} before most cows experience spontaneous ovulation. The optimal time to administer the final GnRH administration is probably somewhere between the standard 48 h after PGF_{2 α} and 72 h in order to minimize spontaneous ovulation before FTAI. Portaluppi and Stevenson (2005) compared three different Ovsynch protocols. At two different farms, the standard Ovsynch protocol, an Ovsynch protocol with FTAI occurring at the final GnRH administration, and an Ovsynch protocol with the second administration of GnRH and FTAI both occurring 72 h after the last PGF_{2 α} administration. Cows receiving GnRH and FTAI at 72 h after PGF_{2 α} had significantly higher CR than when GnRH is given 48 h post PGF_{2 α} and FTAI occurred at the same time or 24 h after the GnRH administration, 35, 23, and 29%, respectively ($P < 0.05$).

TIMING OF AI

Artificial insemination following detected estrus has generally occurred following the a.m. / p.m. guideline. Farms submit cows displaying estrus in the morning for AI in the evening and vice versa (Trimberger et al., 1948). Cows found displaying estrus would usually receive AI at the subsequent milking time. Nebel et al. (1994) found a fixed once-a-day AI time, in the middle of the day, would produce acceptable CR compared to breeding twice-a-day. Sixty-day non-return rates for once daily and a.m. / p.m. AI were 64.6 and 65.6%, respectively. Dalton et al. (2001) investigated the timing of AI at times 0, 12, and 24 h after the onset of estrus monitored by the Heatwatch system. They concluded that AI 12 h after the onset of estrus provided a compromise between functional sperm loss at 0 h and embryo quality at 24 h. In the initial Ovsynch study by Pursley et al. (1995), AI occurred 24 h after the final administration of GnRH. All cows on the study ovulated within 26 to 32 h after the last GnRH administration. This FTAI allowed sufficient time for sperm to reach the ovum for potential fertilization. Another study has evaluated several specific times for AI after the final GnRH administration. Pursley et al. (1998) randomly assigned cows ($n = 732$) to one of five AI times: 0, 8, 16, 24, or 32 h after the final GnRH administration. The PR per AI of 32%

was different at 32 h after GnRH administration than for AI at 0, 8, 16, and 24 h; 37, 41, 45, and 41%, respectively ($P < 0.05$). Although PR per AI for AI at 0, 8, 16, and 24 h after GnRH are not different, there was a quadratic effect among the five AI times with AI at 16 h after administration of GnRH having the greatest success of 45% ($P < 0.05$). Insemination at times 0, 8, 16, and 24 h produced the same calving percentages with only FTAI at 32 h becoming lower; 31, 31, 33, 29, and 20%, respectively ($P < 0.05$). Many dairy farms have adopted an AI time that fits the daily schedule of other cow activities. Insemination at the time of GnRH administration provides the benefit of reduction in labor because of handling cows only once. Reproductive research during the past 10 yr has allowed dairies to become more efficient by designing protocols for FTAI with hormone administrations and AI occurring during only two days of the week (Figure 3).

SPERM TRANSPORT

Most of the dairy cows in the United States receive AI by on-farm trained technicians who target deposition of semen in the uterine body following detected estrus or at a FTAI after synchronization. The deposited sperm must begin to move anteriorly through the reproductive tract toward the site of fertilization, the ampullary-isthmus junction, for conception to occur. Hawk (1983) reviewed the fate of sperm after AI. Two different modes of movement categorize spermatozoa that began to ascend the reproductive tract. A rapid phase movement of spermatozoa occurs within minutes of AI. Spermatozoa that experience this rapid phase of transport are damaged or non-motile and non-significant for fertilization and may even move through the reproductive tract and pass into the peritoneal cavity. Spermatozoa involved with conception undergo a sustained phase of transport where the spermatozoa began to ascend the reproductive tract over the next few hours. Wilmut and Hunter (1984) investigated the time required for spermatozoa transport into the oviducts. Heifers received AI within 8 h after the onset of estrus. The researchers ligated the oviducts at 6, 8, 10, and 12 h after AI in order to establish a minimum amount of time required for sustained sperm transport. Wilmut and Hunter (1984) recovered embryos and ova from the heifers 2 to 4 d after AI. The CR were different for the surgery times 6, 8, 10, and 12 hours post AI, 9, 40, 42, and 70%, respectively ($P < 0.05$). They suggested that a minimum of 6 h may be required for a viable population of spermatozoa to enter the oviduct capable of fertilization and the

process may still be incomplete as long as 12 h after AI because a significantly lower CR was obtained prior to ligation of the oviduct 12 h post AI. Hunter and Wilmut (1984) reported the fate of sperm that reach the oviduct by ligation and embryo or ova recovery. The viable sperm that entered the oviduct did not progress into the ampulla but remain in the caudal portion of the oviduct until ovulation. Suarez (2002) describes the formation and possible functions of a sperm reservoir in the oviduct. A variety of mammalian species has an oviductal sperm reservoir. As spermatozoa enter the isthmus through the uterotubal junction, which is about the width of a sperm head, they began to stick to the mucosal epithelium. One function of the reservoir may be to prevent polyspermy, fertilization by multiple sperm, by allowing only a few sperm to reach the oocyte in the ampulla. The reservoir may also maintain the fertility of sperm until ovulation occurs. A third function of the reservoir may be to regulate hyperactivation at the time of ovulation. Hyperactivation involves extreme flagellar movements and irregular patterns of travel in order for the spermatozoon to meet an ovum for the ultimate goal of fertilization. Ho and Suarez (2001) reviewed several studies concerning the hyperactivation of spermatozoa from the oviductal reservoir. The mechanisms causing hyperactivation of spermatozoa remain unanswered but are probably a result of interactions from hormones, ions, and epithelia secretions.

Dobrowolski and Hafez (1970) examined the movement of spermatozoa for three times after AI at the external os of the cervix of heifers following detected estrus. The researchers sacrificed beef heifers at 1, 8, and 24 h after AI. The average range of spermatozoa recovered from the entire reproductive tract decreased from 13.4 to 0.9% of 2×10^9 spermatozoa from 1 to 24 h after AI. Mean numbers of spermatozoa recovered at each time in the vagina, cervix, and oviducts decreased as the time increased from AI. The uterus and uterotubal junctions experienced a gradual increase in mean spermatozoa numbers at 8 h after AI and decrease by 24 h after AI. Spermatozoa in the reproductive tract gradually decreased following AI and sperm numbers recovered from the uterotubal junction reach a maximum approximately 8 h similar to Wilmut and Hunter (1984). Of the total percentage of spermatozoa recovered at each time, the majority of spermatozoa was in the vagina and ranged from 51 to 86%. Mitchell et al. (1985) conducted two experiments that examined the fate of inseminate in the reproductive tract. The

researchers deposited 1.012×10^9 sperm (approximately 70% abnormal) in the uterus of 10 non-lactating Holstein cows in experiment 1. Only $6.3 \pm 1.4\%$ of the total inseminate was recovered 12 h after AI. Sperm located in the vagina accounted for nearly 92% of the total inseminate. In experiment two discharged mucous and urine was collected every 2 h for 12 h post AI. Mitchell et al. (1985) recovered 73% of the inseminated sperm in experiment two. Mucous recovery accounted for the largest amount of sperm, 60.7%. Similar to experiment one, the researchers recovered only $6.5 \pm 1.6\%$ of the sperm from the entire reproductive tract. Mitchell et al. (1985) evaluated leukocyte concentrations from discharged mucous. Leukocyte concentrations ranged from $3.9 \pm 9.4 \times 10^6$ in the first 2 h post AI to $31.8 \pm 8.1 \times 10^6$ although mean numbers of leukocytes observed at 2, 4, 6, 8, 10, and 12 h post AI were not different ($P = 0.44$). Phagocytosis by leukocytes occurred for some of the 27% of inseminate not recovered. Mitchell et al. (1985) summarized that if this sperm loss is actually occurring, recovering only 7.3% of the total inseminate anterior of the cervix, then a conventional AI dose (15×10^6 sperm) may represent an unrecognized factor that influences fertility because sufficient numbers of sperm may not travel towards the site of fertilization. Nadir et al. (1993) determined that dosage of spermatozoa is critical to the number of accessory sperm of an embryo or ovum. Cows ($n = 40$) were inseminated with 20 or 100×10^6 spermatozoa. The median number of accessory sperm, which would represent competition among potential fertilizing sperm, for the high dose AI was nine-fold greater than the low dose AI although the variation is large ($P < 0.05$). More importantly, increasing the number of spermatozoa seemed to have a positive relationship with fertilization status and embryo quality. This factor may have implication with the current AI dose levels. Cryopreservation or freezing semen had no effect on fertilization rates or motility compared to fresh semen. The AI industry made significant advances in freezing methods to not compromise the spermatozoon viability.

In summary, the fate of spermatozoa has four possibilities. The majority of AI spermatozoa experience retrograde movement from the uterine body back into the cervix and vagina. The initial rapid phase transports a small percentage of sperm into the peritoneal cavity just after AI. The remaining sperm begin a sustained phase of transport that will begin to colonize the oviductal reservoir around 8 to 10 h later or undergo

phagocytosis by leukocytes. The ultimate fate of one hyperactivated spermatozoa will be fertilization of an ovum. Insufficient numbers of spermatozoa at AI compromise the fertilization rate and embryo quality.

SIRE FERTILITY

Dairy herd improvement calculates estimates of sire fertility or ERCR as a measure of individual sire fertility relative to all other sires of the same breed. A particular breed expresses the ERCR value as a deviation from the average of zero for all sires of a particular breed (Pecsok et al. 1994). A sire with a +3 ERCR value has a 3% greater chance of producing a successful pregnancy than the average sire. A sire with an ERCR value of -1 has 1% less chance of producing a successful pregnancy than the average sire. Sires with ERCR values differing by approximately 3% are different (Pecsok et al., 1994). Clay and McDaniel (2001) published results for the predictability of ERCR values. Seventy day non-return rate (NR70) calculated ERCR values using data available from DRMS including herd, month, and year of insemination, energy corrected milk yield, parity of the cow, and day of first AI over a 10 yr period from more than 2,000,000 breedings. Stevenson (2004) criticized using a NR70 for estimating a successful pregnancy. A NR70 assumes the cow is pregnant if AI did not occur 70 d after the last AI. Reasons given for absence of subsequent AI besides pregnancy may include culling, decision to no longer breed, or death. Stevenson (2004) suggests that confirmed pregnancies by veterinarians would be advantageous when data are available. Clay and McDaniel (2001) reported that ERCR values predicted from a minimum of 300 breedings for an individual sire accurately predict a later ERCR when a greater number of breedings occur. Time shows no change in the fertility score for an individual sire. A minimum of 1000 breeding computed early and later ERCR values for 326 sires. Eighty-one percent of the sires changed two points or less and fewer than 8% of the sires changed more than three points. Production traits and profit indices correlated with ERCR values. A sire's type production index and pounds of protein had significant but low positive correlations with ERCR values. Milk, fat, productive life, somatic cell score, and semen price were not significantly correlated with the ERCR value of a sire. Pecsok et al. (1994) also evaluated the economic value of a one-point increase in an ERCR value for an individual sire. A herd suffering from minimal PR could pay more

than five dollars per unit of semen for a one-point increase in ERCR value. The economic benefit is from a greater number of pregnant cows and subsequently more cows returning to peak milk yield. A two-dollar premium pays for a one point increase in ERCR value per unit of semen in moderate or average CR herds (Pecsok et al., 1994).

In summary, Clay and McDaniel (2001) reported that the fertility of a sire was predictable and repeatable if the sire had a minimum of 300 breedings. The ERCR value of an individual sire is likely not to change more than two points throughout his productive life. Pecsok et al. (1994) estimated a two-dollar premium per unit of semen for a sire with one point increase in ERCR value. It is also possible to select sires with high production or profit indices and above average fertility because of significant positive correlation coefficients; however, selection of ERCR values is secondary after production traits or profit indices (Clay and McDaniel, 2001). Estimated relative conception rate values can determine extremely low fertility sires that significantly reduce the chances of pregnancy and subsequently fewer pounds of milk sold per cow over the productive life of a cow (Clay and McDaniel, 2001). The differences between the AI sires achieving a successful pregnancy are small but when a conception advantage is needed fertility indices such as ERCR are beneficial (Stevenson, 2004).

DeJarnette et al. (2004) recently produced the question: Do sires of different fertility perform differently in today's FTAI programs that are commonly used on dairy farms. Their obvious answer was yes, but that insufficient data exist to determine how timing of AI may affect fertility groups. The partial answer may lie in the timing of insemination relative to ovulation for different fertility sires. Saacke et al. (1994) in a review of the literature discussed differences in seminal deficiencies among sires. Seminal deficiencies have compensable and un-compensable characteristics. Semen quality differences among sires that are compensable may be eliminated or minimized by increasing the number of sperm cells per AI dose. Subfertile sires with un-compensable seminal traits cannot attain the normal fertility of the female population by increasing sperm cell numbers in the AI dose. Although the seminal deficiencies of a particular sire that comprise compensable and un-compensable traits are primarily unknown semen differences can be measured microscopically for morphology and viability and by analysis of chromatin structure. Subfertile sires that have higher un-compensable

characteristics represent below average sires on a fertility index such as ERCR. Dalton et al. (2001) reported that inseminating cows closer to the time of ovulation and increasing accessory sperm affect embryo quality because of increased competition among potential fertilizing sperm. The researchers inseminated cows at 0, 12, or 24 h after the onset of estrus. Fertilization rate increased as did the median accessory sperm numbers as the time of AI occurred closer to ovulation. For 117 embryos/ovum classified as excellent/good, fair/poor, and degenerate the median accessory sperm increased as AI became further from the onset of estrus; 1 at 0 h, 2 at 12 h, and 4 at 24 h when embryos were recovered 6 d later ($P < 0.05$). The mode number of accessory sperm was zero suggesting only one spermatozoon is involved in the fertilization process most of the time. Using only embryos from the same data Saacke et al. (2000) reviewed a model that required between 10 and 20 accessory sperm per embryo to optimize embryo quality. Less than 10 accessory sperm per embryo decrease the quality of the embryo. Low accessory sperm numbers could be representative of using specific sires that have uncompensable seminal traits for sustaining normal embryogenesis. Low cell numbers may also represent the possibility of fertilization failure due to compensable seminal traits. Accessory sperm may be unique to a given male and provide insight to the capabilities of fertilizing sperm. Saacke et al. (1994) summarized data from 4 sires displaying the uniqueness of the sperm from an individual sire to reach the site of fertilization. Using a minimum of 10 embryos/ ovum recovered per sire the median accessory sperm number was 34 for sire A, 3 for sire B, 2 for sire C, and 0 for sire D when cows received AI with 20×10^6 sperm cells. These trends were similar when the same sires were used to inseminate cows at 100×10^6 cells per dose with sire A achieving the highest number of accessory sperm (45) and sire D achieving the lowest number of accessory sperm (6). Saacke et al. (1994) report the difference in accessory sperm numbers were not explained by semen viability differences among the 4 sires. Compensable seminal traits does not seem to be a problem in the AI industry today because of normal dosage rates are 20×10^6 spermatozoa which is two to 20 times above the minimum threshold require for acceptable insemination rates (DeJarnette et al., 2004). Saacke et al. (2000) also discussed many attempts taken to increase the number of accessory sperm. These methods include blocking retrograde sperm loss, fresh versus

frozen semen, semen extender composition (milk and egg yolk), sperm microencapsulation, using specific sires, increasing sperm cell dosage, and seminal plasma effects (Saacke et al., 1994). Only the uses of specific sires and increasing sperm cell dose have shown positive effects towards increasing the accessory sperm number per embryo/ ovum. Although fertilization rates increase when AI occurs closer to the time of ovulation, disadvantages of cows inseminated at 24 h after the onset of estrus were found in the quality of embryos (Dalton, 2001). A higher percentage of excellent/good embryos occurred when AI was performed at the onset of estrus, a higher percentage of fair/poor embryos were received when cows were inseminated 12 h after the onset of estrus, and the highest percentage of degenerate embryos were recovered from cows inseminated 24 h after the onset of estrus. These results are probably due to fertilization of an aging ovum or less selection of competent sperm at 24 h AI. The aging ovum would represent a huge factor in proficient embryogenesis. Wilmut and Hunter (1984) reported that sufficient sperm transport to colonize the oviduct takes between 6 and 12 h in the bovine. Greater than two-thirds of all cows undergo ovulation by 33 h after the onset of estrus (Walker et al., 1994). Insemination at 24 h after the onset of estrus would not allow colonization of the oviduct until 30 h after the onset of estrus undoubtedly later than ovulation in most cows. Dalton et al. (2001) reported a greater number of accessory sperm and CR trending higher as the interval from onset of estrus to AI increased, but decreasing embryo quality when AI occurred closer to ovulation suggesting that a successful pregnancy is a compromise between early and later insemination. Early AI results in decreased fertilization rates because of limited sperm life or retention in the oviduct while AI at 24 h represents decreased PR because of poor embryo quality or less sperm selection. These results generally explain the basis for the a.m. / p.m. AI guideline for estrus detection and a quadratic effect of highest PR at 16 h in a FTAI protocol (Pursley et al. 1998). Greater embryo quality may also be reflected by breeding at the onset of estrus; resulting in a greater number of competent spermatozoa through natural selection producing a more favorable fertilization (Saacke et al., 2000). Comparison between high and low fertility sires at extreme AI times, 0 or 24 h after the onset of estrus, may result in different PR because of sire differences. Early insemination may be more favorable for a lower fertility sire because of a greater time allowance for a more

competent spermatozoa selection. If this model holds true then higher fertility sires may perform better than low fertility sires in an insemination protocol 24 h after insemination because less time is needed for competent sperm selection unless fertilization of an aging ovum occurs.

Sire fertility effects on timing of insemination in a FTAI protocol may be answered by Saacke et al. (2000) and Dalton et al. (2001). Using the Ovsynch protocol Pursley et al. (1995) reported more than half of the cows ovulated by 28 h after the last administration of GnRH similar to the time required for ovulation after the onset of estrus of 27.6 hours (Walker et al. 1996). The results from Dalton et al. (2001) would support that sires with low uncompensable seminal characteristics, higher fertility, would perform the same at early or late AI because of the decreased selection pressure for competent spermatozoa. On the other hand, sires with high uncompensable seminal characteristics, lower fertility, should perform as well as a sire with higher fertility at early AI because of ample time to select competent spermatozoa capable of fertilization and embryo maintenance. However, these lower fertility sires may suffer at later AI times due to decreased spermatozoa selection.

The only known study that investigates CR based on sire fertility differences at varying stages of estrus was conducted under New Zealand conditions (MacMillan and Watson, 1975). Sires from three different fertility levels, above average, average, and below average, were selected based on 1000 insemination and 49 d non-return rates. Cows received AI at four different times relative to ovulation, early, mid-, late, and post-estrus. Sires were at least 2% above, within, or below the New Zealand Livestock Improvement Associations' average CR for herd sires. Over 6,000 inseminations were recorded and the average CR was 70.7%. Individual sire fertility groups produced CR similar to selection characteristics. Groups of high, average, and low fertility sires had CR of 72.9, 70.5, and 68.3%, respectively. Although the overall CR of sires were significantly different ($P < 0.01$), the difference in CR for each stage of estrus was greater ($P < 0.001$). Conception rates for early, mid-, late, and post-estrus were 65.7, 69.3, 75.1, and 72.8%, respectively. These results support the work by Dalton et al. (2001) by producing higher fertilization rates closer to the time of ovulation. Sire fertility classification did not influence CR for inseminations of cows in either late or in post-estrus. Sire fertility

levels did influence CR when AI occurred in cows in either early or mid-estrus. Conception rates for cows inseminated in early estrus for either above average, average, or below average sire fertility groups were 74.3, 62.7, and 58.4%, respectively ($P < 0.01$). Conception rates for cows inseminated during mid-estrus for above average, average, and below average sire fertility groups were 71.1, 70.7, and 65.8%, respectively ($P < 0.05$). Fertility differences magnified PR occurring at varying stages of estrus relative to ovulation (MacMillan and Watson, 1975). Conception rates of sires of high fertility are not compromised at any stage of estrus when insemination occurs. Sires of average fertility experience depressed CR when AI occurs in early estrus and sires of below average fertility experienced depressed CR when inseminated to cows in during either early or mid-estrus. Saacke et al. (2000) reported PR is affected by lower sperm life or retention in the oviduct by less competent sperm of lower fertility sires. Sires of different fertility perform the same closer to the time of ovulation suggesting PR failure lies in an aging ovum, not in sperm selection (MacMillan and Watson, 2001). Dalton et al. (2001) reported that sires of lower fertility or uncompensable seminal characteristics may suffer depressed CR when AI occurred during early estrus because of inadequate sperm life. Their results would support data reported by MacMillan and Watson (1975) because of decreased CR for lower fertility sires when AI occurs closer to the onset of estrus. Decreased CR did not apply to high fertility sires when AI occurred closer to the onset of estrus. There are concerns for accepting MacMillan and Watson (1975) data and applying it to present conditions. MacMillan and Watson (1975) inseminated cows using 2.5×10^6 non-frozen spermatozoa in cattle in New Zealand which typically have unlimited access to grazing pasture. The sires were chosen based on fertility classification were of varying numbers and different breeds which may present challenges in interpreting data. The heat detection was the responsibility of each of the 63 herd owners and the variation in the accuracy of heat detection may be large, thus compromising the diagnosis of the stage of estrus at AI. Clearly, the AI methods and dairying conditions of New Zealand are not similar to typical North American dairy production nearly 30 yr later.

RESYNCHRONIZATION AND CIDR INSERTS

A CIDR insert was approved by the FDA for use as a reproductive tool in lactating dairy cows in 2003. A CIDR insert is placed into the vagina of a dairy cow to continuously release progesterone (P_4). Because the CIDR insert is safe for use in food animal's two studies have investigated the effects of a CIDR insert on the return to estrus of previously inseminated cows. The normal estrous cycle of the cow is 21 d with a range of 18 to 24 d. Because an increasing number of dairy farms have adopted the use of FTAI protocols at the first AI focus is placed on cows that remain in a non-pregnant status and receive subsequent AI. In order to identify non-pregnant cows in estrus 18 to 24 d after the first AI, management must rely solely on the detection of estrus. The ability of the farms to accurately identify these non-pregnant cows has become burdensome (Lucy, 2001). Chenault et al. (2003) and El-Zarkouny and Stevenson (2004) conducted studies to determine the effect of CIDR inserts on synchronization rates. Chenault et al. (2003) placed CIDR inserts in 887 cows and 867 cows served as controls and did not receive a CIDR insert. The CIDR inserts were placed in the vagina 14 ± 1 d after AI. The CIDR inserts were removed 7 d later and estrus detection occurred during the subsequent 3 d. A greater proportion of cows receiving a CIDR insert returned to estrus over the next 3 d than cows receiving no CIDR insert, 34.1 and 19.3%, respectively ($P < 0.001$). More cows receiving a CIDR insert returned to estrus during a period beginning 2 d after CIDR insert removal until 6 d after CIDR insert removal than cows not receiving a CIDR insert over a period of 9 d, 18 to 26 d after breeding, 42.8 and 36.4%, respectively ($P = 0.031$). A disadvantage for cows receiving a CIDR insert was a lower CR to the previous AI than for cows not receiving a CIDR insert, 32.7 and 36.7%, respectively ($P = 0.044$). Cows receiving a second AI after CIDR insert removal did not experience different PR than cows receiving a subsequent AI serving as controls, 11.5 and 11.1%, respectively ($P = 0.903$). El-Zarkouny and Stevenson (2004) found similar and contradictory results compared to Chenault et al. (2003). Cows receiving a CIDR insert for 7 d experience a greater amount of estrus synchrony 2 and 3 d after CIDR insert removal than control cows not receiving a CIDR insert where estrus expression was more uniformly distributed over a period of 6 d. However, a once-used CIDR insert failed to return a greater proportion of non-pregnant cows to estrus over a 6 d period compared to

control cows that did not receive a CIDR insert, 33.9 and 28.7%, respectively ($P > 0.05$). El-Zarkouny and Stevenson (2004) did not find a lower CR to a previous AI between cows receiving CIDR insert receiving and control cows, 42.1 and 38.2%, respectively ($P > 0.05$). Similar to Chenault et al. (2003) El-Zarkouny and Stevenson (2004) did not find an advantage of AI to resynchronized estrus in cows receiving a CIDR insert and control cows inseminated on detected estrus over the 6 d period, 20.0 and 31.6%, respectively ($P > 0.05$).

In summary, the CIDR insert is a successful reproductive tool for returning non-pregnant cows to estrus in a fewer number of days. Chenault et al. (2003) and El-Zarkouny et al. (2004) reported that cows receiving a CIDR insert will return a greater number of cows to estrus in during a limited interval, 2 to 3 d following CIDR insert removal. However, the CIDR insert may not increase the proportion of cows that will show estrus in the expected period 18 to 24 d after AI. Based on the results a farm manager must make the decision on whether or not to use CIDR inserts. Farms that have a history of poor estrus detection rates may benefit by being able to focus estrus detection in a period of 2 or 3 d after using a CIDR insert. There have not been reported benefits for CR of a first AI or AI resulting from detected estrus for cows where a CIDR insert was utilized for a period of 7 d, 14 ± 1 d after AI.

OBJECTIVES

The objectives of this study were to determine if FTAI at 2 different times, 0 or 24 h after GnRH, in a Presynch protocol influenced the CR when high (≥ 3 ERCR) and average (-1 ERCR) fertility sires were used. Additionally, a second experiment was conducted to determine the effectiveness of CIDR inserts to allow for resynchronization of estrous in a FAST BACKSM protocol for cows that did not conceive or maintain the conceptus at FTAI.

MATERIALS AND METHODS

COWS

Lactating Holstein cows ($n = 1,457$) from two well-managed dairy herds were utilized from April 2004 to March 2005. The farms were located in North Carolina, USA and each location consisted of approximately 1,000 lactating cows. Cows were housed in free stall barns and bedded on rubber mats or sand. Cows on both farms received a TMR twice daily and were milked three times daily. Rolling herd averages for milk were 11,300 and 11,600 kg. Early post-partum protocols included rectal temperatures, intravenous electrolytes, uterine infusion, antibiotics, and drenching.

TREATMENT

In experiment 1, lactating dairy cows ($n = 1,132$) received two administrations of 25 mg of $\text{PGF}_{2\alpha}$, (Lutalyse[®] Pfizer Animal Health, NY, NY) 14 d apart beginning at 38 ± 3 d in milk (DIM) and 100 μg of GnRH 14 d later (Figure 4). Seven days after GnRH administration cows received a third administration of 25 mg of $\text{PGF}_{2\alpha}$. A final administration of 100 μg of GnRH was given 54 h later at 75 ± 3 DIM. Cows were also randomly assigned an AI time, to be inseminated at GnRH administration (time 0) or 24 h after GnRH administration (time 24). Cows received AI to one of three sires chosen for high ($\geq +3$) or one of three sires chosen for average (-1) ERCR values. Half the cows were assigned by computer-generated randomization to receive a CIDR insert 14 d after GnRH administration for a period of 7 d. All semen used during experiment 1 was collected from a single ejaculate from each sire. Table 1 displays initial sire fertility

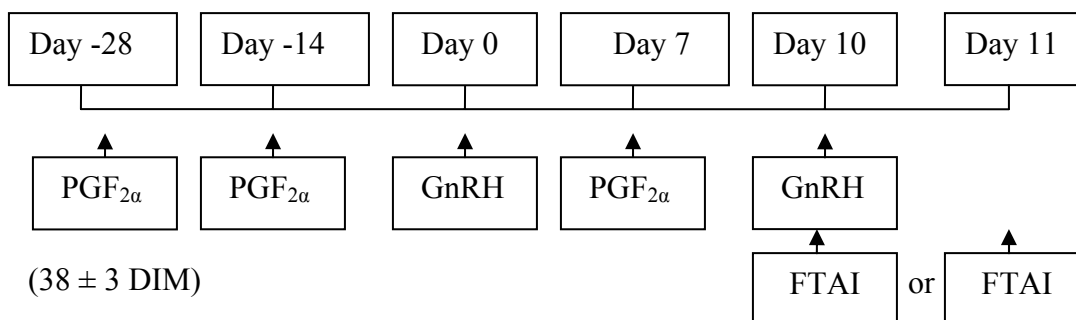


Figure 4: Presynch-Ovsynch protocol for Experiment 1. All cows were administered the final GnRH on d 10, 54 h after $\text{PGF}_{2\alpha}$.

Table 1. Fertility indices from two organizations that measure sire fertility.

	DRMS ^a		ATA ^b	
	ERCR ^c	Repeatability	Relative CR	No. of Breedings
Sire A	-1	97	-1.9	1,535
Sire B	-1	99	-1.6	2,784
Sire C	-1	84	1.4	481
Sire D	3	92	2.5	2,128
Sire E	3	92	4.5	1,189
Sire F	4	70	6.0	4,029

a) Dairy Records Management Systems (Raleigh, NC, USA)

b) Agritech Analytics, LLC (Visalia, CA, USA)

c) Estimated Relative Conception Rate

scores as estimated by two organizations that measure sire fertility for the six sires at the beginning of the study. Dairy Records Management Systems published ERCR values and repeatabilities in November 2003, and Agritech Analytics, LLC (ATA) published relative CR and number of breedings per sire in August 2004. Each mating was assigned to 1 of 6 Holstein sires to minimize inbreeding. Cows without pedigree information were randomly allocated to a sire. Cows were retained by headlocks or an indexing rail to receive hormone treatment or AI during the study. In experiment 1, 106 enrolled cows were culled by farm management before first AI or pregnancy diagnosis was performed and 219 cows were not bred according to the protocol and removed from the analysis. Pregnancy diagnosis was confirmed by a subsequent breeding or rectal palpation by a veterinarian approximately 40 d after AI.

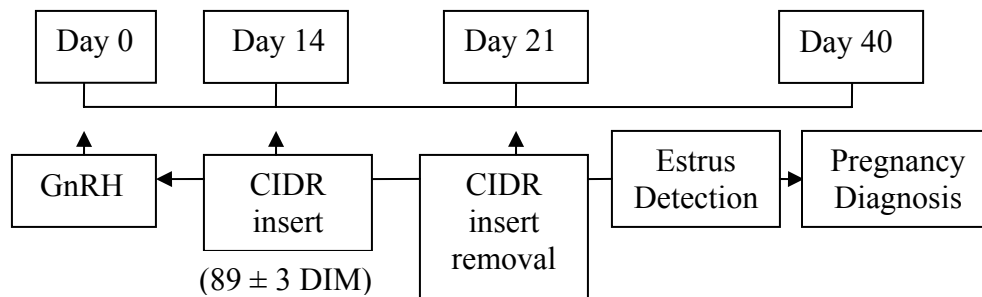
In experiment 2, lactating dairy cows (n = 1,132) were randomly allocated to receive a CIDR insert (FAST BACKSM) (n = 600) or serve as controls (no CIDR insert; n = 532) 14 d after GnRH administration at 89 ± 3 DIM. The CIDR inserts were retained in the cow for 7 d. Herd employees performed routine visual detection of estrus throughout the day. A cow found displaying estrus was submitted for AI by a trained technician at the next milking. Herd managers selected the second AI sire and sire fertility groups were not analyzed in experiment two. Pregnancy was confirmed for 318 (28.1%) cows by rectal palpation to first AI approximately 40 d later and these cows were not included in the analysis for experiment two. During 7 d, 18 to 24 d after GnRH administration, 252 (31.7%) cows were detected in estrus and were utilized to determine the effectiveness of CIDR inserts for resynchronization of estrus (RE) and effects on CR.

After GnRH administration 94 (11.8%) cows were detected in estrus prior to d 18 and removed from the RE analysis for eligible cows. Pregnancy diagnosis for a second AI was confirmed by a subsequent estrus or rectal palpation approximately 40 d after AI. At the time of analysis, 7 (2.6%) cows had an unknown pregnancy status and were removed from the study.

DATA AND STATISTICAL ANALYSIS

Data were analyzed with a statistical software program (SAS Version 9.1 for Windows; SAS Institute, Cary, NC, USA). In experiment 1, first AI PR was analyzed using logistic regression. Fertility group, time of AI, parity, and two-way interactions were forced to remain in the final model. Effects also considered for

Resynchronization – CIDR insert (Fast BackSM)



Control

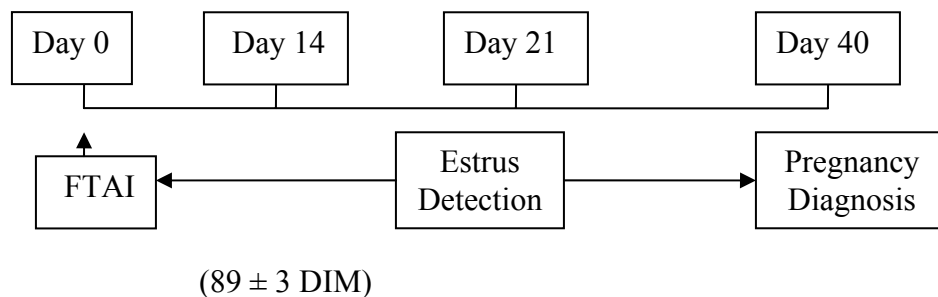


Figure 5: CIDR protocol (FAST BACKSM) for Experiment 2. Cows were randomly assigned to receive a CIDR insert or no CIDR insert 14 d after GnRH administration of a FTAI protocol.

inclusion in the model were season of first AI, location, CIDR insert treatment and all two-way interactions between effects. The model was built using the backward step-wise elimination process, that removed the least significant variable or interaction for reanalysis of the data until the model contained only forced effects and significant ($P < 0.10$) effects. Significance for discussion was declared at $P \leq 0.05$ and tendencies were evaluated at $P \leq 0.10$. The final logistic regression model, after backward stepwise elimination, used to evaluate PR for first AI was:

$$Y_{ijklm} = \mu + F_i + T_j + P_k + FT_{ij} + FP_{ik} + TP_{jk} + C_l + S_m + PC_{kl} + e_{ijklm}$$

where:

Y = pregnancy outcome for a cow diagnosed approximately 40 d after FTAI (0, 1)

μ = overall mean

F_i = the effect of the i^{th} fertility group (1, 2)

T_j = the effect of the j^{th} time of AI (1, 2)

P_k = the effect of the k^{th} parity (1, 2, 3)

FT_{ij} = the interaction of the i^{th} fertility group and j^{th} time of AI

FP_{ik} = the interaction of the i^{th} fertility group and k^{th} parity

TP_{jk} = the interaction of the j^{th} time of AI and k^{th} parity

C_l = the effect of l^{th} CIDR insert treatment (0, 1)

S_m = the effect of the m^{th} season of AI (1, 2, 3)

PC_{kl} = the interaction of the k^{th} parity and l^{th} CIDR insert treatment

e_{ijklm} = random error

Linear contrasts were used to evaluate differences between subclasses of fertility group, TAI, parity, season of AI, CIDR insert treatment and CIDR insert by parity interaction. The results of logistic analyses are presented as odds ratios and 95% confidence intervals for fertility group, time of AI, parity, season of AI, CIDR insert, and CIDR insert by parity interaction. Least squares means for PR were computed using a general linear model (GLM procedure, SAS) similar to that used for the final logistic regression analysis, but included AI sires nested within fertility groups and the interaction of AI sires nested and timing of AI. Fertility groups were classified as follows: Average = Sire A, B, C; High = Sire D, E, F. Timing of AI was one of two times: at the final GnRH administration (0), or 24 h after the final GnRH (24). Parity was categorized into three

classifications: first (1), second (2), or third and greater (3+). Three different seasons of AI were categorized by the month of AI as follows: 1 = April, May, and June; 2 = July, August, and September; and 3 = October, November, December, and January.

In experiment 2, the effect of RS was analyzed using a chi-square test of independence to determine whether the distribution of RS was similar between CIDR insert and control treatments. After determining the number of breedings by day after GnRH administration of the Ovsynch protocol, RE was defined as two periods; 18 to 20 d and 21 to 24 d after GnRH administration of a FTAI protocol. Logistic regression analysis was used to analyze second AI CR in a model forced to include treatment (CIDR insert versus no CIDR insert), and RE period (1, 2). Effects also considered for inclusion in the model were parity (1, 2, or 3+), location (1, 2), and season of second AI (1 = May and June; 2 = July, August, and September; 3 = October, November, and December; and 4 = January, February, and March) and two-way interactions between all effects. The model was built using the backward step-wise elimination process until the model contained significant fixed effects and significant interactions of fixed effects. The *P* value was set at < 0.10 for inclusion. Significance was declared at $P \leq 0.05$ and tendencies were evaluated at $P \leq 0.10$. The final model used to evaluate CR to second AI was:

$$Y_{ijkl} = \mu + C_i + S_j + L_k + e_{ijk}$$

where:

Y_{ijkl} = pregnancy outcome for a cow diagnosed approximately 40 d after second AI during RE (0, 1)

μ = overall mean

C_i = the effect of the i^{th} CIDR insert treatment (0, 1)

R_j = the effect of the j^{th} resynchronization of estrus period (1, 2)

L_k = the effect of the k^{th} location (1, 2)

e_{ijk} = random error

Linear contrasts were used to evaluate differences between subclasses of treatment, RE, and location. The results of logistic analyses are presented as odds ratios and 95% confidence intervals for treatment, RE, and location. Least squares means for CR were

computed using a general linear model that included the effects forced and remaining in the logistic regression analysis.

RESULTS

In Experiment 1, effects of fertility groups, parity, season of AI, and an interaction of CIDR insert by parity were found to affect ($P \leq 0.05$) first AI PR (Table 2). Although differences among individual sires are not reported, least squares means for PR of individual sires are shown in Figure 6. First AI PR for average and high fertility groups were 24.1 ± 2.3 and $29.2 \pm 2.3\%$, respectively ($P \leq 0.05$, Table 2). The conclusion from the analysis of odds ratios was that the high fertility sires were 15% more likely to achieve pregnancy at first AI than sires of the average fertility group (Table 2). Timing of AI did not influence first AI PR ($P > 0.10$). Pregnancy rates for AI at time 0 and 24 were 27.6 ± 1.8 and $25.7 \pm 2.0\%$, respectively (Table 2). There was no significant interaction between fertility groups and TAI ($P > 0.10$). Pregnancy rates for cows bred to average fertility sires at time 0 or 24 were 25.1 ± 2.6 and $23.1 \pm 2.8\%$, respectively (Figure 7). Pregnancy rates for cows bred to high fertility sires at time 0 or 24 were 30.0 ± 2.6 and $28.4 \pm 2.9\%$, respectively (Figure 7). Significant parity effects were found to influence first AI PR. Cows in parity 1 were different from cows in parity 2 and 3+. Pregnancy rates for parity 1, 2, and 3+ were 32.2 ± 2.1 , 25.5 ± 2.5 , and $22.2 \pm 2.5\%$, respectively (Table 2 and Figure 8). There was no difference between cows in parity 2 and 3+. Conclusions from the analysis of odds ratio were that cows in parity 1 were 29% more likely to achieve pregnancy at first AI than cows in parity 2 and were 25% more likely to achieve pregnancy at first AI than cows in parity 3+ (Table 2). Season of first AI remained in the final model. Cows receiving AI during season 3 were more likely to become pregnant than cows receiving AI during season 1. Pregnancy rates for season 1, 2, and 3 were 22.7 ± 2.6 , 26.2 ± 2.5 , and $31.1 \pm 2.0\%$, respectively (Table 2 and Figure 9). Conclusions from the analyses of odds ratios were that cows bred during season 3 were 24% more likely to become pregnant than cows bred during season 1 (Table 2). There were no differences between cows bred in season 2 and season 1 or in season 3 and season 2. Although there was no difference between CIDR insert treatments, there was an interaction of CIDR insert by parity ($P \leq 0.05$, Table 2). Pregnancy rates for second AI cows receiving a CIDR insert or and those not receiving

Table 2. Logistic binomial regression of pregnancy on fertility group, timing of AI, parity, season of AI, CIDR insert, and CIDR insert by parity interaction on PR at first AI for cows bred to either sires of -1 (average fertility) or $\geq 3</math> (high fertility) estimated relative conception rate at either the same time of the final GnRH administration (Time 0) and 24 h (Time 24) after the final GnRH administration.$

Source	Category	AI (no.)	Percent pregnant (%)	Odds Ratio ¹	95% Confidence Interval ²
Fertility Group	Average	574	24.1	1.00 ^a
	High	558	29.2	1.15 ^b	(1.00, 1.32)
Timing of AI	Time 0	620	27.6	1.05	(0.92, 1.20)
	Time 24	512	25.7	1.00
Parity	1	469	32.2	1.00 ^a
	2	330	25.5	0.71 ^b	(0.52, 0.98)
	3+	333	22.2	0.75 ^b	(0.63, 0.90)
Season	One	309	22.7	1.00 ^a
	Two	318	26.2	1.22 ^{a,b}	(0.85, 1.76)
	Three	505	31.1	1.24 ^b	(1.00, 1.52)
CIDR insert	Control	600	26.4	0.99	(0.86, 1.14)
	CIDR insert	532	26.9	1.00
CIDR insert by parity	Parity 1 - no CIDR insert	239	31.0	0.94	(0.78, 1.14)
	Parity 1 - CIDR insert	230	33.3	1.00
	Parity 2 - no CIDR insert	172	21.1	0.79	(0.62, 1.02)
	Parity 2 - CIDR insert	158	29.9	1.00
	Parity 3+ - no CIDR insert	189	27.0	0.99	(0.86, 1.14)
	Parity 3+ - CIDR insert	144	17.5	1.00

- 1) Odds ratio is the estimated chance of pregnancy at AI in a single category relative to the baseline category, considering other explanatory variables included in the model. The baseline for a particular category is represented as 1 and other effects in that category >1 increase the probability of pregnancy whereas other effects in that category <1 decrease the probability of pregnancy. Odds ratios with different superscripts (a, b) are different from each other within each source of variation ($P \leq 0.05$).
- 2) Odds ratios with confidence intervals that do not include 1.00 are different from 1.00 at $P \leq 0.05$.

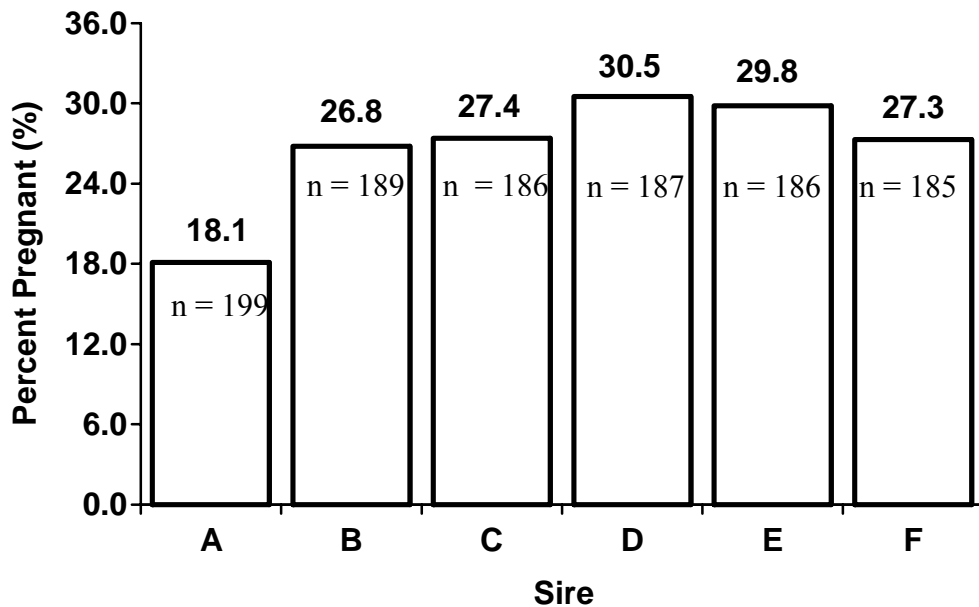


Figure 6. Percent pregnant at first AI for individual AI sires bred to cows at either the same time of the final GnRH administration (Time 0) or 24 h (Time 24) after the final GnRH administration.

an insert were 26.4 ± 1.9 and $26.9 \pm 2.0\%$, respectively (Table 2). Treatment of CIDR insert tended to be different for cows only in parity 2 ($P = 0.07$, Table 2). Pregnancy rates for cows in parity 2 receiving a CIDR insert or no CIDR insert were 29.9 ± 3.6 and $21.1 \pm 3.4\%$, respectively (Table 2, Figure 10). There was no effect of location or interactions with location, therefore the effects were eliminated from the model. There were no significant two-way interactions with season of AI, eliminating these effects from the model.

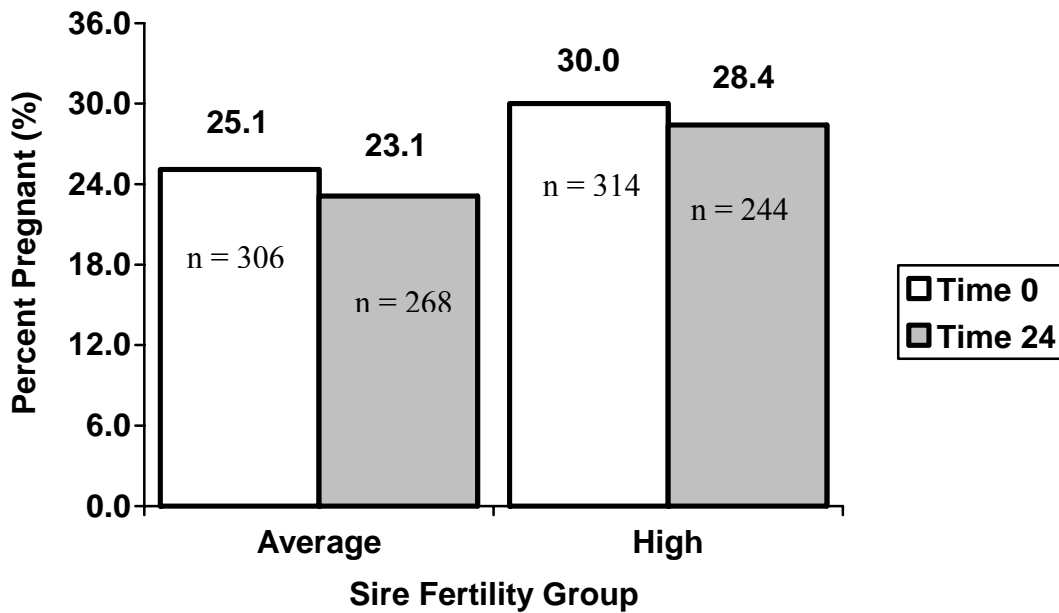


Figure 7. Effect of fertility groups on PR at first AI for cows bred at either the time of the final GnRH administration (Time 0) or 24 h (Time 24) after the final GnRH administration.

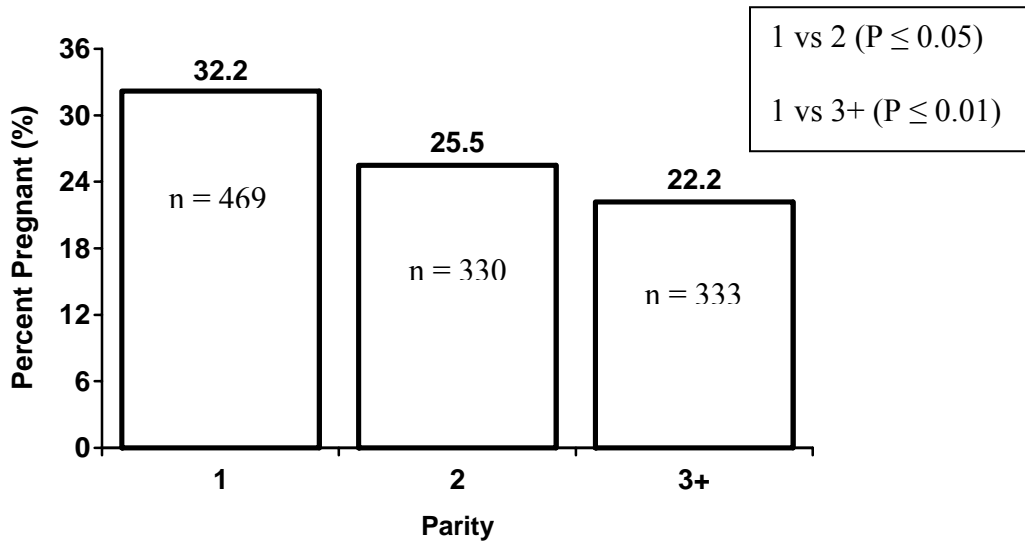


Figure 8. Effect on parity of PR at first AI for cows bred to -1 (Average) or

In experiment 2, the percentage of cows determined to be in estrus 17 to 25 d after GnRH administration of the Ovsynch protocol are presented in Figure 11 by treatment. There was an effect of treatment for RE (Table 3). Percentages of treated and control cows determined to be in estrus 18 to 20 d after GnRH administration of the Ovsynch protocol were 7.5 and 37.2%, respectively (Table 3). Percentages of treated and control cows determined to be in estrus during d 21 to 24 after GnRH administration of the Ovsynch protocol were 92.5 and 62.8%, respectively (Table 3). Treatment did not have an effect on CR of second AI. Least squares means for CR of second AI for cows receiving a CIDR insert and for control cows were 20.7 ± 4.9 and $19.5 \pm 3.6\%$, respectively (Table 4). There were no differences in CR for cows bred 18 to 20 d and 21 to 24 d, 15.5 ± 5.7 and $24.7 \pm 3.1\%$ respectively (Table 4). Locations tended ($P = 0.09$) to be different for CR to second AI. Locations 1 and 2 had a CR of 24.9 ± 4.2 and $15.4 \pm 4.2\%$, respectively (Table 4). There were no effects or interaction with parity or season of AI and these effects were eliminated from the model by backwards elimination.

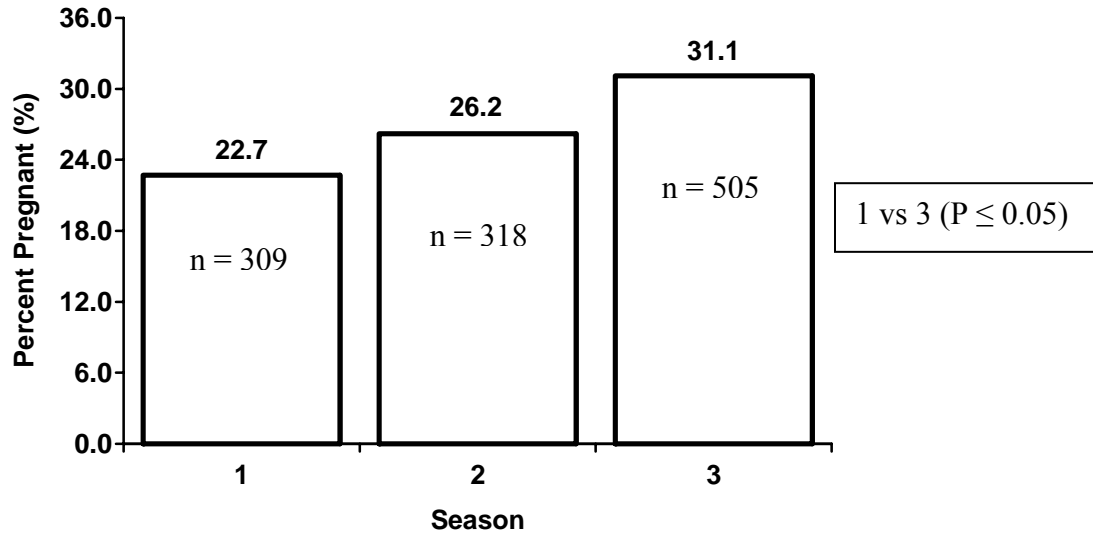


Figure 9. Effect of season on PR at first AI for cows bred to -1 (Average) or ≥ 3 (High) estimated relative conception rate sires at either the same time of the final GnRH administration (Time 0) and 24 h (Time 24) after the final GnRH administration.

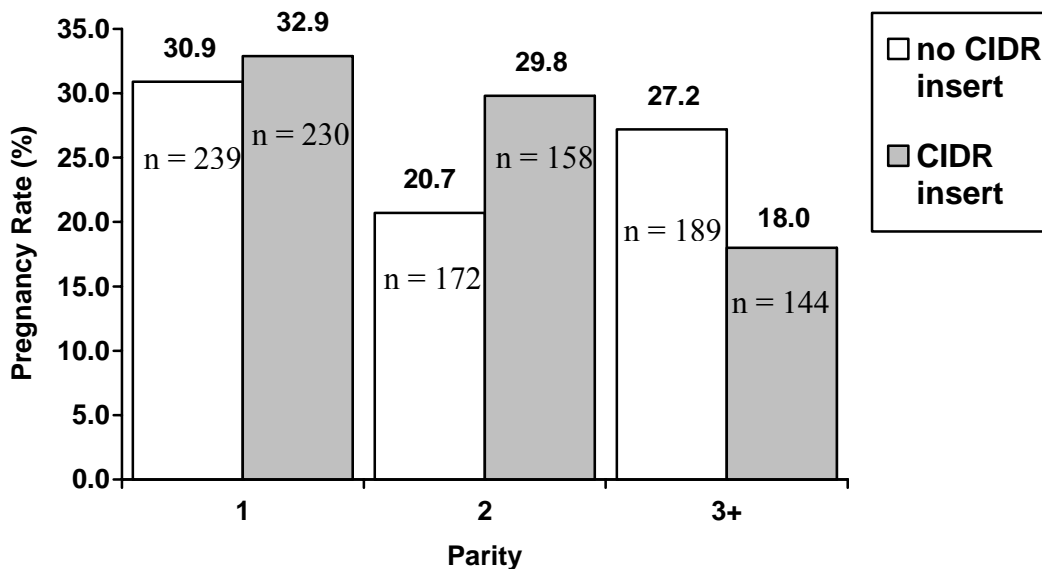


Figure 10. Effect of parity on PR at first AI for cows either receiving a CIDR insert or no CIDR insert 14 d following GnRH administration of a FTAI protocol.

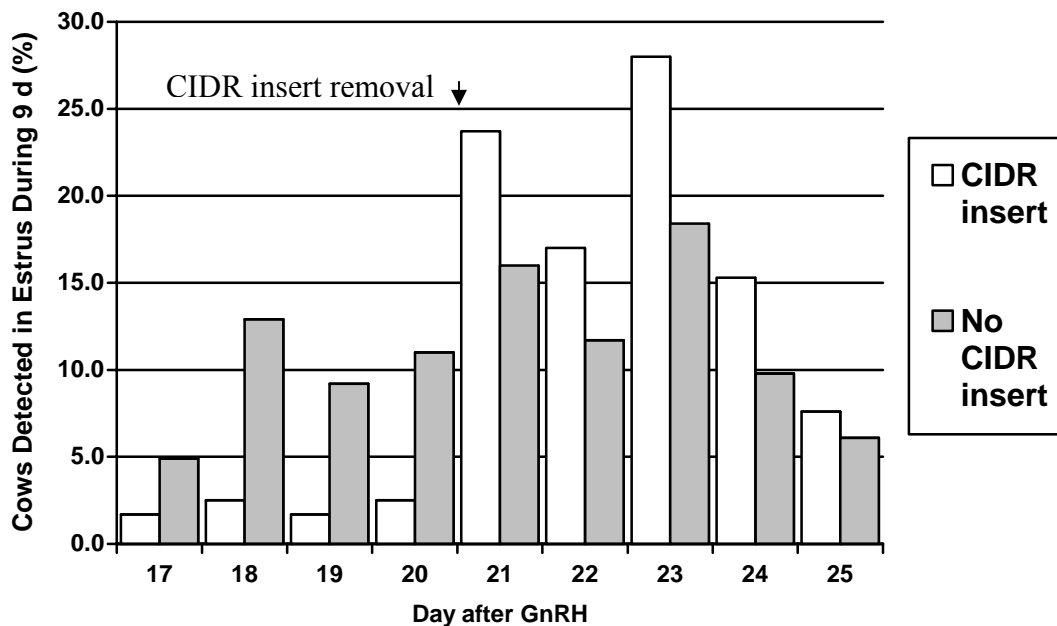


Figure 11. Percentage of cows detected in estrus during 9 d after GnRH administration of a FTAI protocol. Cows were randomly allocated to receive a CIDR insert 14 d after GnRH administration of a FTAI protocol for 7 d or serve as controls (no CIDR insert).

Table 3. Percentage of eligible cows detected in estrus 18 to 20 and 21 to 24 d after GnRH of a FTAI protocol.

Treatment	Eligible ^a (n)	% detected		% of estrus periods detected ^b	
		< 18 d	18 to 24 d	18 to 20 d	21 to 24 d
CIDR insert	371	10.8	28.8	7.5	92.5
Control	424	12.7	34.2	37.2	62.8

- a) The number of cows not pregnant at first AI for each treatment
b) The return to estrus is not independent of treatment (Chi-Square, $P \leq 0.001$)

Table 4. Logistic binomial regression for effects of CIDR insert, resynchronization of estrus (RE), and location (1, 2) for cows randomly allocated to receive a CIDR insert for 7 d or no CIDR insert 14 d after GnRH administration of a FTAI protocol.

Source	Category	AI (no.)	Percent pregnant (%)	Odds Ratio ¹	95%
					Confidence Interval ²
Treatment	CIDR insert	107	20.7	1.00
	No CIDR insert	145	19.5	0.93	(0.48, 1.80)
RE	18 to 20 d	62	15.5	0.56	(0.25, 1.27)
	21 to 24 d	190	24.7	1.00
Location	One	118	24.9	1.74 ^a	(0.92, 3.28)
	Two	134	15.4	1.00 ^b

- 1) Odds ratio is the estimated chance of pregnancy at AI in a single category relative to the baseline for that category considering other explanatory variables included in the model. The baseline for a particular category is represented as 1 and other effects in that category >1 increase the probability of pregnancy whereas other effects in that category <1 decrease the probability of pregnancy. Odds ratios with different superscripts (a, b) tend to be different from the baseline (1.00) in each category at $P = 0.09$.
2) Odds ratios with confidence intervals that do not include 1.00 are different from 1.00 at $P \leq 0.05$.

DISCUSSION

The time interval of 48 h between the final GnRH and PGF_{2α} administration in the standard Ovsynch protocol was increased to 54 h to accommodate the three times a day milking routine of the herds. Portaluppi and Stevenson (2005) reported an increase in PR when the final GnRH administration was administered 72 h after PGF_{2α} compared to GnRH administration 48 h after PGF_{2α}. The major reason cited for improved PR was a greater time allowance for development of a mature ovulatory follicle, allowing for improved subsequent luteal function. Peters and Pursley (2003) suggested that an ovulatory follicle of 14 mm is large enough for consistently high CR. Walker et al. (1996) used the Heatwatch system to determine the onset of estrus, being defined as the first standing mount with a minimum of 3 standing mounts in 4 h, after administration of PGF_{2α} to be 73.1 ± 2.8 h. Their data suggest a greater time allowance than 48 h is needed for development of a mature follicle.

As expected, fertility groups differed for first AI PR ($P \leq 0.05$). Sires were selected based on ERCR values (Average, -1 versus High, $\geq +3$) to compare fertility groups interaction with timing of AI in a Presynch-Ovsynch protocol. The values of ERCR are expressed as a deviation from the average of zero of a particular breed (Pecsok et al., 1994). Repeatability of ERCR values are calculated by the number of breedings and estimated genetic standard deviation of ERCR. A more accurate future ERCR value can be predicted when repeatability is high. The three average sires were selected to be at least 4 ERCR points below the three high fertility sires. Least squares means for PR in this study for average and high fertility groups were 24.1 and 29.2%, respectively; and differed by 5.1% numerically, a slightly greater difference than expected. The decreased performance of the average fertility group may be attributed to Sire A where an overall PR was 18.1, compared to Sire B that had the second lowest overall PR of 26.8% (Figure 6). It has been noted that a criticism for the use of ERCR values is that 70 d non-return rates are used in its calculation in contrast to palpated pregnancies that are more accurate (Stevenson, 2004). AgriTech Analytics, LLC uses confirmed palpations to calculate relative CR. The fertility indices (ERCR and ATA) were similar for the ranking of the six sires when the study was initiated (Table 1). Clay and McDaniel (2001) found that ERCR values can be accurately computed from a minimum of 300 breedings and can

accurately predict future ERCR values when a greater number of breedings occur. Early and later ERCR values were computed for 326 sires from a minimum of 1000 breedings per sire. Of the 326 sires, 81% changed less than two ERCR points and less than 8% of the sires changed more than three ERCR points. Estimated relative conception rates are reported in May and November of each year by DRMS. Five of six sires utilized in the study deviated at least 1 ERCR point from the original ERCR value at the start of the study during one six mo period while the study was being performed. The most notable change in ERCR value during the study was for Sire F. Sire F was selected with a +4 ERCR and a repeatability of 70% in November 2003. In May of 2004 an ERCR value for Sire F was +1 and repeatability of 89%, decreasing by three ERCR points and becoming more reliable. The ERCR value reported in November of 2004 for Sire F was +2 and repeatability of 94%. Thus, Sire F may not represent a high fertility sire based on the selection criterion of this study. Sire B, with a repeatability of 99%, was the only sire not to deviate from the ERCR value during a 6 mo period after the start of the study.

When fertility advantages are needed, selection for sires with above average ERCR values is a practical recommendation. Semen from high fertility sires has economic value beyond genetic potential. Pecsok et al. (1994) found that on average a one ERCR point increase is worth two dollars per unit of semen to the producer. The type production index (TPI) and pounds of protein had significant but low positive correlations with ERCR values (Clay and McDaniel, 2001). However, milk, fat, productive life, somatic cell score, and semen price were not significantly correlated with the ERCR value of a sire.

There was no difference in first AI PR by time of AI. Time 0 and 24 had similar PR of 27.6 and 25.7%, respectively. However, the question was asked by DeJarnette et al. (2004): “Do sires of different fertility perform differently in FTAI protocols commonly used at first AI on dairy farms?” The primary hypothesis of this study was to determine if sire fertility differed by timing of AI. In fact, the respective sire fertility groups performed similarly at each time of AI (Figure 7). These results were unexpected because MacMillan and Watson (1975) reported that sires of different fertility perform differently depending on the time of AI relative to estrus. MacMillan and Watson (1975) selected groups of sires of different fertility to perform AI at different stages of estrus.

Although sire fertility groups were different, stage of estrus at AI were found to have greater effects. In their study, CR was not compromised for high fertility sires at any stage of estrus, although sires of low and average fertility produced depressed CR when AI occurred during early stages of estrus.

Dalton et al. (2001) stated that differences in early (time 0) and late (time 24) AI may affect CR because of natural selection of competent sperm prior to ovulation. Dalton et al. (2001) contended that AI at time 0 may result in lower fertilization rates because of a longer duration of sperm residence in the female reproductive tract prior to ovulation allowing for selection pressure favoring sperm that are more competent. Insemination at time 24 (closer to ovulation) resulted in higher fertilization rates, but decreased embryo quality possibly representing inadequate time for competent sperm selection capable of sustaining embryogenesis (Dalton et al. 2001). A review by Saacke et al. (2000) stated that incompetent fertilizing sperm that can be labeled as un-compensable have been shown to produce a higher number of low quality embryos and lower fertilization rates. DeJarnette et al. (2004) stated that compensable seminal traits do not seem to be a problem in the AI industry today because normal dosage rates are 20×10^6 spermatozoa which is 2 to 20 times above the minimum threshold required for acceptable AI rates. Pregnancy rates may improve at early AI with semen from sires that have a higher percentage of un-compensable traits because of natural selection of a more competent sperm. However, decreased fertility for AI at time 24 may not be representative of using specific sires, but the result of fertilization of an aging ovum (Dalton et al., 2001). Pursely et al. (1995) reported that cows ovulated 26 to 32 h after GnRH administration. Wilmut and Hunter (1984) reported that sperm transport may take 6 to 12 h for a viable number of sperm to populate the oviducts of heifers after AI. Insemination 24 h after GnRH administration may result in decreased CR if sperm are not present at the site of fertilization when the ovum is nearby. Although no difference among fertility groups and timing of AI was found, Sire F produced the greatest numerical difference between AI time 0 and 24. Pregnancy rates at time 0 and 24 for Sire F were 31.9 ± 4.5 and $22.7 \pm 4.9\%$, respectively. Sire F was selected as a high fertility sire; however, the accuracy of his ERCR value was the lowest of the six sires having a repeatability of 70% at the initiation of the study. Sire A produced the lowest PR and

may have the highest percentage of uncompensable spermatozoa. Pregnancy rates for Sire A at time 0 and 24 were 22.1 ± 4.5 and $14.1 \pm 4.6\%$, respectively. Sire D had the highest numerical PR of $30.5 \pm 3.3\%$ and may represent the sire with the lowest percentage of uncompensable spermatozoa. Pregnancy rates for Sire D at time 0 and 24 were 30.3 ± 4.5 and $30.7 \pm 4.9\%$, respectively and represented the lowest numerical difference between AI PR for all sires. Although semen characteristics are not reported for these sires, interpreting the fertility of a sire based on compensable or uncompensable seminal traits alone is difficult because semen from an individual sire may contain both, compensable and uncompensable seminal characteristics, and each to a different degree (Saacke et al. 1994).

An explanation for not finding an interaction between sire fertility groups and time of AI may lie in the relatively narrow interval of ovulation time of a FTAI protocol. Pursley et al. (1995) reported that ovulation occurred in 18 cows 26 to 32 h after GnRH administration with the greatest number of cows ovulating at 28 h after GnRH administration. In contrast, MacMillan and Watson (1975) compared CR obtained by AI technicians in a once-a-day AI protocol. The AI technicians inseminated cows during the morning for cows detected in estrus during the previous 24 h. Time of initial estrus detection during the 24 h period was used to categorize the stage of estrus at AI. Estrus detection was the responsibility of 63 herd owners and the variation in detection of the onset of estrus was unknown. The variation in the average time of ovulation from the onset of estrus activity was reported to be 27.6 ± 5.4 h (Walker et al., 1996). The variation among cows allowed to experience spontaneous ovulation is greater than synchronized ovulation reported by Pursley et al. (1995) possibly compromising the interpretation of results obtained by MacMillan and Watson (1975).

Other studies have reported no difference in the time of AI after GnRH administration when AI occurred at the same time or 24 h after GnRH administration (Pursley et al., 1998; Portaluppi and Stevenson, 2005). Pursley et al. (1998) reported a quadratic effect for cows receiving AI at 0, 8, 16, or 24 h after GnRH. The highest CR, 45%, occurred when AI was performed 16 h after the final GnRH administration. Dalton et al. (2001) discussed explanations for the quadratic effect found by Pursley et al. (1998). Dalton et al. (2001) contended that AI at 12 h after the onset of estrus provided a

compromise between potentially lower fertilization rates for AI occurring at the onset of estrus and reduced embryo quality due to increased degenerate embryos when AI occurred 24 h after the onset of estrus. Dalton et al. (2001) reported that the median number of accessory sperm per embryo (ovum) was related to the time of AI, increasing the fertilization rate as AI occurred closer to ovulation. Saacke et al. (1994) suggested that the number of accessory sperm per ovum is unique to a given sire. Saacke et al. (1994) summarized data from four sires displaying the uniqueness of the sperm from an individual sire to reach the site of fertilization. Using a minimum of 10 embryos (ovum) recovered per sire, the median accessory sperm number was 34 for sire A, 3 for sire B, 2 for sire C, and 0 for sire D when cows were inseminated with 20×10^6 sperm cells. These trends were similar when the same sires were used to inseminate cows at 100×10^6 cells per dose, with sire A achieving the highest number of accessory sperm (45) and sire D achieving the lowest number of accessory sperm (6). Saacke et al. (1994) could not explain the difference in accessory sperm numbers by semen viability characteristics for the 4 sires. Saacke et al. (2000) also discussed many attempts taken to increase the number of accessory sperm. These methods include blocking retrograde sperm loss, fresh versus frozen semen, semen extender composition (milk and egg yolk), sperm microencapsulation, using specific sires, increasing sperm cell dosage, and seminal plasma effects (Saacke et al., 1994). Only specific sires and increasing sperm cell dose had increased the accessory sperm number per embryo (ovum). This study would suggest that timing of AI simultaneously with administration of GnRH 54 h after PGF_{2α} or 24 h later is unrelated to CR and no interaction should exist between timing of AI and CR of sire fertility groups. Differences of sire fertility groups affected PR in this study suggesting that the difference may be the ability of semen from high fertility sires to provide an advantage for reaching the site of fertilization and providing competent sperm capable of sustaining embryogenesis.

Parity differences were also revealed in this study ($P \leq 0.01$). The results disagree with the review by Lucy (2001) citing animals in the first parity contribute to lower fertility at first AI and with Navanukraw et al. (2004) where there was no difference among parities in an Ovsynch or Presynch-Ovsynch protocol. However, Cartmill et al. (2001) reported parity effects where first parity animals had higher CR than multiple

parity cows and Portaluppi and Stevenson (2005) report first parity cows tend to be higher than multiple parity animals.

Seasonal differences in this study ($P \leq 0.05$) may not be explained by environmental factors, but by compliance of administration sequence in the Presynch-Ovsynch protocol and AI at the prescribed time in the protocol. Of the 219 cows not bred according to the first AI protocol 93 were removed in season 1 compared to 51 in season 3. Highest percentage of cows bred off protocol occurred in season 1, 23%, when PR was lower compared to 9% in season 3. Only season 1 and 3 experienced different PR ($P \leq 0.05$). Removing these cows from the study was the responsibility of the farm employees. Insemination of cows detected in estrus following one of the initial $\text{PGF}_{2\alpha}$ administrations was the major error in compliance, but did improve as the study progressed. This practice of removing cows detected in estrus probably increased the percentage of anestrous cows following completion of all hormone administrations and FTAI and may explain the lower PR obtained during season 1 when compared to season 3.

The PR was not different for cows receiving a CIDR insert 14 d following FTAI than cows that did not. In contrast, Chenault et al. (2003) reported that cows receiving a CIDR insert were less likely to become pregnant to first AI when compared to cows that did not receive a CIDR insert. A CIDR insert by parity interaction was not expected and can not be explained biologically ($P \leq 0.05$). Least square means for PR of cows receiving either a CIDR insert or no CIDR insert were 26.9 and 26.4%. Only cows in second parity tended to be different among cows receiving a CIDR insert and cows not receiving a CIDR insert ($P = 0.07$). There were no differences for other parity comparisons for use of CIDR inserts post AI.

The normal estrous cycle ranges from 18 to 24 d with 21 d considered to be the mean estrous cycle length. Administration of a CIDR insert 14 d after GnRH for a period of 7 d should inhibit the expression of estrus during the period of insertion, because of the negative feedback of progesterone released from the device inhibits the release of gonadotrophin and thus follicular growth. During the time (d 14 to 20) of CIDR insert administration 14 (3.7%) treated cows were detected in estrus and received AI compared to 77 (17.7%) control cows. Chenault et al. (2003) reported that 2 cows received AI

during the period that CIDR inserts were utilized and across the eight farms retention rates of the CIDR inserts ranged from 91.9 to 100.0%. If the CIDR insert was lost from a cow during the administration period or the cow never received a CIDR insert, exhibition of estrus by a non-pregnant cow would be expected. The compliance and retention rates of CIDR inserts for the two locations on this study were not measured. However, compliance and/or retention rates may be lower than those reported by Chenault et al. (2003) because of a greater percent of treated cows detected in estrus during the CIDR insert administration period. Chenault et al. (2003) found that 7 cows displayed estrus on the day of CIDR insert removal, but three of these cows had lost the CIDR insert prior to manual CIDR insert removal on d 7. A total of 28 (7.4%) treated cows were detected in estrus on the day of CIDR insert removal during this study. It is not known whether the 28 treated cows detected in estrus on the day of CIDR insert removal actually had CIDR inserts removed that day or it was a compliance or retention issue.

The CIDR insert did resynchronize the return to estrus during a period of 4 d ($P \leq 0.001$) for more treated than control cows. A higher proportion of cows receiving CIDR inserts was found to display estrus than controls during the 4 d period, 21 to 24 d, after GnRH administration of the Ovsynch protocol, 92.5 and 62.8% respectively (Table 3). As expected, a higher proportion ($P \leq 0.001$) of control cows was detected in estrus 18 to 20 d after GnRH administration of the Ovsynch protocol than treated cows, 37.2 and 7.5% respectively (Table 3). It is also logical and practical to evaluate the percentage of control cows detected in estrus over a 7 d period of the expected return to estrus, 18 to 24 d after final GnRH administration. Most dairy herds detect estrus daily to identify as many non-pregnant cows as possible to receive subsequent AI. The percent of available cows detected in estrus over the 7 d period for treated and control cows was 31.5 and 38.3%, respectively (Table 3). Thus, herd management was delaying the estrus period of cows receiving CIDR inserts and unable to detect enough cows in estrus during a 4 d RE to offset the delay of the return to estrus when compared to control cows.

There was no effect of treatment on second AI CR ($P > 0.10$). Conception rates for second AI of cows receiving CIDR inserts and control cows were 20.7 ± 4.9 and $19.5 \pm 3.6\%$, respectively. There was no effect of RE on CR at second AI ($P > 0.10$). Conception rates for RE at second AI during 18 to 20 and 21 to 24 were 15.5 ± 5.7 and

24.7 ± 3.1, respectively. During the 7 d RE period 24.6% of the cows detected in estrus were detected in estrus during the 18 to 20 d interval compared to 75.4% of the cows detected in estrus during the later 21 to 24 d interval. Chenault et al. (2003) discussed the possibility for improved detection of estrus when cohorts of cows are displaying estrus simultaneously. This may explain the increased occurrence for detection of estrus in treated cows on d 21 because treated and control cows were housed together. The expected mean day for return to estrus for control cows was d 21 and the expected mean day of the return to estrus for treated cows was d 23. Day 21 had the second highest number of cows detected in estrus for treated and control cows over the 9 d period, 23.7 and 16.0% respectively (Figure 11). Day 23 had the highest number of cows detected in estrus for treated and control cows over the 9 d period, 28.8 and 18.4% respectively (Figure 11). Cohorts of treated and control cows that were found displaying estrus simultaneously may create more accurate estrus detection resulting in higher CR. The expected return to estrus on d 21 for control cows may have allowed more cows receiving a CIDR insert to be detected in estrus and the expected return to estrus on d 23 for treated cows may have allowed more control cows to be detected in estrus.

Although locations tended to be different ($P = 0.09$) there was no effect of a treatment or RE by location interaction on second AI CR. Return AI CR for location 1 and 2 were 24.9 ± 4.2 and $15.4 \pm 4.2\%$, respectively. The reason for the difference in CR between locations for cows bred during the 7 d return period to estrus is not known. During the 7 d RE location 1 bred 118 (32.4%) of the eligible cows and location 2 bred 134 (29.7%) of the eligible cows.

Chenault et al. (2003) reported incidence of cows that received a CIDR insert returning to estrus on the day of CIDR removal to be 7 of 887 (< 1%). However, 3 of the 7 cows had lost the CIDR insert prior to the appointed time of CIDR insert removal. Detection of estrus on the same day of CIDR insert removal for cows receiving a CIDR insert was found on both locations. Location 1 detected 6 (14.6%) and location 2 detected 22 (28.6%) cows in estrus on the same day as CIDR insert removal over the 9 d period. Whether these cows actually received a CIDR insert, lost the CIDR insert, or were misdiagnosed in estrus was not known. Although, there was not a treatment by location interaction for second AI CR, the fact that location 2 tended to breed more cows

on the day of CIDR insert removal cannot be ignored and most likely explains part of the decreased second AI CR.

Chenault et al. (2003) evaluated the return to estrus for cows receiving a CIDR insert during the 3 d immediately following CIDR insert removal. Chenault et al. (2003) reported that 34.1% of cows detected in estrus over a 9 d period that received a CIDR insert exhibited estrus during the 3 d period with more than 50% of those cows exhibiting estrus 2 d after CIDR insert removal. Similar results were found for this study as well. The highest proportion of cows detected in estrus over the 9 d period, 28.0%, of cows that received a CIDR insert were detected in estrus 2 d after CIDR insert removal (Figure 11).

CONCLUSIONS

The results of this study indicate that the use of high fertility sires is a practical recommendation for improving PR at first AI. There was no difference in timing of AI for fertility groups in a FTAI protocol. However, previous studies found that AI at 12 to 16 h prior to ovulation resulted in a compromise between functional sperm loss and an aging ovum, probably resulting in less selection of competent sperm. Extreme AI times were evaluated during this study, thus it would be assumed that average and high fertility sires perform better when AI occurs at 12 to 16 h after GnRH administration. The ranking of the two fertility groups did not change when AI occurred at either 0 or 24 h after GnRH administration. High fertility sires remained superior to average fertility sires at both times of AI.

Results of this study dispute the validity of the use for CIDR inserts for the RE for cows inseminated previously. Although the CIDR insert effectively increased the proportion of cows detected in estrus during a 4 d period compared to control cows, a greater proportion of cows receiving CIDR inserts were detected in estrus or received subsequent AI later than control cows over the 7 d RE. Thus, the CIDR insert may return a greater proportion of cows to estrus during a fewer number of days; however, during the normal 7 d return to estrus period no difference in detection of estrus was achieved for control cows or cows resynchronized with CIDR inserts. Reproductive research should begin to focus on ways of improving rates for the detection of estrus following first AI. The results of this study found that 56.5% (449) of the cows non-pregnant to

first AI were detected in estrus or received subsequent AI more than 24 d later and 63% (283) of those cows were detected in estrus or received subsequent AI \geq 40 d after first AI. This data supports the fact that detection of estrus is a challenge and an area for improvement for the majority of today's dairy farms.

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VITA

JEFFREY MAX CORNWELL

Shelby, North Carolina

My passion for the bovine began at an early age working beef cattle owned by my family and community. During high school, I participated in livestock judging as a primary FFA event. I also showed beef cattle at the Cleveland County Fair. I chose to remain in an agriculture field at college earning a BS degree in Agricultural Business Management and a minor in Animal Science from North Carolina State University. During college, I worked for the NCSU University Farms at various jobs, including one year working with the beef cattle farm at Reedy Creek, one year working for the farm crew at Lake Wheeler, and two years working for the dairy farm at Lake Wheeler. Internships were held during the summer of 2001 and 2002 at Rocky Creek Dairy, Inc. in Olin, NC and Deerview Jerseys in Mocksville, NC. After completion of my MS in Dairy Science, my wife, Mary, and I will return to Cleveland County and milk around 100 Holsteins.

Education:

High School:

Burns High School Lawndale, NC	1999
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College:

North Carolina State University Bachelor of Science in Agricultural Business Management Minor in Animal Science	2003
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Virginia Polytechnic Institute and State University Master of Science in Dairy Science (in progress)	2005
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Publications:

Kasimanickam, R., J. M. Cornwell, and R. L. Nebel. 2005. Fertility following fixed-time AI or insemination at observed estrus in ovsynch and heatsynch programs in lactating dairy cows. *Theriogenology*. Article in press.

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