

ABSTRACT

WHITLEY, JUSTIN THOMAS. Effect of CIDR-Ovsynch Estrous Synchronization Protocol on Pregnancy Rates and Progesterone Concentrations in Lactating Dairy Cows. (Under the direction of Dr. C.S. Whisnant).

Pregnancy rates of high producing, lactating, dairy cows in the United States have been on a steady decline over the last few decades. It has been suggested that this could be due to insufficient concentrations of serum progesterone at critical times of the estrous cycle as well as after insemination. The objective of this study was to establish an estrous synchronization protocol including exogenous progesterone to increase these serum progesterone concentrations in an attempt to also increase pregnancy rates. The control group (n=27 lactating Holsteins) was subjected to the standard Presynch-OvSynch protocol. Presynchronization was done using two injections of PGF_{2α}, the first being at day -36 and the second at day -22 relative to the date of insemination. The OvSynch protocol was started with GnRH on day -10, followed on day -3 by PGF_{2α}, then a second dose of GnRH administered on day -1, and timed Artificial Insemination (TAI) was done on day 0 at 16 hours after GnRH. Cows assigned to the treatment group received a Controlled Internal Drug Releasing insert (CIDR®) containing 1.38g of Progesterone inserted intravaginally on day -20 which remained in place for 7 days and were given PGF_{2α} on day -13 at CIDR removal. Seventy-two hours later on day -10, the OvSynch protocol was started and followed the same as the control group. Blood samples were collected at all times of injection and at TAI. Pregnancy was done via rectal palpation at regular herd checks by a veterinarian. Pregnancy rate was not different between groups (28.2 ± 2.4% CIDR Protocol, 26.9 ± 2.5% Presynch - OvSynch). Progesterone concentrations throughout both treatments confirmed that both presynchronization methods were able to achieve luteal regression. The

CIDR Protocol was successful at achieving equal pregnancy rates as the Presynch - Ovsynch protocol, however, the study should be repeated with greater cow numbers to determine if one is better than the other.

In the interest of cost analysis of the synchronization protocol a second study was done to determine if the CIDR could be used twice. Both Holstein heifers and lactating Holstein cows had a once used 1.38g CIDR inserted vaginally for 7 days and blood samples collected at insertion, 1 hour, 24 hours, and 7 days later. Serum P4 concentrations were not different between types of CIDR in either heifers or cows. Serum P4 concentrations increased ($P < 0.01$) from time of insertion to 1 hour later and remained elevated at all other times. The commercially available CIDR in the United States can be used for at least two 7 day periods without change in serum P4 concentrations.

Effect of CIDR-Ovsych Estrous Synchronization Protocol on Pregnancy Rates and Progesterone Concentrations in Lactating Dairy Cows

by
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A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Master of Science

Animal Science

Raleigh, North Carolina

2011

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BIOGRAPHY

Justin Thomas Whitley was born July 3, 1987 to Jackie and Wendy Whitley. He was raised most of his life in rural Wilson, North Carolina. His family history in farming and his love and passion for showing and judging livestock in 4-H and FFA instilled a desire to one day have a career in the agricultural industry.

After graduating from James B. Hunt Jr. High School he knew the only place he wanted to go was the College of Agriculture and Live Sciences at North Carolina State University. While an undergraduate at NCSU he was active in the Animal Science Club, Alpha Zeta Honors Fraternity, intramural sports, and attended every basketball and football game possible. His initial intention was to attend the College of Veterinary Medicine and become a large animal vet, but after working in the research labs of Dr. Matt Poore and Dr. Edgar Oviedo the focus changed more towards research. After completing his Bachelor of Science in Animal Science in the spring of 2009 he decided to stay in school and pursue a Master's of Science in Animal Science and further study his favorite topic of Reproductive Physiology. He had always been fascinated by reproduction, endocrinology, and assisted reproductive technologies.

While a graduate student in the Animal Science department, Justin was a member of the American Society of Animal Science, the Society for the Study of Reproduction and the North Carolina Cattlemen's Association. He remained active as an alumni of Alpha Zeta and continued his loyal support of Wolfpack Athletics.

Justin will receive a Master's of Science in Animal Science in the fall of 2011. In June of 2011 he began working as a Farm Manager Trainee with Prestage Farms and has recently moved to Newton Grove, NC. Keeping the possibility of continuing his education and obtaining a Doctorate of Philosophy in the same area of study, he continues to look for his dream job of working in reproductive technologies in the cattle industry.

ACKNOWLEDGMENTS

I would like to thank Dr. Scott Whisnant, Dr. Peter Farin, and Dr. Steven Washburn for serving on my graduate committee. A huge thanks goes to George Elias for all of his help in the lab running my progesterone assays. Thank you to all of the staff at the Dairy Educational Unit for always having the cows/heifers caught up and ready when we needed them. Thanks a million times to Marian Correll for keeping me and all my paperwork straight over the last two years.

Last, but certainly not least, I have to thank all of my family and friends for their love and support throughout this process.

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Review of Literature

Previous research has shown that increasing serum progesterone levels in dairy cows through administration of a controlled internal drug releasing device (CIDR) containing progesterone can result in increased conception and calving rates (Folman et al., 1990; Melendez et al., 2006). Progesterone exposure at different times during the estrous cycle has shown both a positive response (El-Zarkouny et al., 2004, Melendez et al., 2006) as well as no significant difference (Xu and Burton 1998; Lima et al., 2009; Stevenson, 2011). Previous studies have primarily focused on exposure after insemination with hopes of providing the level of progesterone required to maintain pregnancy (Mann and Lamming, 1999). For this study, supplemental progesterone was supplied prior to insemination as an addition to a formerly established estrous synchronization protocol known as Presynch-Ovsynch. Another study was conducted to compare the serum progesterone concentrations produced by a new vs. a once used CIDR in heifers and lactating Holstein cows. To understand the background and rationale for these experiments this review of the literature was conducted.

Progesterone

Progesterone (P4, pregn-4-ene-3,20-dione) is a very active and essential hormone in regulating the proper function of the female reproductive system. It is the major hormone in a class of hormones known as progestogens. In 1934, four independent laboratories, Wintersteiner and Allen, Slotta et al, Butenandt and Westphal, and Hartmann and Wettstein, claimed to isolate the pure crystalline hormone secreted by the corpus luteum (CL) and

named it Progesterone (Allen, 1935; Slotta et al., 1934; Butenandt and Westphal, 1934; Hartmann and Westtstein, 1934). Progesterone is an unsaturated diketone with the empirical formula $C_{21}H_{20}O_2$ which, like all steroid hormones, is derived from cholesterol (Ruzicka, 1936). It was first discovered as being a hormone synthesized in the ovary, or more specifically, in the CL on the ovary. It was not until 1973 when Piziak and Gawienowski discovered that the placenta of guinea pigs could produce progesterone from endogenous precursor (Piziak and Gawienowski, 1973).

Role of Progesterone in the Female Reproductive System

Progesterone is an undeniably important and necessary hormone in the mammalian female reproductive cycle beginning with regulation of the estrous cycle and ovulation. Progesterone is synthesized in the ovary from the granulosa cells that give rise to the eventual corpus luteum. If pregnancy is achieved, the CL persists, and continues to produce P4 for the duration of pregnancy in some species or until the placenta takes over in other species. If there is no mating and conception, the CL will remain for a period of time and P4 levels will remain high, until it is regressed by Prostaglandin $F2\alpha$. While the P4 levels are high, follicles will be growing on the ovary, but until P4 levels drop there will be no ovulation and the dominant follicle will become atretic, giving way to another growing follicle. Once the CL regresses and P4 levels drop, there is a rise in estradiol secretion as well as in Gonadatropin Releasing Hormone (GnRH) which causes Luteinizing Hormone (LH) to surge until LH levels become high enough to for ovulation to occur, thereby completing the cycle (Hansel and Echternkamp, 1973).

Progesterone is important for the entire estrous cycle, and without it there would be no ovulation of follicles. Lydon et al. showed that in progesterone receptor knockout (PR KO) mice, there were functional abnormalities in the ovaries. While size, weight, and overall appearance of the ovaries were no different than controls, the PR KO females did not ovulate despite having preovulatory follicles containing apparently mature oocytes (Lydon et al., 1995). There was also a distinct absence of any CL on the ovaries of the KO mice suggesting that they had never ovulated. It was also shown that the granulosa cells of expected preovulatory follicles in such mice lacked the ability to mature and luteinize regardless of prolonged exposure to gonadotropins. Those mice also had a marked lack of lordosis and never showed any signs of estrus or sexual behavior (Lydon et al., 1995). The observation that P4 is critical for ovulation to occur is supported by a study in primates showing that administration of a progesterone synthesis inhibitor in midcycle prevents ovulation (Stouffer, 1996). The macaques were given gonadotropins to stimulate the growth of multiple preovulatory follicles, followed by human chorionic gonadotropin (hCG) administration to promote oocyte (nuclear) maturation, ovulation, and follicular luteinization. Animals that were in the treatment group receiving trilostane (TRL), a steroid synthesis inhibitor had ovulation abolished. It was progestins, not androgens that resumed ovulation in these animals (Stouffer, 1996). Cows need progesterone exposure to show estrus, as the first ovulation postpartum or at puberty is often without much estrous behavior or "silent". These silent ovulations are thought to be the result of a state of refractoriness to estradiol at the hypothalamus due to the high concentrations of estradiol at the end of pregnancy (Allrich, 1994). Progesterone produced by the corpus luteum from this silent ovulation removes the

refractory state so that the second ovulation is associated with estrus (Carrick and Shelton, 1969).

In mature, lactating dairy cows, subluteal phase serum progesterone levels at less than luteal phase concentrations have been shown to decrease or inhibit the LH surge and thereby inhibiting ovulation (Hayes, 2008). The cows were subjected to a presynchronization protocol consisting of two injections of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) at days -34 and -20 (day 0 being day of insemination) followed 12 days later by an injection of GnRH to ovulate/leutenize any preovulatory follicles and start a new follicular wave. CIDRs were put in with differing concentrations of progesterone (new or incubated in another cow for 14 or 28 days). During the follicular phase (h 72–108), the mean progesterone concentration was different ($P \leq 0.04$) in each treatment group (control: $0.21 \pm 0.12\text{ng/ml}$; CIDR-28: $0.58 \pm 0.10\text{ng/ml}$; CIDR-14: $0.87 \pm 0.12\text{ng/ml}$; CIDR-0: $1.46 \pm 0.11\text{ng/ml}$). Ovulation occurred in 3/7 of the CIDR-28 treated cows, with the lowest P4 concentration, but there was no ovulation in any of the CIDR-0 or CIDR-14 treated cows. Preovulatory follicles were present and most persisted through day 14 and grew to $>20\text{mm}$, but were not ovulated due to the decreased or lack of LH surge frequency (Hatler et al., 2008). This shows that progesterone is necessary for ovulation, but the concentrations and timing of release are extremely important to successful reproductive performance. The regression of the corpus luteum and subsequent fall in serum progesterone levels is also important for ovulation.

It has also been suggested that progesterone is responsible for an increase in relaxin. This increase in relaxin may be a facilitator of follicular rupture and ovulation by increasing collagenase and other proteases that break down the follicular wall and cause it to rupture

(Graham, 1997). This is supported by studies in mice where lowered serum progesterone levels resulted in a decrease in serine proteases, kallikrein, and plasminogen activator and suppresses ovulation. Treatment with exogenous progesterone relieved the suppression suggesting a strong correlation (Loutradis et al., 1991).

Another role of progesterone during the estrous cycle is the control of proliferation of uterine tissues. Changes in levels of tissue growth can be directly correlated to levels of progesterone and estrogen, with estrogen stimulating proliferation and progesterone inhibiting the actions of estrogen, thereby inhibiting proliferation (Clarke and Sutherland, 1990). During the estrogen driven follicular phase of the cycle there is an increase in proliferation of epithelial and stromal cells. Once the follicle is ovulated and the luteal phase begins, there is a decrease in proliferation driven by progesterone. In contrast, during the late luteal phase there is an increase in proliferation directly correlated with high serum levels of progesterone (Graham, 1997).

Cattle Progesterone Levels

Certain serum progesterone levels are necessary at different stages of the cycle as well as gestation to achieve optimum fertility. In the early stages the CL provides this progesterone and it was Ewing et al. in 1968 that first suggested monitoring peripheral levels of progesterone as a means to study CL function (Ewing et al., 1969). Six cows were monitored over seven complete estrous cycles to determine the daily progesterone profile throughout the cycle. They found a rapid increase in P4 from day 3 to day 8 and a slower rate of increase from day 8 to day 17 and noted a decrease of more than 50% from the

previous day on days 18, 19, and 21 depending on the cow. Peak P4 levels in this group ranged from 6.1-10.2 ng/ml, while baseline levels at estrus averaged 0.4 ng/ml. A decline from 4.7 ng/ml on day 19 to 1.5 ng/ml or less on day 20 was observed in cows failing to conceive (Ewing et al., 1969).

For some cows their first postpartum estrous cycle will be a short cycle which results in a short lived CL which may be necessary for resumption of estrous cycles of a standard length of 21 days (McKee et al., 1980). In that study, 2,854 Angus cows were used to monitor the occurrence of short cycling. It was found that estrous cycles of 7-10 days were the most common of short cycling females ($P < 0.005$). In part two of the study, calves were weaned from 25 Simmental cows and those cows came into estrus (10 to 25 days after weaning) at a higher rate than cows still nursing and also had a higher incidence of 7-10 day short cycles. No cows were able to conceive to the artificial insemination at the short cycle estrus. Serum P4 levels increased ($P < 0.01$) before the second estrus when compared to the short cycle and 61.5% conceived at that second estrus (McKee et al., 1980). Since this first estrus does not appear to be fertile, exogenously simulating a rise in progesterone from the short lived CL may be beneficial to first service conception rates in cows that do not exhibit a short cycle. Cows that experienced a transient increase in progesterone, defined as an increase in P4 to >1 ng/ml serum, 6-7 days before the first postpartum estrus conceived earlier (104 ± 4 d compared to 119 ± 5 d, $P = 0.02$) than those that did not have a transient increase (Deutscher et al., 1996). Cows also had a sustained P4 increase sooner than cows that had no transient increase in P4 before the first estrus (101 ± 3 d compared to 114 ± 5 d, $P = 0.02$). Such a transient increase did not occur in 28.5% of the cows before the first postpartum estrus

(Deutscher et al., 1996). Cows that fall into that group could likely benefit from some supplemental P4 before breeding to establish a more fertile estrous. The conclusion of this study was that progesterone likely has a role in preparing the entire reproductive tract for conception and pregnancy.

Progesterone secretion is also required for the preparation of the onset of puberty. Microscopic evaluation of ovaries from heifers that had experienced one or two increases in progesterone showed areas of compact luteal tissue (Berardinelli et al., 1979). Small luteal tissues were 1.5 to 6 mm in diameter and were located within the ovary, assumed to be the leutenization of small follicles, and not palpable from the exterior of the ovary. Polat et al. (2009) reported that progesterone supplementation with a progesterone releasing intravaginal device (PRID) for 12 days in heifers that had delayed onset of puberty resulted in 93.9% of the heifers being cyclic ($P4 \geq 1$ ng/ml) within 72 hours of PRID removal. Fixed-time artificial insemination at 48 and 72 hours after withdrawing the PRID produced 54.6% combined pregnancy rate at 60 days post-insemination.

It is standard to use 1ng/ml serum progesterone as the benchmark to indicate ovulation and the presence of a functional corpus luteum. Perry et al., however, reported that the serum progesterone concentration in a group of 13 Hereford x Angus cows reached a maximum of 0.7 ± 0.2 ng/ml after the first postpartum ovulation, with a range in peak concentrations of 0.3 to 1.2 ng/ml (Perry et al., 1991). They suggested that when 1ng/ml of serum progesterone is used to indicate ovulation, the first short luteal phase may be undetected if peak levels of P4 are < 1 ng/ml. The patterns of P4 concentrations were similar during the first 4 days after the first ovulation and the second ovulation, but from day 5 to

day 8 serum P4 concentrations were higher after the second ovulation ($P < 0.005$). Diameters of CL were measured via transrectal ultrasonography to relate P4 concentrations to CL size. CL diameters followed the same pattern as serum P4 concentrations between first and second ovulations (Perry et al., 1991).

Foster et al. reported that suckling has an effect on postpartum progesterone levels and whether or not the cow has a short cycle. Short cycles, which they called “silent estrus”, were more common in cows that were suckled either twice a day or ad libitum than in cows that weren’t suckled at all (Foster et al., 1981). The study doesn’t mention if the presence of the calf at suckling was necessary to see this effect because it was done with beef cattle. It has since been proven, however, that twice daily suckling, and not just milking with the calf present prolongs postpartum anovulation (Lamb et al., 1999). That would suggest that twice daily milking of a lactating dairy cow with no calf presence should have no detrimental effect on rise of progesterone levels or the interval to first postpartum ovulation.

It is difficult to distinguish and treat progesterone issues because not all cows have the same progesterone profiles. Meier et al. (2009) compared subpopulations of progesterone profiles using cluster analysis and found three shape-based cluster profiles defined as either peaked profile, structured profile (exhibiting a wave like pattern), or flat top profile. Estrous cycles of Holstein-Friesian cows were synchronized at 25-30 days postpartum. The second estrous cycle postpartum resulting in a spontaneous ovulation was the cycle reported. There was no difference between the three groups between days 5 and 7 of the estrous cycle, however, the days that plasma progesterone concentrations were $>2\text{ng/ml}$ were greater in cows with flat top profiles and structured profiles than cows with peaked profiles (Meier et

al., 2009). Similarly, total progesterone released was greater for the flat top and structured profiles groups compared with peaked profiles. Slight differences in progesterone concentrations during the estrous cycle can cause reproductive processes to be disturbed (Inskeep, 2004).

Progesterone Effect on Fertility

Progesterone is indisputably required for the growth, development, and survival of the mammalian oocyte and conceptus. It is the primary hormone responsible for maintaining pregnancy as supported by studies showing that ovariectomized cows are capable of having a successful pregnancy when supplemented with only progesterone (Hawk et al., 1963; Inskeep and Baker 1985). It is important for follicular growth and development (Britt, 1992; Ulberg et al., 1951; Meisterling and Dailey, 1987), and equally as important during the early post-ovulatory period (Hommeida et al., 2004; McNeill et al., 2006a; Mirzaei and Kafi, 2010). During the luteal phase of the estrous cycle, high levels of progesterone inhibit LH secretion thereby causing low frequency pulses that do not support follicle growth and lead to atresia of the dominant follicle and start another follicular wave. When progesterone levels are low, LH pulses are more frequent and sustain growth of the dominant follicle which will secrete more estradiol-17 β to maintain its own growth. When the corpus luteum is present and actively producing progesterone ovulation cannot occur, however, at the onset of luteolysis the largest follicle present will likely ovulate at the next estrus (Inskeep and Dailey, 2005).

Low concentrations of progesterone during the estrous cycle preceding inseminations have been shown to reduce fertility in dairy cows (Folman et al., 1973; Meisterling and Dailey, 1987). Townson et al. (2002) reported that dairy cows having three follicular waves during their cycle were more likely to conceive when ovulating the third follicle which had developed for a shorter period, when compared to the more common occurrence which was cows that had only two follicular waves ($P < 0.01$; 30% cows with three waves vs 68% with two waves). This study showed a higher pregnancy rate in cows with three follicular waves versus cows with only two (81% vs 63% respectively $P=0.058$). These pregnancy rates were significantly higher than the herd average and was attributed to frequent handling and intensive estrus detection as well as the exclusion of any cows with reproductive abnormalities. Ovulatory follicles have been shown to be 1.5 days older and 1.2 mm larger when ovulating from the second wave compared to the third (Inskeep and Dailey, 2005). There is an apparent positive linear relationship between progesterone concentrations on the day of luteolysis and subsequent embryo survival rate (Diskin et al., 2002). Low peripheral progesterone can also lead to persistent dominant follicles (dominant for > 9 days) that are subsequently at a more mature stage of development at ovulation which can markedly reduce pregnancy rates (Mihm et al., 1999). Shaham-Abalancy et al. (2001) reported that low progesterone concentrations resulting in persistent follicles has a delayed stimulatory effect on uterine responsiveness to oxytocin during the late luteal phase of the subsequent cycle. They suggest that the resulting increase in $\text{PGF2}\alpha$ could interfere with maintenance of the corpus luteum during the early embryo stages of pregnancy. The more

probable issue, however, is a low progesterone level causing persistent follicles to mature too far too fast resulting in decreased fertility (Diskin et al., 2006).

Taylor et al. (2003) reported that primiparous high yielding cows with progesterone profiles that suggested persistent corpus luteum had no detrimental effects on fertility or production parameters when compared to cows with normal progesterone profiles. They propose that it may be incorrect to refer to persistent CL and delayed ovulation progesterone profiles as abnormal, and that it is possible that these profiles indicate an appropriate response to a nutritional state or physiological situation such as losing body condition or contracting a uterine infection post-partum (Taylor et al., 2003). Cows with a prolonged luteal phase (PLP) post-partum have been shown to have a first ovulation earlier than cows with normal ovarian activity. This was also related to milk production because for cows with a progesterone concentration $\geq 1\text{ng/ml}$ on day 24 after calving there was an increase in risk of PLP by 1.1 for each 1kg increase in mean peak milk yield during the first 75 days after calving (Mirzaei and Kafi, 2010). Smith and Wallace (1998) reported that primiparous cows with luteal activity before 21 days post-partum did not have reduced fertility parameters. However multiparous cows with luteal activity before 21 days post-partum had longer calving-to-conception interval, more services per cow, and lower conception rates to all inseminations. The difference between the primiparous and multiparous cows could be due to the fact that first calf cows complete uterine involution and recovery at a faster rate than multiparous cows (Zain et al., 1995) and the multiparous cows lost more body condition over the observation period due to their increased milk yield over the primiparous group.

Effects of different levels of progesterone after ovulation and insemination have been more closely studied. Both sub- and supra-optimal progesterone concentrations during the early luteal phase post-insemination have been associated with decreased embryo survival (Stronge et al., 2005; Starbuck et al., 1999). Shelton et al. (1990) reported low serum progesterone levels before day 6 post-insemination in sub fertile cows. They linked that relationship to age of the cows because they were comparing to heifers, however, it is more likely due to the lactation status of the cows. There is a strong correlation between milk progesterone levels on days 5, 6, and 7 post-insemination and embryo survival (Stronge et al., 2005). Optimum milk progesterone concentrations for embryo survival were 7.4, 13.2, and 16.8 ng/ml on days 5, 6, and 7 respectively. The optimum rate of increase in milk progesterone on days 4-7 for maximum embryo survival was 4.7 ng/ml/day. Similarly, McNeill et al (2006b) found a significant ($P < 0.05$) linear and quadratic relationship between the milk progesterone concentrations on days 4-6 and embryo survival, while levels on days 0-3, 7, and 8 showed no significant relationship. Ahmad et al. (1996) established a significant difference ($P < 0.01$) between progesterone concentrations of pregnant and non-pregnant cows on days 6-7 after artificial insemination with the pregnant cows having much higher circulating P4. In contrast Bulman and Lamming (1978) found no difference in milk progesterone between pregnant and non-pregnant cows until day 13 post insemination.

It is not only the level of progesterone that is important, but the timing of the rise in luteal activity. A delay in the luteal phase rise in progesterone concentrations after insemination has been associated with decreased embryo survival (Larson et al., 1997; Darwash and Lamming 1998; Starbuck et al., 2001). Peripheral progesterone concentrations

should start to rise by day 4 post-insemination and reach maximal concentrations by days 8-10 (Niswender et al., 2000; Robinson et al., 2008). In dairy cows previously established as sub-fertile, the rise in post-ovulatory progesterone concentrations is delayed ($P = 0.01$) and occurs more slowly ($P = 0.001$) than in heifers (Shelton et al., 1990).

Lower progesterone levels during days 28-37 post-insemination has also been associated with late embryonic mortality (Inskeep and Dailey, 2005). In heifers that had embryonic death between days 25 and 40 luteal regression was detected at least 3 (range 3-42) days after the embryo was lost. When luteal regression occurred first, embryonic death happened rapidly suggesting that the loss of progesterone being produced by the CL was the cause of conceptus loss (Kastelic et al., 1991). Other studies have reported that all late embryonic death preceded luteal regression, however, the possibility that luteal function had been compromised before embryo death was not ruled out (Inskeep and Dailey, 2005). Starbuck and colleagues (2004) observed pregnancy retention between weeks 5-9 of gestation and found a positive correlation between progesterone concentrations at week 5 and retention of pregnancy ($P \leq 0.05$). If luteal function is compromised after maternal recognition of pregnancy it is possible that regressing the non-functional CL and inducing a new one with progesterone supplementation in between could help maintain the pregnancy. Bridges et al. studied this concept and found cows that had CL that were induced on the ovary ipsilateral to the pregnant uterine horn after day 36 maintained their pregnancies. However the survival rate dropped to 50% when the CL was induced on or before day 36 of gestation (Bridges et al., 2000).

A delay in the rise of progesterone concentrations (day 5.6 ± 0.4 vs. day 4.1 ± 0.1) was also linked to poorly developed embryos that produced insufficient amounts of interferon tau (IFN- τ) (Mann and Lamming, 2001). IFN- τ is a protein secreted by the preimplantation embryo that is responsible for the maintenance of the pregnancy (Roberts, 1996). Progesterone is directly responsible for preventing luteolysis initially by down regulating the oxytocin receptors of the luminal epithelium (Lamming and Mann, 1995; Wathes et al., 1996) until IFN- τ takes over to inhibit PGF 2α secretion by inhibiting the oxytocin receptors (Robinson et al., 1999). IFN- τ also prevents luteolysis through the induction of a prostaglandin synthesis inhibitor (Thatcher et al., 1995). The sufficient production of IFN- τ is dependent on an appropriate timing of increase and level of maternal progesterone secretion (Mann and Lamming, 2001).

There is apparently a range of progesterone concentrations during the early luteal phase that lead to maximum embryo survival. Concentrations of progesterone exceeding 3 ng/ml result in increased embryo survival, but concentrations greater than 9 ng/ml result in a decline in survival (Starbuck et al., 1999). It has been previously established that progesterone plays a role in preparing the uterine environment for pregnancy, so it is possible that these supra-optimum concentration levels result in an advanced uterine environment that would not match up with the maturation level of the embryo.

Effect of High Milk Production on Progesterone

Washburn et al. examined reproductive performance in southeastern Holstein and Jersey cows over a 24 year period (1976-1999). Milk yield increased dramatically over that

time period, while average days open and services per conception likewise increased (Washburn et al., 2002). Higher producing dairy cows have a higher volume of luteal tissue, but lower levels of circulating serum progesterone than low producers and heifers (Fricke et al., 2005, Sartori et al., 2004). Primiparous cows also have lower first service conception rate from that of their maiden fertility as heifers (Taylor et al., 2003). This is likely due, not to poor corpus luteum function, but rather increased metabolism of steroids that comes with increased feed intake and metabolism (Rhinehart et al., 2009). Fricke et al. reported that milk production for the 7 days after estrus was negatively correlated with concentrations of progesterone. They also observed a much higher incidence of multiple ovulations in the high producing cows and speculated that low circulating P4 during the preovulatory waves could have allowed for higher LH and FSH levels, possibly leading to an increased number of selected follicles (Fricke et al., 2005). Multiple ovulations in dairy cattle are undesirable because they lead to increased incidence of twinning which decreases reproductive efficiency and profitability (Nielen et al., 1989). Morris and colleagues also found a negative relationship between progesterone concentration and milk yield at the time of insemination (Morris et al., 2005). Genetic selection for high production appears to have a negative impact on fertility regardless of actual levels of milk production. In vitro cultured oocytes from high genetic merit cows resulted in significantly lower blastocyst yields (Snijders et al., 2000).

High producing dairy cows are often in a negative energy balance (NEB), meaning that their energy requirements for body maintenance and milk production are higher than the amount of energy that they consume from their diet. Such NEB is receiving the most

attention and research into why hormone levels in these high producing cows are lower, leading to poor reproductive performance. Wiltbank et al. (2005) suggest two possible explanations for these lower circulating hormone levels. First, that follicles and CL of lactating cows are less steroidogenically active due to inadequate circulating stimulatory hormones, inadequate substrate available, or lack of intracellular steroidogenic activity. The second and more likely explanation is that lactating dairy cows have increased metabolism of steroid hormones as milk production and feed intake increase (Wiltbank et al 2005). High feed intake increases liver blood flow and consequently steroid metabolism. Prior to feeding, liver blood flow in lactating versus non-lactating Holstein cows was 1561 ± 57 L/h and 747 ± 47 L/h respectively (Sangsritavong et al., 2002). Immediately after feeding any amount, liver blood flow and metabolism of progesterone increased and the steroid metabolism was 2.3 times greater in lactating than in non-lactating animals (Sangsritavong et al., 2002). Hommeida et al. also showed that both milk yield and dry matter intake were higher in cows that had sub-optimal progesterone concentrations than those that had normal progesterone profiles (Hommeida et al., 2004). Similarly, an embryo survival study showed a negative linear relationship between average milk yield and milk progesterone concentrations on post-insemination days 4-7 (Stronge et al., 2005).

High producing cows have much higher energy requirements than non-lactating cows (a cow producing 50 kg of milk/day will require 53 Mcal/day whereas a non-lactating cow requires 12.5 Mcal/day for body maintenance) (Wiltbank et al., 2005). Acute feeding of a total mixed ration (TMR) to lactating Holstein cows has been shown to reduce circulating progesterone (Vasconcelos et al., 2003). In this study cows were fed either 100% of the

TMR, 50% of TMR every 12h, 25% of TMR every 6h, or left unfed for an additional 12h. Feeding 100 or 50% of TMR decreased serum progesterone by 1h post feeding and took 8-9h to recover to pre-feeding levels. Feeding 25% of TMR every 6h, however did not reduce circulating P4 ($P < 0.05$), a management technique that should perhaps be considered on dairies with the extra labor available. In contrast to other reports, that study showed no significant effect of lactation status on serum P4 concentration (Vasconcelos et al., 2003). Ad libitum pasture fed cows with a high dry matter intake (DMI) also had lower circulating P4 concentrations (1.08ng/ml) when compared to cows restricted to pasture for 2h a day (1.71ng/ml $P = 0.05$) (Rabiee et al., 2001a). Outside of a research setting it is difficult to determine which cows are in a positive energy balance and which are in a negative energy balance. Just because a cow is offered enough feed to meet her needs doesn't mean she can or is consuming that amount (Villa-Godoy et al., 1988). This makes treatment of sub-optimal levels of hormones difficult as well, and typically leads to a broad spectrum treatment attempt.

It has been suggested that the developmental competence of the oocyte and the steroidogenic capacity of the follicle in high producing dairy cows is determined by their biochemical environment during the extended period of follicular growth before ovulation, up to 80 days (Britt, 1992, Leroy et al., 2008). This means that a cow being in a NEB during the early postpartum period could negatively affect the quality of primary follicles that will be ovulated later. A NEB status could also result in inadequate corpus luteum function leading to low progesterone concentrations post-ovulation (Leroy et al., 2008). Villa-Godoy et al. showed that lactating cows in a NEB status postpartum had lower progesterone during

the first three estrous cycles after calving (Villa-Godoy et al., 1988). Timely supplementation of progesterone and possibly other steroid hormones affected by the increased metabolism such as estradiol, as well as potentially decreasing the amount or activity of specific steroid-metabolizing liver enzymes are potential solutions to this reproductive issue (Wiltbank et al., 2005).

In contrast, Rabiee et al. (2001b) reported no difference in milk or fecal progesterone metabolite concentrations during the estrous cycle of lactating dairy cows with differing milk yields. In that study, endogenous progesterone was blocked by a GnRH-antagonist implant and exogenous progesterone was supplied through a controlled internal drug releasing device (CIDR) for 11 days (Rabiee et al., 2001b). In a similar study those same researchers only used a CIDR for estrous synchronization, and did not interrupt endogenous hormone secretion and reported the same results of no difference in plasma, milk, or fecal progesterone secretion. The milk yield difference between the high producing and low producing cows in that study was an average of 41% or 30.8 and 21.91 kg/day respectively, however, the daily feed intake did not differ greatly between the groups and fecal output was similar (Rabiee et al., 2002a).

Rabiee et al. (2002b) also reported on the effects of different levels of feeding on progesterone concentrations and fecal progesterone metabolite output. Non-lactating Holstein-Friesian cows were ovariectomised and assigned at random to four treatments: (i) 1x CIDR and restricted access to pasture; (ii) 1x CIDR and ad libitum access to pasture; (iii) 2x CIDRs and restricted access to pasture; (iv) 2x CIDRs and ad libitum access to pasture. Plasma progesterone concentrations were negatively influenced by the increased level of

feeding in the ad libitum group although the amount of progesterone released from the CIDR was higher in the ad libitum group ($P = 0.04$) (Rabiee et al., 2002b). Another study by the same lab reported differences in dry matter intake and metabolisable energy (ME) had no effect on plasma or milk progesterone concentrations despite the fact that higher ME increased daily milk yield (Rabiee et al., 2002c).

Pre-ovulatory Progesterone Supplementation

There have been numerous studies that have examined the possible advantages of pre-ovulatory exogenous progestin supplementation on fertility and pregnancy rates in lactating dairy cows. Researchers have used progesterone as a component of estrous synchronization protocols with varying degrees of success. It is difficult to compare those studies, however, because they mostly use different timing of supplementation along with different synchronization protocols. Some have reported an increase in conception rates (Folman et al., 1990; El-Zarkouny et al., 2004; Melendez et al., 2006) while others have shown no advantage of supplemental progesterone (Xu and Burton 1998; Lima et al., 2009; Stevenson 2011)

Ovsynch is a common timed artificial insemination (TAI) synchronization protocol used in the dairy industry and consists of administration of gonadotropin-releasing hormone (GnRH) on day 0 followed 7 days later by $\text{PGF}_2\alpha$ and then a second injection of GnRH 48 hours later. TAI is performed 16 hours after the second GnRH. Stevenson and colleagues included a CIDR in the Ovsynch protocol for 7 days beginning at the first GnRH injection. Overall pregnancy rates at 28 (40% vs. 50%) and 56 days (33% vs. 38%) were higher in the

Ovsynch + CIDR group than the control (only Ovsynch), however, there was a treatment x location interaction presenting varying results on different farms across the country (Stevenson et al., 2006). Ovsynch + CIDR was more effective at increasing conception rate in cows that had low progesterone concentrations at the time of PGF2 α injection. El-Zarkouny et al. in a similar study using a CIDR with Ovsynch reported pregnancy rates at 29 days (59.3% vs. 36.3%) and 57 days (45.1% vs. 19.8%) with Ovsynch + CIDR vs. Ovsynch alone (El-Zarkouny et al., 2004). In the second experiment of this study CIDR + Ovsynch after a presynchronization protocol of two injections of PGF2 α 14 days apart, the CIDR inserts had no effect on pregnancy rates. Melendez et al., however, found that CIDR + Ovsynch after the same presynchronization protocol did in fact improve pregnancy rates as well as increase progesterone concentrations post-artificial insemination (Melendez et al., 2006).

When used in conjunction with Ovsynch, CIDRs seem to be effective at increasing pregnancy rates, however, other synchronization protocols haven't reflected the same improvement. Lima et al. (2009) used two injections of PGF2 α 14 days apart as a presynchronization followed 11 days later by GnRH on day 0 of the trial, PGF2 α on day 7, estradiol cypionate on day 8 and artificial insemination on day 10 as the control. The two treatments included either one CIDR (containing 1.38g progesterone) or two CIDRs. Plasma concentration of progesterone increased linearly with number of CIDRs used by 0.9 ng/ml per CIDR, however, the inserts did not affect pregnancy rate or pregnancy loss at 38 ± 3 or 66 ± 3 days after AI (Lima et al., 2009). Progesterone has also been used as a presynchronization method. Administering a CIDR for 7 days plus PGF2 α at time of

removal, with removal being either 10 days or 3 days before initiation of TAI wasn't successful at improving ovulation rate, luteolysis, or synchronization rate (Stevenson, 2011). The TAI program in this study was Cosynch (injection of GnRH 7 days before and 72 hours after PGF2 α with the insemination occurring 72 hours after PGF2 α). Using the CIDR alone is also an effective method of synchronizing estrus with 92.9% of cows in estrus within 7 days after removal (Xu and Burton, 1998). This study also found that estrus synchronization with a CIDR inserted for 12 days shortened the time from the start of the breeding season to conception with no negative impact on fertility at the synchronized estrus (Xu and Burton, 1998).

It appears likely that exogenous progesterone, primarily the CIDR, has the potential to make significant improvements to overall pregnancy rates in the dairy industry. It seems to just be a matter of finding the right combination of timing and hormones within the protocol. The next foreseeable research step would be to figure out how and why these individual estrous synchronization protocols work. In vitro production of progesterone by CLs collected on days 6-8, 13-15, 19-20 did not differ between Ovsynch, CIDR, or no synchronization (Aali et al., 2004). However, other labs have reported decreased CL sensitivity to luteotropic factors and lower progesterone secretion after estrous synchronization (Potocka et al., 2009). For corpus luteum tissue incubated for 6 hours with progesterone the concentration of progesterone is over three times higher than that of a control, suggesting that progesterone supports its own synthesis particularly in the early stage of CL development (Kotwica et al, 1996).

Post-ovulatory Progesterone Supplementation

Progesterone supplementation after insemination has been a research focus for much longer than pre-ovulatory supplementation, however, the reported results remain inconsistent. There are reports of increases, decreases, and no change in pregnancy rates when exogenous progesterone is provided. In the early work of Herrick, Dawson, and Wiltbank et al., intramuscular progesterone was provided to repeat breeder cows (cows that did not conceive to the first insemination) and while each study showed a consistent increase in pregnancy rates after supplementation, none of these increases were statistically different from controls primarily because the number of cows in the studies were low (Herrick, 1953; Dawson, 1954; Wiltbank et al., 1956). When those data were collated and analyzed, there was a significant increase in pregnancy rate from 23% in control animals to 42.5% in the treated groups (Morris and Diskin, 2008).

Mann and Lamming (1999) conducted a review of a number of studies carried out in a wide variety of cattle in a number of different environments to investigate the effects of progesterone supplementation on pregnancy rates. Nearly all of the studies reported a trend for an increase in pregnancy rates, however, many studies had too few cows to establish statistical relevance. They combined all the numbers from the studies and found that the start day of progesterone supplementation post-insemination was important. When supplementation started before day 6 post-insemination there was a 10.3% increase in pregnancy rates in treated cows versus controls ($P < 0.001$), and when treatment was started after day 6 there was no significant increase. It was also concluded that when the control group had a pregnancy rate of $< 50\%$ there was a 19.3% increase in pregnancy rate in

progesterone treated cows, but when the controls had pregnancy rates of > 50% there was no significant difference (Mann and Lamming, 1999). Those data reinforces the idea that timing of supplementation is very important and that there are groups of cattle that will be more responsive to treatment than others.

The greatest increase in pregnancy rates of the studies reviewed by Mann and Lamming (1999) reported pregnancy rates of 60% for treated cows and 30% for control cows. Robinson et al., used progesterone-releasing intravaginal devices (PRID) in lactating cows from days 5-12 or 10-17 post-insemination. Pregnancy rates were the same between the two groups; however, the PRID 10-17 group had suppressed endogenous production of progesterone (Robinson et al., 1989). An increase in pregnancy rate in the PRID 10-17 group was likely due to the very low initial pregnancy rates. Other studies starting supplementation on day 10 showed either a small increase (4.3%; Sreenan and Diskin, 1983) or even a decrease (-2.7%; Macmillan et al., 1991) in pregnancy rates. To try and avoid the suppression of endogenous progesterone seen in the Robinson study, Mann et al. (2001) used CIDRs containing 50% of the progesterone in normal CIDRs (i.e. 0.95g instead of 1.9g per device). During the days of treatment (10-17 post-insemination) plasma progesterone was higher in the CIDR group than in the controls (10.3 ± 0.8 ng/ml and 7.4 ± 0.6 ng/ml respectively). After CIDR removal plasma progesterone returned to levels similar to controls indicating that endogenous production had not been affected, however, the conception rates were also not significantly different being 53.3% for control and 56.0% for treated cows (Mann et al., 2001).

Supplementation too early in the estrous cycle has been shown to suppress fertility. A new or used CIDR inserted for days 1-8 or 2-9 respectively decreased pregnancy rates in heifers from 46.4% in controls to 18.2% in the treated groups (Van Cleeff et al., 1996). This can likely be explained by the fact that under a normal progesterone profile, serum progesterone concentrations begin to rise around day 4 post-ovulation (Niswender et al., 2000; Robinson et al., 2008). To try and match the optimum timing of progesterone rise, Larson et al. (2007), started progesterone supplementation with a once-used CIDR on day 3.5 post- artificial insemination and removed the device on day 10. Treated cows had increased concentrations of progesterone by 0.7 ng/ml ($P < 0.05$) and increased pregnancy rates from 35% to 48% ($P = 0.068$). The treatment effect was greater in first and second lactation cows where pregnancy rates were 33% in controls and 51% in CIDR-treated cows ($P = 0.03$) (Larson et al., 2007).

Researchers have varied not only the start day of progesterone supplementation, but also the length of exposure to exogenous sources. Arndt et al. (2009) used CIDRs containing 1.38g of progesterone inserted on day 4 post-insemination and removed on day 18. Day 4 was chosen because this is when circulating levels of progesterone should rise in the pregnant cow, and day 18 was chosen as the removal day because it shortly follows the time of maternal recognition of pregnancy. They reported seeing no increase in progesterone concentrations in the CIDR cows after 30 min, 1 hour, or days 4, 5, 18, or 19. They also saw no increase in pregnancy rates or decrease in pregnancy loss in the CIDR group (Arndt et al., 2009). The lack of increase in progesterone concentrations in this study was attributed to

increased progesterone metabolism in high producing dairy cows (Sangsrivong et al., 2002).

Other exogenous hormones can also be used to indirectly increase progesterone. In multiparous cows having an initial progesterone level between 2 and 4 ng/ml, 1500 iu of human chorionic gonadotrophin (hCG) given on day 5 post-artificial insemination increased pregnancy rates (Kendall et al., 2009). In the same study, treatment with a 1.9g CIDR for 7 days starting on either day 5 or 6 had no impact on pregnancy rate. Bech-Sabat et al. (2009) used GnRH in an attempt to stimulate the release of LH from the anterior pituitary gland, enhancing the action of an existing corpus luteum or inducing the formation of a new corpus luteum. In this study the treatment was started on the day of pregnancy diagnosis (days 28-34) and consisted of either a progesterone releasing intravaginal device for 28 days or a onetime injection of 100 µg GnRH. The PRID treatment increased plasma progesterone concentrations 7 days after treatment, whereas progesterone levels for GnRH and control cows were the same. In cows with one fetus and one CL, PRID decreased the likelihood of early fetal loss by a factor of 0.51 compared to GnRH. In cows with two or more CL progesterone supplementation increased the likelihood of pregnancy loss three times compared to those receiving GnRH, likely due to high plasma progesterone levels causing excessive negative LH feedback reducing endogenous luteal progesterone production. They suggested that treatment at the time of pregnancy diagnosis with PRID in cows with one CL and with GnRH in cows with two or more CL should offer considerable benefits to herds with high incidence of early fetal loss of a non-infectious nature (Bech-Sabat et al., 2009).

Use of Previously Used Controlled Internal Drug Releasing Device in Cattle

The CIDR was first marketed in New Zealand in 1987 and contains 1.9g of progesterone, whereas the CIDR approved in the United States contains only 1.38g, but is still effective. The use of CIDRs for estrous synchronization in cattle in the United States was approved by the Food and Drug Administration in May of 2002. The current cost of an Eazi-Breed CIDR cattle insert produced by Pfizer Animal Health is roughly \$10 per insert. Though only approved for single use, it becomes a much more cost effective synchronization tool if the CIDR can be used more than once. Comparisons have been made between new and used CIDRs of both the 1.9g and 1.38g doses.

In ovariectomized non-suckling beef cows, once-used 1.9g CIDRs have been shown to be still effective to produce luteal phase plasma progesterone concentrations. Twice-used CIDRs, however, were only able to increase plasma progesterone to 0.9 ± 0.1 ng/ml at removal on day 7 (Long et al., 2009). Similarly Van Cleeff et al. (1992), found that in ovariectomized, lactating, dairy cows a new 1.9g CIDR inserted for 9 days increased plasma progesterone more than a used CIDR, but both were effective at producing luteal phase progesterone levels (maximum P4 3.4 ± 0.23 for new and 1.9 ± 0.15 for used CIDR) (Van Cleef et al., 1991). In a study of pregnancy rates of primiparous beef cows, low body condition score combined with a fixed time artificial insemination with a second use CIDR resulted in lower pregnancy rate (Looney et al., 2009)

Re-use of CIDRs containing 1.38g of progesterone has also been reported to provide effective levels of plasma progesterone to suppress estrus for at least an additional 7 days (Stevenson et al., 2003; Cerri et al., 2005). High-pressure steam sterilization (autoclaving)

has been used to sterilize CIDRs between uses and been reported to increase progesterone concentrations of especially during the 8 hours immediately after insertion when compared to CIDRs washed and soaked in chlorhexidine gluconate solution (Zuluaga and Williams, 2007). Mean serum concentrations of progesterone for the 7-day period of insertion were greater ($P < 0.03$) for new (3.7 ± 0.2) and autoclaved (3.4 ± 0.3) than for washed CIDR (2.8 ± 0.2), though all produced luteal phase concentrations of progesterone. Cerri et al. reported that new CIDR tended to increase progesterone concentrations faster ($P = 0.10$) than a used autoclaved CIDR, but both reached a plateau at 90 minutes. Cows in this study were anovular at the start of the trial and both the new and used CIDRs were effective at inducing onset of estrous cycles (Cerri et al., 2008).

Based on the reviewed literature, it was concluded that adding a CIDR as a presynchronization tool before timed artificial insemination with the standard Ovsynch protocol may be beneficial to reproductive performance in high producing lactating dairy cows. To make the proposed synchronization protocol more cost effective it was also hypothesized that a once used CIDR could be washed and used a second time in both lactating dairy cows and virgin heifers. The following experiments were designed to test these hypotheses.

**EFFECT OF CIDR-OVSYNCH ESTRUS
SYNCHRONIZATION PROTOCOL ON PREGNANCY RATES
AND PROGESTERONE CONCENTRATIONS IN
LACTATING DAIRY COWS**

Materials and Methods

The North Carolina State University Institutional Animal Care and Use Committee approved all procedures described in the following experiments.

Animals

Lactating Holstein dairy cows (n = 54) at various stages of lactation located at the North Carolina State University Dairy Educational Unit were used in this study. Cows were randomly assigned to the treatment (n = 27) or control (n = 27) groups. Cows were inseminated by trained AI technicians at the DEU according to study protocol as outlined below. Cows were fed a total mixed ration, provided water ad libitum, housed in a free stall barn, and milked twice daily.

Treatment Groups

The control group (Figure 1) was subjected to the standard Presynch-OvSynch protocol. Presynchronization was done using two injections of PGF_{2α} (Lutalyse®, 5ml i.m.; Pfizer Animal Health, New York, NY), the first being at day -36 and the second at day -22 relative to the planned day of insemination. The OvSynch protocol was started with GnRH (Fertagyl®, 100µg i.m.; Intervet Inc., Millsboro DE) on day -10, followed on day -3 by PGF_{2α}, then a second dose of GnRH (100µg i.m.) administered on day -1, and timed Artificial Insemination (TAI) was done on day 0 at 16 hours after GnRH. Cows assigned to the treatment group (Figure 2) received a Controlled Internal Drug Releasing insert (CIDR®)

containing 1.38g of Progesterone (P4) (Eazi-Breed™; Pfizer Animal Health, New York, NY) inserted intravaginally on day -20 which remained in place for 7 days and were given PGF_{2α} on day -13 at CIDR removal. Seventy-two hours later on day -10, the OvSynch protocol was started and followed the same as the control group. Estroject™ patches (Rockway Inc., Spring Valley, WI) were applied to the tailhead of all cows to determine cows in standing estrus. Cows that were seen in estrus were bred at ~12 hours after onset of estrus and all other cows underwent TAI on day 0.

Sample Collection

Blood samples were taken by coccygeal venipuncture and collected into Vacutainer® (Becton, Dickinson and Company) tubes and blood was stored overnight at 4°C until centrifuged for 15 min. Serum was stored frozen at -20°C until assayed. In the control group blood samples were taken at -36, -22, -10, -3, -1, and 0 days. In the CIDR treatment group blood samples were taken at -20, -13, -10, -3, -1, and 0 days.

Progesterone Analysis

Serum progesterone concentrations were determined using a commercial radioimmunoassay kit (Coat-a-Count, Siemens Medical, Los Angeles, CA) previously validated for use with bovine serum in our laboratory (Whisnant and Burns, 2002). Intra- and inter-assay coefficients of variation were 4.8% and 6.3% respectively.

Statistical Analyses

Pregnancy rates were compared between treatments via Chi-square using PROC FREQ of SAS (SAS 9.2, Cary, NC). Progesterone concentrations were compared between groups using PROC GLM with treatment (with or without CIDR) and time (sample date) in the model statement.

Results

Pregnancy was confirmed at regularly scheduled herd checks by veterinarians at NCSU CVM. Pregnancy rate was not different between groups ($28.2 \pm 2.4\%$ CIDR Protocol, $26.9 \pm 2.5\%$ Presynch - Ovsynch). On days -36 and -22 (days of presynchronization with $\text{PGF}_{2\alpha}$) mean serum P4 concentrations in the control cows were 1.8 ± 0.3 ng/mL and 2.6 ± 0.5 ng/mL, respectively. Concentrations in individual cows included some variation with some having serum P4 concentrations greater than 1 ng/mL and others having < 1 ng/mL. Serum P4 concentration on day -20 and -13 for the CIDR Protocol cows were 1.7 ± 0.4 ng/mL and 3.1 ± 0.6 ng/mL, respectively. Concentrations on day -10 were 2.4 ± 0.5 and 0.6 ± 0.1 ng/mL for the Presynch - Ovsynch and the CIDR Protocol cows, respectively (Figure 3). Serum P4 concentrations on days -3, -1 and 0 were 2.2 ± 0.4 , 0.8 ± 0.3 and 0.6 ± 0.2 ng/mL for the Presynch – Ovsynch group and 2.9 ± 0.4 , 0.5 ± 0.2 and 0.4 ± 0.2 ng/mL for the CIDR Protocol cows (Figure 3), respectively.

Discussion

The hypothesis of this study was that exogenous progesterone prior to Ovsynch would improve follicular development and ready the uterine environment for pregnancy especially in high producing lactating cows having low endogenous progesterone levels. Based on the success of Stevenson et al. (2006) and El-Zarkouny et al. (2004), using a CIDR in conjunction with Ovsynch seemed to be a good combination that may need an adjustment to the timing of treatments. Adding the CIDR at the beginning, before the start of Ovsynch, should increase serum progesterone levels as well as provide some presynchronization. The alternative estrous synchronization protocol including presynchronization using a CIDR insert produced similar results when compared to the standard Presynch-Ovsynch protocol where the presynchronization includes two injections of $\text{PGF}_{2\alpha}$. The experiment should be repeated with larger a larger sample size in both groups.

Progesterone concentrations throughout the Ovsynch protocol indicate that both forms of presynchronization achieved the desired with results with cows having high P4 concentrations on d -3 when PGF was administered and low P4 on days -1 and 0 indicating luteal regression was successfully achieved. The main difference between treatment groups was on d -10 when the CIDR protocol cows had serum P4 concentrations below 1 ng/mL while the Presynch-Ovsynch treated cows had serum P4 concentrations > 1 ng/mL. This difference was expected as PGF was administered on d -13 to the CIDR Protocol group and it was hypothesized that this would result in a greater ovulatory response to the first GnRH

injection of the Ovsynch protocol. Ovulation was not measured in this study directly and more cows would be needed to test whether or not the CIDR Protocol resulted in a higher pregnancy rate. However based on serum P4 concentrations most cows in the CIDR Protocol did respond to GnRH with ovulation.

It is important to know the cost of the synchronization protocol being used on a farm so as to minimize the cost per pregnancy. Number of injections per pregnancy should be minimized as well when possible. Assuming a cost of \$3 per GnRH dose and \$2 per PGF_{2α} the Presynch-Ovsynch protocol would cost around \$12 and include 5 total injections. Assuming the same costs per dose and including a CIDR at around \$10, the CIDR Protocol would cost around \$20 and include 4 total injections. The CIDR is often used more than once which would drastically help offset the cost and make the CIDR Protocol more economical. This would be especially true if the pregnancy rates can be shown to be significantly higher. The following experiment demonstrates that the 1.38g CIDR available in the United States can be used at least twice to achieve luteal levels of serum progesterone.

Control: Presynch-Ovsynch

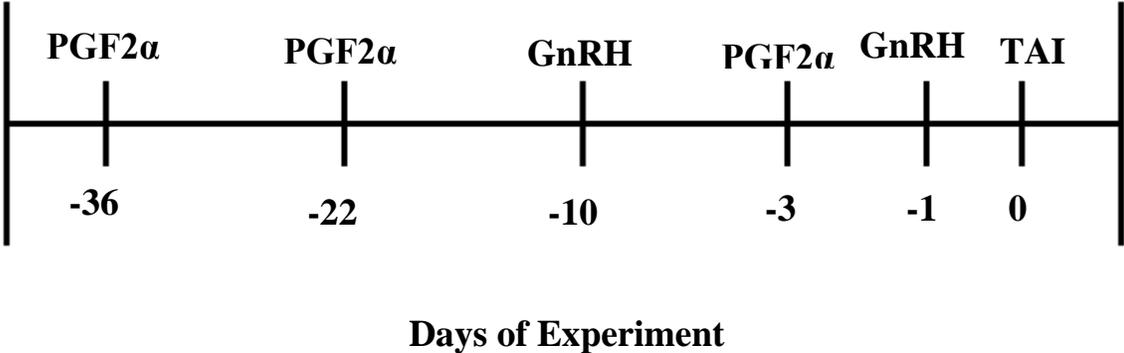


Figure 1: Presynch-Ovsynch estrus synchronization protocol. PGF2α was administered on day -36 and again fourteen days later on day -22 to establish presynchronization of estrous. Ovsynch protocol was started on day -10 consisting of GnRH followed three days later by PGF2α. On day -1 a second GnRH injection was administered followed by TAI on day 0.

Treatment: CIDR + Ovsynch

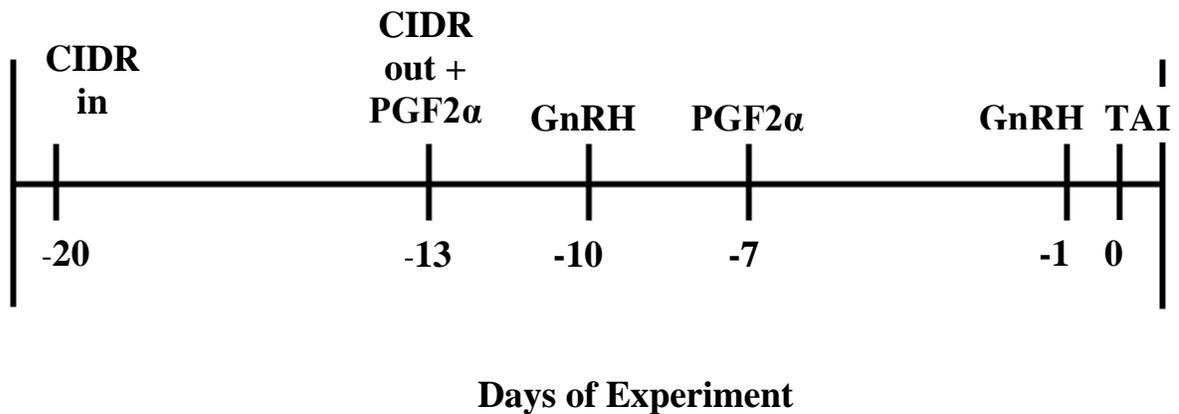


Figure 2: CIDR + Ovsynch estrus synchronization protocol. CIDR was inserted vaginally on day -20. CIDR was removed and PGF2 α was administered on day -13. Ovsynch protocol was started on day -10 consisting of GnRH followed three days later by PGF2 α . On day -1 a second GnRH injection was administered followed by TAI on day 0.

Serum Progesterone Comparison throughout Ovsynch

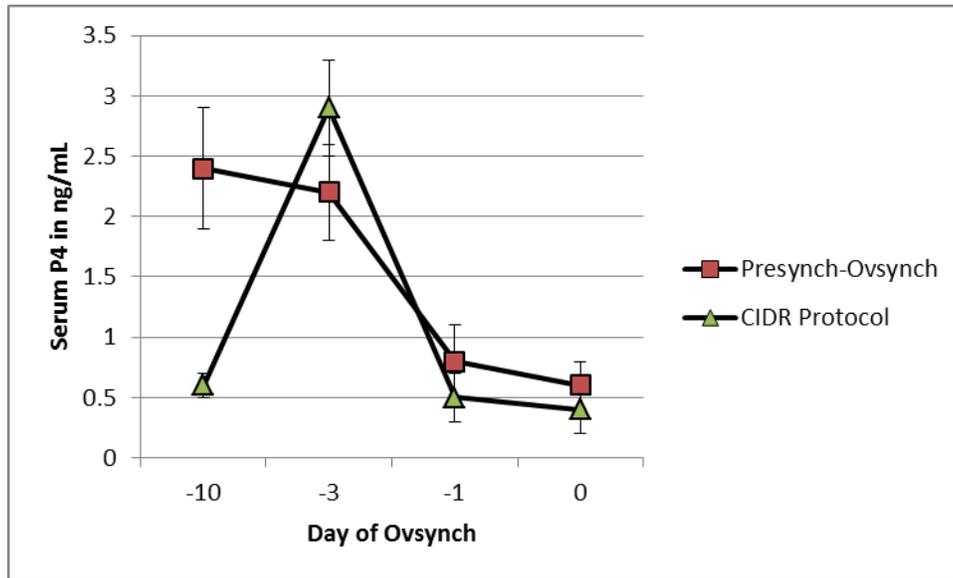


Figure 3: Comparison of serum P4 concentrations at every injection time during the Ovsynch Protocol for both the control and CIDR groups.

**COMPARISON OF SERUM PROGESTERONE
CONCENTRATIONS FROM NEW AND USED CIDR
DEVICES IN HOLSTEIN HEIFERS AND COWS**

Materials and Methods

The North Carolina State University Institutional Animal Care and Use Committee approved all procedures described in the following experiments.

Animals

Holstein heifers (nonlactating $n = 7$ per group) and lactating cows ($n = 5$ per group) were kept at the North Carolina State Dairy Educational Unit. Heifers were kept on pasture, fed a supplemental mixed ration, and provided water ad libitum. Cows were fed a silage based total mixed ration, provided water ad libitum, housed in a free stall barn, and milked twice daily.

Treatment Groups

Heifers and cows were randomly assigned to receive either a new or once used CIDR device. A used CIDR in this study was one that was previously placed in a Holstein cow for a period of 7 days, removed, washed, and stored in a drawer in a temperature controlled room. Washing was done by first rinsing with water followed by rinsing with Nolvasan and being allowed to air dry.

Animals were given 25 mg Prostaglandin F₂ alpha 24 h before CIDR insertion. At day 0, CIDRs were inserted and left in place for a period of 7 days. On day 7 CIDRs were removed and Estroject™ patches (Rockway Inc., Spring Valley, WI) were applied to the tailhead of all heifers and cows to determine animals in standing estrus.

Sample Collection and Analysis

Blood samples were taken by coccygeal venipuncture and collected into Vacutainer® (Becton, Dickinson and Company) tubes and blood was stored overnight at 4°C until centrifuged for 15 min. Serum was stored frozen at -20°C until assayed. In all groups, samples were collected just before the CIDR was inserted, 1 h later, 24 h later and finally when the CIDR was removed at the end of 7 days. Progesterone concentrations were determined by previously validated RIA (Whisnant and Burns, 2002).

Statistical Analyses

Data were analyzed using PROC GLM of SAS 9.2 for type of CIDR (new vs. used), time and the interaction of time by type of CIDR interaction.

Results

Serum P4 concentrations were not different between types of CIDR in either heifers (Figure 4) or cows (Figure 5). Serum P4 concentrations increased ($P < 0.01$) from time of insertion to 1 hour later and remained elevated at all other times. Progesterone levels maintained above 5.5 ng/ml for heifers and above 1.5 ng/ml for cows. There was a significant difference between P4 concentrations in heifers and cows regardless of CIDR type (Figure 6).

Correlating mean milk production, for the seven days when the CIDR was in place, with the increase in serum progesterone concentrations over baseline (sample 1) to the 24 hour sample produced a correlation of -0.61 between milk production and increase in serum P4 concentrations. This was different at the significance level of $P = 0.08$ indicating a trend for higher milk production to be associated with lower P4 concentrations. Milk production means ranged from 35.9 kg/day to 55.9 kg/day in individual cows. There was no difference in milk production between cows receiving new vs. used CIDR (46.3 ± 3.1 kg/day New vs. 41.8 ± 4.1 kg/day Used).

Discussion

Controlled internal drug releasing devices (CIDR) containing progesterone are used in some estrous synchronization protocols in the dairy industry. While the commercially available CIDR is only approved for single use, in the industry it is often used more than once to help offset the cost of the device. Other studies have shown that the 1.38g CIDR available in the United States can be used for a second 7 day period (Stevenson et al., 2003; Cerri et al., 2005), however, it hasn't been shown in heifers and the comparison of use between cows and heifers has not been made to our knowledge. In this study both new and used CIDR provided a rapid and sustained increase in serum P4 concentration in Holstein heifers.

Although not a direct planned comparison progesterone concentrations were higher in heifers than in lactating cows. This supports the hypothesis that cows have lower progesterone concentrations due to the higher metabolic rate associated with lactation (Sangsritavong et al., 2002). Lower progesterone concentrations could be one factor responsible for lower pregnancy rates in dairy cows compared to heifers (Wiltbank et al., 2005).

Future research should be done to determine the threshold for number of uses for a CIDR in heifers and in lactating cows. The utilization in cows and heifers is obviously different so this number of uses for these two would likely be different as well. Storage time and method of washing could also potentially affect the effectiveness of the used CIDR.

New vs. Used CIDR in Heifers

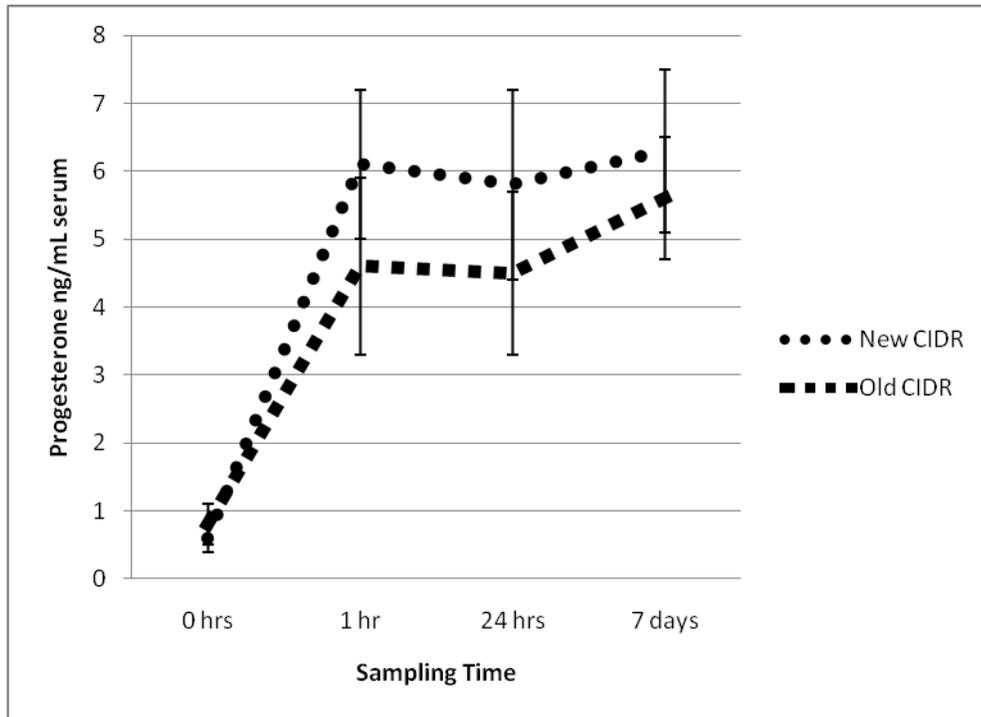


Figure 4: New CIDR vs old CIDR in heifers shows no significant difference between the two over a 7-day period.

New vs. Used CIDR in Cows

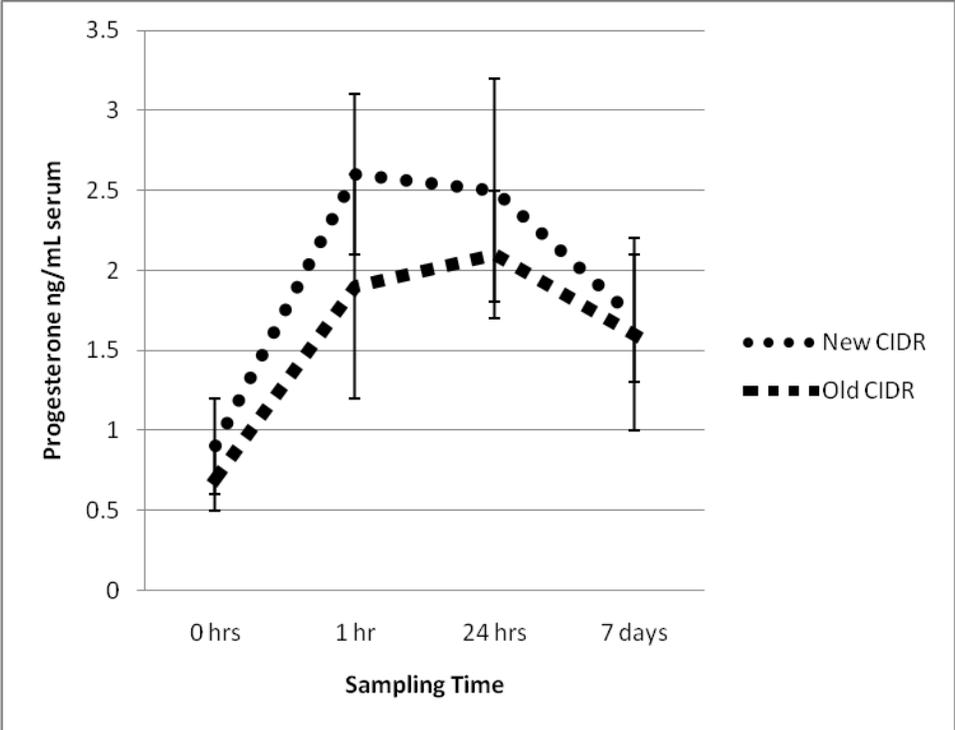


Figure 5: New CIDR vs old CIDR in cows shows no significant difference between the two over a 7-day period.

Heifer vs. Cow Progesterone

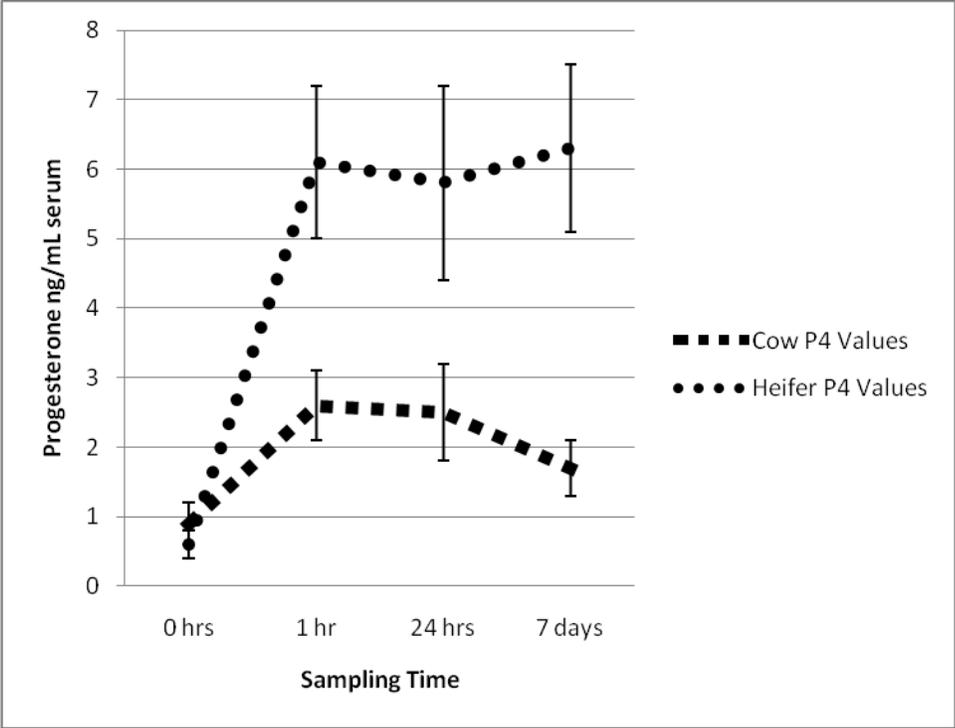


Figure 6: Progesterone concentrations in heifers vs cows regardless of CIDR type show that cows have a significantly lower P4 level during the 7-day period.

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