FERTILITY AFTER TIMED ARTIFICAL INSEMINATION IN RESPONSE TO A CONTROLLED INTERNAL DRUG RELEASE (CIDR) INSERT IN LACTATING DAIRY COWS

by

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B. S., University of New Hampshire, Durham, 2004

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Animal Sciences and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2008

Approved by:

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Abstract

Lactating dairy cows from 2 Kansas farms were used to determine the effectiveness of exogenous progesterone in the form of an intravaginal insert (controlled internal drug release; CIDR) in conjunction with an ovulation-synchronization protocol. Cows were enrolled in a Presynch + Ovsynch protocol after parturition, where they received 2 injections of $PGF_{2\alpha}$, 14 d apart (Presynch) beginning between 30 and 36 DIM. Cows (n = 155) detected in estrus after the second $PGF_{2\alpha}$ injection of Presynch were inseminated (early AI). Remaining cows were assigned randomly to be treated with the Cosynch-72 protocol (GnRH 12 d after last Presynch $PGF_{2\alpha}$ injection, $PGF_{2\alpha}$ 7 d after GnRH, and timed AI + GnRH injection 72 h later) and served as controls (n = 159), or to be treated with the Cosynch-72 protocol and receive a progesterone insert (Ovsynch + CIDR; n = 175) for 7 d between GnRH and $PGF_{2\alpha}$. Blood was collected at d −22 and −10 (relative to TAI at d 0) to determine cycling status based on progesterone concentrations and again at d 11 post AI to determine luteal competency. Treated cows were assigned body condition scores (BCS) on d −22 and −10. Pregnancy status was confirmed by palpation of the uterus per rectum and its contents on d 38 post-timed AI and verified again 4 wk later. Treatment with the progesterone insert increased timed AI pregnancies per AI in Cosynch-72 + CIDR-treated cows when compared with controls (38 vs. 24%), but did not differ from early AI cows (38%). Pregnancy loss was numerically less in progesterone-treated cows than in controls (4.4 vs. 11.8%). Our study shows that increased pregnancies per AI can be achieved by the use of a progesterone insert in a reduced population of cows not yet inseminated, but treated with a progesterone insert.

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Acknowledgments

First, I thank my husband Chris for dropping everything back in New Hampshire to move to Kansas so I could complete my M.S. degree. Chris has been my biggest supporter in helping me get through the everyday challenges. I thank him for his love and understanding he has shown me throughout this time. I thank my parents; Arthur and Dorothy Blodgett for bringing me into this world to work in a field that comes naturally to me. I thank my mom, for helping me through some of life's most difficult times and never giving up on me, particularly after the passing of my father. I thank my family, all eleven sisters and brothers for making me a strong individual.

I also express great appreciation to my mentor and friend, Dr. Jeff Stevenson, for his guidance and support during my time at Kansas State University. I also thank Dr. Ernie Minton and Dr. Barry Bradford for serving on my graduate supervisory committee. I am also grateful to Jamie Wilson for spending countless hours working on the assays for my experiment, as well as teaching me how to conduct radioimmunoassays.

I thank everyone that helped collect samples during my experiment: Brad Buttrey, Matt Burns, and Billy Brown. Thanks to Ohlde's Dairy and Meier's Dairy for allowing the use of their cows to complete this experiment without their help this project could not have been done.

Finally, I thank all my fellow graduate students with whom I shared countless hours during class, and in our office, where we shared so many good times. I am forever grateful to everyone who has helped me through this long journey.

Dedication

I dedicate this work to my father, Arthur Blodgett, who passed away during my freshman year at the University of New Hampshire. I also dedicate this to my husband, Chris Martel, my mother, Dorothy Blodgett, and my sister, Cathy Blodgett. Thank you for all the encouragement and support you have given to me during this time. I love you all.

CHAPTER 1 - REVIEW OF LITERATURE

Introduction

Exceptional reproductive performance is the ultimate goal of any dairy producer. Since the 1970's, fertility in lactating dairy cows has decreased more than 50% (Butler and Smith, 1989). Decades ago, the mature lactating dairy cow had an average productive life expectancy of more than 10 yrs. As the dairy industry changed, cows are now being culled at approximately 5 yr of age after 3.2 lactations. Many events account for the early demise of a dairy cow; with reproductive failure at the top of the list. Failure of the dairy cow to initiate normal estrous cycles in a timely fashion determines her economic profitability. Only after parturition will estrus and ovulation resume when progesterone decreases to nearly undetectable concentrations. Therefore, if the lactating dairy cow fails to resume a normal estrous cycle by 50 to 60 d postpartum, reproductive efficiency is reduced (Chebel et al., 2006), including poorer pregnancies per AI (P/AI). Poor conception ranks as one of the most limiting factors to profitability (Stevenson et al., 2006). A positive correlation between serum progesterone before AI and subsequent P/AI showed that progesterone is vital for fertility (Fonseca et al., 1983; Folman et al., 1990). The fate of a dairy cow lies in her ability to reproduce and produce profitable amounts of milk.

Progesterone is a key hormone secreted by the corpus luteum (CL), which is critical for facilitating estrous behavior via an interaction with estrogen. Progesterone facilitates preparation of the reproductive tract for implantation of the embryo and maintenance of pregnancy. Administration of exogenous progesterone or endogenously secreted progesterone can affect release of gonadotropin-releasing hormone (GnRH) and ultimately alter follicular growth and

development. To completely understand the mechanisms by which progesterone affects follicular growth and development, the stage of the estrous cycle must be known.

Today the primary method for breeding dairy cattle is artificial insemination (AI). However, with increased use of AI comes the importance for effective detection of estrus or ovulation and synchronization protocols. A result of producers failing to apply successfully one or more of the previously cited techniques, reproductive performance has declined. Researchers have developed protocols to help producers induce estrus, ovulation, or both, in cows before AI by synchronizing follicular maturation with luteal regression.

Understanding more about follicular dynamics, luteal regression, and ovulation led to the development of the Ovsynch protocol. The Ovsynch protocol is fairly effective in controlling follicular dynamics and synchronizing a follicular wave with subsequent luteal regression before AI at an appointed time. In addition to Ovsynch, many producers also have implemented a presynchronization (Presynch) protocol before the onset of Ovsynch to synchronize the estrous cycle to a specific stage (d 5 to 12) before applying the Ovsynch protocol. Others have combined the Ovsynch protocol with a progesterone-releasing intravaginal insert (progesterone containing controlled internal drug release; CIDR). The progesterone insert delays estrus until its removal. The combination of Presynch with Ovsynch, and in some cases a CIDR insert, has been shown to increase P/AI. Application of these protocols has given dairy producers an advantage in which estrous detection is not necessary (Pursley et al., 1997a) or of less importance to conception.

Role of Progesterone in Timed AI Protocols

Endogenous Progesterone

Progesterone is a 21-carbon steroid secreted by the CL and placenta to maintain pregnancy and regulate follicular development. Endogenous progesterone secreted by the CL during diestrus inhibits the release of GnRH. Inhibition of GnRH in turn inhibits tonic pulse secretion and the surge of luteinizing hormone (LH). Developing follicles are prevented from maturing to ovulation and estrus activity is inhibited, because progesterone controls or limits pulse secretion of GnRH, and thus, LH release. Progesterone is required for pregnancy maintenance in cattle. Within 4 to 5 d after ovulation, concentrations of progesterone are elevated, and remain so throughout gestation. A direct link can be made between low progesterone and infertility (Lucy, 2001). Elevated concentrations of progesterone can be measured by simple blood and milk tests.

Exogenous Progesterone

Exogenous progesterone applied in the form of a progesterone insert (controlled internal drug release; CIDR) delays estrus until the removal of the CIDR. Ultimately the CIDR affects the sequence of events that occur after the release of prostaglandin $F_{2\alpha}$ and regression of the CL. The CIDR is an intravaginal progesterone insert that contains 1.38 g of progesterone for application during a 7-d period. Once inserted into the vagina, progesterone is absorbed by the vaginal mucosa and enters into the vasculature of the vagina. The progesterone gets released from the CIDR and absorbed into the cow, once in the blood stream, acts at the hypothalamus to suppress release of GnRH (Senger, 2005). Once removed, an injection of $PGF_{2\alpha}$ is administrated to initiate luteal regression. The $PGF_{2\alpha}$ causes a rapid decline in concentrations of progesterone when a CL is present, followed by proestrus and synchronized estrus in 2 to 4 d.

Exogenous progesterone not only suppresses GnRH, but also LH and FSH release are inhibited, and as a result, follicular development and maturation is limited and ovulation is suppressed until the exogenous source of progesterone is removed. The effective dose of progesterone released from a CIDR is not equivalent to similar effects of progesterone secreted by a fully mature CL. Concentrations of progesterone produced by the CIDR insert are approximately 1.5 ng/mL, whereas a mature CL will produce concentrations of progesterone as high as 4 to 7 ng/mL range (Lucy et al., 1992a). To a limited extent, administration of progesterone serves as an "artificial CL", but more than 1 CIDR insert is usually necessary to equal the effects of progesterone secreted by the CL (Senger, 2005). Robinson et al. (1989) reported that insertion of a progesterone-releasing intravaginal device in late diestrus caused decreased endogenous progesterone production. The observations were consistent with Schams and Berisha (2002) concept that the CL has receptors for progesterone. The receptors allow for self-autoregulation of progesterone biosynthesis, which influences the peripheral progesterone concentrations and clearance rates regulated by the liver (Sangritavong et al., 2002).

Follicle Growth

Through use of transrectal ultrasonography, major advances were made in understanding the growth and development of follicles during follicular waves of the estrous cycle. A dairy cow is like most mammals in that she is born with several thousand primordial follicles, each containing an oocyte, to last through her entire reproductive life time. Even though thousands of follicles exist in each ovary, after puberty, generally only one will ovulate at the end of each estrus. The estrous cycle of the cow is comprised of 2 to 3 recurring follicular waves per cycle that are regulated by the release of several hormones. Age, breed, and parity of the cow, as well as environmental conditions, can affect the occurrence of 2 or 3 wave cycles (Celik et al., 2005).

Each wave of growing follicles proceeds through several developmental stages that include selection, deviation, dominance, and ovulation or atresia. Recruitment of a new wave of antral follicles occurs every 8 to 10 d during the estrous cycle. A surge of FSH initiates recruitment a cohort or wave of follicles, although only 1 follicle will be selected to undergo deviation to become the dominant follicle (Ginther et al., 1996). Research by Ginther et al. (1996) showed that emergence of each dominant follicle either preceded or coincided with a transient rise in FSH and was followed by a rise in estradiol-17ß (E2). The rise in E2 reached peak concentrations when the dominant follicle was near maximal diameter.

The dominant follicle has a unique characteristic that sets it apart from all other follicles. It has the ability to escape atresia, and if exposed to the LH surge, its cells will differentiate into the CL after ovulation of the oocyte (Lucy, 2007). Some dominant follicles can become atretic, however, if they mature during the luteal phase and are never exposed to the LH surge (Valdez et al., 2005). Growth of the dominant follicle greater than 4 mm is FSH-dependent, whereby maintenance of the dominant follicle is dependent on changes in progesterone and LH. Any subordinate follicle in each cohort or wave during this time will terminate growth and undergo atresia. Ovulation of the dominant follicle is dependent on whether the follicle has become completely LH dependent and generally occurs when progesterone is low and E2 is high, on d 20 to 23 of the estrous cycle, unless a pharmacological dose of GnRH is exogenously administered or a gonadotropin such as LH or human chorionic gonadotropin (hCG) is given. A study by Mee et al. (1993) showed that an injection of GnRH at estrus increased the ratio of large to small luteal cells in the CL and increased concentrations of progesterone in serum throughout the luteal phase of the estrous cycle and up to 40 d of pregnancy.

Increased E2 combined with the decline in progesterone induces behavioral estrus and triggers an LH surge. This LH surge will cause ovulation in the cow about 27 h later if a mature follicle is present (Walker et al., 1996). Because progesterone inhibits estrus, administration of progesterone during the follicular phase can alter follicular development. Administration of progesterone at intermediate concentrations (0.5 to 2 ng/mL) will block the LH surge, prevent ovulation, and cause dominant follicles to increase to diameters larger than normal (Sirois and Fortune, 1990; Stock and Fortune, 1993). Administration of subluteal concentrations of progesterone during the follicular phase can disrupt normal follicular development and cause the dominant follicle to become persistently larger until the source of progesterone is eliminated. During the third phase of preovulatory development, shortly before ovulation, a sharp increase in progesterone concentrations occurs in the fluid of the preovulatory follicles (Dieleman et al., 1983; Dieleman and Blankenstein, 1985).

Excessive weight loss associated with negative energy balance (NEB) can have negative effects on follicular growth and development by disrupting LH and insulin-like growth factor I (IGF-I; Lucy, 2001). Pulses of LH and plasma concentrations of IGF-I are decreased in cows during NEB. Insulin-like growth factor-1 is primarily produced by the liver, several tissues and cells in the body including the bovine reproductive tract (Dupont and Holzenberger, 2003). During the stages of preimplantation development both the oviduct (Schmidt et al., 1994; Pushpakumara et al., 2002) and the uterus (Geisert et al., 1991; Robinson et al., 2000) express IGF-1, and the actions are mediated by the IGF-1 receptor found on the developing embryo. Elevated IGF-1 concentrations circulating throughout the body have been found to enhance survival of the developing embryo.

Persistent Follicles

Follicular development is regulated by a negative feedback mechanism, in which the pulse frequency of LH secretion is determined by concentrations of progesterone (Kinder et al., 1996). Ovarian cysts seem to be formed from an endocrine imbalance, resulting from a failure during the preovulatory LH surge and correct timing of follicular maturation (Hamilton et al., 1995). Administration of exogenous progesterone causes regression of both persistent follicles maintained by low concentrations of progesterone and ovarian follicular cysts, by suppressing LH pulse frequency (Hatler et al., 2003). When a CL is not present in the ovary, greater frequency of LH pulses resulted when progestins are administered at doses used to synchronize estrus (Kojima et al., 1992; Sanchez et al., 1995). A single injection of progesterone or exposure to a progesterone-releasing intravaginal insert regressed cysts and persistent follicles (Stock and Fortune, 1993; Anderson and Day, 1994; Calder et al., 1999). Follicles developing in the presence of intermediate concentrations (0.1 to 1.0 ng/mL) of progesterone generally formed cysts (Hatler et al., 2003), indicating that intermediate concentrations of progesterone are commonly associated with cysts or may predispose subsequent follicles to form cysts and contribute to the phenomenon of "cyst turnover."

Persistent follicles can occur in one or both ovaries and may persist with a CL. Characteristics that define a persistent follicle include more granulosa cells and more theca mass than dominant follicles (Bigelow et al., 1998). Formation of persistent follicles can be associated with high LH pulse frequency. Development of persistent follicles occurs because LH pulse frequency is not reduced sufficiently in the presence of small doses of progesterone. Small doses of progesterone are capable of blocking the progesterone-induced preovulatory LH surge. Small doses, however, dampen incompletely normal tonic LH pulse frequency (Hatler et al., 2003),

because amount of LH receptors in the granulosa and theca cells of persistent follicles is greater than those of dominant follicles (Cupp et al., 1993). Small doses of progestin cannot reduce tonic LH pulse frequency as occurs when a CL is present, but sustain follicles and prolong the preovulatory secretion of E2. Because oocytes are then exposed to prolonged elevated concentrations of E2, chances increase for abnormal embryonic development and poor fertility (Ahmad et al., 1995). Administration of progesterone for 24 h in heifers suppressed LH and progesterone secretion and induced atresia of persistent follicles. In contrast, when heifers were treated for 6 h with progesterone, persistent follicles recovered from short exposure and reclaimed their dominance (McDowell et al., 1998).

A large dose of progesterone suppressed the dominant follicle more than a physiological dose, but only when given during its growth phase, and not after (Adams et al., 1992). When the dominant follicle was exposed to progesterone during the growing phase, progesterone inhibited growth in a dose-dependent manner. The dose-dependent manner in which progesterone was administered accounted for the size of the dominant follicle during the first wave of the estrous cycle. Dominant follicles had a longer growth phase and grew to a larger diameter in response to the dose of progesterone received. Growth was less affected by small doses than by large doses during the first wave. The oversized dominant follicle in the first wave then delayed emergence of the second wave. It was concluded that during follicular growth phases, small concentrations of progesterone were associated with enhanced follicular growth, whereas larger concentrations of progesterone suppressed follicle growth (Adams et al., 1992).

A few studies established that larger than normal persistent follicles were capable of retaining their capacity to trigger an LH surge and ovulate, however, the oocytes that were ovulated were less healthy (Revah and Butler, 1996; Mihm et al., 1999; Roche et al., 1999). The

exact reasoning behind why health of the oocyte was poor has not been completely studied; researchers believe that the interaction between the persistent follicle and unhealthy oocyte occurred due to the premature activation of the oocyte. Adams et al. (1992) found that heifers reacted differently to continued exposure to progesterone than cows, their results showed heifers had continued periodic emergence of anovulatory follicular waves.

Onset of Estrous Cycles

For a period of time after calving, the lactating cow is anestrous or anovulatory and the uterus is involuting. An anestrous cow fails to ovulate or has ovulated in the absence of behavioral estrus before or after the voluntary waiting period (VWP). During this period the uterus regains its nonpregnant size and function, and returns to a healthy state capable of sustaining a new pregnancy. Reinitiating normal estrous cycles and ovulation occur during uterine involution. At this time the ovaries reestablish normal cyclic hormonal secretion of E2 and progesterone. Research indicates that 20% of dairy cows have not displayed estrus or ovulated before the end of the VWP (Opsomer et al., 2000; Moreira et al., 2001). Failure to display estrus before the end of the VWP is associated with reduced reproductive performance and reproductive failure (Thatcher and Wilcox, 1973).

Use of a progesterone supplement in an anovulatory anestrous cow likely reestablishes hypothalamic signals in response to E2 that is produced by the developing dominant follicle (Walsh et al., 2007). Reestablishing hypothalamic-pituitary-ovarian control bridges the connection between elevated E2 and an LH surge, allowing ovulation of a dominant follicle to occur. Progesterone supplementation in the anovulatory anestrous cow also has an effect on the behavioral center of the brain, thereby increasing the probability of expressed estrus (Rosenberg et al., 1990; Fike et al., 1997). A study by Walsh et al. (2007) examined the use of a

progesterone-releasing intravaginal device (**PRID**) in cows fit with pedometers that failed to display estrus or those found to be anovulatory by the end of a 60-d VWP. In both multiparous and primiparous Holsteins, progesterone-treated cows were inseminated 8 to17 d earlier than controls.

Uterus

Regulation of estrous cycle of the dairy cow is partly dependent on the uterus, because it is the source of $PGF_{2\alpha}$ (Spencer et al., 2004). The uterus of the dairy cow is bicornuate because it is made up of two uterine horns and a small uterine body, which opens into the vagina through a single cervical canal. The uterus consists of 3 layers of tissue, the perimetrium, myometrium, and endometrium, all serving a different function. The myometrium and endometrium serve critical roles in conception, placentation, recognition and maintenance of pregnancy, and parturition.

The myometrium is important in providing motility to the uterus in the form of contractions. Contractions provide transport of sperm and mucus once in the vagina. When the myometrium is under the influence of high progesterone and low E2, the uterus lacks muscle tone and palpates limp or soft. The endometrium is the inner layer of the uterus responsible for secreting essential substances (uterine milk) into the lumen to enhance embryo development and sperm viability. Uterine glands are specialized portions of the endometrium that secrete nutrients that are important for survival and function of the preimplantation embryo (Senger, 2005). Under the influence of progesterone, uterine glands develop from the mucosal and submucosa layer of the endometrium. The endometrium is responsible for releasing oxytocin-induced luteolytic pulses of $PGF_{2\alpha}$ that induce luteolysis during the estrous cycle (Spencer et al., 2004). After formation of the CL, progesterone stimulates the endometrium to support blastocyst growth and its

elongation to become a filamentous conceptus (Spencer et al., 2007). Progesterone from the newly formed CL during early diestrus stimulates accumulation of phospholipids in endometrial luminal epithelium and superficial glandular epithelium, which liberate arachidonic acid for the synthesis and secretion of $PGF_{2\alpha}$ (Spencer et al., 2004). It is during this time that concentrations of progesterone increase and act through the progesterone receptor to block expression of epithelial estrogen receptor and oxytocin receptor in the endometrial luminal epithelium and superficial glandular epithelium. Robinson et al. (2001) examined the effects of progesterone receptors in the uterus and reported that progesterone increased on d 3 after estrus and reached maximum concentrations on d 12 to18 and decreased on d 19 of the estrous cycle.

Growth and development of the conceptus requires progesterone and placental hormones to act on the uterus to regulate the endometrial differentiation and function, pregnancy recognition signaling, uterine receptivity for blastocyst implantation, and conceptus-uterine interactions (Spencer and Bazer, 2002; Bagchi et al., 2003; Spencer at al., 2004). Before the cow becomes pregnant, progesterone prepares the uterus for pregnancy. Progesterone prevents the cow from returning to estrus by suppressing pulses of FSH and LH secreted by the pituitary gland. Progesterone will block the LH surge in a pregnant cow and LH pulses support progesterone production by the CL.

Factors that Influence fertility

Days Postpartum

An antagonistic genetic relationship exists between milk production and reproduction (Nebel and McGilliard, 1993), but under proper management, this negative relationship may be partly overcome. According to the USDA, as milk production in the U.S. has increased by

approximately 20% during the last 10 yr, reproductive performance has decreased. Selection for increased yield has been shown to increase days open at a rate of 1.1 d/yr from 1980 to 1993 (Abdallah and McDaniel, 2000). Many factors play a critical role in the expression of estrus and return to estrus. Days in milk (DIM) and milk yield impact reproduction. High milk-yielding cows have reduced P/AI, in part, caused by delayed resumption of ovarian activity, thus limiting the number of prebreeding estrous cycles (Butler and Smith, 1989). However, one study found cows with above average milk production were reported to have greater fertility at TAI, when compared with herd mates having lesser milk production (Peters and Pursley, 2002). A study by Stevenson and Britt (1979) showed a positive relationship between increased milk yield and days to first ovulation. Before application of controlled-breeding programs, cows were bred AI according to DIM or first estrus. Greater milk yield or greater fat-corrected milk (**FCM**) during early lactation is associated with more prolonged intervals to first ovulation. Increased FCM can reduce expression of estrus and a successful pregnancy up to 150 DIM (Westwood et al., 2002).

Increased P/AI was reported after longer VWP (Whitmore et al., 1974; Britt, 1977; Stevenson et al., 1983). Tenhagen et al. (2003) reported that lengthening the VWP by 3 wk in low- and high-producing dairy cows improved TAI P/AI associated with the Ovsynch protocol. When days to first service were delayed by 3 wk in low-producing cows (VWP was extended from 53 to 59 to 73 to 81 DIM), timed AI P/AI increased from 14.4 to 34.5%, respectively. When VWP in high-producing cows was delayed by 3 wk from 73 to 81 DIM to 94 to 102 DIM, P/AI increased from 28.2 to 41.4%, respectively. Cows inseminated after 75 DIM had greater P/AI than those inseminated before 60 DIM (Pursley et al., 1997b; Stevenson and Phatak, 2005). Furthermore, cows that are inseminated earlier postpartum generally require more services per

pregnancy. Unfortunately, according to Dechow et al. (2004), fertility is unfavorably correlated with yield.

Body Condition

Poor fertility in dairy cows can be caused by a number of factors. In dairy cows, too little or too much body fat at parturition can be associated with a reduction in subsequent milk production and increased health and reproductive problems (Waltner et al., 1993). Body condition can play a role in the return to estrus in addition to negative energy balance (**NEB**) and metabolic disorders that may antagonize health and prolong recovery. Assessing body condition scores (**BCS**) is a method used to determine body reserves (adipose and some muscle tissue) of dairy cattle. A score of 1 (thin) to 5 (obese) is based on the body reserves on back and pelvic regions of the dairy cow. High-producing dairy herds tend to have poorer BCS because of the nutrient demands of milk production. As genetic merit for production increases in dairy herds, so does mobilization of body reserves (Pryce et al., 2001). In the postpartum dairy cow, ovarian activity is directly related to both nutrient intake and milk production (Butler and Smith, 1989). Studies have shown a relationship between loss in BCS and decreased P/AI. For cows enrolled in a timed AI protocol, for every 1-unit increase in BCS during 46 to 66 DIM (16 d before AI) P/AI increased by 8.6 ± 4% (Stevenson et al., 1999). Cows that increased in BCS also had greater concentrations of progesterone.

Nutrition

Nutrition can have both positive and negative effects on fertility. The dairy cow survives by feeding the microorganisms that inhabit the rumen. Inability of the diet to sustain the correct requirements for the rumen organisms to survive can prove detrimental. Poor nutrition during

the dry and early postpartum period correlates with delayed expression of estrus. Feed intake in a prepartum dairy cow is suppressed as a result of the impending parturition. During the last trimester of pregnancy the neonatal calf takes priority, and as a result, fat reserves in the dam become depleted. After parturition, lactation takes priority over reproduction and milk secretion, and both milk yield and feed intake increase, but at different rates. Peak milk production occurs at approximately 60 DIM in older cows, but much later than 100 DIM in first lactation cows, with maximum feed intake occurring several weeks later. The difference in peak milk yield is a result of NEB (Senatore et al., 1996). This delay can endure from 4 to 12 wk of lactation (Butler et al., 1981). As milk production increases in the individual cow it can be accompanied by an increase in both feed intake and overall metabolic concentrations rate such that it causes concentrations of progesterone and estradiol circulating in the blood to decrease. Alterations in steroid balance may be correlated to a reduction in early embryonic development (Moore and Thatcher, 2006).

Effective nutritional management on the farm can affect return to first estrus. Poor nutrition during the dry and early postpartum periods reduces concentrations of glucose, insulin, IGF-I, and also reduced LH pulse frequency (Roche, 2006), ultimately delaying estrus and ovulation. Diets high in nutritional quality can increase concentrations of key steroid hormones such as progesterone. Although diets high in urea concentration, have shown that cows are less likely to show estrus and highly rumen degradable diets negatively affect P/AI (Westwood et al., 2002).

Negative Energy Balance

Early in lactation the dairy cow experiences a NEB, because the energy required for maintenance, growth (in younger cows) and milk yield cannot be supported entirely by nutrients consumed in the diet. A good indicator of NEB can be assessed by BCS because loss of BCS is

correlated with fat mobilization (Wright and Russel., 1984; Komaragiri Madhav et al., 1998; de Vries et al., 2000). Dechow et al. (2002) found that genetic selection for BCS resulted in less loss in body condition during early lactation, indicating that high genetic merit is associated with less severe cases of NEB. Metabolizable body reserves are used to support milk production when dietary inputs are lacking. After parturition, NEB occurs because energy associated with producing milk exceeds the energy consumed in the diet. A direct relationship is observed between NEB and milk production during the first 3 wk of lactation (Butler et al., 1981; Canfield et al., 1990). Greater milk yields have been found to be commonly associated with greater NEB and longer postpartum intervals to first ovulation. However, high milk production should not be confused with NEB, since cows are undergoing a normal process of nutrient partitioning and adipose tissue metabolism during early lactation (Bauman and Currie, 1980). Studies disagree regarding effects of a positive or negative relationship between energy balance, high milk production, and delayed days to first ovulation (positive relationship: Butler et al., 1981; Staples et al., 1990; Canfield et al., 1990; Canfield and Butler, 1990; Canfield and Butler, 1991; Lucy et al., 1992b; Spicer et al., 1993; Whitaker et al., 1993; negative relationship: Ducker et al., 1985; Villa-Godoy et al., 1988; Harrison et al., 1990).

Energy balance influences reproductive processes. Canfield and Butler (1991) suggested that EB may serve 2 functions in the initiation of ovulatory cycles: modulation of pulsatile LH secretion and alteration of ovarian responsiveness to LH signaling. Negative EB can delay processes that lead to the reinitiation of postpartum estrous cycles and normal fertility. Greater NEB has been genetically linked to increased days to the start of luteal activity after calving (Veerkamp et al., 2000). First postpartum ovulation usually occurs between d 15 and 49 (Stevenson and Britt, 1979; Lamming et al., 1982; Butler and Smith, 1989). In addition to

progesterone, NEB and severe weight loss can have negative effects on follicular growth and development. Negative EB affects the follicle by disrupting secretion of LH and IGF-I (Lucy, 2001) as well as their influence on progesterone secretion by the CL (Nebel and McGilliard, 1993). Negative EB was shown to modify follicular populations and affect the average number of follicles during the first 25 d postpartum (Lucy et al., 1991). Pulses of LH and plasma concentrations of IGF-I are decreased in cows during NEB.

Timed Artificial Insemination Protocols

Ovsynch

The Ovsynch protocol was developed by reproductive physiologists at the University of Wisconsin in the mid 1990's as a reproductive tool to synchronize follicular growth and maturation with luteolysis before inducing ovulation before a timed AI (Pursley et al., 1995). Administration of GnRH 7 d before and 48 h after $PGF_{2\alpha}$ resulted in a synchronized ovulation before a TAI. The Ovsynch protocol was designed to inject cows with GnRH at random stages of their estrous cycle once they are eligible to be inseminated after the VWP. If a dominant follicle is present in the ovary (follicle $\geq$ 10 mm), it will ovulate in response to GnRH and a CL will form. Seven days after the GnRH, $PGF_{2\alpha}$ is administered, which causes luteolysis and the follicular phase resumes. After luteolysis, a follicle will ovulate in response to the second injection of GnRH given 48 to 56 h after $PGF_{2\alpha}$. The follicle that ovulates may be a new follicle that developed subsequent to ovulation that was induced by the first GnRH injection. Timed AI can be performed without detection of estrus between 0 and 32 h after the second GnRH injection (Pursley et al., 1998). Best P/AI were achieved when AI occurred about 16 h after GnRH. Since the first report of the Ovsynch protocol, many dairy producers have followed the

recommended protocol of TAI at 16 h after the second GnRH injection. The second injection of GnRH subsequently induced ovulation in 87% of the cows.

As with the second injection of GnRH, the first GnRH injection of the Ovsynch protocol induces release of LH. Vasconcelos et al. (1999) reported that 64% of 156 cows ovulated in response to the first GnRH injection when administered at varying stages of the estrous cycle. Because the first GnRH injection is given at random stages of the estrous cycle, not all cows will ovulate in response to the first GnRH injection or it may be administered when immature follicles are present that are incapable of ovulation. Further, if cows are not cycling, GnRH will not initiate ovulation in every cow. As a result, P/AI may be reduced in some cows. Ovulation or luteinization of the dominant follicle may occur, thus inducing initiation of a new follicular wave.

Ensuring the best outcome from the Ovsynch protocol depends partly on the stage of the estrous cycle at which it is begun. Initiating the protocol during early-to-middiestrus (d 5 to 12) produces greater P/AI than when initiated on other days of the estrous cycle (Vasconcelos et al., 1999). When the Ovsynch protocol was started between d 5 and 12 of the estrous cycle, P/AI were greatest because of greater incidences of ovulation after the first GnRH injection (Vasconcelos et al., 1999; Cartmill et al., 2001). In addition, initiation of Ovsynch during mid-cycle resulted in smaller ovulatory follicles and greater P/AI (Vasconcelos et al., 1999). The Ovsynch protocol increases P/AI by 10 to 12 percentage points when implemented during favorable stages of the estrous cycle (Moreira et al., 2001; Cartmill et al., 2001; El-Zarkouny et al., 2004). An experiment by Wittke et al. (2003) reported that no relationship existed between P/AI and size of the ovulatory follicle for cows treated with Ovsynch during various stages of the estrous cycle. Follicle size in many cows was dependent on the stage of the estrous cycle.

Interval between the first GnRH injection and $PGF_{2\alpha}$ has been studied to determine if there is a chance for increased P/AI depending on the duration of time between injections. The standard for Ovsynch has been to administer injections of GnRH on d 0, $PGF_{2\alpha}$ on d 7 with the second GnRH injection 48 h later to allow for induction of ovulation and timed AI. Delaying the $PGF_{2\alpha}$ injection of Ovsynch by 24 h to d 8 had little effect on preovulatory follicle growth and concentrations of E2 (Stevenson et al., 2007).

Administration of the second GnRH injection 48 h after $PGF_{2\alpha}$ resulted in greater P/AI than when given at 24 and 0 h (55%, 46%, and 11%, respectively; Pursley et al., 1998). Pursley et al. (1997b) investigated the effects of 2 protocols on lactating dairy cows and heifers to look at the effect on P/AI when the interval between $PGF_{2\alpha}$ was altered. The experimental protocol used by Pursley and others assigned cows and heifers to 2 treatments. Control cows received 25 mg of $PGF_{2\alpha}$ and were inseminated according to the a.m.-p.m. rule only following detected estrus. All controls that were not detected in estrus following the initial $PGF_{2\alpha}$ received 2 more injections of $PGF_{2\alpha}$ at 14 d intervals, followed by TAI at 72 to 80 h after the third injection, if not already inseminated after the second or third injection of $PGF_{2\alpha}$. The treated cows and heifers were compared with controls that received a modified Ovsynch protocol. These females were injected with 100 μg of GnRH at random stages of the estrous cycle. Seven days later, the animals received 25 mg of $PGF_{2\alpha}$, and 30 to 36 h later, a second, 100-μg injection of GnRH was administered so TAI could occur 16 to 20 after the second GnRH. Results indicated that P/AI were similar for controls (38.9%) and Ovsynch (37.8%) treatments. Heifers, however, had dramatically different P/AI (control = 74.4% and Ovsynch = 35.1%). Regardless of varying concentrations of progesterone at the time of $PGF_{2\alpha}$, concentrations had no effect on P/AI for cows. In contrast, results revealed that heifers with lesser progesterone concentrations at $PGF_{2\alpha}$

had lower P/AI. Pursley et al. (1997b) suggested that the reasoning behind lesser P/AI in heifers after Ovsynch was that follicular maturation in heifers was poorly synchronized. A successful outcome from the Ovsynch protocol depends on synchrony of the ovulatory follicle with induced CL regression, and subsequent timing of ovulation and AI after the second GnRH injection.

Stevenson et al. (1996) experimented with GnRH injections at 30 to 32 h after $PGF_{2\alpha}$ with a resulting outcome of reduced P/AI when compared with cows treated with only $PGF_{2\alpha}$ with AI after detection of estrus (35.3 vs. 47.1%). When 2 Ovsynch protocols were tested in which the second GnRH injection was given at 33 or 48 h after the $PGF_{2\alpha}$: P/AI were 22.1 vs. 35.6%, respectively (Stevenson et al., 1999). Previous research indicated that P/AI to a TAI protocol (Ovsynch) resulted in similar P/AI as those in which cows inseminated after observed estrus (Burke et al., 1996; Pursley et al., 1997a,b; Britt and Gaska, 1998; de la Sota et al., 1998; Momcilovic et al., 1998; Stevenson et al., 1999).

Presynch + Ovsynch Protocol

Applying the Ovsynch protocol at known stages of the estrous cycle was found to influence reproductive outcomes by improving P/AI (Vasconcelos et al., 1999). A presynchronization protocol was developed in response to the findings that P/AI of cows treated with the Ovsynch protocol were better when cows started the protocol between d 5 and 12 of the estrous cycle. To start the Ovsynch protocol between d 5 and 12, it is necessary to presynchronize the estrous cycle. Use of a Presynch protocol (2 doses of $PGF_{2\alpha}$, 14 d apart), with the second $PGF_{2\alpha}$ injection administered 12 d before starting the Ovsynch protocol, synchronizes a larger number of cows to be on d 5 to 12 of the cycle (Moreira et al., 2001). El-Zarkouny et al. (2004) reported that more than 70% of cows were early to middiestrus when starting Ovsynch after the Presynch protocol compared with 53% of untreated controls starting Ovsynch. More than 90% of cyclic

cows are expected to have synchronized estrus in response to the Presynch protocol within 1 wk after the second injection of $PGF_{2\alpha}$. More simplified protocols in which 1 injection of $PGF_{2\alpha}$ given 10 or 12 d before the initiation of the Ovsynch protocol resulted in better or similar P/AI than after Ovsynch alone (LeBlanc and Leslie, 2003; Cartmill et al., 2001). Cartmill et al. (2001) reported that the single injection of $PGF_{2\alpha}$ in multiparous cows increased P/AI from 28 to 42% when the injection was given 12 d before the start of Ovsynch.

Moreira et al. (2001) reported a 10 to 12% increase in P/AI when 2 injections of $PGF_{2\alpha}$ were applied 14 d apart in the standard Presynch protocol. The Presynch protocol developed by Moreira et al. (2001) has limited efficacy in anestrous cows, because effectiveness of $PGF_{2\alpha}$ depends on the presence of a responsive CL (Chebel et al., 2006).

Portaluppi and Stevenson (2005) conducted an experiment that determined P/AI for cows in a Presynch + Ovsynch protocol, in which timing of the second GnRH injection was altered. Cows in 2 herds were assigned randomly to 3 treatments, in which GnRH and TAI both occurred at 48 h, GnRH 48 h and TAI 72 h, or GnRH and TAI at 72 h. Any cows detected in estrus before their scheduled TAI were not inseminated until their scheduled breeding time. Overall P/AI recorded between the 2 herds were similar for cows that received GnRH injections at 48 h (22.8 vs. 23.5%). Cows that were assigned to GnRH at 72 h and TAI 72 h later achieved greater P/AI (31.4%). Portaluppi and Stevenson (2005) reported pregnancy per AI in first lactation cows to be greater than in older cows (28.5 vs. 24.1%). Other studies that implemented Presynch before Ovsynch reported that first-lactation cows were more fertile at first AI than older cows (Cartmill et al., 2001; El-Zarkouny et al, 2004). However a study by Navanukraw et al. (2004) did not detect an effect of lactation number on pregnancy rate after TAI. Peters and Pursley (2002) examined whether the use of Presynch + Ovsynch protocol during diestrus would improve P/AI

to TAI when compared with Ovsynch alone. Pregnancies per AI after TAI were similar between Presynch + Ovsynch and Ovsynch (41.5% vs. 38.3%), however, greater P/AI were detected for cows of above-average milk production (above-average milk = 45.8% vs. low production = 33.8%; Peters and Pursley, 2002).

Ovulation-Synchronization Protocols Using Exogenous Progesterone

Introduction of a CIDR insert in the late 1990's was first marketed by Pharmacia and Upjohn to improve the timing and efficiency of an AI program. The U.S. Food and Drug Administration (FDA) first approved the use of a CIDR for synchronizing estrus in beef cattle and dairy heifers. The FDA also approved its use in postpartum anestrous beef cows and prepubertal beef heifers, as a means to advance first estrus. When the Ovsynch plus CIDR protocol was used in suckled cows it increased P/AI greater than 50% (Lamb et al., 2001; Larson et al., 2004) compared with Ovsynch alone. Use of the CIDR with the Ovsynch protocol in heifers improved P/AI (Ambrose et al., 2005). As a result of FDA approval in beef cows and heifers and research to confirm increases in P/AI, research was initiated with the CIDR in lactating dairy cows.

The CIDR was tested in protocols that included Ovsynch or Presynch + Ovsynch in attempts to achieve greater P/AI. Results achieved have been both positive and no effect. When used in combination with the Ovsynch protocol, the CIDR is administered for 7 d beginning at the first injection of GnRH and removed when $PGF_{2\alpha}$ injected. In some studies the combination of the Ovsynch plus CIDR protocol has improved TAI P/AI in lactating cows (El-Zarkouny et al., 2004; Stevenson et al., 2006; Melendez et al., 2006) when compared with Ovsynch alone or AI at estrus. A number of studies were published in 2004 that examined effects of a CIDR insert during subsequent use of either the Ovsynch protocol or Presynch + Ovsynch protocol. El-Zarkouny et al. (2004) reported results from a study that reported P/AI in dairy cows after

synchronized ovulation regimens with or without presynchronization and an exogenous source of progesterone. Two experimental treatments were tested; the first being the use of a CIDR in conjunction with an Ovsynch. They reported increased P/AI at 29 d (59.3 vs. 36.3%) and 57 d (45.1 vs. 19.8%) after TAI for Ovsynch + CIDR compared with cows treated with Ovsynch alone in their first experiment. They found that before initiating the Ovsynch protocol, 80% of cows were cyclic. Presynchronization with the 2 injections of $PGF_{2\alpha}$ before initiating Ovsynch increased pregnancy at 29 d after TAI, but the use of the CIDR insert had no effect on pregnancy (46.8 vs. 37.5 %). El-Zarkouny et al. (2004) concluded that use of the CIDR in the Ovsynch protocol improved conception and embryo survival in experiment 1 but not in experiment 2, in part due to differing proportions of cyclic cows before the onset of treatments.

A similar study was performed in Central Mexico during June through September, 2001. Moreira et al. (2004a) evaluated whether the addition of the CIDR increased first service P/AI in lactating cows enrolled in the Ovsynch protocol. A treatment by parity interaction was detected. The interaction indicated that P/AI was increased in first-lactation cows treated with Ovsynch + CIDR compared with Ovsynch (38.2 and 20%). In contrast, no difference in P/AI was detected in older lactating cows (22.3 and 27.5%). Assessing cycling activity based on progesterone concentrations from 2 blood samples collected 7 d before and at the first GnRH injection; Ovsynch-treated anestrous cows had a P/AI of 18.8 % compared with 18.4 % for Ovsynch + CIDR cows. In cyclic cows, P/AI were 23.5 % for Ovsynch and 29.1 % for Ovsynch + CIDR. Moreira et al. (2004a) suggested that because the study was conducted during summer months that heat stress played a role in the effects on embryonic survival.

Moreira et al. (2004b) conducted another study in Central Mexico to examine the effect of a CIDR on first-service P/AI of lactating dairy cows enrolled in a Presynch + Ovsynch protocol

and a subsequent resynchronization (Resynch) of ovulation in nonpregnant cows before second service. Their objective was to evaluate the effects of the CIDR during a Presynch + Ovsynch protocol and what effects it might have on second-service estrus detection rates and P/AI. Presynch was initiated at 25 ± 3 DIM with Ovsynch initiated 14 d after the second Presynch $PGF_{2\alpha}$ injection. A CIDR was inserted at the first GnRH injection for 7 d. The CIDR-treated cows had increased P/AI compared with no CIDR cows (43 vs. 37%).

Cows were further enrolled in a Resynch protocol with or without CIDR insert. A CIDR insert was applied during 7 d at 14 d after first service or no CIDR was applied in controls (no additional treatments). Estrus-detection rate between treatments did not differ, however P/AI in first-lactation cows decreased for Resynch compared with controls (18 vs. 26 %). Older lactating cows treated with a CIDR insert during Resynch had increased PR compared with controls (21 vs. 11%). Moreira et al. (2004b) concluded that use of a CIDR with a Presynch + Ovsynch protocol increased first-service P/AI. In contrast, incorporating the CIDR in the Resynch protocol had no effect on estrus-detection rate. Negative effects on P/AI of first-lactation cows were observed, but a positive effect on P/AI occurred in older cows.

Galvão et al. (2004) offered results on P/AI in cows enrolled in a study in which CIDR inserts were incorporated into a Presynch protocol and a modified Ovsynch protocol. Cows received the standard 2 Presynch injections of $PGF_{2\alpha}$ 14 d apart then were started on the Ovsynch protocol. The second GnRH injection of the Ovsynch protocol, however, was replaced with estradiol cypionate (ECP) before TAI. A number of responses were examined during this study: how the CIDR affected estrus-detection rate, ovulation, P/AI, and late embryonic loss. When compared with the control, the CIDR-treated cows had similar rates of detected estrus (77.2 vs. 73.8 %) after the $PGF_{2\alpha}$ injection of Ovsynch, similar ovulation incidence (85.6 vs.

86.6%), and similar P/AI at 27 d (35.8 vs. 38.8 %) and 41 d (29.3 vs. 32.3%) after AI. Late embryonic loss did not differ between 27 and 41 d after AI for CIDR and control cows (18.3 vs. 16.8 %). Rhodes et al. (2003) found that treating the anovulatory cow with a CIDR for 7 d improved detection of estrus, incidence of ovulation, and P/AI. Use of a CIDR was found to eliminate cows in estrus before the $PGF_{2\alpha}$ injection of Ovsynch, thus decreasing the proportion of cows having a CL at the last $PGF_{2\alpha}$ injection because of less ovulatory response to the GnRH and spontaneous CL regression during CIDR treatment. Cyclic cows had greater P/AI than anovulatory cows at d 41 after AI (33.8 vs. 20.4 %), because late embryonic loss was decreased (16 vs. 30.3 %). Cyclic cows that responded to the Presynch protocol had the greatest P/AI, however, incorporation of a CIDR into a Presynch + Ovsynch protocol and using ECP to induce estrus and ovulation did not improve P/AI in lactating dairy cows. Improved P/AI also was reported for anovulatory dairy cows treated with a CIDR in an Ovsynch protocol (Stevenson et al., 2006).

Research conducted in Chihuahua, Mexico by Melendez et al. (2006) evaluated what effects progesterone might have on P/AI when the CIDR was applied with the Ovsynch protocol cows had failed to show estrus after a standard Presynch protocol started at 47 DIM. Any cow detected in estrus during presynchronization period of 28 d was inseminated according to the a.m. – p.m. rule. After 28 d of estrus detection and AI, the remaining cows were treated with the Ovsynch protocol, of which one-half also received CIDR inserts containing 1.9 g of progesterone for 7 d. The authors concluded that progesterone-supplemented cows had greater P/AI compared with cows treated with Ovsynch alone (31.2 vs. 22.7%). Blood samples were collected randomly from 55 cows per treatment at TAI and 14 d later to measure progesterone. Plasma progesterone concentrations at AI were <1 ng/mL and found not to differ between treatments. In contrast, in

both pregnant and nonpregnant cows on d 14 after TAI, plasma progesterone was increased in cows previously treated with progesterone. These results indicate that progesterone in the CIDR influenced the follicle (predecessor to the CL) and possibly the oocyte and uterus that resulted in better fertility.

Summary

Today's dairy producer is exposed to a rapidly changing industry, and the ability to make a profit depends on excellent management skills. A successful dairy producer must be able to manage nutrition, reproduction, and health of all cows on the farm, with the biggest challenge occurring in the lactating cows. For the lactating cow to remain economically profitable to the producer, she must resume a normal estrous cycle during the first 50 to 60 d after parturition. With the nutrient demand for milk synthesis, major setbacks in reproduction may occur, because requirements for maintenance, growth, and milk yield have greater priorities for energy sources than does the initiation of pregnancy.

As herd size increases, key areas in reproduction must not be neglected. Because of TAI programs, producers may be able to omit detection of estrus from everyday routines and allow more time to be devoted to other areas of management. Use of programs such as Ovsynch or Presynch + Ovsynch, (with or without a CIDR) has achieved acceptable P/AI in some experiments. Some studies have proven that similar or greater P/AI after TAI can be achieved when compared with AI after detected estrus. The key to greater improvement, when using TAI protocols is to achieve greater success in synchronizing ovulation with AI by increasing incidence of ovulation and CL regression.

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CHAPTER 2 - Fertility after timed AI insemination in response to a controlled internal drug release (CIDR) insert in lactating dairy cows

ABSTRACT

Lactating dairy cows from 2 Kansas farms were used to determine the effectiveness of exogenous progesterone in the form of an intravaginal insert (controlled internal drug release; CIDR) in conjunction with an ovulation-synchronization protocol. Cows were enrolled in a Presynch + Ovsynch protocol after parturition, where they received 2 injections of $PGF_{2\alpha}$, 14 d apart (Presynch) beginning between 30 and 36 DIM. Cows (n = 155) detected in estrus after the second $PGF_{2\alpha}$ injection of Presynch were inseminated (early AI). Remaining cows were assigned randomly to be treated with the Cosynch-72 protocol (GnRH 12 d after last Presynch $PGF_{2\alpha}$ injection, $PGF_{2\alpha}$ 7 d after GnRH, and timed AI + GnRH injection 72 h later) and served as controls (n = 159), or to be treated with the Cosynch-72 protocol and receive a progesterone insert (Ovsynch + CIDR; n = 175) for 7 d between GnRH and $PGF_{2\alpha}$. Blood was collected at d −22 and −10 (relative to TAI at d 0) to determine cycling status based on progesterone concentrations and again at d 11 post AI to determine luteal competency. Treated cows were assigned body condition scores (BCS) on d −22 and −10. Pregnancy status was confirmed by palpation of the uterus per rectum and its contents on d 38 post-timed AI and verified again 4 wk later. Treatment with the progesterone insert increased timed AI pregnancies per AI in Cosynch-72 + CIDR-treated cows when compared with controls (38 vs. 24%), but did not differ from early AI cows (38%). Pregnancy loss was numerically less in progesterone-treated cows than in controls (4.4 vs. 11.8%). Our study shows that increased pregnancies per AI can be achieved by

the use of a progesterone insert in a reduced population of cows not yet inseminated, but treated with a progesterone insert.

INTRODUCTION

The dairy industry is constantly adopting new technologies and attempting to survive fluctuations in milk price. It can be said that the fate of a dairy cow lies in her ability to reproduce. Unfortunately, reproductive performance in lactating cows has dramatically declined during the past decade. As a means to address the problem of reduced AI submission rates resulting from poor rates of estrus detection, estrus- and ovulation-synchronization protocols were developed to facilitate timed AI (**TAI**) programs. Development of the Ovsynch protocol in the mid 1990's by reproductive physiologists at the University of Wisconsin opened new doors for dairy producers. The Ovsynch protocol is initiated with an injection of GnRH to induce ovulation of a follicle followed by an injection of $PGF_{2\alpha}$ 7 d later. Ovulation is induced after luteolysis by using a second GnRH injection given approximately 48 h after $PGF_{2\alpha}$. Studies using the Ovsynch protocol have reported pregnancies per AI (**P/AI**) to range between 30 and 40% (Pursley et al., 1995; Burke et al., 1996; Stevenson et al., 1999).

Because GnRH is used to control follicular development and induce ovulation of a dominant follicle and $PGF_{2\alpha}$ causes the regression of the corpus luteum (**CL**), variations in the Ovsynch protocol have been tested to synchronize ovulation by altering timing of GnRH and $PGF_{2\alpha}$ injections. Pursley et al. (1998) demonstrated that altering the timing of the second GnRH injection relative to the timing of AI impacted P/AI. Other alterations to the Ovsynch protocol have been made during the last few years, one of which has been the introduction of an exogenous source of progesterone in attempt to help improve P/AI.

Follicular development and early maintenance of pregnancy requires progesterone secretion by the CL. Progesterone plays an important role in preventing the return of estrus and to synchronize estrus upon its return to baseline concentrations after demise of the CL. Previous studies that used an intravaginal progesterone-releasing insert (controlled internal drug release; CIDR) in conjunction with the Ovsynch protocol, found that P/AI in progesterone-treated cows were improved after TAI in some (Exp. 1 in El-Zarkouny et al., 2004; Moreira et al., 2004b; Stevenson et al., 2006; Melendez et al., 2006), but not in all studies (Exp. 2 in El-Zarkouny et al., 2004; Galvão et al. 2004; Moreira et al., 2004a). Galvão et al. (2004) showed no improvement in P/AI when a CIDR insert was incorporated into a TAI protocol and estradiol cypionate (ECP) was substituted for the second GnRH injection in the Ovsynch protocol to induce estrus and ovulation.

The hypothesis of the present study was that cows that have failed to show estrus following the second of 2 $PGF_{2\alpha}$ injections given 14 d apart (Presynch protocol) would have improved P/AI after a timed AI protocol if progesterone (via CIDR insert) was provided in combination with the TAI protocol. The objective of this study was to determine the effectiveness of applying a CIDR insert in conjunction with a TAI protocol in lactating dairy cows not previously inseminated after a period of detected estrus.

MATERIALS AND METHODS

From January to July 2007, lactating dairy cows from 2 commercial Kansas dairy farms were enrolled in the present study. Characteristics of cows at each location are summarized in Table 2.1. Cows at both locations were housed in free-stall pens and separated according to stage of lactation and lactation number. Each pen consisted of sand-bedded stalls and feed-line

headlocks. Headlocks were set during all procedures, including administrations of hormones, blood sampling, CIDR insertions, AI, and pregnancy diagnosis.

Cows were fed a TMR twice daily that met or exceeded the National Research Council (2001) recommendations for lactating cows. Cows had ad libitum access to fresh water at 3 places in each pen of approximately 100 cows.

Experimental Design

All cows were enrolled in a Presynch + Cosynch-72 protocol after parturition. Following the second Presynch $PGF_{2\alpha}$ injection, cows were observed for estrus based on tail chalking and classical symptoms of sexual behavior (e.g., mucous discharge, bellowing, increased nervousness and activity, walking fence line, licking, sniffing, swelling and reddening of the vulva, mounting other cows, lower milk yield, or standing estrus). At location 2, cows were fitted with activity monitors to assess increased activity associated with estrus. When cows failed to be detected in estrus and were not inseminated after the second of 2 Presynch ($PGF_{2\alpha}$) injections, cows were assigned to a randomized block design based on lactation number (1 or 2+) to receive either of 2 treatments: CIDR or no CIDR at the onset of the Cosynch-72 protocol (Figure 2.1). A modified Ovsynch protocol (Cosynch-72) consisted of injecting i.m. 100 μg of GnRH (Cystorelin, Merial Ltd., Iselin, NJ) and either receiving a progesterone insert containing 1.38 g of progesterone (EAZI-BREED CIDR inserts, Pfizer Animal Health, New York, NY) for 7 d or served as controls. After CIDR insert removal all cows received an injection of $PGF_{2\alpha}$. Cows received a TAI 72 h after $PGF_{2\alpha}$ and were given a second injection of GnRH. Therefore, 3 treatments of cows consisted of those cows inseminated before Cosynch-72 based on signs of estrus (early AI), control, and CIDR. Cows were diagnosed weekly for pregnancy by transrectal

palpation beginning 38 d after AI and reconfirmed 4 wk later. Any pregnancy loss was then determined between the 2 pregnancy diagnoses.

Blood Sampling and Radioimmunoassay of Progesterone

Blood samples were collected via puncture of a coccygeal blood vessel on d –22 (TAI was d 0) and BCS (1=thin and 5=fat) was assigned. For cows subsequently inseminated after this sample (early AI cows), the blood sample collected on d –22 was discarded and no further blood collection occurred. In cows not inseminated in the early AI treatment, blood was collected on d –10 and again 11 d post-TAI. Blood was stored at 5°C for 24 h until serum was harvested by centrifugation. Serum samples were then stored at –20°C until assayed for progesterone by RIA (Skaggs et al., 1986). All samples from each cow were analyzed in duplicate in the same assay. Inter- and intra- assay coefficients of variation for 14 assays were 7.7 and 7.6%, respectively, for pooled sample that averaged 3.7 ± 0.5 ng/mL.

Samples for determining concentrations of progesterone collected on d –22 and –10 were used to determine cycling status of each cow before treatment. When progesterone was ≥1 ng/mL in either of the 2 samples, cows were assumed to have already initiated estrous cycles (cycling). In contrast, those having concentrations in both samples <1 ng/mL were assumed to be anestrous (not cycling). Blood collected on d 11 post-TAI was used to determine any post-treatment effect of the progesterone insert on subsequent luteal function.

Body condition scores (< 1.75, 2.0, or ≥2.25) of cows were divided into 3 ranges of scores. Average FCM (<37.5, 37.5 to 47, or ≥48 kg/d) representing the first 3 test-day milk samples were divided into terciles and used in various statistical models described later.

Statistical Analyses

Data were analyzed by using ANOVA (procedure GLM in the Statistical Analysis System (SAS Institute Inc., Cary, NC). Preliminary models indicated that a treatment by cycling status interaction was significant for P/AI at d 38 but not for pregnancy loss or concentrations of progesterone at d 11 after TAI. Four models were constructed to determine effects of treatment on P/AI at d 38. Each of 4 models differed according to the combination of fixed effects or covariables that were incorporated into the analyses. Because of the interaction, each model included 5 treatments: early AI, CIDR-cycling, CIDR-noncycling, control-cycling, and control-noncycling in addition to lactation number (1 or 2+), treatment by lactation interaction, location, and a treatment by location interaction. Model 1 included the regression (covariable) effects of average FCM and BCS. Model 2 differed from model 1 by considering the fixed effects of body condition (< 1.75, $= 2.00$, or ≥ 2.25) and milk (< 37.5, 37.5 to 48, or ≥ 48 kg/d). Model 3 combined the regression effect of BCS and the fixed effect of average FCM, whereas model 4 combined the regression effect of average FCM and the fixed effect of BCS.

To assess pregnancy loss between d 38 and 4 wk later, the 4 models included 3 treatments (early AI, CIDR, and control), lactation number (1 or 2+), treatment by lactation interaction, location, and a treatment by location interaction, plus the combinations of fixed effects of BCS or covariables described previously.

These combinations of fixed and covariable effects provided varying model R^2 values. Statistical analyses that generated the best fit model was model 2. Model 2 consistently produced the greatest R^2 values and contained the fixed effects of BCS and milk production.

To assess differences in serum concentrations of progesterone collected at d 11 post-TAI, concentrations were analyzed by ANOVA using a model consisting of treatment (early AI,

CIDR, and control), lactation number (1 vs. 2+), location, fixed effects of body condition (< 1.75, = 2.00, or ≥ 2.25) and milk (< 37.5, 37.5 to 48, or ≥ 48 kg/d), pregnancy status, and all 2-way interactions with treatment.

RESULTS

A total of 512 cows at both locations were subjected to the Presynch protocol. Before the first GnRH injection of the Cosynch-72 protocol, 156 cows were identified in estrus and inseminated (early AI). The remaining 356 cows not observed in estrus and inseminated received the first GnRH injection of Cosynch-72 and were randomly assigned to 2 treatments (CIDR or Control). Of 356 cows treated, 22 cows were eliminated from overall analyses because of culling before or after AI or pregnancy diagnosis, disease, death, early insemination during the Cosynch-72 protocol, not inseminated, or repeat AI 1 to 2 d after the TAI. As a result of cows being eliminated from the study for various reasons, a total of 334 cows were treated: 175 in the CIDR treatment and 159 served as controls.

Cycling Status

More than 78% (387/490) of the cows were cycling based on blood serum collected on d –22 and –10 in CIDR and control cows plus early AI cows (assumed to be cycling because they were observed in estrus and inseminated). At location 1, 32.9 % of the cows were inseminated early between the second Presynch $PGF_{2\alpha}$ injection and the onset of the Cosynch-72 protocol and 38.9% were inseminated early at location 2. Of the remaining 334 cows, 231 (69.2%) were found to have elevated progesterone in either or both samples collected before treatment, indicating that about 30% of the treated cows were not cycling or anovulatory before treatment.

Pregnancies per AI

Pregnancies per AI summarized by location, cycling status, lactation number, BCS, and milk yield are shown in Table 2.2. Overall P/AI did not differ between locations (32 vs. 33.6%). No significant difference in P/AI was detected between pre-treatment cycling and non-cycling status (34.4 vs. 26.2%).

First-lactation cows had P/AI that did not differ ($P = 0.12$) from that of older cows (36.6 vs. 29.8%; Table 2.2). Cows having more body condition had greater ($P < 0.05$) P/AI. Cows having a BCS < 1.75 averaged 12.5 percentage points less in conception rate than those cows having BCS 2.25 or greater (Table 2.2). Although P/AI did not differ among test-day milk terciles, P/AI was numerically less in cows having greater FCM (Table 2.2).

Specific treatment effects on P/AI are illustrated in Figure 2.2. No difference was detected **($P = 0.81$)** in P/AI between early AI and CIDR-treated cows (35.9 vs. 37.1%). In contrast, P/AI in control cows were approximately 12.6 percentage points less than in CIDR-treated cows and 11.4 percentage points less than early AI cows. Among cycling cows, those treated with the CIDR did not differ **(**$P = 0.17$) from controls. In contrast, among noncycling cows, CIDR-treated cows had more **(**$P = 0.002$) P/AI than controls.

No lactation by treatment interaction was detected (Table 2.3), but numerically, first-lactation cows had greater P/AI, with early AI cows having the highest P/AI of 46.7% compared with CIDR and control cows (40.3 and 24.7%). In addition, no location by treatment effect was detected. Location 1, however, seemed to have fewer early AI cows conceive at first service than location 2 (32.9 vs. 38.9%). Lowest P/AI was observed consistently in control cows at both locations (Table 2.3).

Pregnancies per AI based on average FCM by treatment were examined (Figure 2.3). Early AI cows showed the greatest P/AI (43.8%) when the average FCM milk was <37.5 kg. As average FCM increased to >48 kg in the early AI cows, P/AI numerically decreased by 14.5 percentage points. Little variation in P/AI was observed in CIDR-treated cows in the 3 FCM terciles. In control cows, FCM >48 kg seemed to reduce P/AI to its lowest value. When FCM was <37.5 kg, P/AI was numerically greatest in the early AI groups (43.8%) compared with that of controls (26.4 %). The CIDR-treated cows had their numerically greatest P/AI when FCM was >48 kg. In contrast, at that same level of FCM, control cows had their lowest P/AI (40.8 vs. 19.6%), a difference of 21.2 percentage points. Fat corrected milk was found to have no effect on P/AI even after the addition of the CIDR.

Figure 2.4 shows P/AI based on BCS that were assigned at the time of the second Presynch $PGF_{2\alpha}$ injection. Early AI cows showed no significant difference in P/AI for cows grouped <1.75 and > 2.25 BCS (32.6 vs. 34.9%). Numerically early AI cows had the highest P/AI when BCS was 2.0. Pregnancies per AI increased in CIDR-treated cows as BCS increased. Control cows had a similar relationship between BCS and P/AI as CIDR-treated cows, because as BCS increased, P/AI increased; however, P/AI did not differ between cows having <1.75 and 2 BCS (18.6 vs. 19.1%). By regression analysis, P/AI increased ($P < 0.05$) 10.9 ± 5.2% for each unit increase in BCS (range of 1.25 to 4.0). In summary, thinner cows had poorer TAI P/AI.

Pregnancy Loss

Pregnancy loss between d 38 and 66 after AI was not significantly affected by treatment. Pregnancy loss, however, was least in CIDR-treated cows (4.6%) compared with control and early AI cows (12.8 vs. 7.3%), respectively.

Serum Progesterone

Serum progesterone concentrations were analyzed in blood samples collected on d 11 post-TAI. Although concentrations of progesterone were greater ($P < 0.05$) in pregnant than nonpregnant cows (5.0 ± 0.3 vs. 4.3 ± 0.2 ng/mL), concentrations of progesterone in CIDR-treated cow did not differ from those in controls (Figure 2.5).

DISCUSSION

The objective of this study was to determine the effectiveness of exogenous progesterone in the form of an intravaginal insert (CIDR) in conjunction with an ovulation-synchronization protocol in lactating dairy cows. Before the present study, a few studies had been conducted to test the effectiveness of supplementing progesterone. Before 2004, a number of experiments were conducted that looked at the effects of Ovsynch on P/AI, however, it wasn't until 2004 when studies addressed the benefit of combining a CIDR into a ovulation-synchronization protocol on P/AI (El-Zarkouny et al., 2004; Galvão et al., 2004; Moreira et al. 2004a,b; Stevenson et al., 2006). El-Zarkouny et al (2004) reported P/AI in cows treated in 2 experiments that examined P/AI after synchronization of ovulation with Ovsynch or a Presynch + Ovsynch protocol. The first experiment confirmed that P/AI increased when progesterone via a CIDR insert was added to the Ovsynch protocol. At d 29 P/AI increased from 36.3 to 59.3 % and 19 to 45.1 % on d 57, between Ovsynch vs. Ovsynch + CIDR. In contrast, in the second experiment (El-Zarkouny et al., 2004) where they presynchronized the estrous cycle before the start of the Ovsynch or Ovsynch + CIDR protocol with 2 injections of $PGF_{2\alpha}$ 14 d apart, they concluded that the use of the CIDR insert had no effect on P/AI. Presynchronization with $PGF_{2\alpha}$ vs. Ovsynch alone was found to increase P/AI from 37.5 to 46.8%.

A similar protocol to the previous one (El-Zarkouny et al., 2004) determined whether progesterone supplementation during Ovsynch would enhance fertility in lactating dairy cows (Stevenson et al., 2006). Overall P/AI at d 28 and 56 for Ovsynch alone were 40 and 33%. They reported that use of the CIDR increased P/AI from Ovsynch alone 50% on d 28 and 38% for d 56 after TAI. A similar study to Stevenson et al. (2006) tested the use of a CIDR in collaboration with a Presynch and TAI protocol with the use of estradiol cypionate in the place of the second GnRH injection of Ovsynch to induce estrus and ovulation (Galvão et al., 2004). However, their results did not show improvement in P/AI in response to the CIDR insert. The 2 studies by Moreira et al. (2004a,b) showed that use of a CIDR insert in conjunction with the ovulation-synchronization improved P/AI. In one study (Moreira et al., 2004a), addition of the CIDR only increased first-service pregnancy rates in primiparous but not in multiparous cows submitted to the Ovsynch + CIDR protocol. The second Moreira et al. (2004b) study found that the use of a CIDR in conjunction with a Presynch protocol improved PR (43 % vs. 37 %) when compared to a Presynch only protocol. The present study found that P/AI did not differ between primiparous and multiparous cows (36.6 vs. 29.8 %).

A study was conducted in Chihuahua, Mexico where 8,650 Holstein milking cows were enrolled in a Presynch + Ovsynch with or without progesterone protocol (Melendez et al., 2006). Results confirmed that after a 28-d presynchronization period of detected estrus and AI, cows not yet inseminated and subjected to the Ovsynch protocol and supplemented with the CIDR insert had greater P/AI and greater concentrations of progesterone at 14 d after TAI than those subjected to the Ovsynch protocol alone.

In the present study, lactating cows benefitted from the use of the CIDR as P/AI increased from 24.5% in controls to 37.7% in CIDR-treated cows. A striking finding in the present study

was the P/AI in early AI cows were bred at an average of 56 DIM. The early AI cows were inseminated 15 d earlier in lactation than their counterparts that received a CIDR or were control cows. Even though early AI cows were inseminated 15 d before the treated cows, P/AI did not differ between the CIDR treated (cycling and noncycling) and early AI cows (cycling = 36.9 %, noncycling = 37.7 %, and early AI = 35.9 %). The present study confirmed results of Melendez et al. (2006) in that a progesterone insert could potentially be beneficial to lactating dairy cows, even with the difference in progesterone concentrations from the insert (1.38 g vs. 1.9 g). When a good estrus-detection program is in place, inseminating cows determined to be in estrus during the Presynch protocol may greatly benefit those cows by producing P/AI that may not differ from that after TAI. The use of 2 injections $PGF_{2\alpha}$, administered 14 d apart, constitutes a practical and relatively inexpensive method for presynchronization. More than 70% of the cows began the Ovsynch protocol during early- to mid-diestrus (between d 5 and 12) when Presynch was applied before Ovsynch, compared with 53% of the cows treated with Ovsynch at random stages of the cycle (El-Zarkouny et al., 2004).

Influence of stage of lactation (voluntary waiting period) and milk production on conception rates after TAI following Ovsynch was examined (Tenhagen et al., 2003). They reported that cows inseminated later in lactation had greater first-service P/AI compared with cows inseminated 3 wk earlier, regardless of level of milk production. The present study showed no difference in P/AI when cows were inseminated 15 d later than the early AI cows (early AI = 35.9%, CIDR-cycling = 36.9%, CIDR-noncycling = 39.6%, control-cycling = 29.4%, and control-noncycling = 14%)

Milk production is generally thought to have a negative impact on P/AI (Tenhagen et al., 2003). Many researchers have speculated that greater milk production results in decreased P/AI,

because high milk production inhibits expression of estrus (Tenhagen et al., 2003). The present study, however, is consistent with earlier reports (Platen et al., 1995;Tenhagen et al., 2001; Tenhagen et al., 2003), that high milk production does not influence P/AI no matter what ovulation synchronization protocol was implemented. In contrast, one study found delaying inseminations to later DIM increased subsequent TAI P/AI (Tenhagen et al., 2003).

Progesterone concentrations were slightly higher in CIDR-treated cows compared with control cows, and more progesterone may have benefited the CIDR-treated cows with reduced embryonic loss between the first and second pregnancy diagnosis. Serum progesterone concentrations between cycling CIDR and cycling control cows did not differ, which is consistent with earlier reports (Melendez et al., 2006; Stevenson et al., 2006). Use of a CIDR was found numerically to have an effect on P/AI based on cycling status when comparing the noncycling cows that either received a CIDR or did not. The noncycling CIDR cows had a P/AI of 37.7% compared with 14% for the noncycling control cows. One possible explanation for this result is the progesterone via the CIDR was helping the noncycling cows to become slightly more fertile. Progesterone could possibly be affecting the oocyte quality or the environment in the uterus and its secretions. Although the specific action(s) of progesterone to improve fertility in timed AI programs remains poorly defined, results of the current study and others (El-Zarkouny et al., 2004; Melendez et al., 2006; Moreira et al. 2004a,b; Stevenson et al., 2006) suggest a rather consistent benefit. It is reasonable to speculate that exogenous progesterone may in some way enhance the quality of the uterine environment and improve the likelihood of successful pregnancy. Stevenson et al. (2006) found that progesterone concentrations in cows with or without active CL were only slightly increased by the CIDR insert. Other results (Melendez et al., 2006) confirmed that after the presynchronization period of detected estrus,

cows subjected to the Ovsynch protocol supplemented with the CIDR insert had greater P/AI and than those subjected to the Ovsynch protocol and no CIDR insert (31.2 vs. 22.7%). They also observed greater concentrations of progesterone in pregnant and nonpregnant cows 14 d post-TAI. One reason why the present study does not confirm the increase in progesterone after AI could be the timing of blood sampling. Blood sampling for progesterone concentration after TAI in the present study occurred at d 11 after TAI whereas it occurred at d 14 in their study. Melendez et al. (2006) speculated that an explanation for improved fertility and greater progesterone in the Presynch + Ovsynch + CIDR cows over those cows not receiving a CIDR insert might have occurred because the progesterone supplementation may induce more synchronized ovulation and normal luteal phases, which is consistent with finding from Stevenson et al. (2006). Because a normal luteal phase or greater progesterone after AI is consistently associated with better fertility (Thatcher et al., 2002; Spencer et al., 2004; Moore et al., 2005), that might help to explain the reduced P/AI for the noncycling control cows.

CONCLUSIONS

Treatment of cows with a CIDR insert in conjunction with the Presynch + Cosynch-72 protocol for 7 d was effective in increasing P/AI compared with no CIDR. Accurate detection of estrus during the entire ovulation-synchronization protocol still should be performed, so cows showing signs of estrus are inseminated. An effective estrus-detection program could potentially reduce costs that are involved with the cow continuing through the Ovsynch + CIDR protocol. Elevated circulating progesterone concentration from the CIDR benefitted the cows with increased first-service P/AI. Our study shows that increased P/AI can be achieved by using a progesterone insert in a reduced population of cows not yet inseminated.

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Table 2.1. Location characteristics

	Location	
Item	1	2
Starting date	January 22, 2007	January 23, 2007
No. of cows	614	854
No. of milking cows	525	790
First Presynch $PGF_{2\alpha}$ DIM	37-43	30-36
Second Presynch $PGF_{2\alpha}$, DIM	51-57	44-50
Breed	Holstein	Holstein and Jersey
Milking frequency, times/d	3	3
Average 305ME, kg	12,390	10,800
Days to 1st service	76	68
Services per pregnancy, pregnant cows	2.9	3.2
Services per pregnancy, all cows	4.7	5.9
Calving interval, mo	14.8	13
Days open	169	137

[1] Rolling herd average assessed at the onset of the experimental protocol.

Table 2.2. Pregnancies per AI (P/AI) based on location, cycling status, lactation number, body condition score, and milk yield

Item	No. of cows	P/AI, %
Location		
1	198	32.0
2	292	33.6
Cycling status[1]		
Yes	387	34.4
No	103	26.2
Lactation		
1	205	36.6
2+	285	29.8
Body condition score[2]		
< 1.75	167	26.8[a]
= 2.00	141	32.6[a,b]
≥ 2.25	182	39.0[b]
Average FCM, kg/d		
< 37.5	161	35.4
37.5 to 47	166	33.1
≥ 48	163	29.5

[a-b] Mean percentages having different superscript letters differ ($P < 0.05$).

[1]Based on serum concentrations of progesterone in blood samples collected before the second Presynch $PGF_{2\alpha}$ injection and before the onset of the Ovsynch protocol in progesterone insert and control cows only.

[2]Assessed before the second Presynch $PGF_{2\alpha}$ injection.

Table 2.3. Effects of lactation number and location on pregnancies per AI

	Treatment		
Item	Early AI	CIDR	Control
		% (n)	
Lactation			
1	46.7 (60)	40.3 (72)	24.7 (73)
2+	29.2 (96)	34.9 (103)	24.4 (86)
Location			
1	32.9 (79)	38.3 (60)	22.0 (59)
2	38.9 (77)	36.5 (105)	26.0 (101)

Figure 2.1. Experimental design of treatments. BCS = body condition score; B =blood sample; CIDR = controlled internal drug release insert containing 1.38 g of progesterone; PD1 = pregnancy diagnosis; PD2 = pregnancy diagnosis; and TAI = timed artificial insemination.

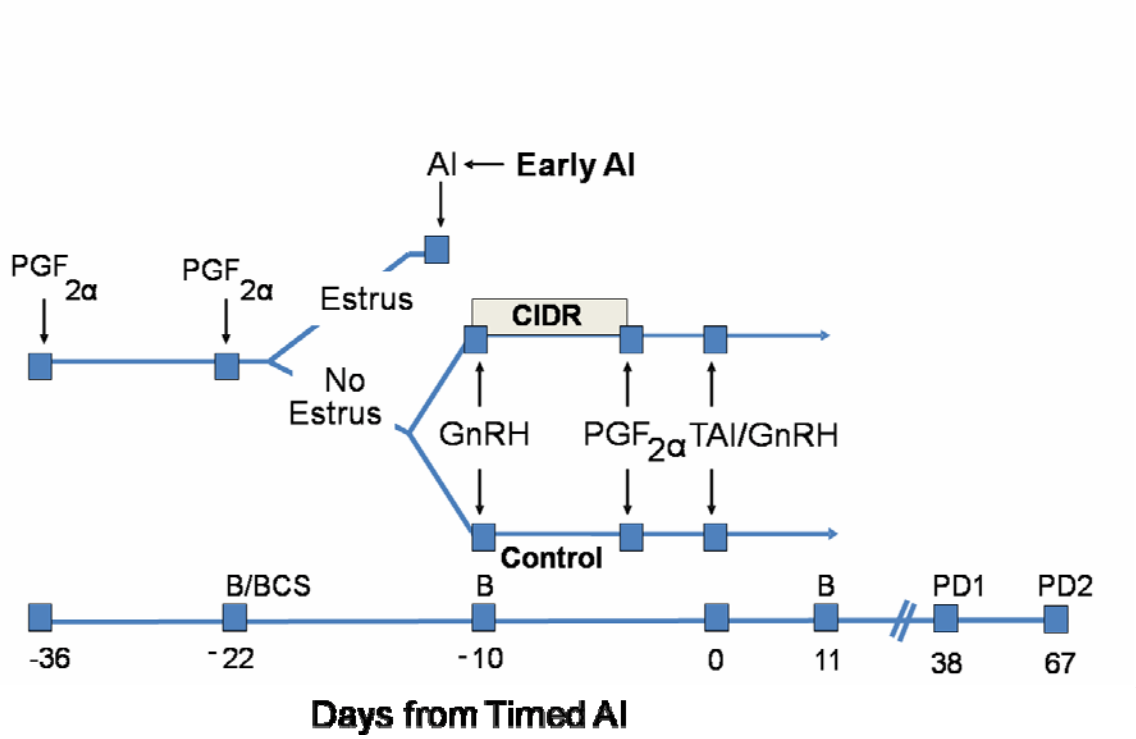

Figure 2.2. Pregnancies per AI (P/AI) based on cycling status. Average DIM at AI for early AI, CIDR, and control cows were, 56 ± 5 (mean ± SD), 71 ± 4, and 72 ± 4. Prior contrasts: early AI vs. CIDR ($P = 0.81$); early AI vs. control ($P = 0.12$); CIDR-cycling vs. control-cycling ($P = 0.17$); and CIDR-noncycling vs. control-noncycling ($P = 0.002$).

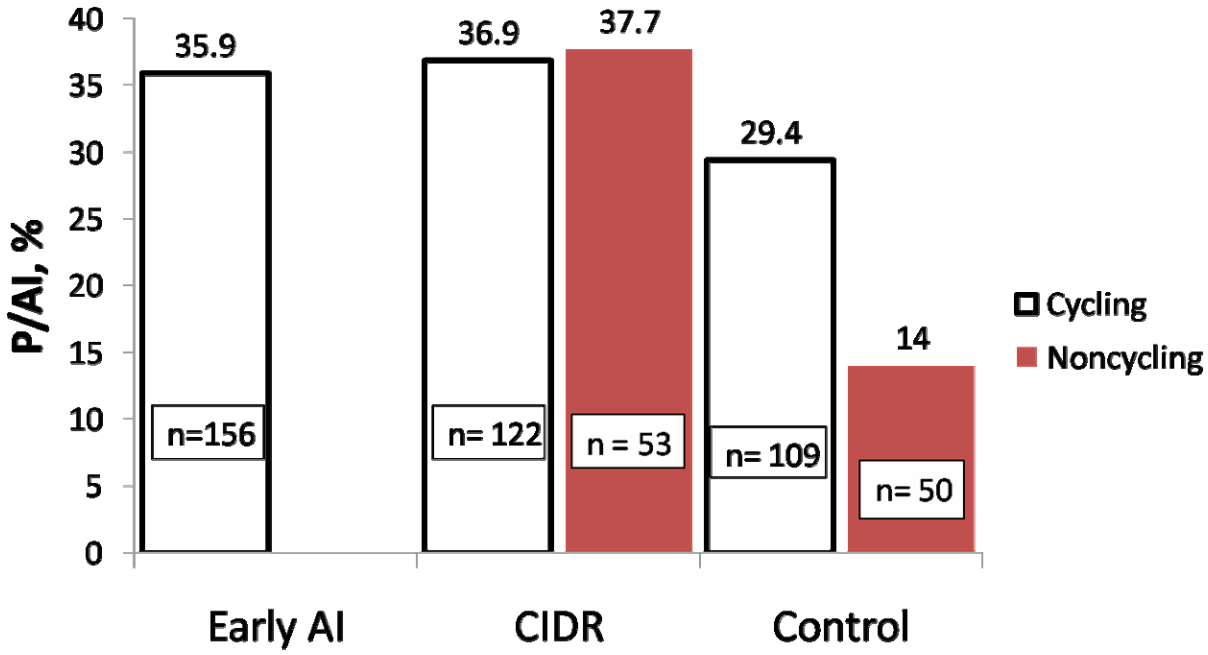

Figure 2.3. Pregnancies per AI based on average fat corrected milk (FCM) divided into terciles. Average FCM represented the mean of the first 3 postpartum test-day samples.

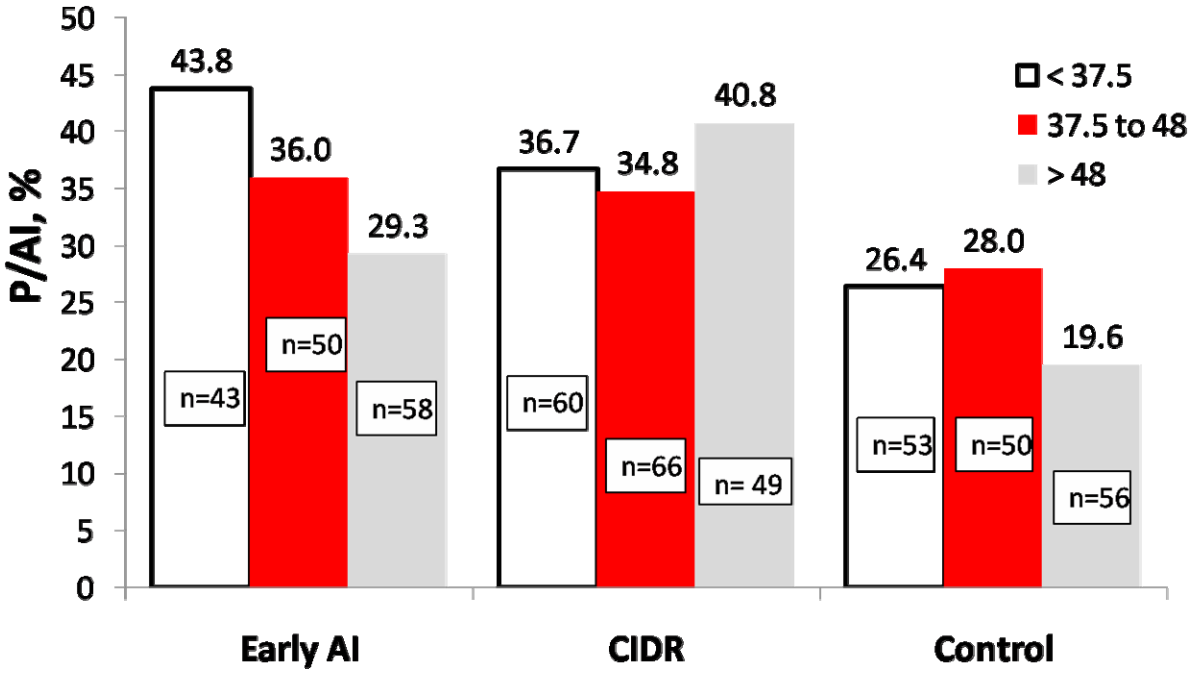

Figure 2.4. Pregnancies per AI (P/AI) based on body condition score assessed at the time of the second Presynch $PGF_{2\alpha}$ injection (44-57 DIM).

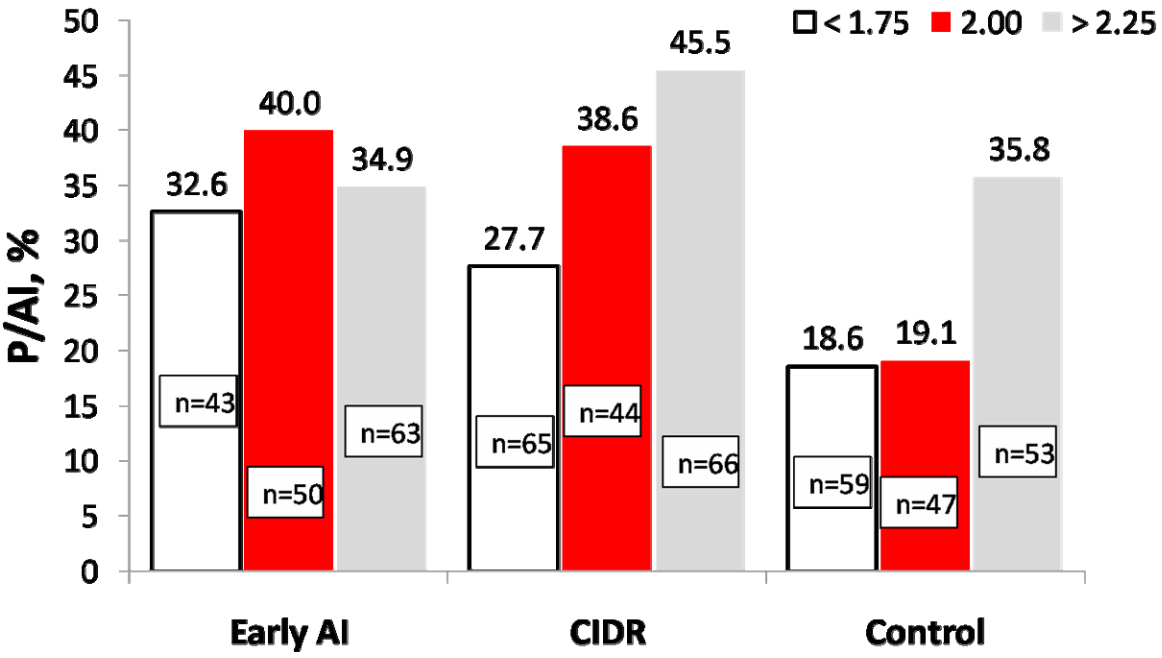

Figure 2.5. Serum concentrations of progesterone after timed AI based on cycling status in CIDR and control cows assessed at d 11 post-TAI.