EVALUATING THE EFFECTIVENESS OF USING THE CONTROLLED INTERNAL DRUG RELEASE (CIDR) INSERT FOR SYNCHRONIZATION OF ESTRUS AND POST-INSEMINATION PROGESTERONE THERAPY TO IMPROVE REPRODUCTIVE PERFORMANCE OF DAIRY CATTLE

by

Jillian L. Fain

(Under the Direction of William Graves)

ABSTRACT

Study 1 combined the CIDR insert for ovulation synchronization and TAI. Animals received 50 µg GnRH (-9 d), CIDR (1.38 g progesterone) (-9 d), 25 mg $PGF_{2\alpha}$ (-3 d), 1 mg ECP (-2 d), -CIDR (-2 d), GnRH (d 0), and TAI (0 d), (OverSynch; n=20) or CIDR (-9 d), $PGF_{2\alpha}$ (-3 d), -CIDR (-2 d), and TAI (0 d) (Control; n=20). Pregnancy rates did not differ ($P > 0.05$); however, estrus activity was increased with OverSynch ($P < 0.05$). Study 2 utilized the CIDR to decrease embryonic loss and for reuse in resynchronization post AI. Supplementation had no effect on pregnancy rates, regardless of season ($P > 0.05$). Progesterone concentrations post AI tend to be higher in heifers than in cows ($P = 0.0651$) and in dairy versus beef heifers ($P < 0.05$). Overall, d 21 progesterone concentrations is positively correlated with d 35 pregnant diagnosis ($P = 0.004$).

INDEX WORDS: Controlled Internal Drug Release (CIDR), Timed AI (TAI), Progesterone, Embryonic Loss, Dairy Cattle

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## DEDICATION

To My Family

Thanks for teaching me the value of hard work, inspiring my dreams, and being my support throughout this journey.

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# CHAPTER 1

# INTRODUCTION AND LITERATURE REVIEW

## A Review of the Estrous Cycle and Endocrinology in Cattle

The bovine is a non-seasonal polyestrous animal that is capable of conception year round. The estrous cycle in cattle is comprised of 21 d, with the first day (d 1) of the cycle being deemed estrus or heat. Estrus occurs when the female is physiologically and psychologically receptive to the male. The estrous cycle is characterized by repeated patterns of ovarian cell proliferation, differentiation, and transformation that aid in follicular development as well as the formation and regression of the corpus luteum (CL). Timing of ovulation is directly correlated with estrus in vertebrates. In the bovine, ovulation occurs in the window of 24 to 32 h after the onset of estrus, which could last from 6 to 24 h (Senger, 2003).

A cascade of events leads to the ovulation of the dominant follicle. The estrous cycle consists of 2 distinct phases termed follicular and luteal, both of which occur within the 21-d cycle. The ability to complete the estrous cycle in this short time frame relies on the adult female's rapidly growing tissues including the follicle and CL, which can both undergo periods of dynamic growth and regression. The follicular phase, the shorter of the 2 phases, consists of the period from regression of the CL to ovulation at which time the dominant follicle is the primary structure. The luteal phase occurs from ovulation to regression of the CL and comprises 80% of the estrous cycle with the CL as the dominant structure (Senger, 2003).

During the luteal phase, there are 2 to 3 waves of growing follicles with each wave culminating at the formation of a large follicle and the eventual selection of the dominant follicle

as seen in Figure 1.1. In a 2-wave cycle, each wave lasts approximately 10 to 11 d; however, in a 3-wave cycle, the second and third waves are much shorter (Stewart, 2004). With these 2 patterns, waves emerge on d 0 and 10 for a 2-wave cycle and d 0, 9, and 16 for a 3-wave cycle. Each wave produces several large follicles 4 to 5 mm in diameter followed by selection and growth of the dominant follicles. All large and dominant follicles will undergo atresia, except the largest dominant follicle in the final wave prior to ovulation (Lucy, 1992). Each wave has a dominant follicle that will achieve the greatest diameter and will suppress the growth of subordinate follicles (Pierson, 1987). This suppression is the result of increasing and decreasing estradiol and inhibin hormonal concentrations. The concentration of luteinizing hormone (LH), however, consistently increases, encouraging growth of the dominant follicle.

Each of these dominant follicles undergo a series of events during their lifespan including 6 d of growth (growing phase), 6 d of no growth (static phase), and followed in the remaining time by regression (regressing phase) (Noseir, 2003). An increase in follicular size of the dominant follicle is directly related to increased estradiol concentrations and decreased progesterone concentrations (Noseir, 2003). Estradiol concentrations peak at the end of the growing phase and begin to decline upon the beginning of the static phase (Bartlewski, 1999). During this time, inhibin secretion is gradually increasing. Increased estradiol and inhibin concentrations suppress follicle stimulating hormone (FSH) release, reducing FSH secretions significantly when selection of the dominant follicle occurs (Bo, 1995). Granulosal cells of atretic follicles show little to no mRNA expression of the estrogen receptor (Bao, 2000). The decrease in estradiol during the static phase initiates the increasing release of FSH and a subsequent follicular wave (Kaneko, 1991). This cycle repeats for each wave that does not produce the preovulatory follicle.

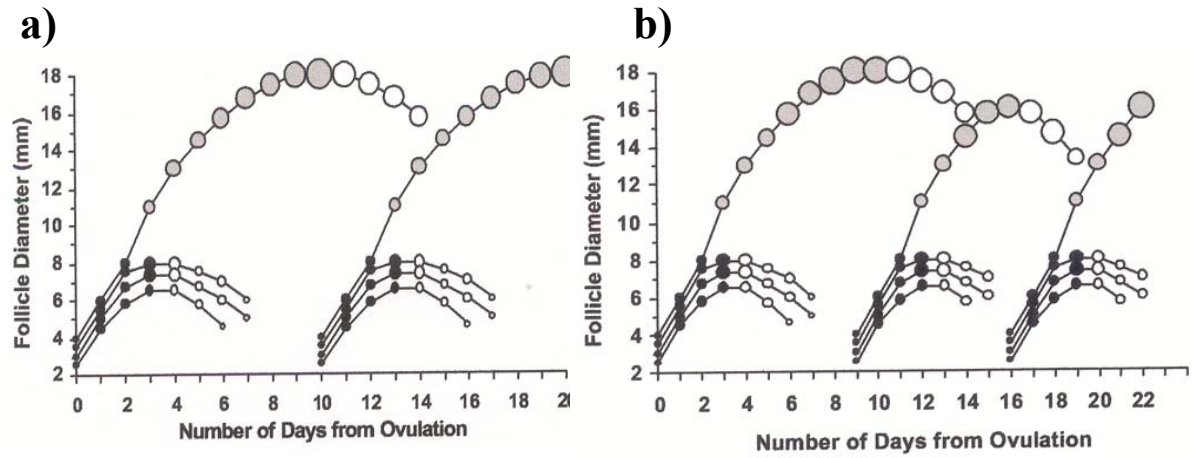


Figure 1.1: Schematic diagram representing 2-wave (a) and 3-wave (b) follicular growth in the bovine estrous cycle. The number of follicular waves is immediately dependent upon the surge of GnRH and subsequent FSH and estradiol. Follicular wave number is also influenced by age, parity, dietary intake, and lactational status with the general guideline of primiparous and multiparous lactating cows having 2 waves and nulliparous heifers have 3 waves (Sartori, 2000). The compensation for increased wave numbers is accounted for with decreased wave time span (Kastelic, 1991). Growing follicles before the selection of the dominant follicle are depicted as black circles, the dominant follicles are the grey circles, and the open circles are atretic follicles undergoing death and regression. (Adapted from Fricke et al., 2001).

In tandem with the follicular dynamics of the luteal phase is the maintenance of the CL and progesterone production. Throughout this time, the CL remains the dominating structure and progesterone synthesis maintains a pulsatile secretion of GnRH from the hypothalamus. Beginning on or around d 14 to 16, the uterus begins to release pulses of prostaglandin into its venous drainage into the ovaries. Within hours, the functional role of the CL has been removed, with the structural portion of the CL often taking more than 12 h to be fully removed. The uterus possesses the most influence on regression of the CL. In cows that have undergone a removal of the uterus (hysterectomy), the functional lifespan of the CL is prolonged in an occurrence of pseudopregnancy (Turner, 1966). By d 17, the CL is fully lysed, and both functional and structural components are removed in a process termed luteolysis that requires a

number of external factors to complete the process (See Figure 1.2). With the regression of the CL and the formation of the preovulatory follicle, the luteal phase has come to an end.

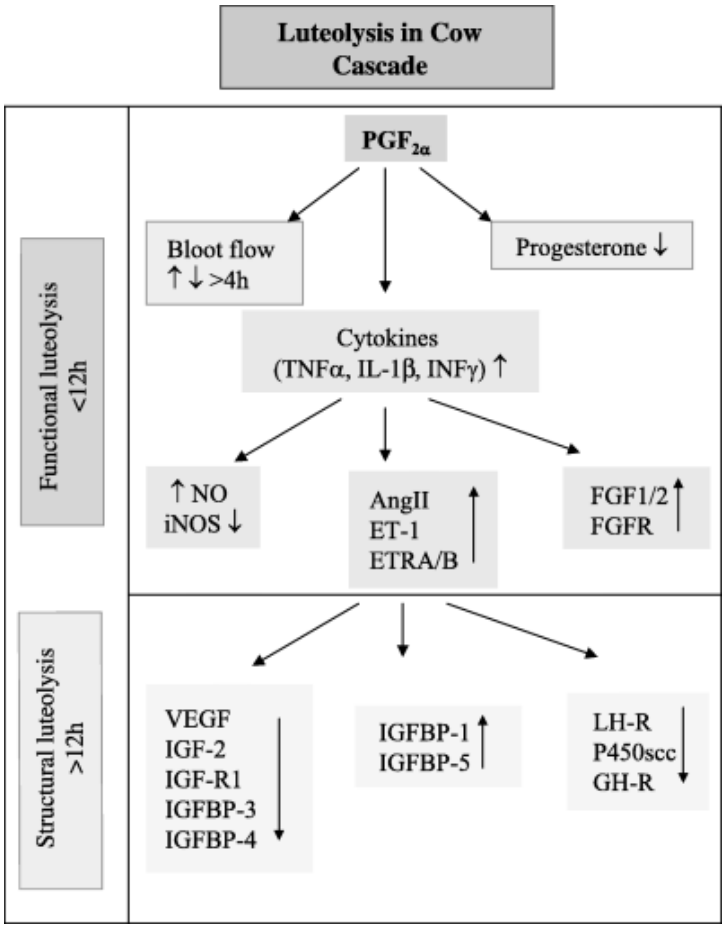


Figure 1.2: Events leading to and causing luteolysis of the corpus luteum. (Adapted from Schams et al., 2004)

With luteolysis eliminating the CL as the dominant structure, the antagonistic actions of progesterone including inhibiting uterine motility, estrus, and ovulation are subsequently removed (Swenson, 1970). At this time, the frequency of GnRH and amplitude of pulses begin to markedly increase. The increase in GnRH simultaneously increases FSH and LH production,

which stimulates growth of the preovulatory follicle formed just prior to the cessation of the luteal phase of the cycle, at which time the follicular phase has begun and will last for 3 to 4 d.

The theca interna cells surrounding the preovulatory follicle secrete estrogen in amounts directly related to the size of the follicle. Subsequently, estradiol plays the dominating hormonal role by positively signaling the hypothalamus and pituitary to secrete low amplitude, high frequency pulses of luteinizing hormone (LH), which increases the concentration of circulating LH driving follicular growth and development until subsequent ovulation (Niswender, 2000). This increase in circulating estrogen secreted by the developing follicle is the stimulant that brings about estrus or heat in the bovine. This is the period of time in the cycle that lasts, on average, from 16 to 18 h during which the bovine is sexually receptive (Swenson, 1970). Estrus is the result of estrogen affecting the nervous system, while the subsequent ovulation is the result of estrogen affecting pituitary gonadotropins (Turner, 1966). Therefore, as the follicle grows larger, estrogen concentrations increase until reaching a threshold level at the hypothalamus. This final growth period of the follicle only lasts from 2 to 3 d with the threshold level being met some 12 to 16 h following the end of estrus. When the threshold level is achieved, a large quantity of GnRH is released, stimulating the consequent release of a large pulse of LH. This preovulatory surge of LH causes ovulation of the growing, dominant follicle. Thus, the cycle begins again with the luteal phase and progesterone as the dominating hormone until regression of the CL or maintenance of the CL with pregnancy.

Hypothalamic-Pituitary-Gonadal Axis

The hypothalamus is the autonomic nervous system hub for the entire network of hormones in the hypothalamic – pituitary – gonadal (HPG) axis that constitutes the network that controls reproduction (See Figure 1.3). This network is the relatively uniform across species and

sexes. The hypothalamus is hub of this network, releasing gonadotropin releasing hormone (GnRH) to the anterior pituitary which in turn controls the secretion of LH and FSH. These 2 hormones in rotation or in combination affect the gonads and signal back to the hypothalamus, regulating ensuing secretions.

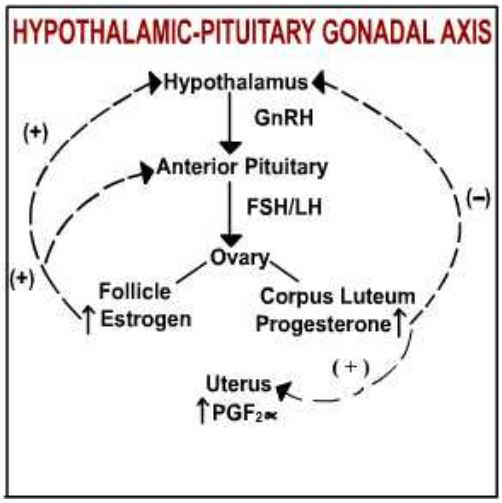


Figure 1.3: Illustration of the network formed from the Hypothalamic-Pituitary-Gonadal axis. Also represented are the main hormonal secretions from each section of the network.

The hypothalamus is composed of connective tissue, nonmedullated axons, and neuroganglia, with the majority of all axons running through the neural stalk to end in the neurohypophysis and with most given rise by 2 pairs of nuclei in the hypothalamus: the supraoptic and the paraventricular (Swenson, 1970). The importance of the portal system is to allow minute quantities of GnRH to act on the pituitary before it is diluted by the systemic blood circulation (Senger, 1997). Once at the pituitary, minute quantities of GnRH are transferred to a second capillary network, where they stimulate other pituitary cells to release their hormones. The pituitary is alone responsible for the secretion of at least 9 hormones, which are protein or

peptide in nature (Turner, 1966). The hormones influencing reproduction are transferred via a capillary network to the gonads. Their influence on the gonads, as well as the release of hormones from the hypothalamus and pituitary, is regulated by negative and positive feedback. The majority of this positive and negative feedback maintains hormonal balance and signals back to the hypothalamus to aid its regulation and production of GnRH. The tonic center within the hypothalamus is most sensitive to negative feedback. In contrast, the surge center, within the hypothalamus, is mainly responsive to positive feedback of estradiol (Senger, 1997). Reasoning stands that without this sensitivity in the surge center, the threshold level of estrogen required for the LH surge and ovulation would increase. It is important to realize that within this system of hormonal control comprised by the HPG axis, no single hormone acts in isolation. Therefore, the axis is the key to hormonal balance, interaction, and cyclicity within the female reproductive system.

The Main Hormonal Regulator

Throughout the luteal and follicular phases of the estrous cycle, GnRH, a decapeptide, plays a constant and active role in cycle regulation. The GnRH neurons represent the final link in the intricate neural network that regulates the secretion and release of LH and FSH through internal and environmental cues (Smith, 2001). The release of GnRH from the hypothalamus is carried through the median eminence to the anterior pituitary via portal vessels. This release is classified into 2 centers within the hypothalamus: the tonic and the surge centers. The tonic secretion of GnRH is a basal secretion that continues throughout the reproductive life of bovines. In addition, the tonic center is responsible for the episodic pulses of GnRH that vary in frequency and amplitude depending on neural activity (Senger, 2003). The second center, the surge center, is also referred to as the “preovulatory” center, in direct correlation with its role in providing the

preovulatory surge of GnRH that stimulates the surge of LH resulting in ovulation. The receptor mRNA is available for LH when the follicle reaches 9 mm in diameter (Martinez, 2003).

Furthermore, GnRH and gonadotroph secretion is highly regulated by estradiol and progesterone feed back to the central nervous system and anterior pituitary to regulate the synthesis and the pattern by which GnRH is released. In 2 separate studies (Beck, 1977 and Kesner, 1981), implantation of high concentrations of estradiol (E2) induced a preovulatory LH surge in ovariectomized cows. The rise in E2 acts on both the hypothalamus and the anterior pituitary and may illicit a response while acting on one center or both (Stumpf, 1991). Estradiol directly influences neurotransmitters in the brain that control pulsatile secretion of GnRH (Smith, 2001). The increase in E2 also increases the number of GnRH receptors on the pituitary. Therefore, it was concluded by Stumpf et al. (1991) that higher E2 stimulated the synthesis and release of LH by increasing the release of GnRH from the hypothalamus in conjunction with increasing the number of GnRH membrane receptors in the pituitary gonadotrophs.

The ability to manipulate production of hormones and alter their follicular and luteal dynamics has been the backbone of designing various estrus synchronization protocols. The first of these studies focused directly on GnRH and its control of LH and FSH. The commercially available analogues of GnRH that are most commonly used for their agonistic properties include Cystorelin (Merial, Athens, GA), Buserelin (Hoechst-Roussel Agri-Vet Company, Somerville, NJ), and Factrel (Fort Dodge Animal Health, Fort Dodge, IA). Analogues of GnRH generally maintain the linear decapeptide structure of GnRH with substitutions of amino acids at position 6 for agonists and positions 2 or 3 for antagonists. The modifications to the chemical structure of natural GnRH for clinical purposes help maintain its stability as well as its capacity to bind to plasma proteins and GnRH receptors (Martinez, 2003). Researchers used GnRH analogues in an

attempt to luteinize the developing antral follicle, induce ovulation, and stimulate formation of a subsequent CL by producing a wave of LH upon GnRH administration (Macmillan, 1991). Research has expanded, utilizing progesterone- and estrogen-like compounds to influence the synthesis and pattern of GnRH release from the hypothalamus.

**Formation of the Corpus Luteum and Progestins**

Corpora (bodies) lutea (yellow) were first accurately described by Regnier de Graaf (1641-1673) who noted their presence before and after parturition in rabbits (Niswender, 2000). Not until 1898 was the function of these yellow bodies fully known. Prenant (1898) described their function as "there can be no doubt ... it (the corpus luteum) acts as a gland, and as a gland of internal secretion" after examining the histology of the CL. Numerous experiments followed with extractions of the biologically active substance produced by the CL. The substance, later deemed a steroid, was named progesterone.

A CL is formed from the cells of the ovulating follicle, and is actually one of only a few adult organs to exhibit growth, development, and regression (Jablonka-Shariff, 1993). Additionally, it is known as one of the fastest growing tissues with progesterone synthesis directly proportional to its size. The CL produces progesterone; however, the expression of mRNA estrogen receptors in the granulosal cells of the CL is required to maintain its survivability (Bao, 2000). The gonadotropin surge that induces ovulation during the estrous cycle also plays a major role in the formation of the CL and maintenance of pregnancy. This surge occurring just prior to ovulation causes the differentiation of the follicular cells and formation of the CL by inducing functional changes in the structure of the thecal and granulosal cells of the ovulating follicle (Peters, 1994). With the LH surge causing luteinization, the thecal and granulosal cells of the follicle begin to differentiate and change functionally until formation

of the CL. The thecal cells have the capability to produce androgens from cholesterol, while the granulosal cells have the ability to produce progesterone and estradiol (Bao, 1998). Functions of the 2 cell types are interdependent for the maintenance of the CL and pregnancy. The thecal cells are responsible for moving cholesterol into the cell and converting it to pregnenolone and/or androgens, while granulosal cells absorb pregnenolone to produce progesterone or the androgens that are subsequently aromatized to estradiol.

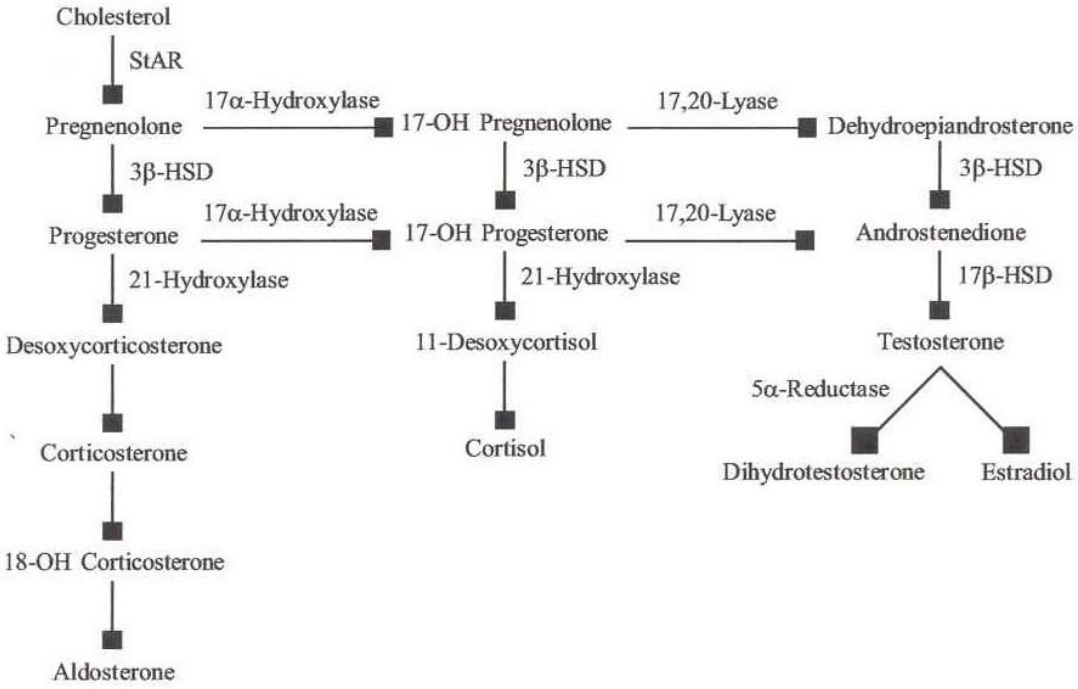


Figure 1.4: Pathways and enzymes needed for the various hormonal productions of the thecal and granulosal cells. Adapted from White and Speiser, 2000.

Prior to ovulation, the thecal and granulosal cells synthesize estradiol in support of granulosal cell proliferation and follicular recruitment (Richards, 1987). Additionally, increased estradiol production forms a positive local loop stimulating increased androgen production by the

thecal cells. Estrogen and its mRNA receptors in the granulosal cells are necessary to maintain follicular growth and maturation (Bao, 2000). However, differentiation during the preovulatory LH surge programs these cells to produce progesterone necessary for pregnancy maintenance. This is accomplished by increased expression of the enzymes needed to convert cholesterol to progesterone including P450 side chain cleavage and 3ß-hydroxysteroid dehydrogenase, in conjunction with decreased expression of those that convert cholesterol to estradiol such as 17α-hydroxylase and aromatase (Bao, 1998). This pathway is described in Figure 1.4.

The luteal cells that form the CL are derived from the granuolosal and thecal cells of the developing follicle and are distinctly different both morphologically and physiologically. The granulosa-derived cells are now called large luteal cells (LLC) while the theca-derived cells are now called small luteal cells (SLC) (Niswender, 2000). The LLC are believed to contain not only steroidogenic abilities but also secretory granules with oxytocin and possibly relaxin, while the SLC are primarily steroidogenic with no secretory granules (Senger, 2003). The LLC and SLC that make up the CL not only have cells capable of producing steroids, but also contain endothelial cells, fibroblasts, pericytes, and other cells from the bloodstream. During the early stages of CL development until the mid-luteal stage, oxytocin, progesterone, and even prostaglandins stimulate luteal cell proliferation and function that is supported by a number of growth factors (Schams, 2004). At this time, the LLC are increasing CL size through hypertrophy, while the SLC are increasing in number by nearly 5-fold, as are the nonsteroidogenic fibroblastic and endothelial cells (Niswender, 2000). The majority of this cellular proliferation comes on or before d 4 (mid-luteal), and hypertrophy continues through d 12 of the estrous cycle (Jablonka-Shariff, 1993). This tremendous and fast growth is similar to that seen in a tumor. Moreover, LH plays a major role in this development by stimulating the

proliferation of LLC while decreasing the ratio of small to large luteal cells (Jablonka-Shariff, 1993). Niswender et al. (1986) reported that in those animals with hypothalamic-pituitary stalk disconnection, progesterone concentrations were similar to control (nondisconnected) animals on d 5, but had decreased concentrations on d 12. When given LH therapy, the stalk disconnected animals had similar progesterone concentrations on d 12. An over abundant amount of LH was given in this particular study, resulting in an increase in LLC number and SLC diameter. The positive effects of LH on the development of the CL is surprising given that one of the main functions of CL progesterone production is to suppress GnRH, LH, and FSH through negative feedback. This intrinsically designed network holds many roles in the fertility of cattle through estrous cycle regulation and maintenance of pregnancy.

**Progesterone's Function in the Estrous Cycle and Pregnancy Maintenance**

Progesterone, produced by the CL, is essential for the secretion and regulation of gonadotropins, uterine preparation for implantation, and maintenance of pregnancy. Within the hypothalamus and pituitary, progesterone secretion during the luteal phase serves a number of functions. First, increasing circulating concentrations of progesterone restrict the frequency of LH pulses and decrease circulating LH concentrations. Progesterone decreases LH secretion by blocking GnRH from the hypothalamus and by reducing the number of receptors for GnRH in the pituitary (Niswender, 2000). In addition to the down regulation of GnRH secretion and reduced GnRH receptors, gene expression is decreased in the ß-subunits that comprise LH and FSH. Also, there is the additional decreased expression of the α-subunit that is a part of all gonadotropins (Brann, 1993). Progesterone also has a direct effect on the concentration of estradiol secretion. Deficiency of progesterone during the luteal phase stimulates increased estradiol concentrations that cause inadequate development and maturation of follicles, impaired

gamete transfer, and inadequate preparation of the uterus for implantation (Wehrman, 1993). As a result, inadequate progesterone prior to AI results in lowered conception rates due to prolonged exposure to estradiol.

The action of progesterone on reproduction is the initiation and maintenance of pregnancy. Deficient concentrations of progesterone in the bovine reproductive tract would hinder functional estrous cycles rendering animals incapable of producing and ovulating a viable follicle in addition to making it difficult to maintain a pregnancy. Furthermore, progesterone establishes estrous cycle length by maintaining CL formation in support of pregnancy or allowing the release of prostaglandin from the endometrium of the uterus when concentrations decrease (Schams, 2004). Elevated concentrations of progesterone during the luteal phase inhibit mitosis from occurring in the endometrium of the uterus while altering the pattern of proteins secreted by the endometrial cells (Niswender, 2000). Additionally, maintenance of normal early embryonic development in mammals requires the secretion of lipid droplets from the endometrium of the uterus, which is increased by progesterone (Wehrman, 1993). Furthermore, progesterone stimulates the maximal secretion of these lipid droplets from the endometrial glands of the uterus while simultaneously decreasing myometrial activity or uterine contractions creating a "quieting" effect of the uterus while the potential conceptus is "free-floating" (Senger, 2003). The shift in protein production and uterus calming creates an environment more conducive to early embryonic development.

After implantation, for pregnancy to be maintained, there must be the appropriate dialogue between the conceptus and the endometrium for maintenance of the CL and pregnancy. The ability of the conceptus to inhibit the production of $PGF_{2\alpha}$ is largely dependent on its production of interferon-tau (IFN-$\tau$). It is estimated that 40% of early embryonic losses occur

from d 8 to 17, when the conceptus begins playing a large role in the inhibition of $PGF_{2\alpha}$ (Thatcher, 1991). The inability of a conceptus to produce sufficient quantities of IFN-τ beginning at the time of embryo elongation around d 14, could largely contribute to this embryonic loss. In most cases, insufficient IFN-τ is a direct result of abnormal development of the embryo or retarded growth. Kerbler et al. (1997) discovered that maternal circulating plasma progesterone concentrations are positively correlated with the IFN-τ synthesis by the developing embryo. This implicates that embryonic death may be minimized with the administration of exogenous progesterone to enhance embryonic development and subsequent IFN-τ secretion (Sumners, 2004).

Species such as the mare and the bitch are fortunate in that the removal of the CL during pregnancy does not result in abortion. In these species, the placenta produces adequate progesterone to sustain pregnancy (Swenson, 1970). Progesterone synthesis by the CL is necessary for maintenance of a pregnancy in the bovine as little or no progesterone is secreted by the placenta. The progesterone production by the CL is capable of blocking the episodic pulses of prostaglandin that would result in lysing of the CL and abortion, while allowing a basal concentration to remain. This leads to the certainty that with premature reduction of the CL, there will be a reduction in progesterone, resulting in the loss of the pregnancy. The final role of progesterone, at the cessation of gestation, is to facilitate mammary alveolar development, thereby allowing the initiation of lactation prior to or at parturition (Senger, 2003). After parturition, the role of progesterone minimizes until cyclicity resumes.

The estrous cycle, reproductive organs, and their respective secretions are a variable network by their own distinction. In addition to the endogenous cyclicity, this process is affected by many exogenous sources. The exogenous sources are capable of manipulating the normal

functioning reproductive process of the female animal. These manipulations have positive or negative influences on the overall fertile productivity of a given animal. The negative impacts create an inconsistent environment within the reproductive system and hinder artificial insemination as well as embryonic development. In contradiction to this, the positive manipulations create a system more conducive to AI and embryonic development.

**The Effects of Heat Stress on Reproduction**

Heat stress has numerous deleterious effects on the dairy industry, though the most profound impact is seen in the area of reproduction. The exposure of dairy cattle to elevated ambient temperatures results in periods of transient infertility that have large economic impacts, which occur prior to, during, and after conception (Howell, 1994). Temperature and humidity are the 2 factors affecting ambient temperatures and are recorded as a temperature-humidity index (THI). Infertility is a significant direct effect of heat stress. However, exposure to increased body temperature, will also illicit indirect responses that will affect reproduction on a less significant level. These responses include but are not limited to a reduction in food intake, respiratory alkalosis, and the redistribution of blood flow throughout the body (Wolfenson, 2000). However, overall conception rates are reduced primarily due to decreased circulating estradiol, compromised follicular growth, and decreased animals serviced to AI.

Circulating hormone concentrations and compromised follicular growth are the direct result of the hyperthermia of organs and tissues, which result in altered functionality and impairments, with these consequences being positively correlated with temperature (Wolfenson, 1995). Dominant follicles in heat stressed cattle are generally smaller and contain less fluid compared with control animals under no heat stress. This decrease in follicular fluid was evident in a study by Wilson et al. (1998) in which there was a significant ($P < 0.01$) decline in growth of

the second wave follicle at d 15 of the estrous cycle. The diameter of this follicle maximized at 15.4 $\pm$ 0.7 mm for thermoneutral heifers compared with 12.4 $\pm$ 0.7 mm for heat stressed heifers. The decrease in follicular development is directly correlated with reduced granulosal cell viability, aromatase activity, and androstenedione production by the theca cells of the dominant follicle (Badinga, 1993). Wolfenson et al. (1995) reported that the dominant follicle in the first wave is greatly reduced in size; however, the pool of large follicles increases. The increase in follicular size, among subordinate follicles, is a direct result of the decreased follicular size of the dominant follicle, which under normal circumstances would impede the growth of smaller follicles. This phenomenon is observed in both heifers and lactating cows (Wilson, 1998). This same pattern occurs with the wave creating the preovulatory follicle, whether it is a second or third wave preovulatory follicle, with both cycles suffering alterations in the dynamics of follicular development. The result is the culmination of many large follicles, which possibly impeded successful ovulation with reduced follicular dominance. Estradiol secretion by the dominant follicle in conjunction with inhibin is responsible for the inhibitory growth effect on subordinate follicles (Findlay, 1993). However, during heat stress, aromatase activity is reduced which consequently reduces estradiol synthesis. In combination with this, there is a reduction in 17α-Hydroxylase enzyme concentrations, which is rate limiting for the biosynthesis of androgens and is responsible for transformation of many substrates for estradiol synthesis by the granulosal cells (Wolfenson, 2000).

The reduction in estradiol not only impedes follicular maturation, but also decreases the signs of estrus, which lead to more missed fertile estrous cycles. Decreased estrous expression during heat stress affects both heifers and cows, and is the main contributor of decreased fertility in heifers, which do not suffer the intensity of hyperthermia and subsequent deleterious effects as

cows (Wilson, 1998). Timed AI (TAI) could plausibly alleviate the occurrences of missed estrous cycles and decrease d open by removing the need to detect estrus (Cartmill, 2001). In addition, this reduction in estradiol plays a critical role in the alteration of dominance in follicular waves of heat stressed animals. This results in altered follicular dynamics, with earlier emergence of the preovulatory follicle, and manifestation of the preovulatory follicle coming 2 to 4 d earlier than observed in normal in non-heat stressed animals (Wolfenson, 1995). This earlier emergence of preovulatory follicles results in the ovulation of older follicles. This incident will potentially reduce the fertility of heat stressed animals as the viability of bovine embryos is only a few d prior to the onset of atresia.

Older follicles are not only ovulated due to early emergence of preovulatory follicles, but also because of possible delayed luteolysis. As previously discussed, there are a series of endocrine events leading to luteolysis including increased concentrations of circulating estradiol, which up regulate oxytocin receptors in the endometrium that are responsible for prostaglandin synthesis. The delay in luteolysis is due to decreased concentrations of circulating estradiol that preclude the cascade of events leading to luteolysis, which is the direct result of damaged follicles incapable of producing estradiol at normal capacity (Wilson, 1998). The delay in the onset of luteolysis in heat stressed cattle increases the duration of the luteal phase, often resulting in follicular waves occurring while progesterone concentrations are high and a greater number of 3 versus 2 follicular waves per cycle in animals subjected to heat stress (Wilson, 1998).

The effects of heat stress on follicular dynamics are also observed into periods without heat stress. This is due primarily to the fact that approximately 40 d are required for bovine follicles to grow through the antral phase. Therefore, many of the negative impacts of heat stress on early follicular development are not seen until 1 to 2 mo later in late summer or early fall

(Thatcher, 1986). Furthermore, those follicles that are damaged by heat stress during the summer months may ovulate an infertile oocyte or develop an under functional CL (Wilson, 1998). All of the aforementioned effects of heat stress prior to and at the time of AI are seen at a much greater extent in lactating cows versus nulliparous heifers. Badinga et al. (1985) collected fertility data on heifers and cows subjected to heat stress from 1975 through 1997 in Monticello, FL. In this study, the decline in conception rates for cows began at 30° C maximum temperature the d after AI; however, the same decline was not seen in heifers until 35° C. Overall conception rates for heifers were 50% during summer months versus 34% for lactating cows. Additionally, a breed difference was detected with Jerseys having the highest conception rate at 45% versus 41% and 39% for Brown Swiss and Holsteins, respectively. Lactation stage was the main contributor to the severity of impact of heat stress on conception rates and embryonic survival.

Numerous studies have been conducted to determine the effects of heat stress on the CL. The main goal of these studies was to observe the effect heat stress has on progesterone synthesis by the CL. Results have varied widely, with researchers finding that progesterone concentrations decreased (Younas, 1993), held constant (Wise, 1988), or increased (Abilay, 1975) during heat stress. Howell et al. (1994) reported similar progesterone concentrations across seasons, with a slight decline in progesterone observed mainly between d 6 and 18 of the estrous cycle in animals during summer. During the past decade, it has become accepted that under conditions of chronic heat stress, circulating progesterone decreases; however, during periods of acute heat stress, progesterone increases (Howell, 1994). Studies conducted by Howell et al. (1994), Wolfenson et al. (2000), and Younas et al. (1993), do not contribute the decreased progesterone to decreased CL size or decreased luteal phase length, but rather to decreased secretory

capability of the CL during chronic heat stress. Wolfenson et al. (1993) initially studied this in vitro, finding that those cells incubated at temperatures 2° C higher than normal, secreted on average 30% less progesterone, with overall cell viability and initial production being lower in those cells collected in summer versus winter.

Sonego et al. (1995) closely studied the secretory patterns of the theca-derived and granulosa-derived luteal cells in vitro. Cells were collected at d 6 of the estrous cycle in summer and winter seasons. They were allowed to incubate for 9 d at 38° C. The large granulosal cells collected during the summer produced slightly lower progesterone concentrations than those collected during winter. However, the rate of increase in progesterone concentrations was reduced for those cells collected during the summer. This was due in majority to the large impact summer heat stress had on the small theca cells with only one fifth of the progesterone secretion compared with those cells collected during the winter.

The final major effect of heat stress lies within the uterine environment and the ability to maintain pregnancy. Abnormal follicular dynamics, impaired CL function, and corresponding progesterone secretion result in alterations of the oviduct and uterine environment that could alter the successful development of an embryo (Wolfenson, 1993). The major effects on the uterine environment contributing to heat stress include production of heat shock proteins by the endometrium, reduced production of interferon-γ by the conceptus, and increased production and release of $PGF_{2\alpha}$ by the endometrium (Wolfenson, 2000). Additionally, all of the uterine modifications previously described for progesterone are compromised during heat stress when concentrations of progesterone are not optimum. A culmination of the subsequent events on reproduction after a prolonged period of heat stress is represented in Figure 1.5.

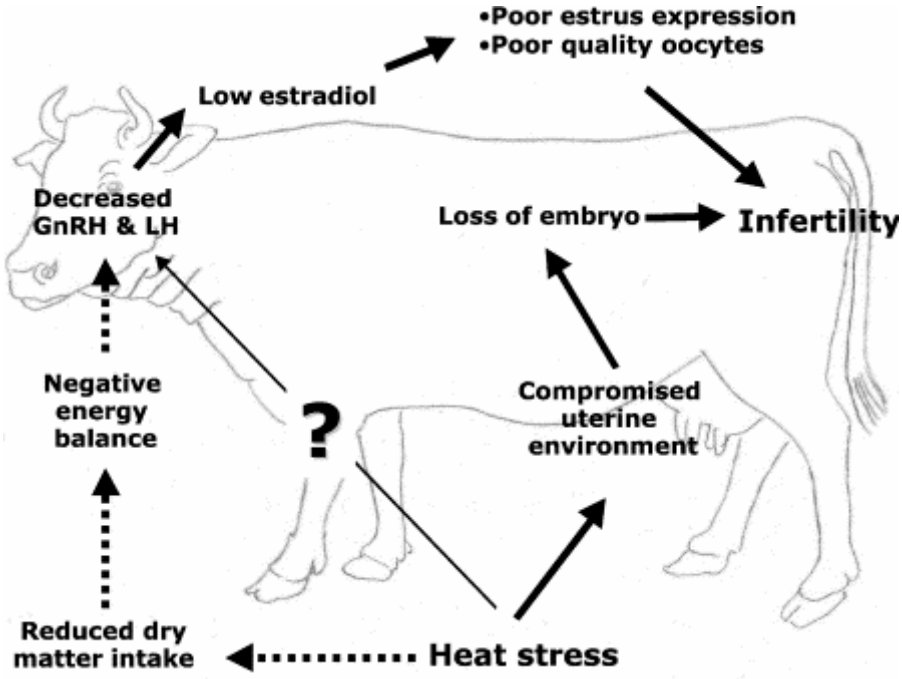


Figure 1.5: Physiological effects of heat stress on cattle. Heat stress can act in a multitude of ways to alter fertility and reproduction in the lactating dairy cow or nonlactating dairy heifer. Reduced dry matter intake can directly cause the inhibition in production of GnRH and LH (indicated by the dashed lines). Associated with the compromised uterine environment is the diminished production of progesterone. Source: Rensis and Scaramuzzi, 2003.

The effects of heat stress are apparent in this simplistic model. However, this model could be improved appreciably with the inclusion of the direct impact heat stress has on ovarian function. As previously described, there are two main structures on the ovary, the follicle and the CL. Heat stress directly impacts the follicle and its ability to produce estradiol and thereby regulate GnRH secretion and estrus. Additionally, heat stress impacts the function of the CL through decreased progesterone needed for normal cyclicty, uterine preparation for implantation, pregnancy maintenance and GnRH release. These must be included to understand the full ramifications of heat stress on reproduction.

**Synchronization of the Estrous Cycle with Progesterone**

Current synchronization protocols for heifers and cows use a variety of methods to effectively synchronize estrus and maximize usage of AI to accelerate genetic progress. Overcoming the variables within the estrous cycle and exogenous sources that are damaging to this cycle make formulating an effective synchronization protocols and implementing it difficult. Unfortunately, with this, the trend in the industry as of 1997 was moving in the direction of increasing mating by natural service rather than by AI in both dairy cows and heifers. This trend is shown in Table 1.1.

Table 1.1: Hoard's Dairyman 1997 census on the percentage of dairy farmers reporting use of a bull for breeding dairy animals.

| | 1990 | 1992 | 1994 | 1996 |
|---|---|---|---|---|
| **Use of Bull for Breeding Dairy Cows** | 34.9 | 36.2 | 38.8 | 41.7 |
| **Use of Bull for Breeding Dairy Heifers** | 44.0 | 46.9 | 47.0 | 49.9 |

Ideally, there needs to be implementation of management processes that will allow for maximal use of AI to see economic and genetic benefit of increased production. An effective management tool to increase AI use would be the implementation of a practical synchronization protocol. Many protocols do not incorporate progesterone into the synchronization method. However, recent research indicates numerous potential benefits for incorporating progesterone in synchronization protocols.

Exogenous Progesterone and Estrogen

Initial use of Estradiol in estrus synchronization was to induce luteolysis; however, in the mid 70's it was concluded that natural prostaglandins were more effective and consistent

luteolytic agents (Burke et al., 1999). Instead, estradiol is most effective in estrous cycle manipulation and synchronization for inducing follicular atresia (Bo et al., 1994). This phenomena, once identified, was thought to possibly overcome the infertility issues associated with estrus synchronization utilizing progesterone. A study to define the effects of progesterone and estrogen together on follicular wave dynamics was conducted by Bo et al. (1991). These researchers used estradiol valerate (EV) and a 6 mg norgestomet progestagen ear implant (Syncro- Mate-B, Sanofi Inc., Overland Park, KS) in conjunction with an intramuscular injection of 3 mg norgestomet. In this particular experiment, they implanted the progestagen norgestomet, injected 3 mg norgestomet i.m. and injected 5 mg EV in tandem shortly after estrus, resulting in suppression of the dominant and next largest follicles for 5 d and then growth of follicles thought to be from the presence of a new follicular wave. Estradiol valerate proved to be a poor form of estrogen for manipulating the estrous cycle due to its long acting effects in cattle.

Following the discontinued used of EV, researchers tried a shorter acting form of estrogen, estradiol 17-ß (E-17ß). Further studies were then conducted by Bo et al. (1994) to test the efficacy of using 5 mg E-17ß and 6 mg Syncro-Mate-B progestogen ear implants. In all heifers with a functional CL, a 500 µg of cloprostenol was administered to lyse the CL and induce estrus prior to beginning the study. When used together, E-17ß and progesterone were much more efficient in suppressing follicular growth and inducing the earlier emergence of a new follicular wave. When used alone, E-17ß induces a surge of LH where FSH drops off quickly and then rapidly increases 12 h later, whereas the combination of E-17ß and progesterone increased FSH gradually over a 24- to 42-h period. The researchers concluded that the suppression of follicular growth in the E-17ß and progesterone treated group was due to the

complete suppression of both LH and FSH secretion, and that this suppression must last for at least 24 h for complete follicular regression.

These 2 studies utilized progestogen implants and various forms of estrogen; however, more recent research utilized estradiol cypionate (ECP), an ester of estradiol, as the exogenous form of estrogen. Estradiol cypionate is a shorter acting form of estrogen and when administered in small doses (0.5mg to 1 mg) is more effective in synchronizing development of a new follicular wave than longer acting forms of estrogen or higher doses (Bo, 1994). Estradiol cypionate doses as high as 2 mg do not increase the duration of estrus in comparison to those animals with spontaneous estrus (Colazo, 2003). Using ECP in small quantities is preferred to other forms of exogenous estrogen as it does not induce excessive estrus activity and its low water solubility that delays its release from the site of injection (Colazo, 2003). However, ECP was removed from the market in the United States in the spring of 2004 by the Food and Drug Administration (FDA).

Estrogen, when combined with progesterone, overcomes the decreased fertility often associated with progesterone therapy. More specifically, estrogen reduces the incidence of a persistent follicle by inducing atresia of the current follicle and allowing the emergence of a new group of follicles (Burke, 1999). This process is also useful in the avoidance of cystic ovaries. The optimum estrogen concentration mimics the naturally occurring threshold level needed to signal the GnRH surge center without excessive estrus or prolonged estrus duration. This also explains the relatively larger proportion of animals observed in estrus (87 vs. 54%) when administered ECP compared with GnRH in a protocol utilizing a CIDR insert (Stevenson, 2004). The reports of these studies suggest that estradiol treatment in conjunction with progesterone

results in the synchronous development of the ovulatory follicle, thereby being more effective for TAI than some more traditional synchronization program approaches.

Exogenous Progesterone and GnRH

Progesterone and GnRH are generally used in conjunction with $PGF_{2\alpha}$. Though this is an expensive method to synchronize estrus, it was believed that the induction of GnRH into the progesterone/ $PGF_{2\alpha}$ protocol would reduce the incidences of persistent follicles with GnRH aiding in follicular turnover. Stevenson et al. (2000) utilized the norgestomet form of progesterone in conjunction with a $PGF_{2\alpha}$ injection 1 d before implant removal and GnRH on the d of AI to test their combined outcome on reproductive performance in heifers. They concluded that although this combination was helpful in inducing some prepubertal heifers into estrus, there was no gain in overall reproductive performance. Conception rates were similar between treatment and a 2x $PGF_{2\alpha}$ control. These researchers concluded that GnRH was incapable of removing the persistent follicle in conjunction with 24-h exposure to progesterone after induction of the luteolytic agent not being able to suppress LH pulse frequency. Another study conducted by Mee et al. (1991) utilized a progestogen-impregnated (6 mg Norgestomet) implant (SANOFI Animal Health, Overland Park, KS) for 6 d with a 50 μg injection of GnRH (SANOFI Animal Health) 72 h after implant removal to test fertility traits on fresh, lactating, Holstein cows. Mee (1991) concluded that this combination of progestogen and GnRH reduced the occurrence of short luteal phases. In addition, the pretreatment of animals with progestogen increased the magnitude of LH release following the GnRH injection. In addition, these animals had increased receptors for LH in both the thecal and granulosal cells. Finally, the progesterone altered the maturation of the largest follicle, leading to increased estradiol production and the isolation of a single, dominant follicle. Therefore, the combination of progesterone and GnRH provides a

more viable dominant follicle via progesterone treatment, and a more substantial LH surge upon GnRH administration. In addition, the preinsemination treatment with progesterone and GnRH not only synchronizes animals to facilitate AI, but also increases the lifespan of the subsequent CL, therefore increasing the probability of conception to parturition success.

Exogenous Progesterone via the CIDR and its Effect on Reproduction

The CIDR was initially manufactured and made available by InterAg in Hamilton, New Zealand. The CIDR approved for use in New Zealand and Canada contains 1.9 g progesterone. However, this CIDR was reengineered for use in the United States to reduce the progesterone load and to reduce the amount of residual progesterone left in the CIDR insert after removal, while still maintaining its biological performance. The CIDR approved for use in the United States is the EAZI-BREED CIDR (Pfizer Animal Health, Kalamazoo, MI) containing 1.38 g progesterone in silicone molded over a nylon spine and is a convenient form of administering P4. The CIDR, without applicator, takes the shape of the letter "T", with wings that are depressed when inserted intravaginally with the CIDR applicator. Expulsion of the CIDR from the applicator allows the wings to straighten, which aids retention by exerting pressure on the vaginal wall. The exposure of the vaginal wall to the wings of the insert allow for the quick transmission of progesterone. The progesterone release from the CIDR can be detected in the bloodstream approximately 12 min after insertion (Rathbone, 2002) and reaches 6 ng/ml within 6 h of insertion (Macmillan, 1993). A study by Rathbone et al. (2002) in ovariectomized cattle found that progesterone declines over the 7-d period of CIDR exposure, never falling below 2.5 ng/ml; however, upon CIDR removal, progesterone concentrations fall to 0.5 ng/ml within 1 h. The CIDR has the capability of initial estrus synchronization, resynchronization, and initiating cyclicity in anovulatory animals (Stewart, 2004).

Administration of exogenous progesterone often has an adverse effect on fertility in those animals that do not have a functional CL at the time of administration. Prolonged exposure to progesterone often results in persistent follicles and subsequently a larger ovulating follicle than normal that can negatively impact conception rates (Austin, 1999). The concentrations of exogenous progesterone administered directly effect suppression of follicular growth in a dose dependent manner (Bo, 1995). However, exogenous progesterone, via the CIDR with the low dose of 1.38 g exogenous progesterone, suppresses follicular growth but does not appear to have these adverse effects on fertility. Thus, the CIDR insert is a method to increase conception rates as well as maintain fertility (Rivera, 2005). Deficiency in luteal function and therefore progesterone production, either before or after insemination, is more often associated with decreased conception rates (El-Zarkouny, 2004). Blood concentrations of progesterone prior to insemination are positively correlated with conception rates (Erb, 1976). Thus, the development of the CIDR is a benefit to an industry striving towards effective synchronization programs that are not detrimental to fertility. The approval of the CIDR insert for synchronization of estrus in beef cows, beef heifers, and dairy heifers occurred in June of 2002. The approval in lactating dairy cattle came over 1 yr later in July of 2003 for use in synchronization of return to estrus. Therefore, the largest proportion of research involving estrus synchronization with the CIDR in the dairy industry has involved its use in heifers.

The current protocol suggested by Pfizer Animal Health, Kalamazoo, MI for use of CIDR inserts in dairy heifers is to insert the CIDR on d 0 for 7 d, inject 5 mL of $PGF_{2\alpha}$ on d 6, and CIDR removal on d 7. Optimal timing of the implementation of a synchronization protocol can differ between animals that vary in stages of the estrous cycle. A single injection of $PGF_{2\alpha}$ does not affect those animals between d 1 and 5 of the estrous cycle, because there is no CL to

regress. The $PGF_{2\alpha}$ in this case has no CL to lyse and therefore has no effect on the estrous cycle. However, when used during the luteal phase of the cycle with a functional CL, $PGF_{2\alpha}$ is an extremely effective synchronization tool. To combat the hindrance that estrous stage has on synchronization protocols, the CIDR may be used to substantiate the growth of the CL for 7 d before lysing by $PGF_{2\alpha}$ on the d of CIDR removal. The increase in efficiency is evident in pregnancy rates where $PGF_{2\alpha}$ alone yields 37% pregnant compared with 45% recorded for CIDR and $PGF_{2\alpha}$ (Lucy, 2001)

The primary problem with use of the CIDR in dairy heifers is the need to heat detect and AI within 4 d following CIDR removal. Unfortunately, many producers choose not to utilize the full advantages of artificially inseminating their heifers due to the lack of labor to adequately heat detect for AI in heifers and the lack of time to supervise AI in heifers (Erven, 1987). Efforts currently are moving towards designing heifer breeding protocols that allow for TAI.

Heifers have not responded well to TAI protocols, as they often do not have suppression of a premature estrus with a basic GnRH or $PGF_{2\alpha}$ based protocol. Ovsynch, a GnRH and $PGF_{2\alpha}$ protocol developed by Pursley et al. (1995), has been proven as an efficient TAI protocol for cows. Ovsynch consists of 100 μg of GnRH, followed 7 d later by 25 mg $PGF_{2\alpha}$, then a second injection of 100 μg GnRH 48 h later with TAI 12 to 16 h after the second GnRH injection. This protocol was deemed extremely effective for TAI in cows. However, it was less effective in heifers compared with breeding based on detected estrus in the study conducted by Pursley et al (1997) as outlined in Table 1.2. In this study, the pregnancy rate of cows was similar for both treatments ($P > 0.10$); however, pregnancy rate was 47.2% lower for heifers in the Ovsynch protocol compared with those bred after detected estrus ($P < 0.01$).

Table 1.2: Pregnancy rates of dairy heifers and cows bred at detected estrus or by TAI using the Ovsynch protocol. Adapted from Pursley et al. (1997).

| | Pregnancy Rate | | |
|---|---|---|---|
| | Control | Ovsynch | *P* |
| | (%) | | |
| Cows | 38.9 (n=154) | 37.8 (n=156) | >0.10 |
| Heifers | 74.4 (n=78) | 35.1 (n=77) | <0.01 |
| *P* | <0.01 | >0.10 | |

With the Ovsynch protocol, the first GnRH injection does not synchronize luteal development in heifers. Follicular growth is much more rapid in heifers, as evident with a 3 wave estrous cycle, and thus it is quite possible that with the Ovsynch protocol, the heifer does not have a dominant follicle to ovulate in response to the second injection of GnRH (Pursley, 1997). It is additionally ineffective due to its inability to suppress premature estrus in at least 18% of treated heifers (Rivera, 2004).

Inclusion of a CIDR insert in ovulation synchronization protocols such as Ovsynch could suppress such variation in estrus. Thereby, the CIDR allows 100% submission rate to TAI while not adversely affecting fertility. However, the CIDR alone with a single injection of $PGF_{2\alpha}$ does not synchronize the estrous cycle tight enough to facilitate TAI (Mapletoft, 2003). Therefore, the CIDR used concurrently with other sources of exogenous hormones, such as GnRH or estradiol, are a way to develop an efficient TAI protocol for heifers.

A study conducted later by Stevenson et al. (2004) included this principle, directly implementing the CIDR insert into the Ovsynch protocol and the Ovsynch protocol with ECP replacing the second GnRH injection in lactating cows. This study found that estrogen, when used in conjunction with a CIDR, induced fewer LH surges and ovulations than the GnRH plus

CIDR protocol. When tested against identical protocols with CIDR vs. no CIDR becoming the only variable, lactating cows synchronized with Ovsynch plus a CIDR had higher d 29 pregnancy rates compared with cows synchronized by Ovsynch alone (El-Zarkouny, 2004). The authors concluded that the difference in pregnancy rates was because the CIDR induced cyclicity in anestrous animals, a function that the Ovsynch protocol less efficiently performs. Rivera et al. (2005), conducted a similar trial with 189 Holstein heifers. The heifers received 100 µg of GnRH on d 0; 25 mg of $PGF_{2\alpha}$ on d 6; and 100 µg of GnRH + TAI on d 8, with or without the inclusion of a CIDR insert. They observed that 24% of heifers without a CIDR insert expressed estrus prior to TAI, compared with no heifers expressing premature estrus when a CIDR insert was used ($P < 0.01$). However, pregnancy rates were comparable ($P > 0.05$) between the treatments when animals were bred on detected estrus prior to TAI.

Garcia et al. (2003) conducted a trial to determine if GnRH or ECP was more successful when used in conjunction with a CIDR insert. Gonadotropin releasing hormone is the direct releasing factor for LH from the pituitary, and is therefore much more efficient at inducing an LH surge than estrogen, which must transverse additional pathways. In this study, 30 Holstein and Jersey heifers received a new CIDR insert (1.38 g progesterone) and 1 mg ECP i.m. to start the protocol. All CIDR inserts were removed 7 d later and were given an injection of $PGF_{2\alpha}$ before being were randomly assigned to receive either ECP 24 h later or GnRH 48 h later. All animals were timed AI 48 to 72 h after CIDR removal. There were no differences in ovulation rates, diameter of ovulatory follicles, and pregnancy rates, between heifers treated with ECP or GnRH.

Inclusion of the CIDR into a protocol utilizing GnRH increases pregnancy rates in cows especially in herds with a significant number of acyclic cows. In addition, CIDR and GnRH

when used in conjunction, have added accuracy in synchronizing estrus in heifers, creating a more applicable and efficient TAI protocol, especially in those herds looking to minimizing heat detection.

The CIDR for Resynchronization

Due to conception failure and embryonic loss, heifers often return to estrus in a much more scattered pattern than do cows (Van Cleeff, 1996). To prevent early return to estrus of previously synchronized, nonpregnant heifers and cows, many researchers have used a CIDR device. In addition, the use of the CIDR to resynchronize estrus in dairy heifers allows a tighter synchrony of estrus, making heat detection less intensive (Rivera, 2005). Fricke et al. (2005) experimented with 81 Holstein heifers to determine the validity of resynchronizing with the CIDR insert from d 14 to 20 post insemination versus breeding at next detected estrus. Inserts were only utilized for 6 d for resynchronization in an attempt to avoid a persistent follicle. These researchers reported that resynchronization with progesterone brought the majority of animals into estrus within a 72-h period following CIDR removal; a significantly higher synchrony rate than observed in heifers receiving no resynchronization ($P < 0.01$). Additionally, pregnancy rate per service was significantly higher in resynchronized heifers than control ($P < 0.05$), a direct result of having a greater proportion of control heifers having more than 2 services. However, Xu et al. (1999) reported that there was a reduced pregnancy rate per service in heifers resynchronized with the CIDR insert from d 16 to 21 after insemination. However, the reproductive performance of the resynchronized animals was higher due to a tighter synchrony of estrus and more opportunities for breeding within a fixed time. Van Cleef et al. (1996) administered a used CIDR insert from d 17 to 22 and reported a return to estrus rate during a 4 d time period of 75 versus 49.3% without resynchronization.

Chenault et al. (2003) attempted resynchronizing even earlier using a new CIDR beginning d 13 to 15 after previous AI and observed increased return rates of nonpregnant lactating cows. El-Zarkouny et al. (2004) conducted a similar study in lactating cows with previously used CIDR insert to cut down on the cost of synchronization and resynchronization. In this particular study, the progesterone treatment did not increase the overall return rates of nonpregnant cows to the first eligible estrus (30.8%) compared with control cows (27%) ($P > 0.05$). However, resynchronization with progesterone via the used CIDR had proportionally more cows diagnosed pregnant on d 29 and re-diagnosed pregnant on d 57 after TAI than controls ($P < 0.01$). These results suggest that resynchronization with a CIDR is multifunctional with it also increasing circulating progesterone concentrations as well as decreasing embryonic mortalities.

Robinson et al. (1989) provided the preliminary evidence needed to support the use of supplemental progesterone post insemination to enhance pregnancy recognition and/or early embryonic survival. These authors reported that supplemental progesterone maintained a short lived CL and increased progesterone production. El-Zarkouny et al. (2004) affirmed the benefits of utilizing progesterone post AI to increase conception rates.

With the multiple purposes of CIDR inserts and the cost related with using them, many have looked into reuse while resynchronizing or supplementing exogenous progesterone. Limited data has been published regarding the efficacy of reusing the CIDR and the amount of progesterone released from them. Beal et al. (personal communication), recently reported that 0.62 $\pm$ 0.02 g progesterone is released in the initial 7 d of use from the reengineered 1.38 g progesterone CIDR, leaving a 0.72$\pm$ 0.02 g of residual progesterone. To further validate how many 7 d periods a CIDR could be used, these researchers used 33 lactating cows and

administered prostaglandin at the time of insertion of a 2nd, 3rd, 4th, 5th, or 6th use CIDR to remove any possible CL. Though the amount of progesterone released from the CIDR diminishes with each reuse, the results of this trial suggest that the CIDR insert may be used up to the fourth time to suppress estrus. After the 4th reuse, the efficiency of suppression of estrus by the CIDR insert declines (Beal et al., personal communication).

The reuse of CIDR inserts is a direct result of their expense in relation to their increasing use in estrus synchronization programs and reproductive management. The popularity of the CIDR remains focused in synchronization; however, its use is branching into resynchronization, and as a source of supplemental progesterone during early embryogenesis.

**Objectives of Studies**

The objectives of the forthcoming studies were to validate multiple uses of the CIDR insert. The initial study utilizes the CIDR in an estrus synchronization protocol in an attempt to increase pregnancy rates of heifers in a TAI protocol. In order to increase the probability of increased pregnancy rates, the CIDR was used in conjunction with multiple other estrus and ovulation synchronization protocols to increase the efficiency of the CIDR for TAI. The second study was conducted in an effort to validate or dismiss the use of progesterone therapy post AI via the CIDR to decrease early embryonic loss. In coordination with this, a d 14 to 21 interval for CIDR administration is the time of resynchronization of estrus according to Pfizer Animal Health's fastback breeding program and was the basis for study 3. Within both of these studies, cows and heifers of beef and dairy breeds were utilized to find the differences that the CIDR plays on circulating blood progesterone concentrations of each animal. Therefore the objectives of study 2 include testing the validity of using the CIDR post AI for decreasing early embryonic loss, the efficiency of reusing a CIDR for resynchronization and the amount of progesterone

released from a used CIDR, as well as to test the actual effects of a CIDR on blood progesterone concentrations in dairy and beef animals of various ages and lactational stages.

# CHAPTER 2

## EFFECT OF COMBINING GnRH AND ECP WITH A CIDR-$PGF_{2\alpha}$ PROTOCOL ON PREGNANCY RATES IN HOLSTEIN HEIFERS SUBMITTED TO TIMED AI[1]

[1] Fain, J.L., W.M. Graves, J.M. Haslett, S.C. Nickerson, and J.K. Bernard. To be submitted to *American Registry of Professional Animal Scientists*..

## ABSTRACT

The objective of this trial was to determine if incorporation of gonadotropin releasing hormone (GnRH) and estradiol cypionate (ECP) into the controlled internal drug release (CIDR)-prostaglandin ($PGF_{2\alpha}$) protocol would increase pregnancy rates of dairy heifers using timed artificial insemination (TAI). This study was conducted over a 6-mo period at the University of Georgia Teaching Dairy in Athens. Forty Holstein heifers with an average age of 16 mo were randomly allocated to 1 of 2 treatment groups. In treatment 1, 20 heifers were synchronized by: 50 µg GnRH (-9 d), CIDR (1.38 g progesterone, -9 d), 25 mg $PGF_{2\alpha}$ (-3 d), 1 mg ECP (-2 d), CIDR removal (-2 d), 50 µg GnRH (d 0), and TAI (0 d), (OverSynch). A second group of 20 heifers (Control) were synchronized by: CIDR (1.38 g progesterone) (-9 d), 25 mg $PGF_{2\alpha}$ (-3 d), CIDR removal (-2 d), and TAI (0 d). Upon CIDR removal, retention rates and discharges were recorded. Estrus activity was monitored using Estru$ Alerts (Universal Cooperatives, Eagan, MN) applied at d -3. Timed AI occurred 48 h after CIDR removal. Pregnancy was determined by ultrasonography at 35 d post AI. For both treatments, CIDR retention rate was 100% and discharge was minimal with no significant effect on pregnancy rate ($P > 0.05$). Pregnancy rates of heifers synchronized by OverSynch (45 %; 9/20) were similar to those in the heifers synchronized with the control protocol (55 %; 11/20) ($P > 0.05$). In the OverSynch protocol, 16 of 20 (80%) heifers had Estru$ Alerts that were all or partially rubbed while only 11 of 20 (55%) were observed in the control group. Additionally, 55% (11/20) of the Estru$ Alerts on heifers in OverSynch were completely rubbed compared with 15% (3/20) in the control. Signs of estrus synchronization through visual appraisal of Estru$ Alerts was significantly higher in the OverSynch heifers ($P < 0.05$). Although the OverSynch protocol did significantly increase estrus activity, it did not increase pregnancy rates with a TAI.

## INTRODUCTION

The goal of developing synchronization protocols for the dairy industry is an effort to increase overall productivity of herds and increase utilization of artificial insemination (AI) for genetic benefits. Animals bred consistently and with accuracy lead to decreased costs per conception and maximization of overall production efficiency. However, many producers are not yet utilizing AI in heifers due to the lack of adequate heat detection and the lack of time to supervise AI in heifers (Erven and Arbaugh, 1987). For this reason, timed AI (TAI) is becoming increasingly popular because relying on estrus detection to identify animals for breeding is time consuming and labor intensive. Therefore, synchronization programs with a short window of required estrus detection or with TAI are greatly needed to increase the use of AI in heifers. Another combating factor is that in general, heifers most frequently exhibit 3 follicular waves that are more difficult to accurately synchronize for TAI than lactating cows that exhibit primarily 2 follicular waves (Sartori, 2000).

Currently, the Ovsynch protocol, developed by Pursley et al. (1995) is an extremely efficient way to utilize TAI techniques in cows. Many producers have used the alternative Heatsynch, which utilizes a more cost effective injection of ECP in place of the second GnRH injection. The Heatsynch protocol results in comparable pregnancy rates in cows to TAI (35.1 $\pm$ 5.0%) as Ovsynch (37.1 $\pm$ 5.8%), ($P > 0.05$) (Pancarci, 2002). However, these pregnancy rates are variable upon the number of anovular cows, because of the reduced ability of Heatsynch to induce ovulation in these animals (Fricke, 2001). However, pregnancy rates for heifers synchronized by Ovsynch of 35.1% are 47.2% less than the pregnancy rates of heifers inseminated after detected estrus (74.4%) (Pursley, 1997). Pregnancy rates in heifers

synchronized with ECP are comparable to those in heifers inseminated after detected estrus both averaging 39.2% (Lopes, 2000).

Until the removal of estrogen from the market, producers were relying on Heatsynch as a new method to synchronize heifers and cows effectively, while others were considering improving the Ovsynch protocol. El-Zarkouny et al. (2004) concluded that in lactating cows, inclusion of a CIDR in the Ovsynch protocol for 7 d between the first injection of GnRH until the only injection of $PGF_{2\alpha}$ has shown to increase d 29 pregnancy rates compared with the Ovsynch protocol alone. Ovsynch is efficient at synchronizing cows with or without a CIDR insert for TAI; however, the Ovsynch protocol in heifers is not feasible due to its inability to suppress premature estrus. Administering exogenous progesterone via the CIDR insert concurrent with the Ovsynch or GPG (GnRH, prostaglandin, GnRH) protocol in heifers results in 100% submission rates for TAI without affecting fertility due to its ability to suppress premature estrus (Rivera, 2005). Rivera et al. (2003) substantiated the use of the CIDR in a GPG system to facilitate AI, noting that though conception rates were similar compared with the basic GPG system, no heifers in the GPG + CIDR protocol were inseminated before TAI. The addition of the exogenous progesterone during synchronization decreases 17ß-estradiol (a factor of premature estrus) and increases conception rates (Wehrman, 1993). No study has examined combining the components of the Ovsynch protocol with the Heatsynch protocol and a CIDR insert into a single synchronization regimen.

The objective of this experiment was to utilize exogenous sources of GnRH, estrogen, progesterone, and prostaglandin to determine their combined effects in an ovulation synchronization protocol on synchronization rate, estrus activity, and pregnancy rates utilizing TAI.

## MATERIALS AND METHODS

Management of Heifers

This trial was conducted as a field study at the University of Georgia Dairy Teaching Facility located in Athens, Georgia. All heifers were fed a TMR containing sorghum silage (32% DM), corn, corn gluten feed, soy hulls, and 12.68% crude protein on a dry matter basis. The ration was fed at a rate of 2.72:9.07 kg concentrate to kg sorghum silage. Heifers had access to free choice high quality Tift 85 bermudagrass hay and were maintained on bermudagrass pastures. Heifers were of adequate breeding age (15 mo) and size averaging 165.1 and 144.78 inches in heart girth and hip height, respectively.

Estrus Synchronization

At the initiation of the breeding period (d 0), 40 Holstein heifers were assigned randomly to 1 of 2 groups. Twenty heifers were administered the control protocol, which consisted of the placement of a CIDR insert (EAZI-BREED CIDR, Pharmacia Animal Health, Kalamazoo, MI) from d 0 to 7 and an injection of 25 mg $PGF_{2\alpha}$ (Lutalyse, Pharmacia Animal Health) on d 6. The remaining 20 heifers received the treatment protocol, which included the control protocol with the addition of 2 50-µg GnRH injections (Cystorelin, Merial, Duluth, GA) on d 0 and d 9 as well as a 1 mg injection of estrogen (ECP, Pharmacia Animal Health) on d 7 upon CIDR removal (OverSynch). This protocol did implement the use of a half-dose treatment of GnRH, which has proven to be as effective as a full-dose and decreases ovulation synchronization costs (Fricke, 1998; McKee, 2004). All heifers received TAI using semen from sires of similar proven fertility at 48 h after CIDR removal. In both protocols, all animal handling including all injections and AI was conducted at 1400 h. Those animals assigned to OverSynch were administered the second GnRH injection at TAI. All GnRH injections and $PGF_{2\alpha}$ injections were given using a

20-gauge 3.81-cm needle. To correctly dose the 1.0 mg ECP, a 1-ml tuberculin syringe was used for all injections. Although no animals were bred based on visual heat detection, a heat mount detector (Estru$ Alert, Western Point Inc., Merrifield, MN) was applied on d 7 at the time of CIDR removal for analysis of estrus activity. Finally, upon insertion of each CIDR, half of the animals (n = 10) in each treatment group received shortened CIDR tail length with the tail being cut 6.35 cm below the tip of the vulva. The remaining animals were left with full CIDR tail intact. The variation in tail length was to determine any retention rate differences based on short or intact CIDR tails. Additionally, each CIDR was inserted using antiseptic lube (Nolvalube®, Fort Dodge Animal Health, Fort Dodge, IA) to minimize discharge and all discharge was scored upon CIDR removal as none or some. This procedure was carried out over 3 seasons, fall, winter, and summer, with 10 control and 7 treatment occurring in fall, 5 control and 6 treatment in winter, and concluding with 5 control and 7 treatment in summer. Artificial insemination throughout this trial was performed by one individual.

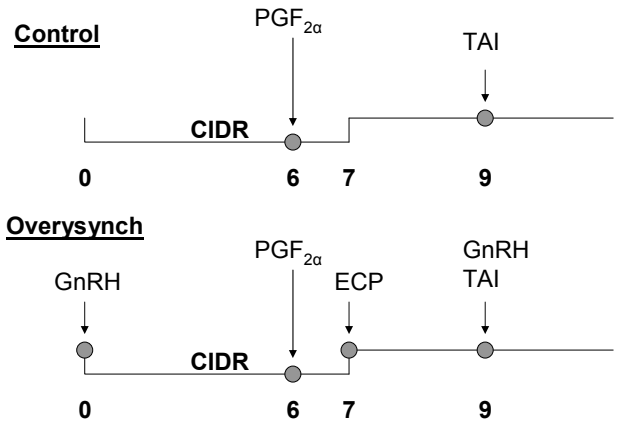


Figure 2.1. Experimental design illustrating the Control and OverSynch protocols to synchronize ovulation to TAI. All injections occurred in the PM. In both experiments, Estrus Alert Heat Mount detectors were applied on d 7 with CIDR removal. CIDR = Controlled Internal Drug Release containing 1.38 g of progesterone.

Determination of Estrus Activity

Estrus activity was monitored in 2 ways. Estru$ Alert heat mount detectors were applied on d 7 upon CIDR removal and were scored for estrus activity on d 9 at the time of AI. Estrus activity was determined by the amount of rubbing found on the Estrus Alerts, as described below, which is a direct result of mounting and possible standing activity. As outlined by the manufacturer, a detector that has had all of its coating rubbed off (All) indicates > 5 mountings, a partial rubbing occurring to the coating (Partial) as 3 to 5 mountings, and a detector with no coating rubbed off (No) indicates 0 to 1 mountings (Western Point Inc., Merrifield, MN). Detectors were scored based on rubbing as an "All", "Partial", or "No" rub. Animals falling into the all or partial rub category were considered to be in estrus at the time of AI. A secondary sign of estrus was the presence (Yes) or absence (No) of mucous at TAI.

Pregnancy Diagnosis and Embryo Survival

Heifers were checked at 35 d post AI for pregnancy status by ultrasonography by one individual. Pregnancy was confirmed at d 60 post AI before heifers were moved into the dry lot pens to ascertain any embryonic loss. A heifer was reported pregnant with detection of fetal fluid and a fetal heartbeat. All animals diagnosed pregnant at 35 d and again at 60 d were considered so until expected calving; therefore, no further heat detection or artificial AI occurred in these animals. At calving, the sex and status (alive or dead) were recorded for future analysis.

Statistical Analyses

Pregnancy rates at d 35 were analyzed as a linear regression model using the LOGISTIC procedure of SAS (Statistical Analysis Systems, v9.1: Cary, NC). The LOGISTIC procedure was utilized because it accounts for binary data with expectations of discontinuous variables such as "event vs. non-event" as well as ordinal variables such as "low, medium, high".

Treatment, season, age of animal, estrus alert status, mucous, and discharge were included as main effects. Backwards selection at the significance level of $P = 0.05$ was used for removal of independent variables from the model.

## RESULTS AND DISCUSSION

### CIDR Retention and Discharge

Retention rate of all CIDR inserts was 100%, regardless of tail length. This exceeds previous studies that reported a 1 to 5% loss (Lucy et al., 2001; Ryan et al., 1995). There was no difference in discharge upon CIDR removal ($P > 0.05$) between the control and treatment group. Additionally, discharge had no effect ($P > 0.05$) on pregnancy rates to TAI. However, discharge did have a tendency to effect estrus activity prior to the time of AI ($P = 0.14$), with those animals experiencing some discharge tending to have decreased estrous activity.

### Estrus Activity

Thirty-nine of 40 heat mount detectors were retained until removal at TAI. Only one animal did not retain the Estru$ Alert until time of AI. This animal was in the treatment group and was recorded as a "no" rub at TAI for estrus activity analysis. Estrus activity between d 7 and d 9 was detected in 27 of 40 (69.2 %) animals in this study. Greater estrus activity in the form of mounting and standing was detected in those animals in the OverSynch treatment ($P = 0.0129$) (80 %) compared with control (55 %). Moreover, 11 were designated "all" rubbed following OverSynch compared with 3 animals in control as illustrated in Figure 2.2. Increased estrus activity with OverSynch is not surprising, as many previous studies conducted utilizing ECP as part of synchronization protocol reported the ECP treated animals as having increased or more incidences of estrous activity (Jordan, 2003; Bo, 1994; Colazo, 2003).

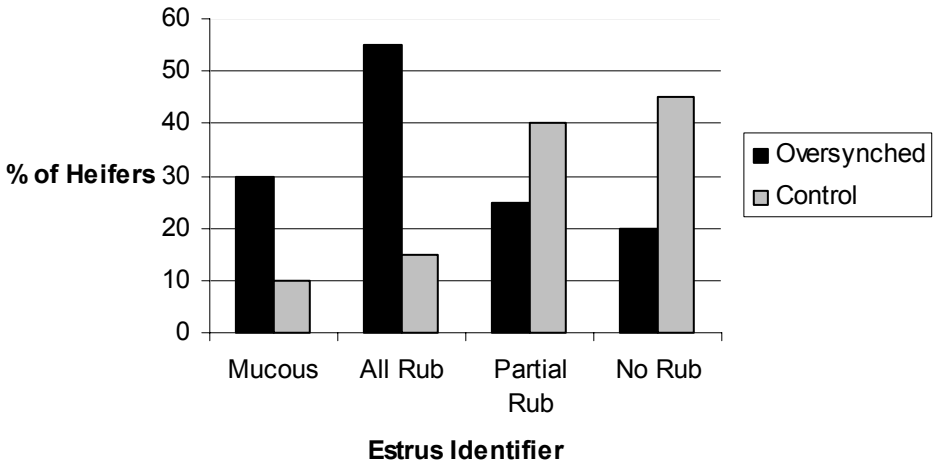


Figure 2.2: Visualization of the difference in estrous activity between the OverSynch and Control heifers.

Estrous activity was also compared with the presence of mucous at TAI. Heifers in the OverSynch group showed numerically higher incidences of mucous at TAI ($P > 0.05$). Heifers with a greater amount of estrous activity during the 48-h period prior to TAI tended ($P = 0.14$) to have greater incidences of mucous at AI. Mucous was estimated by the inseminator and was visual appraisal of a clear secretion in conjunction with lubrication at TAI. Though uterine tone was not evaluated in the current study, Stevenson et al. (2004) reported that the Ovsynch protocol limits secondary signs of estrus including uterine tone, mucous, and mounting activity. This is due to the second injection of GnRH, creating an LH surge that hinders the secretion of estrogen from the preovulatory follicle, a situation that is not observed with use of ECP in the current study. Secondary signs of estrus were apparent in the current study within the treatment group through both mounting behavior and mucous secretion, which increases insemination ease.

Pregnancy Rates

At 35 d post AI, 11 of 20 (55 %) animals were pregnant in the control group versus 9 of 20 (45 %) in the OverSynch group. Though numerically different, these pregnancy rates are not significantly different ($P > 0.05$). The relative pregnancy rates for the OverSynch group can not be decisively explained in the current study as no identical protocol has been tested. However, the pregnancy rate of the OverSynch group is comparable with those from a similar study (48%) in which heifers received GnRH and CIDR insertion on d 0, $PGF_{2\alpha}$ on d 7, with CIDR removal and ECP injection on d 8 and TAI on d 10 (Ambrose, 2003). The pregnancy rates in the treatment group of the current study and those reported by Ambrose et al. (2003) are higher that the 39.2% rate observed by Lopes et al. (2000) when administering GnRH (d 0), MGA (d 1-6; 0.5 mg/d), $PGF_{2\alpha}$ (d 7), and ECP (d 8) with a TAI 48 h later on d 10. However, the current treatment did not result in an increase in pregnancy rates compared with a basic CIDR-$PGF_{2\alpha}$ (control) protocol with TAI. Decreased pregnancy rate in the OverSynch group is plausibly related to the timing of insemination following the CIDR removal and ECP.

The injection of ECP was most critical in coordination with TAI because it was used not only to induce secondary signs of estrus but was also used to attain the threshold level of estrogen needed to induce the LH surge and ovulation (Swenson, 1970). Estradiol cypionate was administered at the time of CIDR removal to reduce labor intensity. However, efficacy of using ECP to induce an LH surge may be increased if given 24 h after CIDR removal in order to have the progesterone clearance necessary to substantiate an LH surge. Circulating progesterone from the CIDR insert takes at least 8 h to clear the bloodstream (Rathbone, 2002). The time for ECP to induce an LH surge and ovulation is not only longer, but is also less effective compared with GnRH (Stevenson , 2004). This is reasonable, realizing that ECP is not the immediate release

factor for LH, whereas GnRH is a direct signal. However, when ECP was utilized via the Heatsynch protocol in conjunction with progesterone in cows, the addition of progesterone tended to shorten the interval to estrus after ECP compared with herdmates not given progesterone (19 $\pm$ 5h vs. 33 $\pm$ 4h, respectively) ($P = 0.06$) (Stevenson, 2004). Estradiol, when used alone, has been shown to decrease LH pulse amplitude in cattle (Price, 1988). Progesterone has been shown to decrease follicular diameter size (Ireland, 1982). When estrogen and progesterone are used together it has been suggested that there is a subsequent suppression of both LH and FSH, with overall follicular size decreasing in a dose dependent manner (Price, 1988). This could account for the decrease surge of LH reported by Stevenson et al. (2004) and also account for the numerically lower pregnancy rates seen in the current study.

A study by Peeler et al. (2004) reaffirmed that the time from ECP injection to ovulation is longer than when utilizing GnRH as the LH inducer. These researchers reported higher pregnancy rates of heifers receiving ECP injection 24 h after CIDR removal with TAI at 56 h compared with insemination given at 48 and 72 h. In their study, heifers ovulated 39.8 $\pm$ 3 h after ECP injection or 63.8 $\pm$ 3 h after CIDR removal. These results are relatively consistent with a study conducted, in which ovulation following simultaneous CIDR removal and ECP injection in cows occurs 58 $\pm$ 3 h later (Stevenson, 2004). Garcia et al. (2003) reported similar times from CIDR removal to ovulation for heifers treated with ECP. The window from CIDR removal and GnRH stands much shorter at a 25 $\pm$ 3 h interval (Stevenson, 2004). A much longer window to ovulation (81.6 $\pm$ 5 h) was reported for beef heifers given an injection of ECP at the time of CIDR removal or 86.4 $\pm$ 3.5 hours when ECP was injected 24 h after CIDR removal. The time of ovulation relative to the ECP injection at the time of CIDR removal or 24 h post CIDR removal is not significant so it can be reasonably hypothesized that CIDR removal in

tandem with ECP in the current study did not alter the timing of ovulation compared with Heatsynch alone. Furthermore, despite the difference in time frames, it is noteworthy that heifers ovulate slightly later than do cows, due to the occurrence of a 3-wave cycle associated with nulliparous heifers compared with the 2-wave cycle of cows.

Studies not including a CIDR insert and utilizing TAI 48 h after injection of ECP report comparable pregnancy rates with Ovsynch utilizing GnRH 12 to 16 h prior to TAI in cows (Thatcher, 2002). Inclusion of the CIDR does not hinder estrus as shown in the current study; however, it does increase the time from estrus to ovulation. This observation is based on the greater proportion of animals in heat prior to TAI without an increase in pregnancy rate. Stevenson et al. (2004) noted that inclusion of the CIDR with ECP increased the interval from estrus to ovulation by only 2 h, which does not account for the decreased pregnancy rate with TAI. However, their study was conducted with cows, not heifers. In the present study, eliciting a 48 h interval from ECP injection to TAI was devised, in part, to allow GnRH to substantiate a follow-up LH surge in those animals not responding to ECP. However, compared with ovulation timing, the inseminated sperm have been in the reproductive tract for 10 to 12 h. The sperm, on average, take 8 h to reach the isthmus portion of the oviduct and become capable of fertilization. Sperm lifespan is 18 to 24 h in the female reproductive tract compared with an ova lifespan of only 8 to 10 h following ovulation. According to recent studies, the sperm viability with insemination prior to ovulation would not be a contributor to decreased pregnancy rate per AI, as the most often contributor is an aged oocyte with insemination occurring after ovulation (Pursley, 1998).

This trial was conducted throughout 3 seasons that varied in relative Temperature Humidity Index (THI). The groups in each season were 10 control and 7 OverSynch in fall, 5

control and 6 OverSynch in winter, and 5 control and 7 OverSynch in summer. The peak THI for these 3 seasons on the d of TAI were 69.7, 64.4, and 85.4, respectively, and were determined by data published by the Climatology Department at the University of Georgia. Pregnancy rate was further analyzed as a function of season. Pregnancy rates for control and OverSynch were determined as follows: 50 % and 57.4 % in fall, 80 % and 66.7 % in winter, and 40 % and 14.8 % in summer, respectively. Pregnancy rates were lower in summer ($P = 0.04$) compared with winter and fall. The lower pregnancy rate observed during the summer was expected as heifers are heat stressed, which can have multiple effects on the estrous cycle, including but not limited to suppression of the dominant follicle, decreased blood progesterone, decreased oocyte quality, and alterations in steroidogenesis (Wolfenson, 2000). Most embryonic mortality related to heat stress occurs within 21 d of conception (Bridges, 2000). Embryos at the stage before d 35 are most susceptible to embryonic mortality; as the embryo ages into a fetus, its susceptibility to heat stress declines (Hansen, 2002). The utilization of a TAI protocol alleviated the deleterious effects of missed estrous cycles and estrus, which are a consequence compromised follicular development and decreased circulating estradiol. A season and treatment interaction was not detected ($P > 0.05$), but might have been anticipated if TAI was not utilized. Estrus activity decreases with heat stress; therefore, pregnancy rates would be expected to decline in the control group with decreased estrus expression (Wolfenson, 2000). This occurrence would not be anticipated in the OverSynch group with an injection of ECP to substantiate estrus, resulting in a greater number of inseminations.

Given the higher estrous activity observed for the OverSynch treatment, it was reasonable to think that this activity possibly influenced pregnancy rate. However, estrous activity was not an accurate predictor ($P > 0.05$) of pregnancy status at d 35. Though nonsignificant

($P > 0.05$), the treatment group was a more accurate predictor of d 35 pregnancy diagnosis to TAI with a p-value approaching 0.10, than was estrous activity. Difference in sire estimated relative conception rates (ERCR) could have confounded differences in pregnancy rates, however, the frequency of each sire was too low for statistical analysis.

All 20 animals (9 in treatment and 11 in control) diagnosed pregnant at d 35 calved, with 3 calves still born. The sex of the calves born alive or dead were used for analysis. Three of the 9 heifers (33.3%) calving in the OverSynch group had female calves compared with 6 of 11 in the control group (54.5%). Given the small group numbers, these differences are numerically but not significantly different ($P > 0.05$). Xu et al. found in 1999 reported a higher proportion of female calves (53.8 %) in heifers synchronized with estrogen, progesterone, and prostaglandin compared with the control heifers (45.7%) ($P < 0.01$) Previous studies have reported that altering the interval from TAI to ovulation can skew the sex ratio (Rivera, 2005). Furthermore, many studies have suggested that insemination $\geq$ 1 d prior to ovulation results in more females, with insemination around the time of ovulation skewing the ratio to males (Pursley, 1998). The studies by Pursely et al. (1998) and Xu et al. (1999) would hypothesize that the sex ratio in the OverSynch group should be skewed towards female calves, relative to synchronization methods and timing of insemination. However, in the current study, the time of insemination relative to ovulation was not determined; therefore, any comments on the skew in the sex ratio seen in the current study would only be assumptions.

## CONCLUSIONS

Incorporating Heatsynch, Ovsynch, and a CIDR insert into a single ovulation synchronization protocol utilizing TAI did not improve pregnancy rates compared with those reported in previous studies utilizing Heatsynch or insemination after detected estrus. However, in the current study, the pregnancy rates of both control (CIDR-$PGF_{2\alpha}$) and treatment (OverSynch) groups were higher than those previously reported for Ovsynch in heifers (Pursley et al., 1997). The OverSynch treatment does increase estrus activity and secondary signs of estrus prior to TAI; however, it does not appear to efficiently facilitate TAI at 48 h after CIDR removal and concurrent ECP injection. At that point, the OverSynch protocol would not be economical given the comparable pregnancy rates of the less costly CIDR-$PGF_{2\alpha}$ or Heatsynch synchronization protocol.

Increasing the time from CIDR removal and ECP to TAI was the next step in this experiment. The window to ovulation following ECP is much longer than anticipated. The signs of estrus are evident sooner but do not appear to be accurate predictors of subsequent ovulation in 12 to16 h. The addition of progesterone in the current study, further lengthens the time to ovulation as reported by Stevenson (2004). Therefore, a 56-h interval from CIDR removal and ECP to TAI would appear to be a viable alternative; however, ECP was removed from the market before this could be tested. With the removal of ECP, neither the OverSynch protocol tested here, nor the Heatsynch protocol can be utilized for TAI in heifers. Development of synchronization protocols at this time must move to the more precise understanding of follicular dynamics in heifers and formulating an efficient TAI protocol without the incorporation of estrogen

# CHAPTER 3

## EFFECTIVENESS OF PROGESTERONE THERAPY POST AI VIA A CIDR INSERT ON DECREASING EARLY EMBRYONIC LOSS AND FOR THE RESYNCHRONIZATION OF ESTRUS IN DAIRY AND BEEF ANIMALS IN THE SOUTHEAST [2]

[2] Fain, J.L., W.M. Graves, J.M. Haslett, S.C. Nickerson, and J.K. Bernard. To be submitted to *Journal of Dairy Science.*

## ABSTRACT

Two experiments were conducted to test the efficacy of using progesterone treatment post AI to decrease embryonic mortality in dairy animals and to resynchronize estrus in dairy and beef heifers. In experiment 1, all animals were synchronized utilizing a single injection of 25 mg $PGF_{2\alpha}$ and were inseminated 12 h after animals were observed in standing estrus. Cows and heifers were randomly assigned to 1) receive post AI progesterone therapy (cow n = 11; heifers n = 13) from d 14 to 21 after AI using the CIDR insert (1.38g progesterone) (treatment) or 2) receive no further treatment post AI (cows n = 5 cows; heifers n = 9) (control). This trial was split and run in the summer and winter seasons to determine progesterone variability. Supplementation of progesterone after AI had no effect on pregnancy rates in heifers or cows, regardless of season ($P > 0.05$). No animals in this experiment diagnosed pregnant at d 35 were diagnosed open on d 60; therefore, no embryonic loss occurred, regardless of treatment. Progesterone concentrations on d 21 in heifers, regardless of treatment, tended to be higher ($P = 0.0651$) than those observed in cows. During both seasons, use of the CIDR maintained progesterone concentrations from d 14 to d 21; however, there were significantly higher progesterone values throughout the winter season when compared with summer ($P = 0.0084$). In experiment 2, beef (n=12) and dairy (n=32) heifers were initially synchronized utilizing a new CIDR insert (1.38 g progesterone) (d -10) with a 5 cc injection of $PGF_{2\alpha}$ at the time of CIDR removal (d -3). Animals were then artificially inseminated at 12 h after detected estrus (d 0). At 14 d post insemination (d 14), all animals received the same previously inserted CIDR for a second 7-d period until removal on d 21, followed by reinsemination occurring 12 h after detected estrus. Pregnancy rate response to initial synchronization was higher in both dairy (52.17%; 12/23) and beef (75%; 3/4) heifers compared with resynchronization, which yielded

pregnancy rates of 40% (4/10) and 50% (3/6), respectively. Use of the new CIDR insert significantly increased ($P = 0.002$) progesterone concentrations from d -10 to d -3 in heifers, whereas the used CIDR did not increase progesterone concentrations from d 14 to d 21 ($P > 0.05$). A mean increase in progesterone concentrations from d 14 to d 21 was a significant positive predictor of pregnancy ($P = 0.0133$). Furthermore, on d 21, progesterone concentrations were positively correlated with incidence of pregnancy at d 35 ($P = 0.004$). The use of exogenous progesterone maintains circulating blood progesterone concentrations in heat stressed heifers and non heat stressed heifers and cows. Although a used CIDR does not appear to maintain progesterone concentrations similar to those with a new CIDR, it did successfully suppress and resynchronize return to estrus.

## INTRODUCTION

Early embryonic loss is a factor that diminishes reproductive efficiency in dairy cattle around the world, with late embryonic or early fetal deaths as high as 10 to 12% in cattle (Van Cleeff, 2001). This loss becomes more prevalent among those operations when cattle are subjected to heat stress in excess of 3 mo of the year. A temperature humidity index (THI) is an accurate method of calculating the amount of heat stress that animals are enduring. A THI between 72 and 79 is indicative of mild heat stress, 80 to 89 is medium heat stress, and greater than 90 is severe heat stress. It is estimated that the U.S. dairy industry suffers losses of 897 million dollars each year on account of decreased performance, increased mortality, and decreased reproduction on account of heat stress (St-Pierre, 2003). One substantial cause for this increased pregnancy loss is the decreased circulating progesterone concentrations which accompanies heat stress (Howell, 1994). Decreased progesterone concentrations often result in increased frequency of LH secretion and consequently, termination of pregnancy. To combat

this event, circulating progesterone concentrations must be maintained in the early embryonic period until maternal recognition and subsequent placental attachment occur. Progesterone supplementation could plausibly decrease embryo mortality by preventing luteolysis in the heat stressed animal. Among the causes of embryonic death are corpus luteum (CL) insufficiency and developmentally delayed embryos that are incapable of maintaining a functional CL (Van Cleef, 1996). Both of these conditions result in lower circulating maternal progesterone, which may be reversed by supplementing progesterone during early embryonic development. Studies report that the majority of embryonic deaths occur between d 8 and 18 (Roche, 1981). This trend coincides with a severe drop in progesterone between d 6 and 18 in heat stressed animals (Howell, 1994). A few studies conducted thus far (Robinson, 1989 and El-Zarkouny, 2004) indicate that post insemination supplementation of progesterone were beneficial to maternal recognition of pregnancy and/or early embryonic survival.

The use of exogenous progesterone to decrease early embryonic loss can also be used to resynchronize cattle. The CIDR insert is reported to efficiently resynchronize estrus and additionally has been proven nondetrimental to fertility in animals previously inseminated (VanCleef, 1996). Unfortunately, with several synchronization methods, many animals that fail to conceive are not identified until the 35 d pregnancy check, resulting in at least a 35-d breeding interval and a missed estrous cycle (Chenault, 2003). Utilizing a CIDR to resynchronize estrus would drastically reduce this interval and result in a more compact breeding window.

The objectives of this study were to 1) to determine if a CIDR insert is capable of increasing circulating blood serum progesterone concentrations in heat stressed and nonheat stressed heifers and cows, 2) ascertain if pregnancy loss can be decreased and d 35 pregnancy rates increased with supplemental progesterone, 3) compare the actual impact that a new and

used CIDR inserts have on circulating progesterone concentrations in heifers, and 4) test the efficiency of implementing a used CIDR insert in a resynchronization protocol.

## MATERIALS AND METHODS

### Experiment 1

Animal Management

This experiment utilized primiparous and multiparous lactating Holstein cows (n=16) and nulliparous Holstein heifers (n=22), and was conducted in 2 replicates, one during summer (June 2004) and the second during the winter (February 2005). The average maximum daily THI for breeding dates in the summer portion of the study was 91. The averaged maximum daily THI for all those animals enrolled in winter was 57. All animals were located at the University of Georgia Teaching Dairy Facility in Athens.

Cows averaged 1.5 lactations and were 132 $\pm$ 32 DIM, and were maintained in free stall barn bedded with sand. Cows were fed a TMR twice per d to meet NRC requirements based milk yield and parity. They were milked twice daily at 0300 and 1500 h and averaged 30$\pm$7 kg of milk on the last test d before the initiation of this trial.

Heifers were maintained in paddocks with established bermudagrass pasture and fed free choice Tift 85 bermudagrass hay. Heifers were supplemented with a mix of ground corn, corn gluten feed, and soy hulls, consisting of 12.68% crude protein on a dry matter basis. The ration was mixed at a rate of 2.72 kg concentrate to 9.07 kg sorghum silage and fed once daily at 1500 h. All animals were a minimum 15 mo of age before initiation of the trial.

Experimental Design

All animals initially enrolled in the trial were synchronized with a single 5 cc injection of Lutalyse (Pfizer Animal Health, Kalamazoo, MI). Animals were fitted with Estru$ Alert

(Western Point Inc., Merrifield, MN) heat mount detectors to aid in estrus detection and were checked for heat twice daily AM/PM. When detected in standing estrus, animals were bred artificially within 12 h. All animals in this trial were bred by a single inseminator. Only those animals that were inseminated to initial synchronization continued on in the trial. All animals inseminated to synchronization in both the summer and winter seasons, were randomly assigned to receive a CIDR insert post AI as the treatment or no further treatment post AI and served as the control. More animals were intentionally allocated to the treatment group in each season and to the summer season. This allocation was done to establish more data on progesterone supplementation and its effects on progesterone tendencies in heifers and cows in time of heat stress. Table 3.1 displays the arrangement of this blocking. The increased age evident in winter heifers compared to summer heifers is the result of 2 outlying older heifers with ages of 35 and 21 mo.

Table 3.1: Blocking of animals for CIDR post AI study based on age and treatment. Age designation is the average lactation number for cows and the average age in mo for heifers.

| | Summer | | Winter | |
|---|---|---|---|---|
| | Cow | Heifer | Cow | Heifer |
| Treatment | 7 | 9 | 4 | 4 |
| Age | 1.71 | 17.78 | 1.75 | 20.25 |
| Control | 4 | 5 | 1 | 4 |
| Age | 1.25 | 16.4 | 1 | 17 |

After AI, animals assigned to the treatment group received a new CIDR insert containing 1.38 g of progesterone on d 14 following insemination. The CIDR remained in place for 7 d until removal on d 21 post insemination. To minimize any possible infection, Nolvalube was applied to each CIDR upon insertion. Additionally, all CIDR tails were cut to 6.35 cm below the

tip of the vulva to maximize retention rates. Animals in the control group received no further treatment.

Blood Sampling

All animals were bled by coccygeal venipuncture on d 0 immediately prior to AI, d 14 immediately prior to CIDR insertion, and d 21 immediately prior to CIDR removal. Samples were collected in Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) and allowed to clot overnight. The clotted samples were centrifuged at 3000 rpm for 15 min within 24 h of collection and serum was harvested and frozen at 0° C until analysis. Blood serum progesterone concentrations were established by radio immunoassay utilizing ImmuChem coated tubes (MP BIOMEDICALS, Costa Mesa, CA). Progesterone concentration was determined by running duplicate samples in a gamma counter calibrated for $^{125}I$. The 2 sample quantities were accepted and averaged allowing the percentage error was below 5.00.

Pregnancy Rate

Pregnancy diagnosis was conducted 35 $\pm$ 2 and 60 $\pm$ 2 d after AI by transrectal ultrasonography. Visualization of intraluminal uterine fluid and detection of a fetal heartbeat were used as positive indicators of pregnancy (Fricke, 2002). Early embryonic loss between d 1 and 35 was established through differences in pregnancy rates. Additional embryonic loss was calculated for the period from d 35 to 60 at the second pregnancy check. At calving, the sex of offspring was determined for analysis.

Statistics

Effect of treatment on d 35 pregnancy rate was determined using the LOGISTIC procedure of SAS (Statistical Analysis Systems, v9.1, Cary, NC ) and analysis of contingency tables. Serum progesterone concentrations between treatment and control animals were analyzed

with GLM. To determine the effects of treatment on circulating blood progesterone concentrations, an F-test analyzing the difference in sample means in combination with a pooled standard deviation estimate was utilized. For reassurance of these results, the same analysis was performed with a transformed variable, providing a more symmetric variable. In further analyses, predictors of d 21 progesterone concentrations were determined with analysis of covariance with the original regression equation including d 14 progesterone concentrations, treatment, season, and age. Interactions were run but not included in the model. All comparisons were deemed significant with a *P*-value less that 0.05.

**Experiment 2**

Heifer Management

This experiment utilized nulliparous dairy and beef heifers and was conducted from March 2005 to June 2005. Averaged maximum daily THI for the breeding dates was 77.4 and ranged from 66.5 to 87.1. All animals were located at the University of Georgia Teaching Dairy Facility and Beef Unit located in Athens.

Nulliparous Holstein dairy heifers (n=32) were maintained on bermudagrass pastures with access to Tift 85 bermudagrass hay. A supplement containing ground corn, corn gluten feed, and soy hulls, and 12.68% crude protein on a dry matter basis was mixed at a rate of 2.72 kg concentrate to 9.07 kg sorghum silage and fed once daily at 1500 h. Heifers averaged 17 mo of age and were all first or second service heifers.

Nulliparous Limousine beef heifers (n = 12) averaged 14 mo of age and were maintained on bermudagrass pastures, bermudagrass hay ad libitum, and were fed a supplemental mixed concentrate composed of corn, corn gluten feed, soy hulls, and cottonseed meal.

Experimental Design

This experiment was conducted from March through early June and was a time period of mild and moderate heat stress for both beef and dairy heifers. The objectives of this experiment were to 1) determine any circulating blood serum progesterone variability occurring between a new and used CIDR insert when utilized for the standard 7-d protocol, 2) compare pregnancy rates between initial synchronization with the new CIDR insert and resynchronization with a used CIDR insert and 3) ascertain any differences in progesterone concentrations between beef and dairy heifers and their subsequent effects.

To test these specific objectives, the new CIDR insert and initial synchronization was Phase 1 (control) and the used CIDR insert and resynchronization was Phase 2 (treatment). This protocol elicited a new CIDR insert for 7 d with $PGF_{2\alpha}$ injection on d 7 upon CIDR removal. All animals were checked for signs of estrus for 4 d and artificially inseminated 12 h after detection of standing estrus. At 14$\pm$ 1 d after AI, used CIDR inserts were inserted intravaginally again for 7 d until removal on d 21. Additionally, any animal not serviced after initial synchronization was injected with 5 cc $PGF_{2\alpha}$ at d 21 with CIDR removal. All animals were again observed for signs of heat for 4 d following used CIDR removal and were bred 12 h after standing estrus.

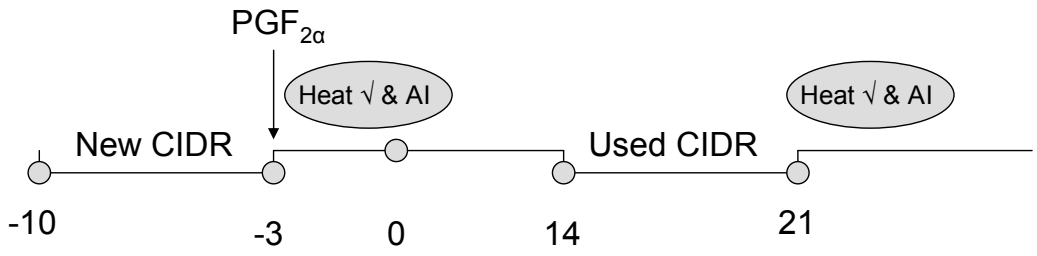


Figure 3.1: Protocol for all animals in CIDR synchronization and resynchronization with CIDR reuse study.

Reuse of each CIDR was conducted in a manner to minimize the incidences of bacterial transmission. Upon initial CIDR removal, all were washed in a Nolvasan solution mixed at a rate of 6.59 ml per L of clean water. The CIDR was then placed on a Ziploc bag numbered according to the heifer it was removed from and allowed to air dry in a cool place. Following drying, each CIDR was stored in a bag with corresponding heifer number until reinsertion at d 14. All estrus activity was determined by visual detection of estrus activity twice daily along with heat mount detectors.

Discharge

Discharge was scored at the time of new and used CIDR removal as an indicator of infection. Incidence of bacteria transmission and infection is a current justification for not reusing CIDR inserts. Scores were assigned by one person throughout the experiment and were recorded as "yes" for discharge or "no" for no discharge present.

Conception Rates

Pregnancy diagnosis of dairy heifers was conducted at 35 $\pm$ 2 d after insemination. Animals in this particular trial were pregnancy checked 35 $\pm$ 2 d after initial insemination and were checked again 35 $\pm$ 2 d if they were inseminated after resynchronization. Therefore, animals could theoretically receive 0, only 1 pregnancy check, or 2 pregnancy checks according to the number of times inseminated. Heifers diagnosed pregnant to an insemination date were then rechecked 25 d later to determine any possible embryonic loss.

Pregnancy diagnosis of beef heifers was conducted once at 67 $\pm$ 2 d after resynchronization (91 $\pm$ 2 d for heifers pregnant to initial breeding). These heifers were turned to pasture with a bull 1 wk after the conclusion of the study. Differentiating pregnancy from synchronization and resynchronization was not an issue as no animals received more than one

service to the synchronization and resynchronization program. Pregnancy from AI versus natural service was determined based on size of the fetus relative to insemination date.

Blood Sampling

All animals enrolled in the trial were bled by coccygeal venipuncture on d -10, -3, 0, 14, 21, and 24. The d 0 and 24 samples were taken just prior to AI, d -10 and 14 samples were taken prior to CIDR insertion, and d -3 and 21 were taken immediately following CIDR removal. Samples were collected in Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) and were allowed to cool and clot overnight. Samples were centrifuged at 3000 rpm for 15 min within 24 h of collection. Serum was harvested and frozen at 0° C until analysis. Blood serum progesterone concentrations were established by radio immunoassay utilizing ImmuChem™ coated tubes (MP BIOMEDICALS, Costa Mesa, CA). Progesterone concentrations were determined by running duplicate samples in a gamma counter calibrated for $^{125}I$. The 2 sample quantities were averaged and the percentage error was below 5.00.

Statistics

Pregnancy rates and differences in progesterone at determined times were analyzed using the LOGISTIC procedure of SAS (Statistical Analysis Systems, v9.1: Cary, NC ). Differences in progesterone concentrations over a time frame were analyzed by the paired difference t-test. To determine if progesterone concentrations were correlated with incidence of pregnancy, the LOGISTIC procedure was used in conjunction with classification tables. The overall fit of the tables was determined by the Hosmer-Lemeshow lack-of-fit table. Chi-squared tests were used to make comparisons between beef and dairy heifers.

## RESULTS AND DISCUSSION

### Experiment 1

Pregnancy Rate

Supplementation of a CIDR insert for d 14 – 21 post AI did not increase ($P > 0.05$) d 35 or d 60 pregnancy rates in heifers or cows in either the summer or winter seasons. With no differences in pregnancy rates, there was assumed to be no difference in early embryonic loss from d 1 to 35 between the control and treatment groups. Additionally, no animals, regardless of season or treatment, suffered embryonic loss between d 35 and 60 in this particular study. One heifer and one cow, both in the treatment group, did abort during the fetal stage. Embryonic deaths were any occurring before d 60 and the 2 fetal abortions occurred at greater than 90 d in gestation. Numerically, overall pregnancy rates were higher for both heifers and cows during the winter season as would be expected, though this difference was nonsignificant ($P > 0.05$). The small number of animals in each group made definitive conclusions difficult.

Although no distinct difference in pregnancy rates was observed in the present study, the analysis went further to examine the effects CIDR treatment was having endogenously. As a group, CIDR treated animals, in both summer and winter, had a d 21 mean progesterone concentration of 4.682 ng/ml (SE = $\pm$ 0.34) which is numerically higher than the control animals with 3.362 ng/ml, though given the small sample sizes of 24 and 14, respectively, these differences were nonsignificant ($P > 0.05$). However, when analyzing the change in progesterone concentrations between d 21 and 14 in treated versus control animals, there was a difference ($P = 0.0132$). Progesterone concentrations in those animals receiving supplemental did not change (-0.15 ng/ml) from d 14 to d 21, while animals in the control group experienced a -3.13 ng/ml (SE = $\pm$ 1.14) decrease in progesterone concentrations ($P < 0.05$).

In recalling the objectives of this experiment, it was important to differentiate the impact of the CIDR insert on heifers versus cows. Furthermore, summer was the critical season for this experiment, as the protocol was designed to decrease embryonic loss during heat stress. Regardless of treatment, heifers and cows experienced no difference in d 14 progesterone concentrations ($P > 0.05$); however, there was a trend ($P = 0.0651$) for heifers to have higher d 21 progesterone concentrations than cows, averaging 2.1 ng/ml more progesterone on d 21. El-Zarkouny et al. (2004) report that the diminished progesterone in cows is associated with the increased dry matter intake needed to substantiate milk production, subsequently causing increased passage of blood through the liver, which increases the metabolization rate of progesterone. Additionally, dairy cows have a higher rate of metabolic heat resulting in conversion of feed energy to milk energy (El-Zarkouny, 2004).

After removing all open animals from the summer analysis, there was no difference ($P > 0.05$) in d 14 or d 21 progesterone concentrations between heifers and cows. However, when progesterone concentrations in animals diagnosed pregnant at d 35 were analyzed as a function of age and treatment, CIDR treated heifers had significantly higher ($P < 0.01$) d 21 progesterone concentrations than control heifers. Conversely the d 21 progesterone concentrations of pregnant cows were higher ($P < 0.05$) for control cows than in CIDR treated cows. Similar results were reported by Burke et al. (1997), suggesting that supplementation of progesterone increases circulating blood progesterone concentrations to a degree that endogenous production is reduced.

Regarding the winter season, there were no differences in progesterone concentrations in cows or heifers on d 14 or d 21 regardless of d 35 pregnancy diagnosis ($P > 0.05$). However, progesterone concentrations were not maintained within the winter group as a whole as observed in summer. There was, on average, a sharp decline in progesterone from d 14 to d 21 regardless

of treatment in open animals in the winter study ($P < 0.05$), a phenomena not seen within the summer open animals. However, as evident from Figure 3.2 the sharp decline in progesterone from d 14 to 21 in open animals is the result of a sharp decline in the control animals and is not a result of CIDR treated animals. Evident in the same figure is the relative consistency of progesterone concentrations from d 14 to 21 in the treated animals. The progesterone concentrations of CIDR treated animals were maintained in both the summer and winter seasons. The decline in the control open animals in the winter season from d 14 to d 21 is a positive indicator of return to estrus. The lack of progesterone decline in the control open animals of the summer season is a direct physiological effect of heat stress. The compromised follicular development is decreasing the secretion of estradiol needed to substantiate surges of GnRH and LH for a subsequent estrus and normal return to the estrous cycle. Graphs located in Figure 3.3 depict the trend in circulating progesterone concentrations based on age of animal and pregnant status.

When analyzing the difference in progesterone concentrations between pregnant animals in the summer and winter season, it is evident that circulating blood progesterone concentrations are higher in those animals in the winter group. Pregnant animals in the winter group are above 7 ng/ml on d 14 and 21, while in summer, all pregnant animals in summer are below 7 ng/ml on these respective dates. The progesterone concentrations on d 14 and 21 in pregnant animals of the winter protocol are significantly higher than those found in the summer protocol, regardless of treatment ($P = .0084$)

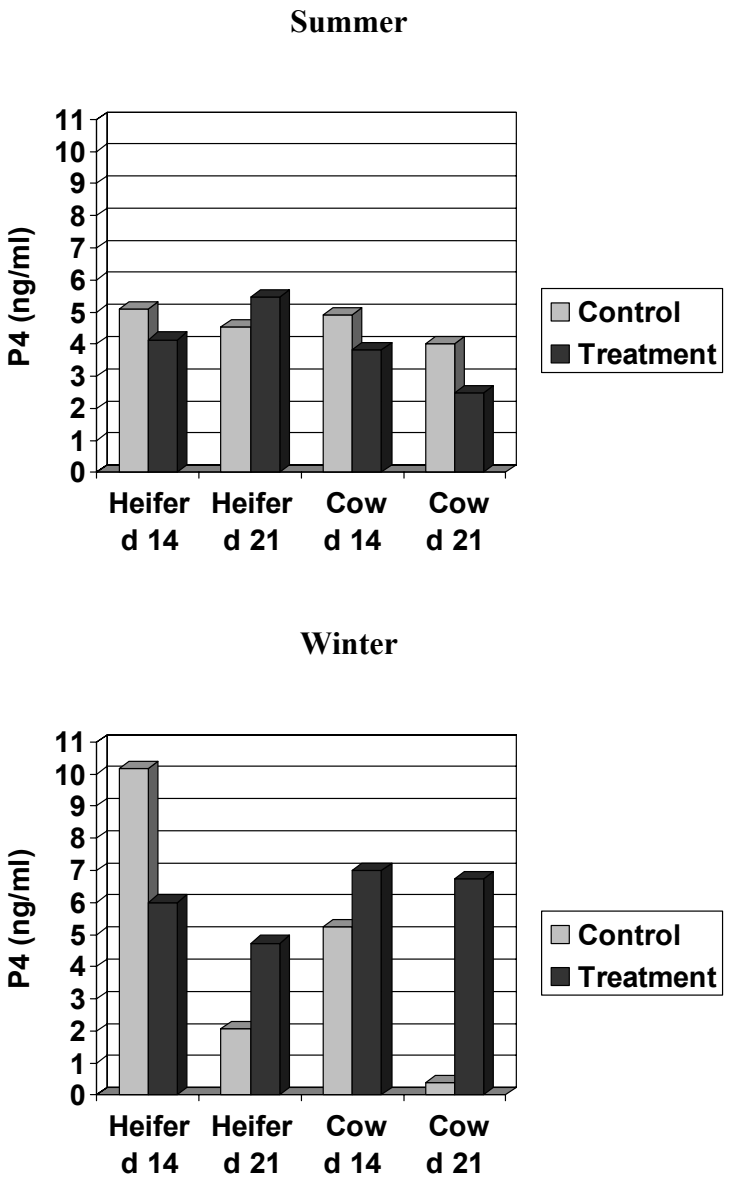


Figure 3.2: Trend in circulating blood progesterone concentrations based on treatment, lactational status, and season. This figure directly depicts that progesterone concentrations are maintained with additional supplementation of a CIDR insert in heat stressed heifers and non heat stressed heifers and cows.

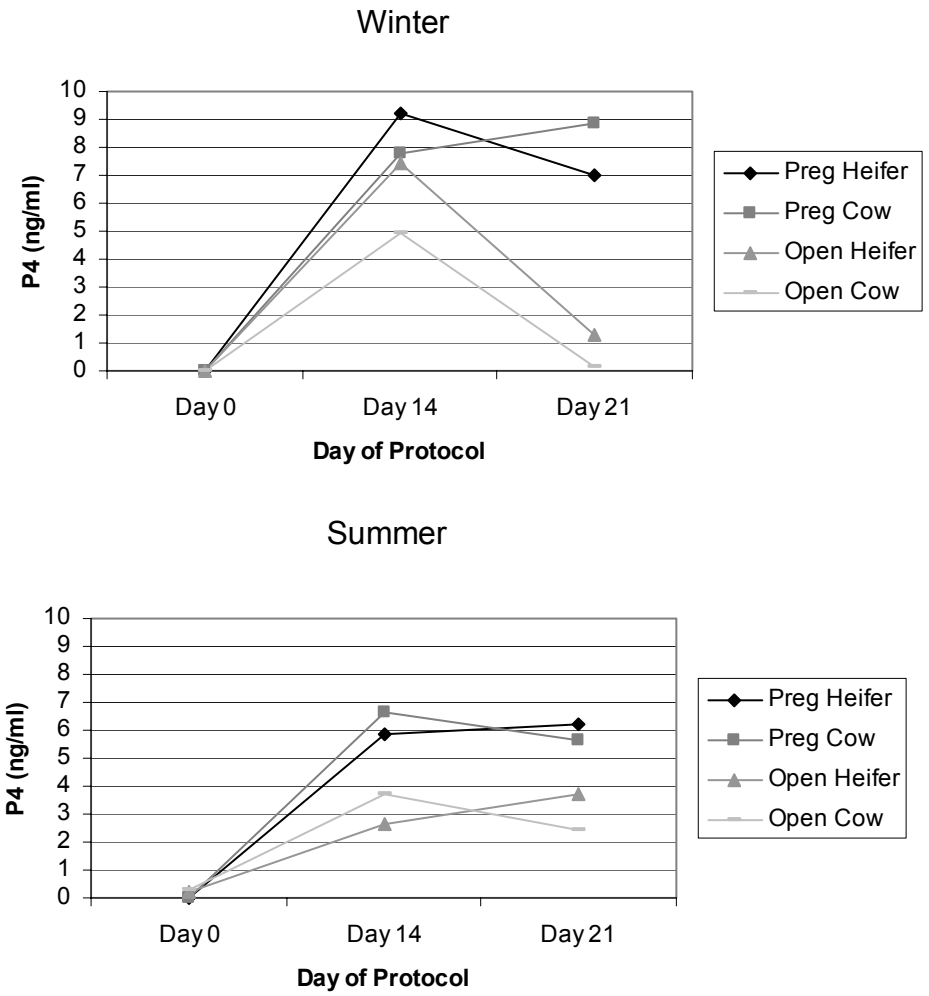


Figure 3.3: Serum progesterone concentrations of cows and heifers based on d 35 pregnancy status. Progesterone concentrations are split according to season and regardless of treatment.

As outlined in Figure 3.3, progesterone concentrations of pregnant cows were lower than those of pregnant heifers ($P > 0.05$). Cows generally experience greater metabolic need and therefore have a higher DMI associated with greater milk yields (El-Zarkouny, 2004). This increased dry matter leads to increased metabolism of progesterone through the liver, resulting in lower circulating progesterone concentrations and decreased progesterone availability (Wiltbank, 2000).

Based on this, analysis was conducted to see if d 14 progesterone concentrations could be used to predict d 21 progesterone concentrations. Initially, analysis of covariance was used to predict d 21 from d 14. The initial regression equation was: Day21 = B0 + B1*Day14 + error. Following this analysis, the study looked further to see if the prediction could be improved appreciably by using information such as treatment, season, and age (heifer versus cow). All factors tended to raise the predicted value of d 21 concentrations from the baseline prediction; however, only the treatment indicator was significant ($P = 0.0350$). See Table 3.2.

Table 3.2: Prediction values of variables used to determine d 21 progesterone concentrations

| Variable | SE | P Value |
|---|---|---|
| **Day 14 Progesterone** | 0.20586 | 0.0056 |
| **Treatment** | 1.06577 | 0.0350 |
| **Season** | 1.21235 | 0.1179 |
| **Age** | 1.00089 | 0.5770 |

Thus, the final equation, including all factors to predict d 21 progesterone concentrations is D21 = -2.21 + 0.609*D14 + 2.34*T + 1.95*S + 0.56*H + error ($P = .0412$), where T, S, and H are indicator variables (0 – 1 variables) which are "1" for treatment, summer, and heifers, respectively. The d 21 concentrations can, therefore, be predicted using d 14 progesterone concentrations with an $r^2$ of 0.25. Though this is not a remarkable predictor, it is evident that it is more accurate to predict d 21 from d 14 than not. With this model, in predicting d 21 progesterone concentrations, the treatment group is approximately 2.34 ng/ml higher compared with the control group with the same beginning d 14 progesterone values

Burke et al. (1997) reported that serum progesterone concentrations increase beginning 2 h post CIDR insertion. However, after 7 d, there was no marked difference between CIDR

treated and control animal blood progesterone concentrations. The researchers concluded that endogenous secretion of progesterone may decrease with the exogenous supplementation. In addition, this effect could be confounded with the increased metabolism of progesterone with increased concentrations.

Sex of Offspring

Of those animals enrolled in the summer, 9 calved. Of these 9 calvings, 4 were female and 5 male. Of the animals carrying females, all had blood progesterone concentrations below 6 ng/ml on d 21. Of the animals carrying males, 4 of 5 had blood progesterone concentrations above 6 ng/ml on d 21. The remaining animal carrying a male had a progesterone concentration of 4.3 ng/ml on d 21. Additionally, 3 of 4 females decreased in progesterone concentrations from d 14 to 21, with one female remaining the same. Conversely, 3 of 5 males increased in progesterone concentrations from d 14 to 21, 1 remained the same, and 1 displayed a 2 ng/ml decrease. The sample sizes are too small to allow adequate statistical evaluation; however, the separation in sex of offspring based on d 21 progesterone concentrations should be noted.

There were even fewer calvings for those animals enrolled in winter and the trend reported in the summer animals was not evident. Of the 4 calvings, 3 were female and 1 male. The animals carrying female offspring had progesterone concentrations on d 21 of 5.2, 5.9, and 12.5 ng/ml. The only animal carrying a male had a blood progesterone concentration of 9.5 ng/ml on d 21. Again, sample sizes were too small to allow for statistical evaluation.

**Experiment 2**

CIDR Disinfection

Efforts were made to minimize the possible transmission of bacteria, a problem that is a limitation in the reuse of CIDR inserts. Only 11.36 % of animals (n = 44) fitted with a new CIDR experienced any discharge. When used CIDR inserts were used, only 13.63 % exhibited

discharge. The differences in rate of discharge resulting from a new or used CIDR insert were not different ($P > 0.05$). The percentages of animals with a discharge was lower than previously reported in a similar study utilizing new CIDR inserts and Nolvalube (21.69%, Graves, 2004).

Conception Rate to First and Second Services

Synchronization and resynchronization rates were determined by the number of animals inseminated by the total number of animals synchronized or resynchronized. Conception rates were determined by dividing the number of animals that were diagnosed pregnant at d 35 post insemination by the number serviced to synchronization or resynchronization. In the dairy heifers, only 12.5 % were not detected in estrus and not serviced at initial synchronization or resynchronization. Twenty-three of the remaining 28 animals were synchronized and serviced after the removal of the new CIDR insert. Of these 23 animals, 47.8% were diagnosed pregnant at d 35, which is comparable to 47% pregnancy rate reported by Van Cleef et al. (1996) when using a new CIDR insert for 9 d with $PGF_{2\alpha}$ on d 7. Ten of the 28 animals were serviced after resynchronization, with 50% being repeat a breeding. Of the 10 serviced at resynchronization, 4 (40%) were diagnosed pregnant at the d 35 check. Again, this conception rate is similar to those reported by Van Cleef with 35.1% pregnant after removal of a used CIDR device from d 17 to 22. Two of the 4 received first service at resynchronization, resulting in an overall pregnancy rate of 46.87% (15/32) for synchronization and resynchronization in dairy heifers. All synchronization rates and conception rates are listed in Table 3.3.

Among beef heifers, 33.3% were bred to initial synchronization with the new CIDR insert and had a conception rate of 75%. Six animals were bred following resynchronization. However, in this case, none of these animals were serviced during the initial synchronization period. The conception rate to resynchronization was 50% (3/6), resulting in an overall

pregnancy rate to synchronization and resynchronization of 50%. The increase in synchronization rates between initial synchronization and resynchronization is possibly an effect of the ability of the CIDR during the initial synchronization to initiate cyclicity in prepubertal animals for resynchronization. All beef heifers were exposed to a bull for 60 d following the trial. Within the 6 heifers not bred to AI, only 2 conceived by natural service, leaving 4 of 12 open after 85 d and a potential 4 estrous cycles. The lower pregnancy rate may be because of delayed sexual maturity, with McLauchlan et al. (2005) reporting that approximately 35% of Limousine crossed beef cattle had not cycled at 15 mo of age. Of the 4 heifers not artificially inseminated or bred by natural service, only one heifer was classified prepubertal with progesterone concentrations on all 6 test dates not exceeding 1 ng/ml

Table 3.3: Synchronization and conception rates between the new and used CIDR insert.

| | Total | Synchronized | | Conception Rate | Resynchronized | | Conception Rate |
|---|---|---|---|---|---|---|---|
| | n | n | % | % | N | % | % |
| **Dairy** | 32 | 23 | 71.9 | 47.8 (11/23) | 10 | 31.3 | 40.0 (4/10) |
| **Beef** | 12 | 4 | 33.3 | 75.0 (3/4) | 6 | 50.0 | 50.0 (3/6) |

Due to the low number of animals for resynchronization, no significant differences in conception rates occurred between synchronization and resynchronization within the dairy or beef heifers ($P > 0.05$). The differences between conception rates to synchronization and resynchronization were lower in dairy compared to beef heifers, reporting a 7.8% decline in dairy heifers and a higher 25% decline in beef heifers. The larger decline in the beef animals is the result of low animal numbers. The overall pregnancy rates of 46.87 and 50% for dairy and beef, respectively, are lower than those reported in a similar study conducted by Martinez et al.

(2001). That particular study utilized beef heifer that were all synchronized and fixed time inseminated and resynchronized with a CIDR insert for 7 d beginning at 13 $\pm$ 1 d post insemination or were fed the progestin melengestrol acetate (MGA) (0.5 mg/d) for 7 d, both groups receiving insemination 6 to 12 h after detection of estrus. The heifers resynchronized utilizing a CIDR insert had conception and pregnancy rates of 65.1 and 61.4, respectively, which are higher than those reported in the current study. Instead, the results of the current study were more similar to those animals fed MGA in the Martinez (2001) study, which had conception and pregnancy rates of 50 and 40%, respectively. The lower conception and pregnancy rates seen in the current study could be accounted for by variations in environment, with heifers in the current study enduring heat stress.

In this trial there were no animals not resynchronized for comparison; however, in both the beef and dairy heifers, 11 of the 17 (64.7%) animals not synchronized to initial AI were resynchronized and returned to estrus within a 4-d time frame. This agrees with the results of VanCleef (1996) who reported without resynchronization, 49.3% returned to estrus within a 4-d period (d 18 to 22) compared with 75% with resynchronization (d 23 to 26).

Embryonic loss was not calculated for the beef heifers as all animals were turned in with a bull at the conclusion of the study for a 60-d period and received only a 67$\pm$2 d pregnancy check. Therefore, the pregnancy rates in beef heifers had already incurred any embryonic loss. At the d 60 pregnancy check in the dairy heifers, no animals had incurred embryonic loss.

Progesterone Concentrations

Unless otherwise stated, progesterone comparisons and results are based on analyzing the beef and dairy heifers together. Progesterone concentrations on d -3, d 14, and d 21, were positively correlated, having $r^2$ values of +0.27 (d -3 to d 14), + 0.42 (d -3 to d 21), and +0.38 (d

14 to d 21) between each of the 3 levels. The highest correlation occurs between d -3 and d 21, both of which occur at the time of CIDR removal. The best linear predictor of d 21 progesterone concentrations can be derived by including the significant d -3 ($P = 0.0030$) and d 14 ($P = 0.0303$) predictors in the equation: D 21= 0.351 + 0.630 * D -3 + 0.387 * D 14 + error. The $r^2$ value for this equation is 0.34 ($P = 0.002$).

Following the indication that progesterone concentrations were related at the time of CIDR removal, the difference in mean progesterone concentrations between insertion of a new (d -3) and used (d 21) CIDR insert was then analyzed. With the use of the new CIDR, the mean progesterone concentration increased by 2.09 ng/ml ($r^2 = +0.324$, $P = 0.002$). Thus, there was an increase in mean progesterone concentrations from d -10 to d -3 when using the new CIDR insert. When examining mean progesterone concentrations from d 14 to d 21 with the used CIDR, mean progesterone concentrations minimally decreased by -0.64 ($r^2 = 0.85$, $P = 0.40$). Therefore, there was no difference in d 14 and d 21 progesterone concentrations, the time period during which a used CIDR insert was utilized.

It was concluded that the difference in progesterone concentrations from d -10 to d -3 was due to the large number of zero values (17/44 = 38.6%) at the d -10 time. The large number of 0 values at d -10 versus no zero values at d 14 is largely due to the manipulation of the estrous cycle that occurred during the initial synchronization, so that the majority of animals in the luteal phase, with subsequent higher progesterone concentrations on d 14 with a used CIDR. However, when analyzing the distribution of d -10 and d -3 values with the UNIVARIATE procedure of SAS, there are no anomalous differences, signifying that the difference in these 2 progesterone values is real.

A second approach to the correlation between progesterone concentrations during this trial, was to determine if these correlations were associated with d 35 pregnancy status. The mean progesterone difference between d 14 and d 21, with a used CIDR, is significantly correlated with d 35 pregnancy status ($P = 0.0133$) (Table 3.4). As such, in the 22 animals that showed increased progesterone concentrations from d 14 to 21, 14 were diagnosed pregnant at d 35 (63.6%). Similarly, of the 22 animals with decreased progesterone during the same time period only 7 (31.8%) were diagnosed pregnant, leaving the remaining 68.2% open. Therefore, increasing progesterone concentrations from d 14 to d 21 is a significant indication of pregnancy.

Table 3.4: Progesterone variation between the new and used CIDR inserts and its significance as a predictor of d 35 pregnancy.

| Variable | Logistic Effect | SE | 2-tailed P-value |
|---|---|---|---|
| New CIDR removal P4 - insertion P4 | -0.0118 | 0.0738 | 0.8729 |
| Used CIDR removal P4 - insertion P4 | +0.212 | 0.0857 | 0.0133 |

When examining all 6 progesterone concentrations individually, there are additional predictors of pregnancy. The LOGISTIC model proves that d -3 progesterone concentrations have a tendency to be a predictor of pregnancy ($P = 0.0618$; SE = 0.1717). Whereas, d 21 progesterone concentrations are a very significant predictor of pregnancy ($P = 0.004$; SE = 0.1417). Van Cleef et al. (1996) conducted a similar study in dairy heifers, administering a used CIDR from d 18 to 22. Their reports confirm the results above indicating that higher progesterone at the time of CIDR removal (in their case on d22) was found in pregnant heifers. The average progesterone concentration in that particular study for pregnant heifers was 9.83 $\pm$ 0.78 vs. 4.27$\pm$ 0.73 ng/ml for non-pregnant heifers. The trends in progesterone concentrations

between dairy and beef heifers are found in Figure 3.4. For the dairy heifers in the current study, average progesterone on d 21 for pregnant heifers was 10.37 vs. 3.79 ng/ml for nonpregnant heifers. The progesterone concentrations on d 21 for pregnant heifers without a CIDR, as reported in the previous study, was 5.3141 ng/ml. The CIDR insert does compensate progesterone concentrations above what is required for the maintenance of pregnancy in heifers. The lower progesterone concentrations on d 21 for nonpregnant heifers was a positive indicator of resynchronization, indicating that luteolysis has been completed and that removal of the exogenous progesterone will induce estrus. Using the prediction equation: $\ln (P/Q) = -0.5801 - 0.3207 * D\text{-}3 + 0.4082 * D21$, and the prediction constant of 0.5, predictions of pregnancy would be correct 70% of the time. This number is an apparent correct classification rate derived from the Hosmer-Lemeshow lack-of-fit table and classification table.

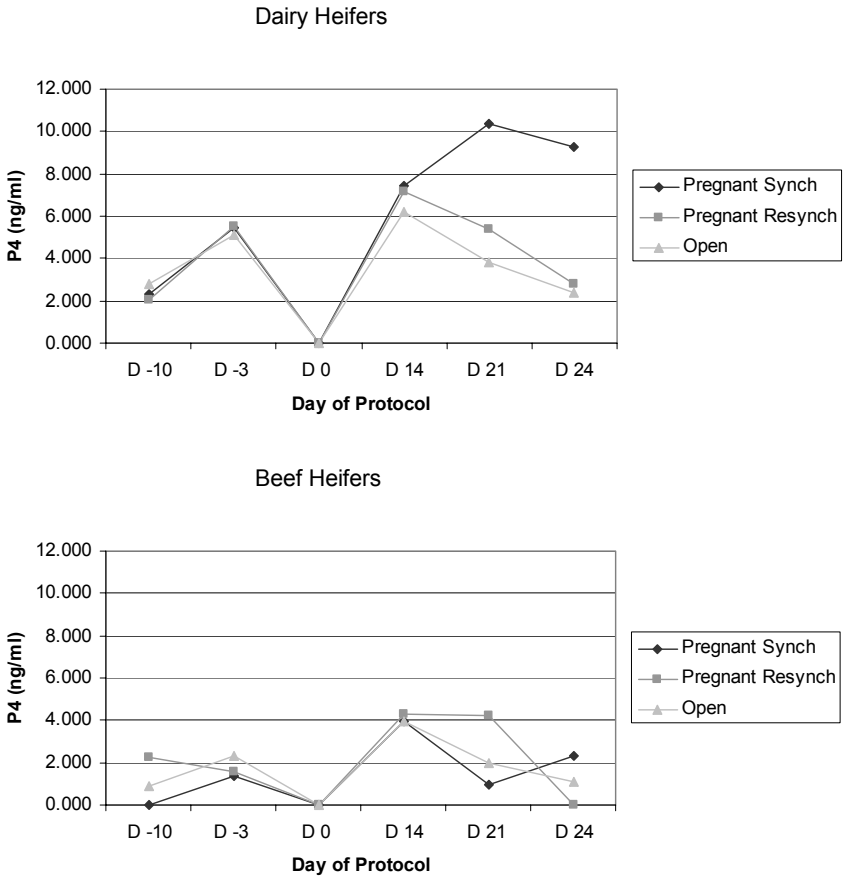


Figure 3.4: Representation of progesterone concentrations between dairy and beef heifers on test dates of protocol based on d 35 pregnancy diagnosis. It is evident that pregnancy in both beef and dairy heifers alters the concentration of progesterone production, however, the degrees of these alterations are different.

Overall, no differences in pregnancy rates ($P > 0.01$) occurred between beef and dairy heifers with use of this synchronization and resynchronization protocol. However, progesterone therapy had significantly different effects on progesterone concentrations in beef versus dairy heifers throughout the trial. Figure 3.5 depicts the average progesterone concentrations on test dates between beef and dairy heifers.

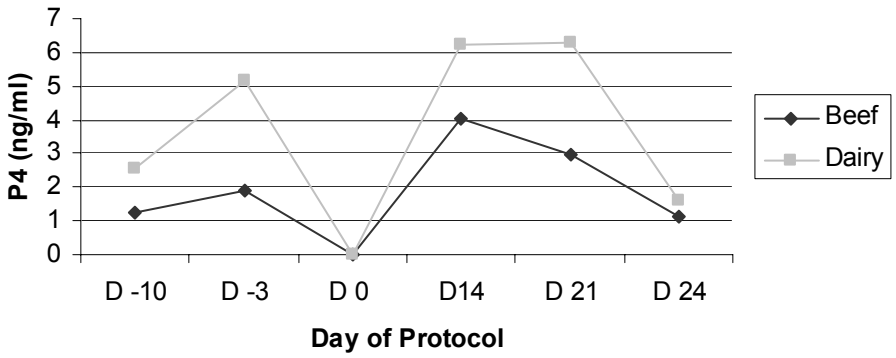


Figure 3.5: Progesterone concentration variation in dairy and beef heifers throughout the protocol, regardless of d 35 pregnancy diagnosis. Differences in metabolization of progesterone were evident in the sampling of progesterone on 6 test dates, depicted in this timeline. Though the shapes of the graphs are the same, the peaks are higher in dairy compared with beef heifers

In general, beef heifers had lower progesterone concentrations than dairy heifers ($P < 0.05$). These concentrations were significantly different at the d -3 ($P = 0.0032$) d 14 ($P = 0.0377$) and d 21 ($P=0.0274$) sampling times. Both the d -3 and d 21 concentrations were -3.29 ng/ml in beef animals. These 2 significant points of variation in progesterone amounts were observed at the time of CIDR removal, as shown in Table 3.5. The greatest differences were recorded on d -3, 14, and 21, however, higher concentrations were consistently recorded in dairy heifers.

Table 3.5: Identifying the most significant progesterone test date differences between dairy and beef heifers.

| P4 Test Date | AVG Beef-Dairy | t-stat | 2-tailed P-value |
|---|---|---|---|
| D -3 | -3.29 | -3.13 | 0.0032 |
| D 14 | -2.73 | -2.21 | 0.0377 |
| D 21 | -3.29 | -2.32 | 0.0274 |

It has been reported by Mann et al. (2003) that the plasma progesterone concentrations of beef heifers appear to be less important in embryonic development than those found in dairy cows. They found little or no difference in progesterone concentrations between pregnant and nonpregnant beef heifers. Referring to Figure 3.4, the same phenomenon is observed, with the beef heifers in the current study having comparable progesterone concentrations throughout the trial regardless of pregnancy status. It is assumed that the increased progesterone concentrations in dairy animals is a direct result of increased corpus luteum cell size and number resulting in increased progesterone. Rational indicates this increase is the product of years of selection and genetic progress through utilization of AI to increase reproductive productivity. The corpus luteum is the essence of the estrous cycle and maintenance of pregnancy and would thus be positively influenced by selection for reproductive traits.

Additionally, it could also be deduced that the variability in constituents and intake of diets between replacement dairy and beef heifers could alter fatty acid concentration with alterations made to cholesterol, which is the precursor for progesterone. Differences could occur from luteal tissue scavenging cholesterol from the blood, positive or negative feedback from esterification of cholesterol from lipoproteins in the blood, or a consequence of progesterone clearance or metabolism through the body. Alterations in steroidogenesis and steroid metabolism could not only account for the progesterone differences seen in beef versus dairy heifers, but could reasonably account for the variations seen throughout the 2 trials.

## CONCLUSIONS

Utilizing a CIDR post insemination at d 14 to d 21 to decrease the incidence of embryonic loss was not validated in the current studies as losses were minimal for both treatment and control animals. Though it is evident from the results of experiment 1 that a CIDR post AI is a substantial contributor to maintaining progesterone concentrations in heat stressed animals its use for lowering embryonic loss needs to be further investigated. Additionally, the difference in the effect of the CIDR on cows versus heifers is clearly defined in the current study. Heifers overall have higher circulating blood progesterone concentrations which is significant in times of heat stress. This is due in part to the fact that cows endure a higher degree of heat stress in conjunction with a greater metabolic demand, subsequently leading to the increased metabolic rate of circulating blood progesterone.

In experiment 2, though the CIDR does not appear to decrease the fertility issues associated with heat stress in heifers, implementing a used CIDR post AI does bring heifers into a tighter synchrony of estrus than using no resynchronization method. Additionally, using the CIDR to resynchronize estrus effectively decreases the interval between breedings and is nondetrimental to those animals previously inseminated. Finally, reusing a CIDR insert has no deleterious effects on reproduction either through synchronization of estrus or pregnancy rates in the current study. The amount of progesterone released from a used CIDR does appear to be decreased in the current study, however, this was not enough to negate its biological activity to suppress estrus.

Overall, the use and reuse of the CIDR insert post AI for progesterone therapy and resynchronization of cattle is both useful for reproductive management and cost effectiveness. The capability of the CIDR to increase progesterone concentrations both in times of heat stress

and not makes it viable for resynchronizing estrus and plausibly aid in embryonic development. The latter effect was not validated in the current studies, however, the benefits of increasing progesterone during embryonic implantation and maternal recognition are viable and have been reported previously. These 2 trials overall validate the use of the CIDR insert in heifers throughout seasons and for synchronization and resynchronization, further research must be conducted to establish its viability for reproductive management in cows.

# CHAPTER 4

# IMPLICATIONS

Administration of 1.38 g of exogenous progesterone via the CIDR insert is an effective way to illicit the biological activities of progesterone without affecting fertility. Use of the CIDR in reproductive management has been reported and validated since it was made available. Initial studies were limited in using the CIDR for estrous cycle synchronization, however, new research is reporting alternative uses. These uses currently include resynchronization and aiding early embryonic development. Therefore, the application of the CIDR can occur prior to and after AI as a method to increase conception rates and maintain fertility.

The use of the CIDR in estrous cycle synchronization protocols has been reported in beef cows, beef heifers, and dairy heifers since its arrival on the market in the United States in June of 2002. In study 1, it was concluded that when combining the CIDR with GnRH and ECP in a TAI protocol for heifers, it is necessary to increase the interval of ECP injection and CIDR removal to TAI. The window to ovulation following ECP is longer than anticipated, regardless of obvious estrus activity prior to 48 h. Implementing a TAI at 56-h interval from ECP and CIDR removal to TAI appears from previous reports (Stevenson, 2004; Peeler, 2004; Garcia, 2003) to be a viable alternative. However, this concept is put on hold currently and possibly indefinitely with the removal of ECP from the market by the FDA.

With the approval of the use of the CIDR insert for synchronization of return to estrus in lactating dairy cattle in July 2003 has been a new release of reports of the efficacy of using them in dairy cows. In experiment 1 of study 2, one of the main objectives was to ascertain the

difference in progesterone utilization from the CIDR in dairy heifers versus cows. These differences were clearly defined with dairy heifers having higher circulating progesterone concentrations than cows, which is significant in times of heat stress. The differences in the metabolic needs and activities of cows versus heifers due to lactational status accounts for the variation in progesterone concentrations

Utilizing a CIDR in reproductive management is often hindered on account of the cost associated with the device. For this reason, many producers and researchers have researched the viability of reusing the CIDR insert to make them more cost effective aids. Experiment 2 of study 2 examined the viability of reusing CIDR inserts for resynchronization in dairy and beef heifers. Although progesterone release from the CIDR is lower in a reuse protocol, the biological activity of the CIDR to suppress estrus is not altered. Furthermore, it is apparent that beef heifers are less dependent on progesterone in early embryonic development, with results that show progesterone concentrations nearly equal between pregnant and non pregnant beef heifers at d 14 and 21 post AI. This could be the product of intense selection of reproductive traits in the dairy industry and/or the differences in dietary constituents and intake between beef and dairy heifers.

Additionally, utilizing the used CIDR to resynchronize estrus was validated with its ability to suppress estrus in addition to decreasing the breeding interval without any detrimental effects on embryonic survivability. Study 2 was instead looking to prove any positive ramifications of progesterone supplementation post AI via a CIDR on embryonic survivability. Unfortunately, this use was not justified in the current studies as embryonic loss, was minimal in control and treated groups.

In conclusion, it is apparent through studies 1 and 2, that the CIDR is an effective tool in the reproductive management of dairy and beef animals. Its ability to be used for estrous cycle synchronization, post-insemination progesterone supplementation, and resynchronization of estrous is validated through these studies. However, more research must be conducted to validate progesterone supplementation for supporting embryonic development.

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