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FIXED-TIME INSEMINATION OF PORCINE LUTEINIZING HORMONE-TREATED SUPEROVULATED BEEF COWS AND THE RESYNCHRONIZATION OF BEEF COWS FOR FIXED-TIME EMBRYO TRANSFER

A Dissertation

by

JOHN STEPHEN NELSON

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2008

Major Subject: Physiology of Reproduction

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Approved by:

Chair of Committee,	David Forrest
Committee Members,	Paul Harms
	Charles Looney
	Duane Kraemer
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ABSTRACT

Fixed-time Insemination of Porcine Luteinizing Hormone-treated Superovulated Beef Cows and the Resynchronization of Beef Cows for Fixed-time Embryo Transfer.

(December 2008)

John Stephen Nelson, B.S., Texas A&M University;

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Chair of Advisory Committee: Dr. David Forrest

Two trials were conducted to compare the effectiveness of fixed-time artificial insemination (AI) to AI based upon visual detection of estrus following superstimulation of donor beef cows. In Trial 1, multiparous beef cows (n = 31) were randomly allotted to one of three treatments following superstimulation and removal of an intravaginal progesterone insert (CIDR). Cows in the Control group were inseminated at 12 and 24 h after onset of estrus. Cows in the Estradiol group were injected with estradiol-17β (1 mg, im) at 12 h and inseminated at 24 and 36 h after CIDR removal. Cows in the pLH36 group were injected with porcine LH (Lutropin, 12.5 mg, im) at 24 h and inseminated at 36 and 48 h after CIDR removal. Mean numbers of viable embryos were 7.8, 3.6 and 9.6 for Control, Estradiol and pLH36 treatment groups, respectively ($P > 0.10$). In Trial 2, multiparous beef cows (n = 22) were randomly allotted to one of three treatments following superstimulation and removal of a CIDR. Sixteen of the cows were superstimulated a second time approximately 50 days later and allotted to one of the two treatments that differed from the initial treatment group. Cows in the Control group were inseminated at 12 and 24 h after onset of estrus. Cows in the two pLH groups were

injected with porcine LH (Lutropin,12.5 mg, im) at 24 h after CIDR removal and were inseminated with either one unit of semen at 36 and 48 h (pLH36) or with two units of semen at 48 h (pLH48) after CIDR removal. Mean numbers of viable embryos were 3.0, 6.4 and 3.8 for Control, pLH36 and pLH48 treatment groups, respectively ($P > 0.10$). These data indicate that administration of pLH can facilitate use of fixed-time AI in superovulated beef cows without sacrificing embryo production.

The second study evaluated the efficacy of resynchronizing beef cow recipients using CIDR devices for only 7 or 14 d. Recipient cows received CIDRs either on the day of transfer (n = 88) or 7 d post-transfer (n = 230). All CIDRs were removed on d 21 and cows were observed for estrus between d 22 and 24. Cows that displayed estrus were ultrasounded on d 30, those cows not pregnant that possessed a CL had an embryo transferred that day. Cows were later examined for pregnancies approximately 23 to 30 d later. There were no differences in pregnancy rates between cows with 7 or 14 d CIDRs and therefore data were combined. Pregnancy rates at two different ranches indicate that beef cow recipients can be successfully resynchronized by insertion of a CIDR without compromising pregnancy rates of transferred embryos. At Center Ranch the pregnancy rate for the first transfer was 57% while the resynchronized group that received the second transfer had a pregnancy rate of 55%. At Mound Creek Ranch the first transfer of embryos produced 59% pregnancy rates while the second transfer had a pregnancy rate of 71%. No significant differences ($P > 0.05$) were observed between the pregnancy rates of the initial transfer and those of the resynchronized transfer using only

CIDRs, indicating that resynchronization using CIDRs can be used without reducing pregnancy rates.

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CHAPTER I

INTRODUCTION

Embryo transfer (ET) has been used commonly for over thirty years in the United States. Initially all collections and transfers were done surgically and later technology would allow for the collection and transfer of embryos using nonsurgical methods. Further advances were made in equipment ranging from collection filters, catheters, and transfer stylets. Hormones used for superovulation would go through several changes as well over the years as new products were being developed. Through all of this there have not been advances made in the number of transferable embryos collected by practitioners over the last twenty years. While input costs have soared, the efficiency of embryo collection and transfer have not markedly changed. Synchronization methods have been developed following those traditionally used in artificial insemination protocols that have allowed for more efficient use of recipients.

The ability to use fixed-time artificial insemination (AI) for superovulated beef females would save both time and labor in embryo transfer programs and could aide in the success of collecting embryos from donor females that usually do not display estrus. This would also allow for more control over the stages of embryos for collection days that are for cryopreservation of embryos.

This dissertation follows the style of Journal of Animal Science.

Recipient females play an integral role in embryo transfer programs. The success of most embryo transfer days hinges on the quality and synchrony of the cows synchronized to receive the embryos. The recipient cows usually represent the greatest cost because of the large numbers of females often required. The incorporation of the resynchronization programs for artificial insemination protocols could decrease the number of days between transfers, decrease the number of days cows are not pregnant and ultimately reduce overall costs to producers with large scale embryo transfer programs.

The hypothesis of the first study was that superstimulated beef cow donors could successfully be inseminated at fixed-time without compromising embryo production. The specific objectives of this study were to compare timing of ovulation and embryo production achieved by traditional superstimulatory and AI methods based upon detection of estrus, with the addition of either porcine LH or estradiol-17β to the protocol in order to facilitate timed AI in superovulated beef cows.

The second study was designed to determine if beef cows could successfully be resynchronized for a second embryo transfer utilizing only CIDRs. The specific objectives were to compare pregnancy rates between: 7- vs. 14-d CIDR duration, fresh vs. frozen embryos transferred, the two ranches, and 1st transfer vs. resynchronized transfer.

CHAPTER II

REVIEW OF LITERATURE

Introduction

The practice of bovine embryo transfer has undergone several changes since commercialization in the early 1970's. At that time, most ET was performed surgically with donor females of Continental breeds in order to produce greater numbers of these cattle while avoiding the time and expense of quarantines associated with importation of cattle into the United States. Use of ET has expanded to nearly every breed of cattle and is almost always performed non-surgically. Embryo transfer continues to be utilized by select ranchers and breeders to propagate genetically valuable offspring.

The actual process of collecting and transferring embryos is the end result of several preceding events. Often, a donor female will first display a reference estrus, then receive superovulatory injections of follicle stimulating hormone (FSH), which will be followed by an injection of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) to induce estrus, and then the female will be AI at 12 and 24 h post-onset of estrus without regard to time of day or night. The onset of estrus is followed by the preovulatory surge of luteinizing hormone (LH) and this is critical to the time of ovulation. Due to the variability in onset of estrus in beef females, this results in AI of cows at all hours of the day and night, especially when groups of eight or more cows are being synchronized to have embryos collected on the same day. The ability to achieve a tighter synchrony and enable breeding of cows at a specific, desired time would have great benefits to those performing the AI for ET.

After embryos are collected they must be either frozen for transfer at a later date or transferred fresh. Transferring of embryos involves the synchronization of recipient females that must be synchronized with the donor females so that the embryos are placed in a uterine environment of the recipient that coincides with the age of the embryos. Maintaining large numbers of recipient cows can become expensive. As the duration that the recipient cows are not pregnant increases, the cost per pregnancy also increases. The recent idea of resynchronization is an attempt to shorten the number of days that females are not pregnant, by resynchronizing all of the females using a technique that does not interrupt pregnancy, and transferring embryos a second time into females that are not pregnant from the first transfer.

Embryo transfer is the common term that usually is used to refer to the entire process from collection of the embryos until the transfer of those embryos. These processes involve very precise timing from: the initial superovulation of the donor female, AI of the donor cow, collection of the embryos, and finally transfer of the embryos into cows at a similar stage of the estrous cycle. Methods to optimize these steps have been studied for over 30 yr. The following is a discussion of embryo transfer and select hormones and products that are involved and/or have been used to control timing of the previously mentioned events and estrous synchrony of beef cows. This review will begin with the estrous cycle and describe the follicular and luteal phases, then review embryo transfer and resynchronization.

Estrous Cycle

Polyestrous females, such as the cow, have continuous cycles throughout the year. The bovine estrus cycle ranges from 18 to 24 d with an average of 21 d. This allows the female to have repeated opportunities throughout the breeding season or year to become pregnant.

The estrous cycle is divided into two phases, the follicular and the luteal phase. Each phase is named after the dominant ovarian structure that is present during that phase. The follicular phase is described as the time from the regression of the previous corpus luteum (CL) to the time of ovulation. This phase in a bovine female is relatively short and comprises of approximately 20% of the length of the cycle. During the follicular phase, the follicle is the predominant structure present on the ovary, and the main steroid hormone that it secretes is estradiol. Initial formation of the CL occurs immediately following ovulation. The follicular cells undergo changes to become luteal cells and transition away from producing estradiol to producing progesterone. The luteal phase will account for 80% of the duration of the estrous cycle. During this time follicles continue to grow and regress, but will not produce high levels of estradiol or ovulate (reviewed by Senger, 2003).

Follicular Phase

In cattle, like most mammalian species, a large pool of primordial follicles exists in the ovary of the female at birth. Erickson (1966) quantified the number of follicles throughout the lifespan of a cow to be greater than 100,000, and there were a few

hundred growing follicles on the ovary at any given time. The stages that occur during each wave of follicular development are recruitment, selection, and dominance. These were the terms that were originally used by Hodgen in 1982 (reviewed by Lucy, 2007). Recruitment was described as the initial cohort of follicles that transitioned from primordial to primary follicles and began growing and producing estradiol. Many of these recruited follicles will undergo atresia, those that survive will be selected and continue to grow. Many of these selected follicles will also regress until in most cases, one follicle becomes dominant. Cattle typically have two or three waves of follicular growth during an estrous cycle. Once the first wave becomes atretic at mid-cycle, under the influence of progesterone, a second wave begins. Cows that possess a two-wave cycle will then have a follicle that reaches dominance and proceeds to ovulate. Cows that have three-wave cycles will have the second wave become atretic and a third wave will begin. A follicle from the third wave will become dominant and eventually ovulate after regression of the CL or removal of progesterone. Two-wave cows generally have shorter estrous cycles because the length of the luteal phase is shorter than those that possess a third wave (Fortune et al., 1991; Lucy et al., 1992).

Increased blood FSH concentrations have been observed coinciding with enhanced follicular growth (Schams et al., 1977). Adams et al. (1992) used ultrasonography to correlate the relationship between follicular waves and the FSH concentrations in the blood. Concentrations of FSH increased during and after the LH surge and the postovulatory surge of FSH initiated the first follicular wave, then a

second wave appeared at midcycle when the first wave became atretic (reviewed by Lucy, 2007).

Initial follicular growth from the primordial to the primary stage is not FSH dependent. Follicle stimulating hormone does play a role after growth to the primary follicle stage as FSH receptors have been shown to be present in primary and secondary follicles (Bao and Garverick, 1998). These FSH receptors emerge soon after formation of the granulosa cell layer (Bao and Garverick, 1998) and the LH receptors appear with the formation of the theca cell layer. Garverick et al. (2002) demonstrated that the bovine follicle can grow to 4 mm in diameter without FSH, but follicular growth thereafter was FSH dependent. Results further indicated that these follicles (> 4 mm in diameter) were those that comprised the cohort that participates in the follicular wave. Increases in blood FSH concentration drive FSH-dependent follicles (4 to 5 mm) into larger diameter size classes and promote estradiol synthesis by the follicles. The enlarged FSH-dependent follicles are subjected to the selection process (Lucy, 2007).

While follicles that participate in the recruitment stage of the follicular wave are FSH-dependent, follicles that are selected to become dominant are dependent upon LH. The FSH receptor continues to be expressed in the granulosa cells during development (Bao et al., 1997). Xu et al. (1995) believed that the shift from FSH to LH dependence was a pivotal point in the development of the dominant follicle as the dominant follicle would then be able to possibly starve the other follicles from further development. Follicle stimulating hormone secretion is inhibited by estradiol and inhibin while LH secretion is not. This mechanism however is overridden in superovulated females as

exogenous FSH is continuously supplied, and therefore more than one follicle is able to establish dominance regardless of the inhibitory effects of estradiol and inhibin on FSH secretion from the anterior pituitary.

Corpus Luteum

The CL is a transient endocrine gland which forms from follicular granulosa and theca cells on the ovary after ovulation. The primary function of the CL is synthesis and secretion of progesterone, which is required for establishment and maintenance of pregnancy in all mammals. Inadequate progesterone production or premature luteolysis causes embryonic mortality and loss. Niswender and Nett (1994) reported that a significant proportion of the 25 to 55% embryonic mortality rate occurring in mammalian females results from inadequate luteal function. Progesterone secreted by the CL has an important role in the reproductive process because it is required for endometrial secretory changes, early embryonic development, implantation, and maintenance of a viable pregnancy (Vega and Devoto, 1997). Luteal regression is necessary for the cyclicity of reproductive processes (Vega and Devoto, 1997). By regulating the serum concentration of progesterone, time of ovulation in livestock species can be controlled (Britt, 1979).

Under the influence of the preovulatory surge of LH from the anterior pituitary gland, the mature follicle ruptures and expels the ovum (reviewed by McCracken et al., 1999). After follicular rupture, there is a dramatic infolding of the follicular wall that presumably facilitates migration of fibroblasts, endothelial cells, and theca interna cells

into the central regions of the developing CL. Tissue remodeling and cellular migration are facilitated by the breakdown of the basement membrane that separates the avascular granulosa layer from the theca interna layer (O'Shea et al., 1980). Granulosa and theca cells that have been luteinized in response to luteotropins transform from estrogen-producing cells to cells primarily producing progesterone (Davis and Rueda, 2002). During luteinization, formation of the CL resembles formation of a tumor in that it increases in weight approximately 20-fold (Smith et al., 1994). Unlike tumor formation, however, the rapid growth is halted during the mid-luteal phase, yet the mechanisms regulating this phenomenon remain unknown.

Morphologically, the CL is comprised of at least four types of cells: small and large steroidogenic cells, capillary endothelial cells, and fibroblasts (Wiltbank, 1994). The steroidogenic cells are classified as either large luteal cells (LLC) or small luteal cells (SLC), as determined by their size. Both of these cell types produce progesterone but are regulated by different mechanisms (Hansel et al., 1991). Small luteal cells are of theca cell origin and respond to LH with increased secretion of progesterone via activation of the protein kinase A second messenger pathway (Wiltbank, 1994). Small luteal cells make up 20% of total CL volume and account for 25% of total luteal cells (Wiltbank, 1994). Large luteal cells, of granulosa cell origin, contain receptors for prostaglandin (PG) $F_{2\alpha}$ and mediate the luteolytic action of this hormone (Fitz et al., 1982; Wiltbank, 1994). Large luteal cells represent nearly 40% of the total volume, but only account for 10% of the cell numbers within the CL (Wiltbank, 1994). Nearly 80% of progesterone is synthesized by LLC (Niswender et al., 1985), which have exceptional

steroidogenic and protein-secreting capacity (Wiltbank, 1994). Capillary endothelial cells represent 10% the volume but nearly 50% of the total cell numbers within the CL (Wiltbank, 1994).

Almost a hundred years ago, Andres Prenant hypothesized that the CL must have an endocrine function because of "its abundant vascularity, a sign by which the histologist characterizes a gland of internal secretion pouring its products into the internal environment of the organism via the blood" (Prenant, 1898; as reviewed by Wiltbank, 1994). New blood vessels are formed by sprouting from established vessels, a process known as angiogenesis. Angiogenesis takes place under physiological conditions such as wound healing, ovarian follicular development, CL formation, and endometrial growth (reviewed by Hanahan and Folkman, 1996). The proliferation of capillaries during angiogenesis involves a number of processes, including basement membrane breakdown, endothelial cell migration, endothelial cell proliferation, and development of capillary lumina (Folkman and Klagsburn, 1987).

The mature CL receives most of the ovarian blood supply and ovarian blood flow is highly correlated with the rate of progesterone secretion (Nett and Niswender, 1981). This high rate of blood flow to the CL could be essential not only for removal of progesterone from the CL into the systemic circulation but also for the efficient delivery of hormones, nutrients, and substrates to the CL (Wiltbank, 1994). The exchange between the luteal cells and the bloodstream is also facilitated by the highly fenestrated nature of the luteal capillaries, which provides substantial permeability to large proteins. Therefore, the high rate of luteal blood flow, the exaggerated plasma membrane surface

area on the vascular side of luteal cells, and the highly permeable nature of the luteal capillaries allow for facile exchange of proteins and hormones between the luteal cells and vasculature (Wiltbank, 1994).

Investigators have speculated that a wide variety of hormones act on the CL by regulating luteal blood flow including LH, estradiol, progesterone, vasopressin, oxytocin (OT), $PGF_{2\alpha}$, PGE_2, catecholamines, prolactin, and histamine (Wiltbank, 1994). The hormone primarily responsible for maintaining function of the CL in the bovine and ovine is LH. Immunoneutralization of LH in sheep caused a significant decrease in total ovarian blood flow (20% reduction by 24 h) and serum progesterone concentrations (40%; Niswender et al., 1976).

Prostaglandin $F_{2\alpha}$ or catecholamines decrease blood flow to the CL (Niswender et al., 1973; Nett et al., 1976; Nett and Niswender, 1981). Some of these effects may be due to constriction of larger arteries or arterioles, and others may be due to alterations in systemic blood pressure. These decreases in blood flow are believed to be important during luteolysis ($PGF_{2\alpha}$) or during moments of stress (catecholamines), but it is still unclear which hormones, if any, cause vasodilation assuring substantial luteal blood flow (Wiltbank, 1994).

Progesterone

Progesterone is essential for establishment and maintenance of pregnancy. Length of the reproductive cycle is also governed in part by progesterone. During the follicular phase, circulating levels of progesterone are low, while rising concentrations of

estradiol act on the hypothalamus and anterior pituitary gland to stimulate high frequency pulses of LH. The elevated serum concentrations of LH drive follicular development to the point of ovulation (reviewed by Lucy et al., 1992). After ovulation, the CL produces high circulating concentrations of progesterone, which block surges of GnRH from the hypothalamus (Kasa-Vubu et al., 1992) and reduce the number of receptors for GnRH by downregulating mRNA encoding the receptor for GnRH in the anterior pituitary gland (Laws et al., 1990; Bauer-Dantoin et al., 1995). The net effect of decreased GnRH secretion and GnRH receptor numbers is restriction of LH secretion to low frequency pulses resulting in reduced mean serum concentrations of LH.

All steroid hormones, including progesterone, are derived from the 27-carbon structure, cholesterol. The most common sources of cholesterol in domestic animals for progesterone are high and low density lipoproteins (HDL and LDL; Ohashi et al., 1982; Pate and Condon, 1982). Once inside the cell, cholesterol can be used for steroidogenesis or esterfied with long-chain fatty acids and stored as cholesterol esters in lipid droplets. When needed for steroidogenesis, free cholesterol is transported to the outer mitochondrial membrane by an intact cytoskeleton and then to the inner mitochondrial membrane by steroidogenic acute regulatory protein (StAR; Clark et al., 1994; Stocco and Clark, 1996; Stocco, 1998).

In the first step in progesterone synthesis, cholesterol (27 C) is converted to pregnenolone (21 C) by cytochrome P450 side chain cleavage complex ($P450_{SCC}$) in the inner mitochondrial membrane. The mRNA encoding cytochrome $P450_{SCC}$ and adrenodoxin, an additional carrier responsible for conveying electrons from reduced

pyridine nucleotides to cytochrome $P450_{SCC}$ and molecular oxygen (reviewed by Saez, 1994), were detected within stage I, II, and III of bovine corpora lutea (early, early-mid, and late-mid luteal phase; Rodgers et al., 1987). Conversion of cholesterol to pregnenolone by side chain cleavage enzyme was originally thought to be the rate-limiting step in progesterone synthesis. Hydroxylated intermediates such as 25-hydroxycholesterol, 22R-hydroxycholesterol, and 20α-hydroxycholesterol, all of which can rapidly diffuse across the mitochondrial membrane to the cytochrome $P450_{SCC}$ enzyme, are freely converted to pregnenolone in steroidogenic cells (Lambeth et al., 1982; Tuckey 1992). Emerging evidence has shown that cholesterol transport across the mitochondrial membrane by StAR is the rate-limiting step in steroidogenesis (Stocco and Clark, 1996). Steady-state concentrations of mRNA encoding StAR or StAR protein peak in the estrous cycle (Juengel et al., 1995; Pescador et al., 1996). Removal of the ovine pituitary during early luteal development severely reduced mRNA encoding StAR and replacement with LH or growth hormone (GH) prevented this decrease (Juengel et al., 1995). Expression of mRNA encoding StAR and StAR protein appear essential for maximal secretion of progesterone in the bovine (Pescador et al., 1996). After cholesterol is converted to pregnenolone, pregnenolone is converted to progesterone by the action of 3β-hydroxysteroid dehydrogenase/Δ^5,Δ^4 isomerase (3β-HSD), in the smooth endoplasmic reticulum and progesterone is released into the bloodstream (reviewed by Niswender et al., 2000).

Stimulation by LH is critical for the long-term steroidogenic capability of luteal cells, including maintenance of normal amounts of mRNA encoding 3β-HSD,

cytochrome $P450_{SCC}$ enzyme, and StAR (Juengel et al., 1995). Wiltbank et al. (1991) reported LH binding to its receptor stimulated progesterone secretion in small luteal cells through the protein kinase A pathway. Binding of LH to its receptor activates adenylate cyclase, which converts adenosine triphosphate to cyclic adenosine monophosphate, which ultimately activates protein kinase A (Wiltbank et al., 1991). Exogenous GH also increases concentrations of progesterone in serum, and GH is necessary for normal luteal development in ewes (Juengel et al., 1995).

The targets of progesterone are the reproductive tract and the hypothalamic-pituitary axis. The actions of progesterone on the reproductive tract are to prepare it for the initiation and maintenance of pregnancy. Progesterone serves as a negative feedback mechanism in the hypothalamus to suppress gonadotropins thereby preventing further follicular development (Kasa-Vubu et al., 1992). In the absence of pregnancy or at the end of pregnancy, the CL will cease to produce progesterone and the tissue mass will decrease in size accompanied by a loss in cellular integrity (Davis and Rueda, 2002).

Progestins

In the 1940s, studies showed that estrous cycles of cows could be altered by the administration of exogenous progesterone. Further studies used progestins delivered by injections, sponges, and feed additives to delay and control estrus of cattle for more concise synchronization. Increasing the duration of progesterone administration was found to result in tighter synchrony and increased rates of synchronization (Odde, 1990); however, administration of these progestins for long durations (greater than 14 d)

compromised pregnancy rates and were no longer considered acceptable (Zimbelman and Smith, 1966). Older studies focused on artificial insemination and natural service of females and not embryo transfer. Progestins were shown to not have a negative affect on embryos transferred by several groups inducing persistent dominant follicles with long-term progestin supplementation (Wehrman et al., 1996; Wehrman et al., 1997; Baruselli et al., 2000; Bo et al., 2002).

Administration of low levels of progesterone result in high frequency LH pulses. Estradiol concentration is higher prior to estrus, and the onset of the preovulatory LH surge occurs earlier after the removal of a low dose supplementation compared to those receiving higher doses of progesterone (Wehrman et al., 1993).

Prostaglandin $F_{2\alpha}$

The effects of $PGF_{2\alpha}$ on the CL have long been recognized as important in regulation of the estrous cycle. Prostaglandin $F_{2\alpha}$ of uterine origin is the hormone responsible for luteal regression in livestock (McCracken, 1971). Hysterectomy of heifers and ewes resulted in delayed luteolysis (Wiltbank and Casida, 1956). Luteal regression consists of two processes, functional and structural luteolysis that differ in their temporal and mechanistic features (Meidan et al., 1999). Functional luteolysis refers to the rapid decline in luteal progesterone, while structural luteolysis describes events leading to the structural demise of the CL (Meidan et al., 1999).

In ruminant species, $PGF_{2\alpha}$ enters the ovarian artery from the utero-ovarian vein, via a countercurrent exchange mechanism. This allows $PGF_{2\alpha}$ to travel to the ovarian

artery without entering the pulmonary circulation where it would be enzymatically inactivated by the lungs (Piper et al., 1970).

Four steps are involved in production of $PGF_{2\alpha}$. First, arachadonic acid must be produced by phospholipases such as phospholipase A_2 (Rosenthal et al., 1995). Then arachadonic acid is converted into PGG_2 by cyclooxygenase (COX) I and II. Next, PGG_2 is converted to PGH_2 by the PGF synthase enzymes PGHS-1 and PGHS-2 (Hershman, 1994; Karim et al., 1996). Finally, conversion of PGH_2 to $PGF_{2\alpha}$ is facilitated by PGF synthase (Watanabe et al., 1988).

Prostaglandin $F_{2\alpha}$ acts by binding to specific receptors localized to large steroidogenic luteal cells where the receptors are tightly coupled to the phosphoinositide signal transduction pathway (Fitz et al., 1982). These receptors belong to the seven-transmembrane family of G protein-coupled receptors. Upon binding to high-affinity receptors, $PGF_{2\alpha}$ induces activation of membrane-bound phospholipase C via a stimulatory G protein. Phospholipase C catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate to inositol 1,4,5-trisphosphate (IP_3) and 1,2-diacylglycerol (DAG; Berridge and Irvine, 1984). Increased cytosolic concentrations of IP_3 result in release of free Ca^{2+} from the smooth endoplasmic reticulum to the cytoplasmic compartment (Berridge and Irvine, 1984). Increased free Ca^{2+} and DAG, localized to the plasma membrane, stimulate the catalytic activity of Ca^{2+}-dependent protein kinase C. Protein kinase C is believed to mediate many of the antisteroidogenic actions of $PGF_{2\alpha}$ in LLC (McGuire et al., 1994). Purified LLC cultured with $PGF_{2\alpha}$ exhibit an influx of extracellular Ca^{2+} to the cytosolic compartment (Wiltbank et al., 1989).

It has been shown that a number of intracellular mechanisms are involved in $PGF_{2\alpha}$ inhibitory effects on progesterone synthesis (Estevez et al., 2002). Carlson et al. (1982) found that structural changes in the cellular membranes during luteolysis in the bovine CL, related to the loss in cellular function. Prostaglandin $F_{2\alpha}$ reduces the amount of sterol carrier protein and rapidly decreases mRNA encoding StAR, which may affect the transport of cholesterol into the mitochondria (McLean et al., 1995). Additionally, $PGF_{2\alpha}$ decreases levels of mRNA encoding 3β-HSD (Hawkins et al., 1993).

Prostaglandin $F_{2\alpha}$ also reduces blood flow to the CL and may cause luteolysis by depriving the gland of nutrients (Phariss et al., 1970; McCracken, 1971). A reduction of blood flow to the ewe CL after administration of $PGF_{2\alpha}$ has been demonstrated (Niswender et al., 1973; Nett et al., 1976). Prostaglandin $F_{2\alpha}$ causes degeneration of luteal endothelial cells (O'Shea et al., 1977), resulting in reduction of capillary density (Braden et al., 1988), thus reducing blood flow to the luteal parenchyma. In large and small luteal cells, morphological changes do not become evident until 24 to 36 h after exposure to $PGF_{2\alpha}$ (Sawyer et al., 1990), even though the steroidogenic capacity of these cells are been reduced by this time. Although uterine $PGF_{2\alpha}$ has been implicated as the primary luteolysin in the ruminant CL, recent studies suggest that other paracrine and autocrine factors in the ovary may be necessary for $PGF_{2\alpha}$-induced luteolysis to occur (Motta et al., 2001).

In the bovine CL, an increase in the number of $PGF_{2\alpha}$ receptors occurs during the late luteal phase, which could explain the reduced luteolytic action of $PGF_{2\alpha}$ when given in the early luteal phase (Rao et al., 1979). However, Wiltbank et al. (1995)

demonstrated that luteolytic mechanisms not functional in the early CL were not due to lack of $PGF_{2\alpha}$ receptors as high affinity $PGF_{2\alpha}$ receptors were present in the early bovine CL. Decreased sensitivity to extragonadal $PGF_{2\alpha}$ in the early CL depends on locally produced PGs, OT, progesterone (Skarzynski and Okuda, 1999; Miyamoto et al., 1993) and(or) nitric oxide (NO; Skarzynski et al., 2000). Luteal OT, $PGF_{2\alpha}$, and progesterone are major components of an autocrine/paracrine positive feedback loop in the bovine CL (Skarzynski et al., 2000), acting to upregulate function of $PGF_{2\alpha}$ receptors and the $PGF_{2\alpha}$-intracellular calcium ($[Ca^{2+}]_i$)-protein kinase C cascade (Skarzynski et al., 2000). This feedback loop may be involved in a protective mechanism against premature luteolysis in the early CL. When cows possessing nonresponsive (4-d) or responsive (11-d) CL were administered $PGF_{2\alpha}$, the concentrations of luteal ascorbate and mRNA encoding 3β-HSD and $PGF_{2\alpha}$ receptors were decreased, while mRNA encoding insulin-like growth factor binding protein-1 was increased in both 4- and 11-d CL. These data indicate that the d 4 CL were not completely unresponsive to $PGF_{2\alpha}$ actions (Tsai and Wiltbank, 1998; Pate, 2003). On d 11, the bovine CL responded to $PGF_{2\alpha}$ with an increase in steady state concentrations of mRNA encoding COX-2, whereas a decrease in mRNA encoding COX-2 was observed in d 4 CL (Tsai and Wiltbank, 1998). Pate (2003) suggested that the ability of the CL to establish a positive autocrine feedback loop of endogenous PG synthesis is a key component for the acquisition of luteolytic capability. These results support the proposal of Milvae (1986) that stimulation of luteal PG synthesis is a critical factor in luteal regression. Indomethacin, a PG synthetase inhibitor that blocks the COX pathway of metabolism of arachadonic acid, was used by

Milvae (1986) in cows 4 to 6 d post-ovulation to demonstrate that blocking luteal PG caused a reduction in cycle length. Griffeth et al. (2002) used indomethacin delivered by atrigel in an intraluteal implant to determine if luteal PGs were needed for luteolysis in ovine. They found that when luteal PGs were blocked structural regression failed to occur, although functional regression, serum progesterone less than 1.0 ng/mL, had occurred.

Oxytocin

Estrogen and progesterone indirectly control endometrial $PGF_{2\alpha}$ secretion by regulating formation of OT receptors in several nonprimate species. Oxytocin is a peptide hormone synthesized as part of a high molecular weight precursor in the hypothalamic magnocellular neurons where OT packaged into secretory granules, stored and released from the posterior pituitary (reviewed by McCracken et al., 1999). Wathes and Swann (1982) were the first to suggest that OT synthesis occurs in the peripheral tissue after high concentrations of OT were found in ovine luteal tissue. Estrogen and progesterone, in addition to regulating uterine $PGF_{2\alpha}$, may also play a role in control of OT secretion from the neurohypophysis during luteolysis, a hypothesis supported by data demonstrating that peaks of OT occurred synchronously with peaks of PG metabolite in the peripheral blood of sheep during luteolysis (Fairclough et al., 1980). There is also evidence that OT is involved in the processes leading to luteal regression. Exogenous $PGF_{2\alpha}$ was observed to elicit a gradual increase in peripheral plasma levels of OT in humans and pigs, suggesting that $PGF_{2\alpha}$ caused a release of OT from the

posterior pituitary (Gillepsie et al., 1972). Wilks et al. (1969) demonstrated that administration of OT early in the estrous cycle of cattle causes luteolysis. At luteolysis, OT receptor concentrations in the endometrium are high, and OT secreted by the CL itself acts on the endometrium to stimulate $PGF_{2\alpha}$ secretion (Flint and Sheldrick, 1982). As $PGF_{2\alpha}$ further stimulates the CL to secrete OT, a positive feedback loop is established between the CL and the uterus (Flint and Sheldrick, 1982). In the early- and mid-luteal phase of the cycle, endometrial OT receptor concentrations are low and $PGF_{2\alpha}$ secretion is inhibited. The appearance of OT in the endometrium causes positive feedback, which results in the episodic secretion of $PGF_{2\alpha}$ (Sheldrick and Flint, 1985).

Luteinizing Hormone

The follicular phase of the normal estrous cycle is characterized by an increase in pulsatile secretion of LH that culminates in the preovulatory surge of LH (Rahe et al., 1990). An increase in the frequency of LH pulse secretion during the follicular phase is important for the final growth and maturation of the ovulatory follicle and its oocyte (Hyttel et al., 1997). The preovulatory surge release of LH is also believed to be the signal for resumption of meiosis in oocytes present in ovulatory follicles, and induces ovulatory follicles to ovulate. This surge of LH is the critical time point that will control the time of ovulation, and therefore determine the exact time at which a female should be mated to optimize fertilization rates. The timing of estrus, the endogenous LH surge, and ovulation are especially variable among superstimulated donor cattle (Callesen et al., 1986; D'Occhio et al., 1997), and a significant inverse relationship has been reported

between ovulation rate and the interval from administration of $PGF_{2\alpha}$ to the LH surge in donors (reviewed by Bo et al., 2006). Control of the LH surge in superstimulated cattle has been studied. The main attempt has been to postpone the LH surge in relation to $PGF_{2\alpha}$ treatment, allowing more follicles to develop and obtain the capacity to ovulate. Several attempts have used GnRH antagonists (Rieger et al., 1989) to block LH and GnRH agonists (D'Occhio et al., 1997) to downregulate and desensitize the pituitary receptors to GnRH. The latter study used Lutropin to mimic the LH surge and cause ovulation after endogenous LH had been downregulated, and found no difference in the number of viable embryos collected between controls and treated donor cows. Although not increasing the number of embryos, this approach does permit more scheduling of the procedures. However, the GnRH agonist implant, Deslorelin, is not available for cattle in most countries (reviewed by Bo et al., 2006).

Manipulation of the Follicular Wave for Superstimulated Donor Cows

Conventional protocols for ovarian superstimulation were most successful when treatments were initiated between 8-12 d after estrus (Bo et al., 1995). Many of these original studies did not evaluate follicular status of animals that were treated with gonadotropins. During these trials, monitoring follicular development using ultrasonography was, in most cases not available in most laboratories (Bo et al., 2006).

Follicular wave emergence occurs 8-12 d after estrus (Ginther et al, 1989). It has been demonstrated that superovulatory responses are highest when gonadotropin treatments were initiated at the time of follicular wave emergence. Follicular wave

emergence has been synchronized using pLH or GnRH, but these methods have resulted in lower numbers of transferable embryos when compared to estradiol treatments or follicular ablation (Deyo et al., 2001). Estradiol 17B has been the most common approach in many countries for synchronizing follicular wave emergence at the time of the administration of the CIDR or other progesterone treatments (Bo et al., 2006). Other estrogen esters have been used as well because of the limitations in the availability of estradiol 17B in some countries.

Fixed-time Insemination of Superstimulated Beef Donors

The ability to detect estrus in any embryo transfer or artificial insemination program is very crucial for success. Detection of estrus can frequently be the cause for failure in these programs because of the difficulty in the ability to accurately detect estrus. In superstimulated cattle, there is greater variability in the timing of the endogenous LH surge and ovulation in cattle (Callasen et al., 1986; D'Occhio et al., 1997) and a significant inverse relationship has been reported between the ovulation rates and the interval from PGF administration to the LH surge in donor cows (Greve et al., 1983; reviewed by Bo et al., 2006). Bo et al. (2006) conducted a series of experiments to evaluate the time breeding of donor cows using GnRH or pLH at various hours after PGF administration and progesterone removal. In these studies it was concluded that cows could be inseminated without regard to onset of estrus at 12 and 24 h after the administration of pLH or GnRH. These studies were done using an 8-shot superovulatory protocol and CIDR removal on either days 6.5, 7, or 7.5 after CIDR

insertion. The number of transferable embryos did not differ between the controls, which were conventionally bred 12 and 24 h after progesterone removal, or the GnRH- or pLH-treated cows. Efforts have also been made to postpone the LH surge in superovulated donor cows. GnRH agonists were used by Reiger et al. (1990) to block the LH surge, and then cattle were given hCG after PGF administration. The number of transferable embryos was not different between control groups and those treated with hCG 72 h after PGF administration.

Most work performed regarding fixed-time insemination of superovulated donor cows has been done in South America using Bos indicus cattle. A protocol named P-36 was developed in Brazil by Nogueira and Barros (2003). In this protocol the progesterone releasing device is removed 36 h post-PGF treatment and ovulation is induced 12 h later using LH. Since ovulation occurred between 24 and 36 h after pLH treatment, fixed-time insemination was performed 12 and 24 h after pLH injection (Noguiera and Barros, 2003). In another study by Baruselli et al. (2005) the administration of pLH was performed at 12 and 24 h post-CIDR removal, and cows were inseminated at 12 and 24 h later. In these studies also using Nelore donors, the number of degenerate embryos increased and the viable embryos decreased when the administration of pLH was delayed until 24 h post-CIDR removal. Baruselli et al. (2008) performed a study in which the P-36 protocol was used where cows were inseminated at 12 and 24 h post-pLH or at 16 h post-pLH with a single insemination. In this study, there were no differences between one or two fixed-time inseminations.

Nogueira et al. (2007) used two different types of progesterone delivery systems and dosages of pLH of 12.5 and 25 mg. After CIDR removal, pLH was administered 12 h later and AI was performed 12 and 24 h post-pLH administration. In these studies using Nelore cattle, an average greater than 13 viable embryos were obtained per collection. This average is well above the accepted averages that range from 6 to 7 viable embryos per collection.

Ultrasonography

The application of transrectal real-time ultrasonography to the study of bovine reproduction represents a technological breakthrough that has revolutionized knowledge of reproductive biology (Fricke, 2002). The non-invasive methodology of ultrasonography allows for the visualization of the reproductive tract during all stages of estrous and menstrual cycles, as well as gestation. Ultrasonography uses high-frequency sound waves that produce images. Liquids do not reflect sound waves back and appear black, where tissues reflect waves and appear as varying shades of gray depending on density (Fricke, 2002).

Early integration of ultrasound technology by the livestock industry included applications such as transvaginal follicular aspiration and oocyte recovery (Pieterse et al., 1988; Pieterse et al., 1991; Meintjes et al., 1993) and as a complimentary technology for embryo transfer procedures (Fricke, 2002).

Embryo Transfer

Embryo transfer is one of the fastest, most economical ways to produce multiple offspring from the same dam and sire. The commercial embryo transfer industry began in the United States in the early 1970's with the introduction of continental breeds of cattle that were at that time in high demand and short supply. The potential of embryo transfer was soon recognized as the techniques improved over the next several years, and the ability to freeze and thaw embryos successfully was soon discovered. Currently over 500,000 embryos are collected and transferred annually (Thibier, 2003). In the early years of embryo transfer embryos were collected surgically and transferred into recipients immediately. Development of nonsurgical techniques to collect embryos from cattle accelerated the growth of a commercial embryo transfer industry. Currently nearly 50% of embryos collected in the United States are frozen (Thibier, 2003).

While the technique offers many benefits, embryo transfer does require more intense management of females. First, the female must be superovulated, which usually involves decreasing dosages of FSH administered over 4 d to rescue follicles that would normally undergo follicular death (atresia). On the third day of FSH injections, $PGF_{2\alpha}$ is administered to cause regression of the corpus luteum. In approximately 2 d, the female will display estrus and will then be AI at 12 and 24 h post-onset of estrus. Seven days after the onset of estrus, embryos are collected. After collection, embryos are classified by stages and grades, and viable embryos are either transferred fresh or frozen to be transferred at a later date. When embryos are transferred into a recipient female, she must be in synchrony with the age of the embryos collected from the donor female. The

uterine environment must be at the correct stage of the estrous cycle to maximize the chances of embryo survival. Therefore, it is critical that synchronization between donor and recipient cows be no more than 24 h ± to maximize success in bovine embryo transfer.

Resynchronization

The greatest economic cost of ET is the procurement and upkeep of recipients (Looney et al., 2006). Not every recipient that receives an embryo will become pregnant. Pregnancy rates for ET can range from the 40 percentile to 90 percentile depending on whether the embryos were transferred fresh or frozen-thawed before transfer, and upon many other physiological and environmental factors. Embryonic death following ET causes enormous economic losses in the embryo transfer and cattle industry. These embryonic losses have been related to the inability of the CL to secrete substantial levels of progesterone to prepare the endometrium for embryo implantation and maintain pregnancy.

A greater concentration of progesterone increases the capacity of the conceptus to produce interferon-τ, a main regulator of pregnancy recognition (Binelli et al., 2001). Progesterone has also been shown to be a suppressor of apoptosis in bovine luteal cells (Okuda et al., 2004). Baruselli et al. (2000) and Nasser et al. (2004) have shown that eCG given on the day of follicular wave emergence increased the number of CLs, plasma progesterone, conception rates, and pregnancy rates in bovine embryo transfer recipients. Similar results have been shown following administration of eCG on day 7

post-insemination (Rajamahendran and Sianangama, 1992) and on the day of embryo transfer. In addition, Binelli et al. (2001) stated that a larger preovulatory follicle may generate a larger CL that will secrete more progesterone and thereby have a positive effect on pregnancy recognition and pregnancy rates following ET (Baruselli et al., 2000). The classical method to obtain a larger preovulatory follicle is to promote follicular growth under low circulating concentrations of progesterone or progestagens (Kinder et al., 1996). Kinder et al. (1996) showed that the administration of progesterone to achieve subluteal concentrations of circulating progesterone (1 to 2 ng/ml of plasma) allowed LH pulses to occur more frequently than during the mid-luteal phase. Luteinizing hormone released in these patterns results in the prolonged growth and maintenance of a dominant follicle and elevated plasma concentrations of estradiol-17β (Sirois and Fortune, 1990).

Cattle that do not become pregnant after embryo transfer typically return to estrus 18 to 24 days after the initial synchronized estrus. To facilitate the synchronized transfer of embryos a second time, the recipients would need to be resynchronized. Resynchronization has been shown to be successful with the use of the intravaginal progesterone-releasing device (Stevenson and Mee, 1991; Purvis and Whittier, 1997; Van Cleef et al., 1996; Chenault et al., 2003; Macmillan and Peterson 1993, and Stevenson et al., 2003) which may also increase conception rates at the resynchronized estrus (Stevenson and Mee, 1991). The CIDRs are usually inserted around d 14 post-estrus and removed on d 21, and the females typically return to estrus 36 h post-CIDR removal. The majority of nonpregnant females display estrus within a 24 h period.

Progestin supplementation is effective in preventing spontaneous estrus before its removal (Stevenson et al., 2003).

A major concern involving resynchronization was whether the first estrus after AI or ET could be resynchronized without having adverse affects on previously established pregnancies. Stevenson et al. (2003) showed that there was no strong evidence suggesting that the implementation of resynchronization protocols was disruptive to established pregnancies in four experiments in both cows and heifers.

Another concern of using resynchronization protocols in which CIDRs remain inserted in females after luteolysis has taken place is the occurrence or possibility of persistent dominant follicles forming and ovulating. The development of a persistent dominant follicle has a detrimental effect on conception rates after artificial insemination due to an altered oviductal environment (Binelli et al., 1999) and/or premature maturation of the oocyte (Mihm et al., 1994; Revah and Butler 1996). However this is not necessarily true for embryo transfer as the oocyte quality is not important as it would not be fertilized. Results have shown that the development of persistent dominant follicles in recipient cows did not alter the pregnancy rates (Wehrman et al., 1996; Wehrman et al., 1997; Baruselli et al., 2000; Bo et al., 2002). Mantovani et al. (2005) showed a decrease in conception rates when using females with persistent dominant follicles that were allowed to ovulate after progesterone removal. However, in this study an embryo was transferred into all cows that possessed CL. In the previously mentioned studies, embryos were only transferred to those recipients that displayed a visual estrus following removal of the CIDR.

CHAPTER III

FIXED-TIME INSEMINATION OF PORCINE LUTEINIZING HORMONE-TREATED SUPEROVULATED BEEF COWS

Introduction

Estrus detection is an important aspect of donor management that is crucial for successful embryo transfer (ET) programs in which cows are superovulated and bred at both embryo transfer facilities and on the farm. Failure of successful detection of estrus can be detrimental to the results of an embryo transfer program. Technologies such as Heatwatch have made estrus detection less complicated and removed much of the guesswork; however, donor females still require insemination usually at two time periods 12 and 24 h after the onset of estrus and in some practices three separate inseminations usually 12 h apart. These practices usually result in insemination technicians performing AI at various hours of the night and morning in order to facilitate the proper time of insemination. In commercial AI work much research has been done

over the past three decades to better synchronize cattle for AI, fixed-time insemination has been developed and is a popular choice now for many commercial and purebred operations. More recently, work has been done to determine ways these same types of practices can be used to AI donor cows that have been superovulated. Most work in this area has primarily been involved with Bos indicus cattle. Several groups (Nogueira and Barros, 2003; Baruselli et al., 2005; Bo et al., 2006) have been able to successfully use endogenous LH or GnRH to fixed-time inseminate superstimulated beef donor cows.

The objectives of this study were to compare timing of ovulation and embryo production between traditional superstimulatory and AI methods and the use of additional treatments of estradiol-17β and pLH to facilitate timed AI in superovulated beef cows.

Materials and Methods

Trial 1

Experimental Design

Multiparous, cross-bred beef cows (n = 31; predominantly Bos taurus, with no more than ¼ Bos indicus influence) weighing approximately 500 to 600 kg and of similar body condition (BCS 5 to 6)) were randomly allotted to one of three treatments: Control (n = 10), Estradiol (n = 11), or pLH36 (n = 10). On Day 0, cows were selected by transrectal ultrasound evaluation for presence of a corpus luteum (> 10 mm) prior to CIDR® (1.38 g progesterone, Pfizer Animal Health, Kalamazoo, MI) insertion. An injection containing 2.5 mg (im) estradiol-17β and 50 mg progesterone (Combo; Med Shop Total Care Pharmacey, Inc., Longview, TX; im) was administered to all cows at CIDR insertion (Day 0; Figure 1). Superstimulatory treatments with Folltropin-V (Bioniche Animal Health, Belleville, Ontario, Canada) began on Day 4 for 3.5 days (236 mg NIH-FSH-P10; im) in decreasing dosages (0700 and 1900 h daily, im). An injection (im) of a $PGF_{2\alpha}$ analogue that contained 625 µg D-cloprostenol (Estrumate®; Schering-Plough, Union, NJ, USA) was administered (im) at 0700 and 1900 h on Day 6, and the CIDR was removed at 0700 h on Day 7, followed by the final injection of Folltropin. In addition, a patch that contained a radio transmitter for recording time and duration of each mount (HeatWatch®; CowChips LLC, Denver, CO, USA) was applied to the rump of the cow to optimize detection of estrus.

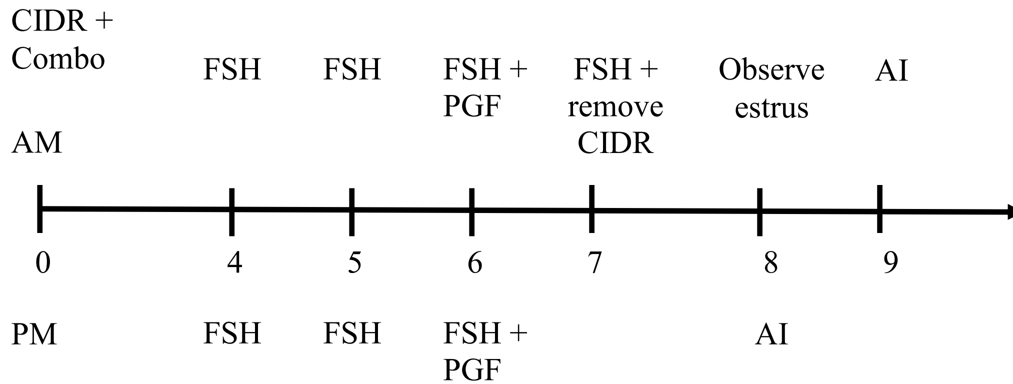
Donors in the Control treatment were inseminated at 12 and 24 h post-onset of estrus (first mount) with frozen-thawed semen. Donors in the Estradiol treatment

received 1 mg estradiol-17β (Med Shop Total Care Pharmacey, Inc., Longview, TX, im) 12 h after CIDR removal and insemination with one unit of semen at 24 and 36 h after CIDR removal. Donors in the pLH36 (Lutropin®-V; Bioniche Animal Health, Belleville, Ontario, Canada) treatment were administered 12.5 mg pLH (2.5 cc, im) at 24 h and were inseminated at 36 h and 48 h after CIDR removal with one unit of semen. All inseminations were performed with semen obtained from a single sire and by the same technician.

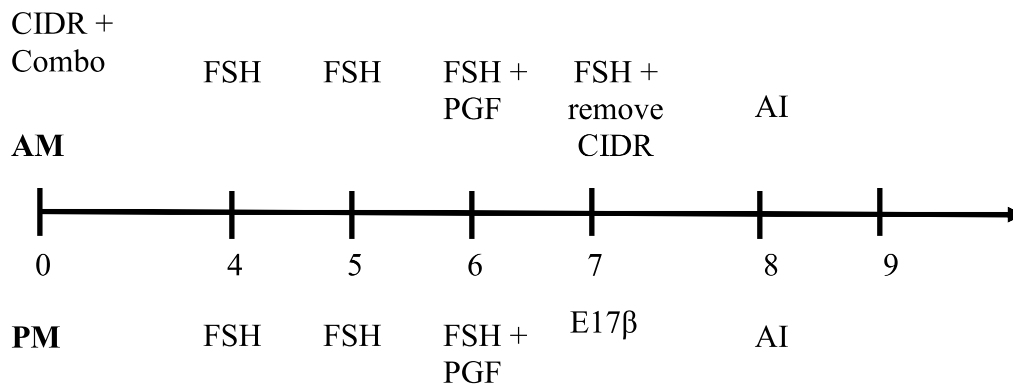
All cows were examined by ultrasonography (Aloka 500v ultrasound console; Corometrics Medical Products; North Wallingford, CT; 7.5 MHz transducer) at 24, 32, 40, 48, 56, 64, and 72 h post-CIDR removal to determine the time and distribution of ovulations. Ovulation was determined by the disappearance of follicles (> 12 mm) from the prior examination. Embryos were collected (EZ way filter w/Y Junction tubing, PETS, Canton, TX; 18 and 20 gauge Foley Catheter, Bardia, Covington, GA; Lactated Ringer's, Hospira Inc., Lake Forest, IL; Bovine Serum Albumin, ICPbio LTD., Auckland, New Zealand) 8 d after CIDR removal, to collect approximately d 7 embryo.

The numbers of unfertilized ova, degenerate embryos, viable embryos (Grades 1 or 2) and total ova were recorded. Treatment effects on intervals to onset of estrus and to ovulation, and on numbers of total ova, degenerate embryo, unfertilized ova, and viable embryos were analyzed by Proc GLM of SAS (SAS; Cary, NC, USA).

Control



E17β



pLH36

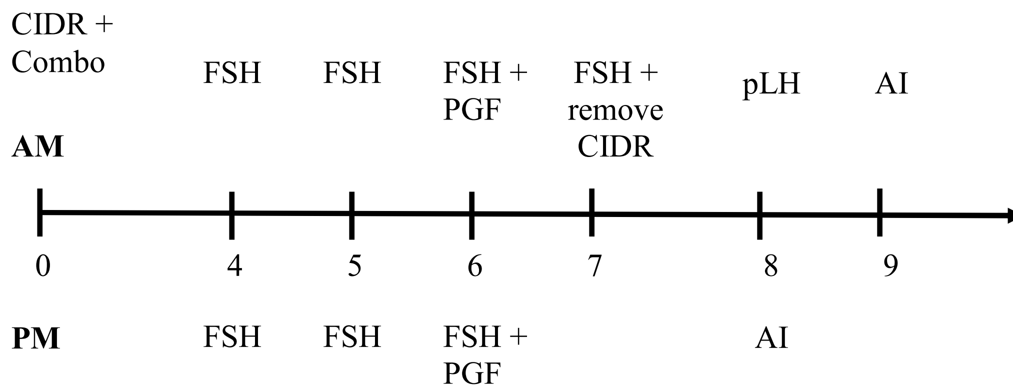


Figure 1. Schematic diagrams of superstimulation protocols for Control, E17β, and pLH36 for Trial 1.

Trial 2

Embryos were collected after superstimulation of multiparous crossbred cows (n = 22; predominantly Bos taurus, with no more than ¼ Bos indicus influence). A second embryo embryo collection was conducted with sixteen of the cows approximately 50 days after the first collection. Cows were randomly allotted to one of three treatments: Control (n = 11), pLH36 (n = 12), or pLH48 (n = 15). On Day 0, cows were selected by transrectal ultrasound evaluation for presence of a corpus luteum (> 10 mm) prior to CIDR (1.38 g progesterone) insertion. An injection containing 2.5 mg (im) estradiol-17β and 50 mg progesterone (im) was administered at the time of CIDR insertion (Figure 2). Superstimulatory treatments with Folltropin-V began on Day 4 for 3.5 days (236 mg NIH-FSH-P10) in decreasing dosages (0700 and 1900 h daily, im). Cows were injected (im) with D-cloprostenol (625 μg) at 0700 and 1900 h on Day 6, and the CIDRs were removed at 0700 h of Day 7, followed by the final injection of Folltropin. In addition, HeatWatch patches were applied to optimize heat detection.

Donors in the Control treatment were inseminated at 12 and 24 h post-onset of

estrus (first mount) with frozen-thawed semen. Donors that were administered pLH were divided into two treatment groups. Donors were administered 12.5 mg of pLH (2.5 cc, im) at 24 h after CIDR removal and were inseminated at 36 and 48 h after CIDR removal with one unit (pLH36) or at 48 h after CIDR removal with two units of semen (pLH48). All inseminations were performed with semen obtained from a single sire and by the same technician. Cows that were superstimulated a second time were equally allotted into one of the two treatment groups that differed from their initial treatment group.

The numbers of unfertilized ova, degenerate embryos, viable embryos (Grades 1 or 2) and total ova were recorded. Treatment effects on intervals to onset of estrus and to ovulation, and on numbers of total ova, degenerate embryos, unfertilized ova, and viable embryos were analyzed by Proc GLM of SAS (SAS; Cary, NC, USA).

Control

AM	CIDR + Combo	FSH	FSH	FSH + PGF	FSH + remove CIDR	Observe estrus	AI
	0	4	5	6	7	8	9
PM		FSH	FSH	FSH + PGF		AI	

pLH36

AM	CIDR + Combo	FSH	FSH	FSH + PGF	FSH + remove CIDR	pLH	AI
	0	4	5	6	7	8	9
PM		FSH	FSH	FSH + PGF		AI	

pLH48

AM	CIDR + Combo	FSH	FSH	FSH + PGF	FSH + remove CIDR	pLH	AI 2X
	0	4	5	6	7	8	9
PM		FSH	FSH	FSH + PGF			

Figure 2. Schematic diagrams of superstimulation protocols for Control, pLH36, and pLH48 for Trial 2.

Results

Trial 1

In the first year significant differences were not detected between the three treatment groups for total ova (P = 0.14), viable embryos (P = 0.12), unfertilized ova ((P = 0.55), and degenerate embryos (P =0.18). There were, however numerical differences between the three treatments for total ova and viable embryos collected. Total ova collected were 11, 6, and 11.6 for Contol, Estradiol, and Lutropin, respectively (Table 1). Viable embryos collected for the three treatments were 7.8, 3.6, and 9.6 for Control, Estradiol, and Lutropin respectively. These data indicate that the Lutropin treated cows may have embryo production comparable to that of the control cows, however, while not significant, the Estradiol treated cows did have numerically lower total numbers of ovulations and fewer viable embryos, however, the number of cows per treatment group were not high enough to detect any significant differences between these treatment groups. The time of estrus post-CIDR removal and ovulation time did not differ between the treatment groups (P = 0.80; Table 2). The distribution of ovulation was calculated as the percentage of follicles that ovulated by the time periods of 48, 56, 64, and 72 h after CIDR removal. The three treatment groups varied in the percentage of follicles that ovulated, by 56 h after CIDR removal, the percentage of follicles observed that had ovulated were 66.6, 67.2, and 83.6% for Control, Estradiol, and Lutropin, respectively (Table 3).

Table 1. Mean (±SE) number of total ova, unfertilized ova, degenerate embryos, and viable embryos by treatment in Trial 1[a]

Treatment	(n)	Total ova	Unfertilized ova	Degenerate embryos	Viable Embryos
Control[b]	10	11.0 ± 2.4	0.9 ± 0.6	2.3 ± 0.7	7.8 ± 2.3
Estradiol[c]	11	6.0 ± 1.1	1.4 ± 0.5	1.0 ± 0.4	3.6 ± 1.2
pLH36[d]	10	11.6 ± 2.8	0.7 ± 0.4	1.3 ± 0.3	9.6 ± 2.6

[a]Means within column do not differ ($P > 0.10$).
[b]Inseminated at 12 and 24 h after onset of estrus.
[c]Estradiol-17β (1 mg, im) 12 h after CIDR removal and inseminated at 24 and 36 h after CIDR removal.
[d]Porcine LH (12.5 mg, im) 24 h after CIDR removal and inseminated at 36 and 48 h after CIDR removal.

Table 2. Mean (±SE) interval from CIDR removal to onset of estrus and to ovulation by treatment in Trial 1[a]

		Interval from CIDR removal to:	
Treatment	(n)	Onset of estrus (h)	Ovulation (h)
Control[b]	10	25.1 ± 1.5	58.5 ± 1.6
Estradiol[c]	11	22.0 ± 1.8	57.5 ± 1.7
pLH36[d]	10	26.3 ± 2.0	57.3 ± 1.0

[a]Means within columns do not differ ($P > 0.10$).
[b]Inseminated at 12 and 24 h after onset of estrus.
[c]Estradiol-17β (1 mg, im) 12 h after CIDR removal and inseminated at 24 and 36 h after CIDR removal.
[d]Porcine LH (12.5 mg, im) 24 h after CIDR removal and inseminated at 36 and 48 h after CIDR removal.

Table 3. Percentage of follicles ovulated at 48, 56, 64, and 72 h after CIDR removal by treatment.

		Percentage of follicles ovulated at each time interval (h)			
Treatment	(n)	48	56	64	72
Control[a]	10	25.9	40.8	28.9	4.4
Estradiol[b]	11	13.3	53.9	27.9	4.9
pLH36[c]	10	7.2	76.4	15.7	0.7

[a]Inseminated at 12 and 24 h after onset of estrus.
[b]Estradiol-17β (1 mg, im) 12 h after CIDR removal and inseminated at 24 and 36 h after CIDR removal.
[c]Porcine LH (12.5 mg, im) 24 h after CIDR removal and inseminated at 36 and 48 h after CIDR removal.

Trial 2

Differences were not detected between the three treatment groups (Table 4) for total ova (P = 0.83), viable embryos (P = 0.21), unfertilized ova (0.41), and degenerate embryos (0.71). Mean numbers of viable embryos did not differ among treatments, but the maximum response was observed in the pLH36 group. Mean numbers of total ova, unfertilized ova, degenerate embryos and viable embryos were not different (P = 0.50) between the two trials. Therefore, the data were combined to compare the two treatments that were common across both trials (Control and pLH36). Mean number of total ova was similar (P = 0.87) between Control and pLH36 groups (Table 5). Although not significantly different (P = 0.15), there was a favorable response in mean number of viable embryos for cows in the pLH36 group compared with the Control group.

Table 4. Mean (±SE) number of total ova, unfertilized ova, degenerate embryos, and viable embryos by treatment in Trial 2[a]

Treatment	(n)	Total ova	Unfertilized Ova	Degenerate embryos	Viable Embryos
Control[b]	11	8.8 ± 2.1	3.4 ± 1.1	2.4 ± 1.1	3.0 ± 1.2
pLH36[c]	15	10.6 ± 2.1	2.0 ± 0.6	2.2 ± 0.9	6.4 ± 1.5
pLH48[d]	12	10.0 ± 2.3	2.5 ± 0.6	3.7 ± 1.4	3.8 ± 1.5

[a]Means within column do not differ (P > 0.10).
[b]Inseminated at 12 and 24 h after onset of estrus.
[c]Porcine LH (12.5 mg, im) 24 h after CIDR removal and inseminated at 36 and 48 h after CIDR removal.
[d]Porcine LH (12.5 mg, im) 24 h after CIDR removal and inseminated with two units of semen at 48 h after CIDR removal

Table 5. Mean (±SE) number of total ova, unfertilized ova, degenerate embryos, and viable embryos pooled across Trials 1 and 2 by treatment[a]

Treatment	(n)	Total ova	Unfertilized ova	Degenerate embryos	Viable embryos
Control[b]	21	9.9 ± 1.6	2.2 ± 0.7	2.4 ± 0.7	5.4 ± 1.4
pLH3	25	11.0 ± 1.6	1.5 ± 0.4	1.8 ± 0.6	7.6 ± 1.4

[a]Means within columns do not differ ($P > 0.10$).
[b]Inseminated at 12 and 24 h after onset of estrus.
[c]Porcine LH (12.5 mg, im) 24 h after CIDR removal and inseminated at 36 and 48 h after CIDR removal.

Discussion

The superovulation and breeding protocols used in these experiments were designed to facilitate the fixed-time breeding of superovulated beef cows. Donor females are typically inseminated 12 and 24 h after the onset of estrus, and there is great variability in the onset of estrus following PGF administration and/or PGF administration and CIDR removal. The wide range in timing of the onset of estrus is accompanied by a wide range in times that females need to be inseminated in order to maximize embryo production. Results of current study indicate pLH36 treatment produced as many or more viable embryos after Fixed-time AI as did the control treatment. Bo et al. (2006) conducted a series of experiments to evaluate timed breeding of Bos indicus donor cows using GnRH or pLH at various hours after PGF administration and progesterone removal. Bo et al. (2006) concluded that cows could be mated without regard to onset of estrus at 12 and 24 h after the administration of pLH or GnRH. Differences between these studies and the present study included an 8-injection superovulatory protocol and CIDR removal on either days 6.5, 7, or 7.5 after CIDR insertion. The number of transferable embryos did not differ between the controls (conventionally inseminated at 12 and 24 h after CIDR removal) and the GnRH or pLH treated cows.

D'Occhio et al. (1999) blocked LH release in superstimulated beef heifers using Deslorelin and induced ovulation with 25 mg of pLH (im) at 60 h post-CIDR removal with a single insemination at 12 h post-onset of estrus compared to control heifers that were inseminated at 0, 12 and 24 h post-onset of estrus. There was no difference

between the fixed time AI and three inseminations at 12 h intervals in heifers. Nogueira et al. (2007) used two different types of progesterone delivery systems and dosages of pLH of 12.5 and 25 mg in Nelore cattle. The average number of viable Nelore embryos exceeded 13 per collection, which is well above the accepted average (range from 6 to 7 viable embryos per collection). The CIDRs were not removed from the Nelore females until the evening of the last day in contrast to the morning removal of CIDRs in the current study. The pLH was administered 12 h after CIDR removal and the Nelore females were inseminated at 12 and 24 h post pLH administration. Estrus is known to be shorter in Bos indicus cattle compared to Bos taurus cattle, and Bos indicus cattle have a greater tendency to display estrus during the night (Bo et al., 2003). In the current study, the administration of pLH was delayed 12 h relative to the previous studies because of the differences in duration of estrus between Bos taurus and Bos indicus cattle.

The aforementioned results are similar to those of the present study in which differences were not detected between the conventional methods of programming donor cows and the protocols designed for insemination at a fixed-time after Lutropin administration. The current study used a 7-injection FSH stimulation protocol with CIDR removal 7 d post-insertion. In the both the first and second trials, numbers of viable embryos for the pLH36 group were at least as equivalent to that of the Controls, Estradiol, and pLH48. As expected, the pLH treatment did not reduce the variability in embryo production among individual cows (range from 0 to 29 viable embryos). There do not appear to be any detrimental effects of fixed-time insemination of donor cows following pLH administration. No differences were observed among the treatment

groups for interval to onset of estrus post- CIDR removal, ovulation time, or number of ovulations.

Noguiera et al (2007) cited unpublished data in which ovulation occurred over a 24-h period beginning 24 to 48 h after the final FSH administration in a standard superovulation protocol, where no exogenous hormones were used to induce ovulation. This large range is believed to be caused by the variation in timing of the LH surge in superstimulated cattle. The reange in timing of the endogenous LH surge has been reported from 22 to 54 h post-PGF administration in superovulated cattle possessing a CL (Callesen et al., 1986) and from 44 to 56 h post-PGF in females containing a progesterone releasing device (D'Occhio et al., 1997). In the current study, ovulations may have occurred early relative to semen delivery in the pLH48 treatment group which could explain the numerically lower viable embryo production compared to the Control and the pLH36 groups. The aim of this study was to control timing of the LH surge and subsequent ovulation to enhance the ability to inseminate donor cows and increase the probability of maximizing embryo production and reducing the number of inseminations.

These data, in combination with prior studies, indicate that it is possible to eliminate or minimize detection of estrus in donor programs and allow for the timed breeding of females during normal employment hours. This would be a significant benefit to both "in-clinic" and "on the farm" embryo transfer businesses and would simplify procedures for clients in managing their own donor cows for embryo collection.

Implications

These results indicate that donor females can be successfully inseminated using a fixed-time system following treatment with a CIDR and an injection of pLH . Numbers of total ova and viable embryos obtained from the pLH36 groups were equal to or greater than those of control females that were inseminated 12 and 24 h after the onset of estrus. Administration of Lutropin resulted in a more efficient method of AI without requiring detection of estrus. In the current studies, inseminations were performed in the pLH group at 1900 and 700 h. This is usually not the case as cows are bred at nearly every hour of the day and night. Without sacrificing embryo production, fixed-time AI can successfully be used to manage donor females and reduce the labor requirement. Under the conditions of the present study, estradiol treatment did not appear to be an option for timed breeding of donors since the lowest number of total ova and viable embryos were produced by this protocol. Additional research is warranted to determine the factors that influence the efficacy of pLH in fixed-time insemination of superstimulated donor females and to determine if bovine females may become refractory to pLH if it is repeatedly used.

CHAPTER IV

RESYNCHRONIZATION OF BEEF COWS FOR FIXED-TIME EMBRYO TRANSFER

Introduction

The maintenance of recipients can in many cases be the most costly part of an embryo transfer program. Cows are often maintained for the sole purpose of serving as recipients and other times are purchased at various times of the year and allowed to calve or in many instances simply purchased not pregnant and entered into the program. There are many advantages and disadvantages to various approaches of maintaining recipient herds. With the increases in input costs the last several years minimizing the days that cows are not pregnant may be very beneficial in reducing costs for ranches or farms that utilize embryo transfer.

Resynchronization has been shown to be successful with the use of the intravaginal progesterone-releasing device (Stevenson and Mee, 1991; Purvis and Whittier, 1997; Van Cleef et al., 1996; Chenault et al., 2003; Macmillan and Peterson 1993, and Stevenson et al., 2003) and in addition to resynchronizing females, may

increase conception rates at the resynchronized estrus (Stevenson and Mee, 1991). Most of this work has been done in AI studies, but the synchronization of estrus is not different between AI and ET. The CIDRs are usually inserted near d 14 post-estrus and removed on d 21, and the females typically return to estrus 36 h post-CIDR removal and the majority of nonpregnant females display estrus within a 24 h period. Progestin supplementation is effective in preventing spontaneous estrus before its removal (Stevenson et al., 2003).

The purpose of this study was to determine if beef cows could successfully be resynchronized for a second embryo transfer utilizing only CIDRs. The specific objectives were to compare pregnancy rates between: 7 vs. 14 d CIDR, fresh vs. frozen embryos transferred, 1st and 2nd transfers between two ranches, and overall 1st transfer vs. resynchronized transfer.

Materials and Methods

Brangus and Brangus x Angus crossbred cows (n=391) maintained on a ranch setting with coastal bermudagrass pasture with supplementation were used in this study. Recipient cows were subjected to a hormonal regimen to synchronize estrus/ovulation to facilitate embryo transfer and then resynchronize the same cows to transfer embryos 23 d later into cows not pregnant in an attempt to reduce the number of days in between transfers observed in common synchronization protocols (Figure 3).

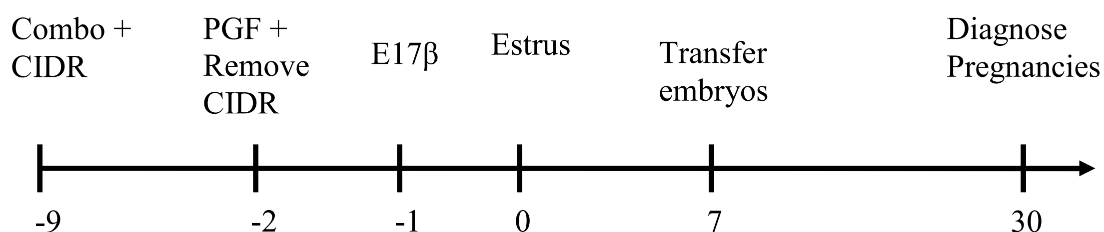


Figure 3. Schematic diagram of estrous synchronization protocol typically used for recipient cows followed by the transfer date and pregnancy determination usually conducted at 30 d pregnant.

Cows were then assigned to one of two treatment groups. Both groups received a CIDR (1.38 g progesterone) insertion plus 2.5 mg (i.m.) estradiol-17β and 50 mg progesterone (Combo; Med Shop Total Care Pharmacy, Inc., Longview, TX; i.m.) on d -9. On d -2 CIDRs were removed and an injection of $PGF_{2\alpha}$ (Lutalyse, Pharmacia & Upjohn Co., Kalamazoo, MI , 25 mg, i.m.) given, followed by an injection of 1 mg

estradiol-17β (Med Shop Total Care Pharmacy, Inc., Longview, TX; i.m.) 24 h later. Cows were expected in estrus the following day, d 0. Seven days post-estrus, cows were evaluated for transfer of embryos if they possessed a palpable corpus luteum. A single embryo, either fresh or frozen in ethylene glycol for direct transfer, was transferred to each eligible recipient cow on day 7. In the first group (n=88; Table 5) at Center Ranch, cows received a CIDR on the day of transfer (Figure 4; Table 6), and the second group (n=94) received a CIDR 7 d post-transfer (Figure 5). All CIDRs were removed after 14 d on d 21 and cows not pregnant were expected to return to estrus on approximately d 23. Cows' tail heads were painted at time of CIDR removal as visual aid indicators and cattle were monitored for estrus in the morning and evening after CIDR removal until d 24. At Mound Creek Ranch all cows had CIDRs inserted on the day of transfer and removed 14 d later. Then all cows were monitored for pregnancy by transrectal ultrasonography on d 30. All cows not pregnant and with a palpable corpus luteum from the second estrus had an embryo transferred. Each cow was then processed for pregnancy detection 40 to 60 days later.

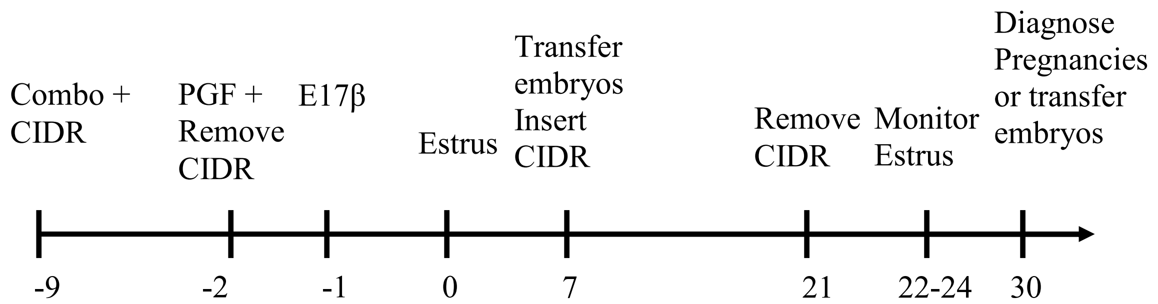


Figure 4. Schematic diagram for synchronization of recipients and the resynchronization of recipients for Group 1 with CIDR insertion the same day as initial embryo transferred, followed by the monitoring of estrus for a second transfer.

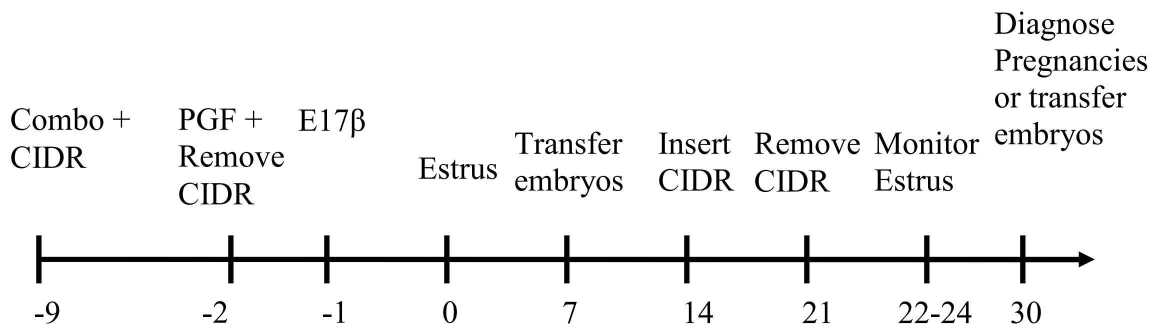


Figure 5. Schematic diagram for synchronization of recipients and the resynchronization of recipients for Group 2 with CIDR insertion 7 d post transfer of initial embryo transferred, followed by the monitoring of estrus for a second transfer.

Table 6. The number of cows at the two locations receiving CIDR treatment for 7 or 14 d.

Ranch	Duration of CIDR treatment (d)	
	7	14
Center Ranch	94	88
Mound Creek Ranch	136	

Data were collected on pregnancy status and used to determine first service and second service pregnancy rates. Overall treatment effects on pregnancy rates of 7 d CIDR, 14 d CIDR were compared to pregnancy rates of initial transfers. Data were analyzed between first and resynchronized transfers and between ranches using Chi Square analysis.

Results

Results from the two Center ranch locations that used 7 or 14 d CIDRs for resynchronization were not different (P = 0.6) and therefore the data sets were combined. The pregnancy rates for the initial transfers at Center Ranch were 57% (Table 7) for fresh embryos transferred over two different days at both locations. The two groups that were resynchronized had a combined pregnancy rate of 55%. There was no significant difference in the pregnancy rates between the first and second transfer at this location (P = 0.68). At the Mound Creek location the pregnancy rate for the initial transfer of embryos was 59% for fresh and frozen embryos combined. For the first and second transfer at Mound Creek Ranch, greater than 50% of the embryos transferred each day were frozen, direct transfer embryos frozen in ethylene glycol. The resynchronized transfer at Mound Creek ranch produced pregnancy rates of 72%. While the numerical number was higher for the percent pregnant, there was no significant difference

Table 7. Resynchronization data from Mound Creek Ranch and Center Ranch comparing the pregnancy rates from the initial transfer of embryos using conventional synchronization methods and the CIDR only resynchronization transfers[a].

Ranch	1st Transfer (%) (n)	2nd Transfer (%) (n)
Mound Creek	59[a] (136)	72[a] (39)
Center Ranch	57[a] (182)	55[a] (55)

[a]Means within and rows do not differ P>0.05.

Table 8. First transfer pregnancy rates compared to second transfer pregnancy rates across pooled data from the two ranches.

	1ST Transfer (n)	2nd Transfer (n)
Center Ranch and Mound Creek Ranch	56 (318)	62 (94)

[a]Means within rows do not differ (P > 0.10)

(P = 0.06). At Mound Creek Ranch cows that had embryos transferred to them the second time were once again administered CIDRs and this group was resynchronized a second time. In this limited recipient group transferred to (n = 9) the pregnancy rate was 77%.

Pregnancy rates were different between the two locations for the initial transfer (P = 0.60) or the resynchronized transfer (P = 0.14), therefore the data sets were pooled together. The pregnancy rates of the first transfer did not differ from the resynchronized transfers (Table 8; P = 0.38). The pregnancy rates for the first transfer for both ranches were 56% compared to 62% for the resynchronized transfers. Stages and grades of embryos were similar across both ranches and across transfers within ranches (Table 9).

At Center Ranch, of the resynchronized cows determined to not be pregnant after the first transfer, 55 recipients possessed an acceptable CL (Table 10), while 22 (29%) cows were rejected for not possessing CL. At Mound Creek Ranch 39 resynchronized

cows were transferred to while 18 (32%) cows were rejected for not having acceptable CL.

Table 9. Comparison of the grades and stages of embryos transferred at Mound Creek Ranch and Center Ranch for the 1st Transfer and the 2nd Transfer expressed as a percentage of the embryos transferred.

	Grades of embryos transferred			Stages of embryos transferred		
Ranch 1st or 2nd Transfer	1	2	3	4[a]	5[b]	6[c]
Mound Creek Ranch 1st Transfer	83	12	5	48	42	10
Mound Creek Ranch 2nd Transfer	72	25	3	45	45	10
Center Ranch 1st Transfer	71	26	4	68	29	3
Center Ranch 2nd Transfer	82	13	5	62	32	6

[a]Morula
[b]Early blastocyst
[c]Blastocyst

Table 10. Resynchronization data from Mound Creek Ranch and Center Ranch evaluating the number of recipient cows used for 2nd embryo transfers and the number of cows rejected for transfers.

Ranch	Received 2nd Embryo	Did Not Receive 2nd Embryo
Mound Creek Ranch	39	18
Center Ranch	55	22

Discussion

The resynchronization protocols used in this study were designed to minimize days between transfer dates and to decrease labor and input costs while not sacrificing pregnancy rates. Resynchronization has been used in AI with success, but published data on resynchronization of cows for embryo transfer remain limited. Several studies have demonstrated AI success rates for the resynchronized AI that were comparable to those of the initial synchronization (Stevenson and Mee, 1991; Purvis and Whittier, 1997; Van Cleef et al., 1996; Chenault et al., 2003; Macmillan and Peterson 1993, and Stevenson et al., 2003). In these studies CIDRs were removed at different times (18 to 21 d) to allow for AI.

The goal of the study was to remove the CIDR on day 21 of the cows' cycle with the thought that those cows that would have naturally possessed 18- to 20-d cycles would not be allowed to ovulate early. When the CIDRs was removed from the cows, those that displayed estrus began approximately 36 h post-CIDR removal and by 72 h post-CIDR removal (day 24 of cycle) all cows that were not pregnant had usually displayed estrus. There were instances in cows that did not display estrus that were not pregnant, but the incidence was never greater than 10% of the non-pregnant females. This window allows for fixed-time embryo transfer to occur on day 30, as all cows displaying estrus would fall into a ± 24 h window.

Differences were not detected at Center Ranch between the first and second transfers. The first transfer consisted of only fresh embryos while the second round of transfers in the resynchronized females had 54% frozen embryos transferred.

Numerically there was only a 2% difference on pregnancy rates and this could easily be accounted for by the number of frozen embryos transferred.

At the Mound Creek Ranch location there was no significant difference between the first or second transfers performed. The initial transfer was 81% frozen direct-transfer embryos and 19 % fresh embryos. The resynchronized recipients that received embryos had 77% frozen direct-transfer and 23% fresh embryos transferred. The pregnancy rate of the first transfer was 59% and the second transfer was 72%. This difference was not statistically different. In addition, this ranch decided to place embryos back into a small group of cows that were not pregnant after the second transfer. Of these 9 cows resynchronized a second time, 7 were pregnant (77%). While the number of transfers was extremely low, it did demonstrate that three transfers could be performed in a 46 d window and it appears that pregnancy rates were not reduced from the first transfer until the third. However, there are other factors involved such as nutrition and weather changes during the spring season of the year which were not accounted for in this study. Cows synchronized to receive embryos at these two locations did receive supplemental feed and the continuous supplementation over an extended period may account for some of the variability in the pregnancy rates. Previous results for synchronizing cows for a second and third transfer of embryos after cows were determined to not be pregnant by conventional synchronization protocols demonstrated a decrease in pregnancy rates of 12% between the first and second transfer and a decrease of 12% between the second and third transfer (Looney et al., 2006). These were large numbers of both fresh and frozen embryos. Perhaps with larger

number we would have seen this trend, but our data indicate that the results should not be lower that those previously reported and the benefit may simply be reducing the number of days in between transfers and subsequently the calving window of the embryo transfer calves. For many producers utilizing embryo transfer there are advantages to having shorter calving windows. The amount of attention devoted to calving can be decreased and many producers that have their own production sales can have cattle more uniform in size and age than if the birth dates are spread out across a greater number of days.

One concerning factor of this type of resynchronization is the cows that were rejected for inadequate CL that were previously transferred to and resynchronized. Unfortunately data was not obtainable on the number of cows rejected at the initial transfer at these ranches; therefore, more general data about cows rejected will be used to compare the rates that were observed. Looney (2006) published that the normal rate at which recipients were synchronized and did not receive embryos was approximately 11-12%. In this study at Center Ranch, of the resynchronized cows determined to not be pregnant after the first transfer, 29% of the cows were rejected for not possessing a CL and at Mound Creek Ranch 32% cows were rejected for not having acceptable CL. This was approximately 18 and 21 percentage points different, respectively. Another concern is that those cows that were initially rejected after the first transfer. These cows could not simply receive another CIDR for resynchronization and be expected to accept embryos for the next transfer. These cows needed to be resorted and set up accordingly to the initial protocol involving CIDR, Combo, PG, and Estradiol. However, in this

short window between transfers, these cows did not work as a group to receive embryos, and less that 25% of these cows would work for the next round of transfers. Many of these cows at the time of the initial transfer possessed follicles that were large enough to ovulate while some were simply anestrous. It may be more beneficial in the future to attempt to administer GnRH to these cows to cause formation of a CL before CIDR are reinserted 7 d later. Otherwise, these cows considered anestrous may need to be left out of the next round of transfers and placed on higher nutrition planes in order to facilitate the acceptance of an embryo at a later transfer date. It was observed that those cows that did not possess CL and had CIDR administered again after rejection, were not successful as a recipient group.

No differences were detected between cows receiving the CIDR on day of transfer or 7 d post transfer. There are benefits to each scheme, the CIDR on day of transfer allows for the cattle to not be handled until 14 d later when the CIDR are removed. This allows only one use of the CIDR though. The 7 d post transfer reinsertion of the CIDR allows for the CIDR to be used a second time if desired, this method does require another trip through the chute for cows and depending on the availability of labor and cost consciousness will probably determine which method producers would use.

Implications

These data indicate that cattle can successfully be resynchronized without reducing pregnancy rates. The pregnancy rates were not lower for the resynchronized cows at either ranch location than for first embryo transfer. This type of recycling of recipients may prove to increase efficiency in the number of days cows are not pregnant. There were problems however as we observed a higher number of rejected recipients among those resynchronized than is typically reported. The ability to perform three rounds of embryo transfer in a 46-d window does have many benefits and could be very useful in production scenarios. Further research is needed to determine more effective methods to utilize the cows that are rejected at the time of the first transfer.

CHAPTER V

SUMMARY

Results from these studies indicate that superovulated donor cows can successfully be fixed-time inseminated without compromising viable embryo production and recipient cows can be resynchronized to decrease the number of days between transfers without decreasing pregnancy rates. These results from the first study indicate that donor females can be successfully inseminated using a fixed-time system following treatment with a CIDR and an injection of pLH . Numbers of total ova and viable embryos obtained from the pLH36 groups were equal to or greater than those of control females that were inseminated 12 and 24 h after the onset of estrus. Administration of Lutropin resulted in a more efficient method of AI without requiring detection of estrus. In the current studies, inseminations were performed in the pLH group at 1900 and 700 h. This is usually not the case as cows are bred at nearly every hour of the day and night. Without sacrificing embryo production, fixed-time AI can successfully be used to manage donor females and reduce the labor requirement. Under the conditions of the present study, estradiol treatment did not appear to be an option for timed breeding of donors since the lowest number of total ova and viable embryos were produced by this protocol. Additional research is warranted to determine the factors that influence the efficacy of pLH in fixed-time insemination of superstimulated donor females and to determine if bovine females may become refractory to pLH if it is repeatedly used.

Data from the second study indicate that cattle can successfully be resynchronized without reducing pregnancy rates. The pregnancy rates were not lower

for the resynchronized cows at either ranch location than for first embryo transfer. This type of recycling of recipients may prove to increase efficiency in the number of days cows are not pregnant. There were problems however as we observed a higher number of rejected recipients among those resynchronized than is typically reported. The ability to perform three rounds of embryo transfer in a 46-d window does have many benefits and could be very useful in production scenarios. Further research is needed to determine more effective methods to utilize the cows that are rejected at the time of the first transfer.

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